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PACIFIC SALMON: A CONDUIT OF MARINE NUTRIENTS
AND ORGANIC MATTER TO STREAM ECOSYSTEMS

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PACIFIC SALMON: A CONDUIT OF MARINE NUTRIENTS AND ORGANIC MATTER TO STREAM ECOSYSTEMS.

By

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ABSTRACT

PACIFIC SALMON: A CONDUIT OF MARINE NUTRIENTS AND ORGANIC MATTER TO STREAM ECOSYSTEMS.

By

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The influence of marine-derived nutrients (MDN), delivered by spawning salmon, on Alaskan streams was determined by measuring the carbon and nitrogen isotopic composition of stream biota (fishes, macroinvertebrates, biofilm). Our main study site, Fish Creek (FC), was divided into two reaches, separated by a salmon barrier, and sampled before, during, and after spawning for two years. In Salmon Creek, we assessed the upstream-downstream study design by comparing two sites also separated by a barrier, but with no MDN influence. Isotopic differences in taxa from these two sites were negligible. All lower FC consumers demonstrated MDN incorporation during and after spawning and a "legacy" effect from previous subsidies. Moreover, primary consumers exhibited a rapid MDN response and an increased MDN incorporation downstream. Macroinvertebrate isotope values are an ideal tool for tracking MDN dynamics, but spatial and temporal variability in MDN incorporation should be considered before isotopes are utilized in fisheries management.

My thesis is *dedicated to my parents* who have made everything possible. You have always supported me to wander, stumble, and dream. I am forever grateful.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The annual return of anadromous salmon (*Oncorhynchus* spp.) has been a conspicuous feature of streams in the Pacific Northwest for thousands of years. Salmon deposit marine-derived nutrients into streams by excretion, gamete release, and subsequent decay of their own carcasses (Krokhin 1967; Brickell and Goering 1970; Mathisen et al. 1988). Therefore, salmon symbolize a biological link between marine, terrestrial and freshwater ecosystems and are a conduit of organic matter and nutrients transfer across ecosystem boundaries. A new paradigm emerges that delineates material flow from marine to freshwater ecosystems, which differs from the concept of material flow from the continents to the oceans being unidirectional (Milliman and Meade 1983).

Although assimilation of MDN by autotrophs occurs by remineralization and uptake, heterotrophic bacteria can obtain MDN via these mechanisms and direct uptake. Both of these groups contribute to biofilm Marine-derived nutrients can be available to macroinvertebrates and fish through trophic transfers initiating with biofilm and/or direct consumption of carcasses or carcass fragments within course particulate organic matter (CPOM) pool (Bilby et al. 1996).

Evidence for the incorporation of MDN into freshwater and terrestrial food webs is derived from a growing base of literature that concentrates on stable isotope analysis (Kline et al. 1990; Kline et al. 1993; Bilby et al. 1996; Johnston et al. 1997; Garman and Macko 1998; Ben-David et al 1997, 1998; Szepanski et al 1999; Hilderbrand et al 1999, Minakawa and Gara 1999). The basis for these studies is the observation that the isotopic composition of an organism reflects its food source. Anadromous salmon have a distinct

marine isotopic signature, typically greater than -20.0 ‰ for $\delta^{13}C$ and 11.0 ‰ for $\delta^{15}N$, which differs from both freshwater and terrestrial inputs that form the base of lotic food webs (Kline et al. 1990; Bilby et al. 1996; Doucett et al 1999; Johnston et al. 1997). As organic matter cycles through the biota there is a trophic fractionation or preferential incorporation of the heavy isotope during assimilation at each successive trophic level (Minagawa and Wada 1984; Peterson and Fry 1987). In general, trophic-related fractionation is about 3-4‰ and 0-1‰ for nitrogen and carbon, respectively (Deniro and Epstein 1978; DeNiro and Epstein 1981; Minagawa and Wada 1984; France 1994). Rapidly growing organisms, including insects, can quickly reflect the isotopic compositions of a new diet (Fry and Arnold 1982, Ostrom et al. 1997). Therefore, if stream organisms are incorporating MDN, and have high tissue turnover, should demonstrate a quick change in their isotope composition to reflect the new MDN diet.

Carbon and nitrogen isotope values have been used in concert to define the (1) estimates of the relative importance of organic matter sources to a consumer's diet at the base of the food web using mass balance equations and (2) trophic structure (McConnaughey and McRoy 1979; Harrigan et al. 1989; Johnston et al. 1987; MacAvoy et al. 2000; see review Phillips and Gregg 2001). Consequently, multiple isotope analysis is a powerful tool to examine marine-derived nutrient flow and incorporation in stream food webs.

Marine-derived nutrient subsidies have important implications for fisheries and ecosystem management. Microbial and algal growth are augmented with increased delivery of marine nutrients and organic carbon to streams (Richey et al. 1975; Wipfli et al. 1998; Fisher Wold and Hershey 1999). This in turn can increase prey abundance,

primarily invertebrates, which are an important component in juvenile fish diets (e.g. juvenile coho salmon). The additional secondary production in stream ecosystems should increase the freshwater survivorship of salmon, ultimately leading to elevated salmon production (Groot and Margolis 1991; Michael 1995; Johnston et al. 1997; Wipfli et al. 1999). Therefore, it has been proposed that salmon runs create marine nutrient subsidies that set forth a natural positive feedback system of nutrient deposition that is critical to the survival of salmon and important in sustaining the Pacific Northwest's oligotrophic freshwater food webs (Mathisen et al. 1988; Cederholm et al.1999). Recently Finney et al. (2001) and Bilby et al. (2001) proposed that a relationship between the abundance of spawning salmon and the stable isotope-based estimates of incorporation of MDN in stream and riparian biota can be used to determine an escapement level of salmon needed to sustain salmon runs.

The importance and urgency of studying MDN subsidies has recently been emphasized by managerial actions implemented in the Pacific Northwest. With limited quantitative guidelines, resource managers have begun to place spawned-out salmon carcasses into streams to presumably elevate stream productivity and ultimately salmon production (Levy 1997; Barnard 2000). In addition, slow-release briquettes (i.e. compressed, processed, salmon byproducts) and slow-release artificial fertilizers are being considered as potential salmon stock restoration tools (Ashley and Slaney 1997; Sterling et al. 2000). However, before we begin to make large-scale management decisions, the relationship between salmon subsidies and ecosystem responses need to be better understood. In order to understand the effects of MDN on stream ecosystems we

need to understand the incorporation of MDN into the food web in both a temporal and spatial context.

The aim of my study was to evaluate the relative importance of salmon on trophic dynamics and energy flow in Southeast Alaskan streams using stable isotope analysis (SIA). I compared the carbon and nitrogen isotope values of stream consumers in an upstream (reference) to downstream (treatment) reach in Fish Creek, AK during pre- and post-spawning periods of 2000 and 2001 and during spawning in 2001. Specifically, my objectives were four-fold: (1) characterize the marine-derived endmember, (2) evaluate the treatment/reference study design, (3) quantify MDN incorporation in major trophic components of stream ecosystems, (4) evaluate longitudinal variation in the isotope values of macroinvertebrates and (5) examine inter-annual variability in MDN incorporation. The ultimate goal of this study was to assess the relative importance of the MDN subsidy to the Fish Creek food web in both a spatial and temporal context.

METHODS

Study Site

Stable isotope samples were collected from two watersheds, Fish Creek and Salmon Creek in southeast Alaska during 2000 and 2001 (Fig. 1). Fish Creek is located on Douglas Island and is the primary focus of this paper. Fish Creek has an impassible waterfall, which prevents the upstream migration of salmon (i.e. salmon barrier). Thus, the stream was separated into an upper reach (reference) and a lower reach (treatment)

(Fig. 2). We termed the portion of the stream above and below the barrier as the upper and lower reach, respectively. Fish Creek experiences annual runs of Pink (*Oncorhynchus gorbuscha*), Chum (*O. keta*), and Chinook (*O. tshawytscha*) salmon. Pink and Chum salmon are the predominant species in terms of both biomass and abundance. Peak spawning occurs from late-July to August, depending upon species and stream discharge.

Salmon Creek provided an opportunity to evaluate the MDN study design. Both Fish and Salmon Creek are 3rd order streams dominated by snow melt and groundwater inputs, have watersheds of comparable sizes, 36 and 26 km^2, respectively, have similar gradients, and have a large amount of woody debris in the stream channels. Longitudinally, Salmon Creek has several waterfall barriers, samples were collected from above and below the second waterfall. These samples enabled us to examine the effects of a barrier without the influence of salmon in a stream that experiences spawning salmon. These returns occur below the first barrier where the stream is heavily influenced by the Douglas Island Pink and Chum (DIPAC) hatchery.

Sample Collection

Fish Creek was sampled intensively on four dates, corresponding to pre-salmon and post-salmon spawning periods during 2000 and 2001 above and below the salmon barrier. Additional sampling periods occurred in Fish Creek prior to spawning in July 2000 and during the salmon run in August 2001. The upper and lower reaches of Fish Creek were further sub-divided into 5 reaches. Therefore, there were 10 sub-reaches

sampled on each sampling period. The distance between the lower sub-reaches was approximately 2 km and the upper sub-reaches were approximately1 km apart. The distance between the sub-reach furthest downstream in the upper reach and the sub-reach furthest upstream in the lower reach were approximately 2km apart. The sub-reach sampled furthest downstream was approximately 1 km from the estuary and was not affected by high tide periods.

Salmon Creek was sampled on two dates, corresponding to pre-spawning and post-spawning periods in 2001. Three sub-reaches were sampled above and below the second barrier in Salmon Creek and the upper and lower study reaches were approximately 3km apart.

In order to analyze the marine-derived isotope signature, adult female Pink and Chum salmon, were sampled using seine nets as they first entered Fish Creek. Hatchery and 'wild' salmon spawn in the streams. Therefore, in addition to assessing isotopic differences between species, we collected adult Pink and Chum female salmon from a local salmon hatchery (DIPAC) for analysis. We also wanted to assess the changes in carbon and nitrogen isotope ratios in returning salmon as they migrate upstream (i.e. residence time in freshwater). Thus, we collected "fresh" adult female salmon from the estuary, spawned out fish, recently deceased fish, and decomposing carcasses from Fish Creek.

After collection, salmon were stored on ice, brought to the lab at the Pacific Northwest Research Station and frozen prior further processing for SIA. Kline et al. 1993 and Masterson 2000 verified that transverse sections of adult salmon are representative of the whole organism, therefore, two cross-sections were sampled from adult salmon at the

mid-section and head region. The cross-sections were dried at 60°C, homogenized (Wig-L-Bug® ball and capsule amalgamator, Crescent Industries), lipid-extracted, and re-homogenized to a fine powder. Lipids were removed by soxhlet extraction using an azeotropic mixture (87:13) of chloroform and methanol for 6 hours (Gould et al. 1997). Lipid extraction was preformed because lipids are depleted in ^{13}C relative to the bulk $\delta^{13}C$ value. Fish can have variable amounts of lipids and the $\delta^{13}C$ lipid can overprint the carbon isotope ratio of the diet (e.g., Kennicutt II et al. 1992; Focken and Becker 1998; Doucett et al. 1999).

Minnow traps, baited with a perforated bag of salmon roe, were used to collect resident freshwater fishes. In 2000, Dolly Varden (*Salvelinus malma*) were sampled from above and below the barrier, but were only captured in the upper reach of Fish Creek in 2001. In addition, benthic-feeding coastrange sculpin (*Cottus aleuticus*) and drift-feeding YOY coho were sampled in lower Fish Creek. Neither of these fish exists above the salmon barrier. YOY coho were sampled pre- and post-spawning in 2000 and at a sampling-interval of approximately every 2 weeks during June to August 2001. Coastrange sculpin were sampled only in 2001 from the lower reach of Fish Creek. Stomach contents of all resident fish were removed immediately by gastric lavage. Fish were stored on ice until processed. If the resident fish were approximately greater than 10cm, cross-sections were sampled and if less than 10cm the whole fish was processed. Fork length and fish species were recorded and all fish samples were freeze dried, homogenized, lipid extracted, and re-homogenized into a fine powder in preparation for SIA.

Aquatic insects were collected using D-frame kick nets. Insects were separated from organic matter in the field and stored on ice until sorted to taxa and associated functional feeding group in the lab. To ensure sufficient size for isotopic analysis, most of our samples represent composites of 10-200 individuals of each taxa from a single sub-reach. In cases of certain rare macroinvertebrate taxa, samples for individual sub-reaches were combined. Insects were identified to the lowest taxonomic group (primarily to family) and associated functional feeding group (Merritt and Cummins 1992). Individual taxa were placed in scintillation vials, and dried at 60°C. The collected taxa represented four macroinvertebrate functional feeding groups, 1) Scrapers or the primary consumers, 2) collector-gathers, 3) shredders, and 4) predators. All macroinvertebrate samples were lipid-extracted, acidified, and homogenized. Insect samples were acidified (1 N HCl) to remove any ^{13}C enriched-carbonates that may have accumulated in stomach tracts and then dried at 60°C.

Biofilm (i.e. epilithon) samples were collected from 10-20 randomly selected large cobbles combined from each sub-reach in Fish Creek in 2000-01 (Kline et al. 1990). Each cobble was removed from a riffle and placed in a pan, scraped on the top surface using a nylon brush, rinsed with stream water, collected in a Whirl-pak®, and transported back to the laboratory on ice. In the laboratory, samples were centrifuged, decanted, and then the concentrated biofilm sample was placed in a scintillation vial and dried at 60°C. Biofilm samples were acidified (1 N HCl) and ground into a fine powder using a mortar and pestle. Course particulate organic matter (CPOM) samples were collected in 2000 and 2001 in areas of retention (i.e. behind woody debris). In final preparation for SIA, CPOM samples were homogenized to a fine powder using a coffee grinder.

Stable Isotope Analysis

Macroinvertebrate and fish samples were analyzed at the Isotope Biogeochemistry Laboratory of Michigan State University using either a Carlo Erba NA 1500 nitrogen/carbon analyzer interfaced to a PRISM (Micromass, Manchester, England) or a Eurovector elemental analyzer interfaced to a ISOPRIME (Micromass) stable isotope mass spectrometer. Biofilm, CPOM, and FPOM samples were processed for SIA by a modified Dumas combustion where the samples are combusted on a slow temperature program (1 h at 850°C and cooled from 700 to 500 0.6° C min^{-1}) (Macko et al. 1987). The evolved CO_2 and N_2 gas produced from combustion were purified cryogenically and the isotope ratios of the resulting gas were measured on a PRISM mass spectrometer (Micromass).

Isotope values are reported relative to an international standard and expressed as:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard})-1] \times 1000$$

where R is the ratio of the heavy light isotope. Standards are V-PDB and N_2 for carbon and nitrogen, respectively. Reproducibility of the measurements is 0.2 ‰.

MDN Quantification

To determine the influence of salmon on the stream ecosystem we quantify the influence of MDN on stream biota using mass balance equations. The following general equation was used to derive the equation used to quantify the degree of MDN incorporation,

$$\delta_{diet} = f_1\delta_1 + f_2\delta_2 \quad (1)$$
$$1 = f_1 + f_2$$

where δ_{diet} is the $\delta_{consumer}$ including the discrimination between the consumer and its diet, f_1 and f_2 are the fraction of food source 1 and 2, and δ_1 and δ_2 are the isotopic signature of food source 1 & 2. As a result of our study design, we were able to compare an organism in the reference reach (i.e. upper reach) to the same organism in the treatment reach (i.e. lower reach) and we assumed the diets of the organisms between the two reaches were similar, except for the contribution of MDN to the lower reach food web (cf. Johnston et al. 1997; Chaloner et al. 2002). As a result, the following equation was used to quantify the degree of MDN incorporation:

$$\% \text{ MDN incorporation} = [(\delta_{Lower} - \delta_{Upper})/((\delta_{MDN} + (TL \times F)) - \delta_{Upper})] \times 100 \quad (2)$$

where δ_{Lower} is the mean $\delta^{15}N$ or $\delta^{13}C$ value of the organism in the lower reach, δ_{Upper} is the $\delta^{15}N$ or $\delta^{13}C$ value of the organism in the upper reach, δ_{MDN} is the isotopic value for an adult salmon, TL is the proposed trophic level of the organism, and F is the degree of fractionation. Two estimates of trophic fractionation were used in our modeling efforts,

2.7 and 3.4 ‰ for nitrogen and 0.7 and 1 ‰ for carbon that are commonly reported in the literature (e.g. DeNiro and Epstein 1978, 1980, Tieszen 1983, Hobson 1992). This resulted in two estimates of % MDN incorporation. Consequently, we consider that the amount of MDN incorporated by consumers to fall within the range bounded by these estimates and report our estimate of % MDN as such. Macroinvertebrate scraper, collector-gather, and shredder functional groups were assigned to trophic level one. Freshwater fishes and the macroinvertebrate predators assigned to trophic level two. Mixing models are highly sensitive to the isotope values incorporated into the model. Therefore, we evaluated uncertainty in our % MDN calculations, including standard errors. We modified the approach Phillips and Gregg (2001) to include our reference/treatment study design. The model is as follows,

$$\sigma^2_{fMDN} = 1/(\delta_{MDN}-\delta_{Upper})^2[\ \sigma^2_{\delta Lower} + f^2_{MDN}\sigma^2_{\delta MDN} + (1- f_{MDN})^2\sigma^2_{\delta Upper}] \quad (3)$$

where $\sigma^2_{\delta MDN}$, $\sigma^2_{\delta Lower}$, and $\sigma^2_{\delta Upper}$ represent variances of the mean isotopic values for adult salmon, and organisms in the lower reach, and the same organism in the upper reach, respectively and f_{MDN} represent the % MDN incorporation calculated from equation 2. δ_{Lower} and δ_{Upper} have been corrected for the associated trophic fractionation and trophic level.

Statistical Analysis

This study consisted of randomly sampling stream organisms for isotope analysis under different conditions over time. Therefore, a repeated measures ANOVA with time as the repeated measure and a random factor. SAS version 8.2 (2001) was used for the repeated measures ANOVA analysis. In other cases, comparisons required a students t-test or one way ANOVA (e.g. comparison among both wild and hatchery, pink and chum salmon). These statistics were performed with STATISTICA (release 6.0).

RESULTS

Characterizing the MDN Endmember and Barrier Effect

The first objective of our study was to characterize the carbon and nitrogen isotope values of the marine endmember. To do so, we compared unspawned hatchery (DIPAC) and 'wild' pink and chum salmon collected from spawning periods during 2000 and 2001 (Table 1). There was no difference between salmon species or between wild and hatchery individuals (ANOVA, $P < 0.01$). Therefore, we used the average carbon and nitrogen isotope values of all adult salmon do derive the MDN endmember: -19.9 ± 0.4 and 11.4 ± 0.4 ‰, respectively. The second objective was to assess whether the freshwater residence time or spawning migration of adult salmon would affect their carbon and nitrogen isotopic composition (Table 1). There was no difference in carbon or nitrogen isotopic compositions between adult salmon collected from the saltwater and dead decaying salmon collected from Fish Creek in 2001 (ANOVA, $P < 0.01$).

An important strategy to evaluate the influence of MDN on the stream biota was to compare organisms in an upstream reach above an impassible waterfall to organisms of the same taxa from the lower reach. We first evaluated the effects of a barrier on the carbon and nitrogen isotopes of stream consumers. In order to do so, organisms of the same taxa were collected from two reaches in Salmon Creek that were not influenced by salmon, but separated by a barrier during August 2001. The macroinvertebrates, Baetidae and *Epeorus*, from above and below the barrier did not significantly differ in $\delta^{15}N$ values collected (Table 3, ANOVA, $p > 0.01$). The isotope values of the single Chloroperlidae *spp.* collected in the upper was very similar to one collected from the lower reach (difference of 0.3‰ or less). Statistically, the *Cinygmula* $\delta^{15}N$ values in the upper reach are enriched in ^{15}N relative to the lower reach of Salmon Creek (ANOVA, $p > 0.01$). However, given the average isotope values (0.5 +/- 0.3 and 1.1 +/- 0.1) and the reproducibility of the measurement 0.2, it is unlikely that the difference in these values is truly significant. Carbon isotope ratios for all taxa sampled were not significantly different between the two reaches (Table 3, ANOVA, $p<0.01$).

Food Web Components

Biofilm and CPOM

There were no significant differences in biofilm isotope values between the upper and lower reaches during any sampling periods in 2000 (ANOVA, $P > 0.01$). Sampling of CPOM was limited. Only two Samples were obtained from upper and lower FC in

2001 after spawning. The $\delta^{13}C$ values of the samples collected in the lower reach differed by only 0.3 ‰ from those from the upper reach. The $\delta^{15}N$ values of the same CPOM samples from the lower reach were approximately 4 ‰ enriched in ^{15}N relative to the CPOM samples collected from upper Fish Creek (Table 1).

Macroinvertebrates

With the exception of Paraleptophlebiids, relative to pre-spawning, $\delta^{13}C$ and $\delta^{15}N$ values of macroinvertebrates in the lower reach shifted towards the MDN signature after spawning occurred in Fish Creek (Table 1, Fig. 3). The one exception, *Paraleptophlebia*, exhibited greater carbon and nitrogen isotope values in pre- relative post-spawning periods in 2001. The *Paraleptophlebia* dataset is limited. Because the dataset consists of one sample for each period sampled, it prevents us from drawing conclusions from this apparently contradictory trend (Table 1). Macroinvertebrates in the upper (reference) reach did not exhibit higher carbon or nitrogen isotope values after spawning relative to pre-spawning periods (Table 1, Fig 4c and 4d). The observation that the carbon and nitrogen isotope values of consumers stayed constant in the upper reach while those of the same consumers increased in the lower reach after spawning is consistent with MDN incorporation. In pre-spawning periods, there are several cases where carbon and nitrogen isotopic variability of the macroinvertebrate predator, Chloroperlidae *spp.*, is high relative to that of other macroinvertebrates. The carbon and nitrogen isotope values of Chloroperlidae *spp.* also did not differ significantly between the upper and lower reaches during the pre-spawning period in 2001 (ANOVA, $p>0.05$) (Table 1). After spawning in

2001, Chloroperlidids differed significantly for only nitrogen (ANOVA, $p<0.01$) and not carbon isotope values (ANOVA, $p>0.5$). In 2001, these macroinvertebrate predators did not significantly differ in either carbon or nitrogen isotope ratios between pre-spawning and post-spawning periods in lower Fish Creek (ANOVA, $p>0.05$) (Table 1).

In addition to the evidence reflecting MDN incorporation in primary consumers, several other salient features appeared in our macroinvertebrate data set. Macroinvertebrates isotope values varied with respect to size of individuals and functional feeding groups. Also, MDN incorporation varied among macroinvertebrates. This was associated with apparent changes in trophic position relative to prespawning periods.. We examined small and large sized *Drunella* from upper and lower Fish Creek sampled in August 2001 (Table 1). Large *Drunella* were more enriched in ^{15}N relative to the smaller individuals (t-test, $p < 0.01$). Carbon isotope values of large and small individuals were not significantly different (t-test, $p > 0.1$).

Macroinvertebrates within the scraper group, *Cinygmula*, *Drunella*, and *Epeorus* differed from each other in their carbon and nitrogen isotopes values regardless of sampling period in both years (Fig. 4, Fig. 5, ANOVA, $p<0.01$). Overall, the average $\delta^{13}C$ value of *Epeorus* was lower than that of the other two scraper taxa regardless of location or sampling period (Table 1, ANOVA, $p<0.01$). *Drunella* samples had the highest $\delta^{15}N$ values during pre-spawning periods compared to the other two scraper taxa collected in 2000 and 2001 (ANOVA, $p<0.01$). After spawning in the lower reach, *Cinygmula* were the most enriched in ^{13}C and ^{15}N relative to the other two scraper taxa during 2000 and 2001.

Qualitatively, differences in the % MDN incorporation appeared to occur between the scraper taxa and among different functional feeding groups (Table 2). Post-spawning data show that *Cinygmula* incorporated more MDN nitrogen than did the other two scraper taxa in both 2000-2001 (56-69 % and 40-57 %, respectively). As for MDN carbon incorporation, *Cinygmula* exhibited the greater % MDN carbon incorporation (71-77 %) than the other two scraper taxa (40-52 %) in 2000 and 2001. As indicated by carbon and nitrogen isotope values, the macroinvertebrate trophic structure (the relative position of an organism within the food web), in upper Fish Creek remained relatively constant over the season and between years (Fig. 4c and 4d). In contrast, the trophic structure of the macroinvertebrate food web changed from the pre-spawning and post-spawning periods in lower Fish Creek, which was a result of the different degrees of MDN incorporation by functional feeding groups (Fig. 4a and 4b).

Macroinvertebrate carbon and nitrogen isotope values were enriched in ^{13}C and ^{15}N in the lower reach relative to the upper reach, regardless of season or year (rmANOVA, <0.005) (Table 1). Evidence for the presence of MDN in lower Fish Creek, in the absence of spawning salmon, is exhibited by the degree of MDN incorporation in the stream biota during the pre-spawning sampling period (Fig. 5a and 5b). The "legacy effect" was more apparent in 2000 relative to 2001. Estimates of % MDN nitrogen incorporation for macroinvertebrate consumers in the lower reach prior to spawning were lower than those based on carbon incorporation for both years (Fig. 5a and 5b). After salmon spawned, the average % MDN carbon and nitrogen incorporation for all macroinvertebrates collected was 44 and 50 % for 2000 and 2001. As for carbon, the

average % MDN incorporation for macroinvertebrates was 59 and 48 % for 2000 and 2001.

Freshwater Fishes

Resident Dolly Varden from the upper reach were significantly depleted in ^{13}C and ^{15}N relative to Dolly Varden collected in the lower reach during 2000 (t-test, $p<0.01$; $\Delta_{\text{lower-upper reaches}}\ \delta^{15}N \cong 6$ ‰, $\Delta_{\text{lower-upper reaches}}\ \delta^{13}C \cong 5$ ‰) (Table 1). In the lower reach in 2000, carbon isotopes values for Dolly Varden appeared higher after spawning than prior to spawning, but this difference was not statistically different (t-test, $p>0.05$). During the same time period, however, the nitrogen isotope values were statistically different between pre- and post-spawning (t-test, $p<0.01$). In 2001, no Dolly Varden samples were caught in minnow traps in either pre- or post-spawning periods.

Estimates of % MDN incorporation were high before spawning; 50-56 % and 49-55 % for carbon and nitrogen, respectively. The high degree of MDN incorporation in Dolly Varden prior to the salmon subsidy could reflect the ability of DV to access other MDN resources during migration to and from the estuary (e.g. estuarine invertebrates). After spawning, the % MDN incorporation for Dolly Varden was 80-91 % and 59-67 % for carbon and nitrogen, respectively. However, because the individuals below the barrier could be accessing MDN from other sources than the salmon subsidy, pre- and post-spawning individuals from the lower reach were used in equation 2 to estimate the % MDN incorporation instead of comparing the upper and lower reaches. In 2000, Dolly Varden incorporated an average of 62 % MDN carbon and 26 % MDN nitrogen after

spawning. In the upper reach, Dolly Varden carbon and nitrogen isotopes were very similar between sampling periods (5.8 to 6.3 ‰ and –26.0 to -27.1 ‰, respectively).

Dolly Varden $\delta^{15}N$ values were not correlated with fork length either prior to or during spawning in the upper reach, but there was a correlation between $\delta^{15}N$ and fork length in lower Fish Creek (Fig. 6). Interestingly, the carbon and nitrogen ratios of the post-spawning Dolly Varden sampled in 2000 from the lower reach are positively correlated. Also, samples from this period exhibit the most enriched isotope values, suggesting that even though these fish are likely to be migratory there is some evidence for MDN incorporation (Fig. 6).

Coastrange sculpin only exist in lower Fish Creek. Because we did not have individuals from the upper reach, sculpin from pre-spawning periods were used in the models to estimate %MDN. All coastrange sculpin were collected in 2001. Carbon and nitrogen isotope values were significantly different in the post-spawning compared to pre-spawning (Table 1, t-test, $p<0.01$). Freshwater sculpin incorporated an average of 83-100 % MDN carbon and 16-19 % MDN nitrogen. Unlike other fish analyzed in this study, the average $\delta^{13}C$ value of post-spawning sculpin (11.8± 0.9 ‰, $n = 10$) was higher than that of the bulk isotopic signature of adult salmon (-18.3 ± 1.2 ‰, $n = 10$) (Table 1). Sculpin had a large degree of variability in $\delta^{13}C$ value from both the pre- and post-spawning periods. The $\delta^{15}N$ values were not positively correlated with fork length.

All YOY coho samples were collected in 2001 from Fish Creek. Carbon and nitrogen isotope values of these YOY coho were higher after spawning relative to pre-spawning (t-test, $p<0.05$, Fig. 7). The average % MDN incorporation for YOY coho was 34-57 % for carbon and 22-28 % for nitrogen. YOY coho $\delta^{13}C$ and $\delta^{15}N$ values were

positively correlated and are a function of the time of sampling. Coho salmon parr showed similar patterns to the YOY coho, including no correlation between $\delta^{15}N$ and fork length, but a positive correlation between carbon and nitrogen isotope ratios that is a function of the time of sampling.

Longitudinal and Inter-Annual Trends

Cinygmula, *Epeorus*, *Drunella*, and Chloroperlidae were collected from a sufficient number of sub-reaches in lower Fish Creek to evaluate longitudinal trends in isotope data. In several cases, these taxa exhibited a relationship of increasing $\delta^{13}C$ and $\delta^{15}N$ values with distance downstream from the salmon barrier during and post-spawning in both years (Fig. 8). The trend was evident for $\delta^{15}N$ values of Chloroperlidae and *Cinygmula* in 2000 and 2001. It was also observed for $\delta^{13}C$ values of *Epeorus* in 2000 and 2001 and for $\delta^{13}C$ data for *Drunella* in 2001. The longitudinal trend did not occur prior to spawning or during any sampling period in the upper reach. The slopes of the relationship between distance downstream from the barrier and a) the carbon isotope values of *Epeorus* and *Drunella* in 2001 and b) the nitrogen isotope values of *Cinygmula* and Chloroperlidae in 2000 and 2001 were similar for during and post-spawning periods, respectively (Fig. 8). During the salmon run in 2001, differences in $\delta^{13}C$ and $\delta^{15}N$ isotope values of *Cinygmula* collected 1.2 kilometers apart (endpoints of our sampling transect) were 1.6 and 3.8 ‰ , respectively. The associated % MDN incorporation estimates ranged from 16 to 36 % (Fig. 9).

Estimates of the salmon escapement between 2000 and 2001 did not differ (D. T. Chanoler, unpublished data) However, the biomass delivered to Fish Creek was slightly greater in 2001, due to a greater contribution by chum, but historically both years experienced low returns (D. T. Chaloner, unpublished data). The "during" spawning period in 2001 occurred approximately two weeks after the arrival of the first salmon migrating upstream. As a result, only live salmon (with no sign of decay) existed in Fish Creek during this sampling period (B. S. Graham personal observation). Isotope values of macroinvertebrates collected during the salmon run exhibited elevated carbon and nitrogen values relative to pre-spawning values from lower Fish Creek (Table 1, Fig. 10). The scraper, predator, and collector-gather functional feeding groups incorporated 25-52 and 24-38 % MDN carbon and nitrogen, respectively, relative to the pre-spawning period (Table 2). The shredder functional feeding group (i.e. utilize CPOM as a food resource) incorporated less MDN during the salmon run at 14-15 and 19-20 % for carbon and nitrogen, respectively. After spawning, estimates of % MDN incorporations were different between 2000 and 2001 for the scraper and predator taxa (Table 2). *Cingymula* and *Epeorus* exhibited similar $\delta^{13}C$ values between years and greater $\delta^{15}N$ values in 2001 relative to 2000 (Fig. 10).

DISCUSSION

A comparison of $\delta^{13}C$ and $\delta^{15}N$ values of stream biota were made between the treatment and reference reach of Fish Creek. Sub-reaches from each of these locations were sampled prior to and after spawning in 2000 and 2001, and during spawning in 2001. Our Fish Creek data set allows us to examine differences (1) between reference and

treatment reaches, (2) among sub-reaches, (3) among pre-spawning and post-spawning periods in 2000, and pre-, during, and post-spawning periods in 2001. The results from this study illustrate that Fish Creek consumers incorporated MDN, but the degree of incorporation was influenced by the spatial and temporal variation in MDN, metabolic turnover rate of the organisms, trophic time lag, the age of the organism, and migratory behavior.

The MDN Endmember and Barrier Effect

The upstream-downstream study design applied in Salmon and Fish Creek is one approach to evaluate MDN subsidies on stream ecosystems (Kline et al. 1993; Bilby et al. 1996; Johnston et al. 1997, D. T. Chanoler et al. 2002). Although this approach is commonly used to evaluate the importance of MDN in stream ecosystems, little has been done to evaluate the issue of pseudoreplication (Hurlbert 1984). In other words, without any effect of MDN, do upper and lower reaches have similar food web structures? Salmon Creek provided an opportunity to examine this issue as there were two barriers (i.e. waterfalls) leaving two study reaches without any MDN influence. Macroinvertebrates collected from such two reaches in Salmon Creek showed no difference in their carbon isotope values (Table 3). *Cinygmula* showed minor differences in its nitrogen isotope values between the two reaches but this was deemed insignificant considering the standard deviation associated with the replicates and error associated with the measurement (Table 3). Overall, in the small discrete watersheds of Salmon Creek,

stream macroinvertebrates showed a high degree of isotopically similarity above and below a barrier.

The carbon and nitrogen isotopic composition of adult pacific salmon did not differ (1) between pink and chum salmon, (2) between wild and hatchery individuals, and (3) among fresh, spawned out, and decomposing salmon. The most likely interpretation for these data is that pink and chum salmon, both wild and hatchery individuals, have similar feeding strategies or subsist at the same trophic position. The data also shows that salmon entering streams retain their marine isotopic signature throughout their freshwater migration upstream and during their subsequent decay. Kline et al. (1993) first reported differences in $\delta^{13}C$ and $\delta^{15}N$ between fresh and spawned-out Pacific salmon and showed that spawned-out individuals had higher $\delta^{13}C$ and $\delta^{15}N$ values relative to fresh individuals. Doucett et al. (1999) suggested $\delta^{13}C$ values increase as a result of energy reserves, primarily lipids, being lost during spawning migration of Atlantic salmon (*Salmo salar*), but $\delta^{15}N$ values seem unaffected by the upstream migration. The discrepancy between our carbon isotope data and those of Kline and Doucett results because our samples were lipid extracted. The removal of lipids produces an increase in the carbon isotope values of the organism because lipids are at least 3 ‰ more depleted in ^{13}C relative to the average bulk tissue $\delta^{13}C$ value (Tiezen et al. 1983). We confirm this in our study by comparing the isotope values of lipid extracted tissue to the non-lipid extracted counterparts of three macroinvertebrates: *Drunella,* Chloroperlidae, *Cinygmula* and *Zapada*. Considering the average difference for all taxa, the lipid-extracted aliquots were depleted in ^{13}C by 1.2 ± 0.4 ‰ relative to the non-lipid extracted sub-samples. No differences in $\delta^{15}N$ values were found between the lipid-extracted and non-lipid extracted

sub-samples ($\delta^{15}N$ values differed by 0.3 ± 0.2 ‰ between the two groups) (Fantle et al. 1999). High $\delta^{13}C$ values observed by Kline et al. (1993) in decomposed salmon are consistent with the loss of ^{13}C depleted lipids. In conclusion, our results confirm lipid content will affect the $\delta^{13}C$ values of adult salmon, but after lipid extraction adult salmon have similar isotopic composition throughout their migration upstream.

Food Web Components

Biofilm

Autotrophic uptake of mineralized MDN could be an initial and crucial link between the MDN subsidy and upper trophic levels in lotic ecosystems. However, both CPOM and biofilm did not show a clear response to the MDN subsidy and their trophic relationship to macroinvertebrates was not clearly discerned based on nitrogen isotope values (Table 1). Several studies have examined $\delta^{13}C$ and $\delta^{15}N$ values of biofilm with respect to MDN subsidies (Kline et al. 1990, Bilby et al. 1996, Johnston et al. 1997, and Chaloner et al. 2002). Whereas Kline et al. (1990) and Bilby et al. (1996) showed MDN incorporation by the biofilm community, this trend was not demonstrated in Chaloner et al. (2002). Chaloner et al. (2002) discuss a variety of mechanisms that can result in isotopic variation and overprinting of the MDN signature of the biofilm community including differences in algal growth, nutrient pool size (Finlay et al. 1998), and, community structure. They suggest that in addition to MDN, the $\delta^{13}C$ values of biofilm could respond to algal growth rates and their influence on ^{13}C discrimination during photosynthesis (Laws et al. 1995). They also point out that isotopic discrimination (i.e.

fractionation) during nitrogen cycling (e.g. nitrification) or the pool size of the dissolved inorganic nitrogen reservoir can influence the $\delta^{15}N$ of algae (Fogel and Cifuentes 1993). The latter seems particularly relevant because like Bilby et al, (1996), large variation in the size of the dissolved inorganic nitrogen pools as a consequence of the MDN subsidy and its subsequent decline have been observed in our study and elsewhere. (Mathisen et al. 1988, Bilby et al. 1996, Mitchell, 2003). Biofilm isotopic values could be an artifact of the relative proportion of heterotrophs to autotrophs (Hamilton et al. 1992, Chaloner et al. 2002). Whereas, both autotrophs and heterotrophs take up dissolved inorganic or organic nitrogen, only heterotrophs are likely to assimilate nitrogen from marine-derived particulate matter (Fenchel et al. 1998). Finally, studies which analyzed filamentous algae (Kline et al. 1990, Johnston et al. 1997) exhibited less carbon and nitrogen isotopic variation than those that analyzed biofilm randomly sampled from rock surfaces (Bilby et al. 1996, Chaloner et al. 2002). Therefore, in the context of nutrient cycling at the base of the stream food web, MDN input is only one among many variables affecting the isotopic composition of biofilm.

Similar to the results of Chaloner et al. (2002) in Margaret Creek, AK, the isotope values of our biofilm in pre-spawning periods in upper fish creek were frequently higher than those of associate macroinvertebrates from the same location and time. Furthermore, $\delta^{15}N$ values of biofilm in pre-spawning period were higher than those during spawning in upper and lower Fish Creek. These observations are consistent with our conclusion above regarding multiple factors controlling the isotope values of biofilm. An important line of future inquiry would be to better characterize the isotopic compositions of the biofilm

(e.g. Hamilton et al. (1992) method) and therefore, provide a more comprehensible link between the biofilm community and primary consumers.

Macroinvertebrates

In stream food webs, macroinvertebrates represent an essential link between the lower trophic levels and fishes and are therefore, crucial to understanding organic matter and nutrient cycling in streams. The increase in isotope values between pre- to post-spawning periods for macroinvertebrates isotope values generally support the established concept of MDN incorporation in stream ecosystems (Fig. 3). Temporal trends in carbon and nitrogen isotope values of Chloroperlidae *spp.* suggest that they experienced MDN incorporation, however differences among sampling periods in both years were not statistically significant. This is, in part, due to a) high variation about the mean isotope values during pre-spawning periods and b) low sample numbers (Table 1, Fig. 4).

The notably high isotopic variability among Chloroperlids could be a consequence of (1) variation in feeding habits with depth in substrate, (2) variation in nitrogen isotope values at the base of the food web, and/or (3) trophic position. Chloroperlidae *spp.* $\delta^{15}N$ values reflect the average ^{15}N trophic level of all their prey (weighted by dietary contribution) plus approximately 3 ‰ (DeNiro and Epstein, 1979). Differences in prey and associated average $\delta^{15}N$ of the diet might occur if the habitat of Chloroperlidae *spp* varies within the stream. For example, Chloroperlids located deeper within the substrate might have access to different prey than those that live closer to the substrate-water column interface. To illustrate that such variation in diet could influence

the isotope values of chloroperlids consider the following example. If a chloroperlid at the surface of the stream bottom consumed 50 % baetidae ($\delta^{15}N$ = 1.5 ‰) and 50 % paraleptophelibidae ($\delta^{15}N$ = 8.3) then the $\delta^{15}N$ value of chloroperlidae should be approximately 7.9 ‰, including a trophic fractionation. Whereas, if a cholorperlidae located deeper in the substrate consumed 10 % baetidae and 90 % paraleptophelibidae then the $\delta^{15}N$ should be approximately 7.6 ‰.

In addition to changes in diet that occur between habitats, the isotopic composition of the base of the food web would also contribute to variation. With increasing depth in the stream profile the available nitrogen pool could be influenced by different nitrogen cycling processes than those occurring in the stream (Ostrom et al. 2002). For example, although nitrification dominates in highly aerated streams resulting in ^{15}N depletion in the production (i.e. NO_3) and ^{15}N enriched values in the residual substrate (NH_4^+) if suboxic localized regions develop in the subsurface, denitrification would dominate. In this case, the residual substrate, NO_3 would be enriched in ^{15}N. Thus, the ^{15}N at the base of the food web would depend on (1) the DIN that supplies nitrogen to the foodweb (NO_3 or NH_4^+) and (2) the predominant process that influences the source.

Chloroperlids were identified to the taxonomic level of family, while most other macroinvertebrates were separated into separate genera. Different genera within the same family group exhibited differences in nitrogen isotope values that could indicate differences in feeding strategies or differences in the isotope value at the base of the food web. If the former is the case, some of the nitrogen isotopic variability could result from different trophic position of the various taxa lumped into the Chloroperlidae *spp.* samples.

Isotope values of macroinvertebrates varied with respect to size and functional feeding groups (Table 1). *Drunella* carbon isotope values were similar between large and small individuals but nitrogen isotope values were higher in larger relative to small individuals. *Drunella* appears to be consuming a similar carbon resource throughout their development. The large and small individuals were collected from the same sub-reaches and at the same time. Therefore, the base of the food web did not change and, thus, can not account for the observed differences in nitrogen isotope values between the two size classes. Instead, differences in $\delta^{15}N$ values between the two size classes may reflect a change in breadth of diet. Even though food acquisition mechanisms remain similar throughout the life stages of scraper taxa, the increase in the size of mouth parts of larger individuals enhances the possibility of accidental ingestion of early instars (Cummins 1980). Ingestion of early instar macroinvertebrates would increase the trophic position and, consequently, the isotope value of *Drunella*.

Functional feeding groups (FFG), a classification system used extensively by stream ecologists, are defined by morpho-behavioral characteristics (Merritt and Cummins 1996). The FFG classification, combined with stomach content analysis are used to examine trophic interactions and structure (e.g. Harrigan et al. 1989). Stomach content analysis provides investigators information biased toward an organism's most recent feeding event and the FFG method designates a general feeding strategy related to the organism's life history. However, the FFG method and stomach content analysis do not always reflect an organism's diet, due to differential digestion and assimilation. SIA is advantageous because 1) it records only the assimilated portion of the diet and 2) it gives a time-integrated view of the diet. Thus, isotope data provides some additional

information to our understanding of food webs based on stomach content analysis and FFG method. The FFG approach indicates that *Cinygmula* and *Epeorus* are scrapers. The stable isotope data (for all sampling periods) suggests that these two taxa likely have different feeding strategies as their $\delta^{13}C$ and $\delta^{15}N$ values deviate from one and another regardless of location. The FFG method indicates scrapers utilize biofilm as their primary food resource. If the different scrapers were indiscriminately feeding on biofilm the isotope values would be similar. The difference in isotope values between the taxa observed here could result if certain scraper taxa assimilate a more labile component of the biofilm, which might have a different $\delta^{13}C$ value than the bulk biofilm $\delta^{13}C$ value (Hamilton et al. 1992). It appears *Cinygmula* and *Epeorus* are either utilizing different components of the biofilm or are supplementing their diets with other food resources that are isotopically distinct.

MDN incorporation varied within scraper functional feeding group prior to and after spawning. In both years, *Cinygmula* exhibited an average of 67 % MDN (C & N) incorporation in the post-spawning period, the greatest for any macroinvertebrate taxa, whereas, *Drunella* and *Epeorus* incorporated approximately 46 and 47 % MDN, respectively. Relative to the other two scrapers, *Cinygmula* could be utilizing a food resource more heavily influenced by MDN by either (1) selectively grazing a portion of the biofilm community that incorporated more MDN, (2) feeding directly on or accidental ingestion of MDN particles, or (3) could be located in a habitat where the base of the food web is ^{15}N enriched. Finally, it is important to note that a common strategy in organisms is mixotrophy and invariably this can apply to macroinvertebrates and would lead to variability in the carbon and nitrogen isotopic data. It is clear, however, that

scraper taxa, in particular *Cinygmula* is utilizing a food resource that is highly influenced by the MDN subsidy.

The high MDN incorporation estimates exhibited by macroinvertebrates could reflect fast tissue turnover rates and a larger degree of isotopic disparity between the MDN subsidy and the stream-derived diet. The rate that an organism's isotope value changes is a function of net growth efficiency or in other words, the degree of growth relative to the degree of assimilation (Fry and Arnold 1982, Tiezen et al. 1983). Invertebrates have rapid isotopic turnover (i.e. within 30 days, Fry and Arnold 1982, Ostrom et al. 1997), therefore, changes in diet should have been observed during our sampling period of 5 months. In addition, the MDN subsidy, which is isotopically distinct from the stream diet, should lead to a conspicuous shift in isotopic values. Thus, a response to changes in diet is more likely to be detected in an organism with fast tissue turnover rate and short-lived compared to an organism that is longer-lived with a slow tissue turnover rate (Hesslein et al. 1983). Furthermore, long-lived stream organisms such as Chlroperlidae, relative to other macroinvertebrates with a shorter life span would experience multiple pulses of MDN and their tissues would represent a time-integrated diet.

The Legacy Effect

Lower Fish Creek food web demonstrated a legacy of marine-derived nutrients retained from the previous year's spawning salmon. Accordingly, anadromous salmon may represent a subsidy that sustains stream productivity throughout the year. Marine-

derived nutrients and organic matter can be retained in the stream by physical retention in large-woody debris (LWD) (Bilby and Likens 1980) or by subsurface retention (Edwards 2000). The degree of retention depends on many factors, including the number and intensity of spate events, the amount of LWD, the retention and utilization of MDN in ground water or the hyporheic zone, the degree of absorption of MDN onto particles in the sub-surface, and biotic uptake turnover time.

Relative to upper Fish Creek, several groups of macroinvertebrates exhibited elevated carbon and nitrogen isotope values prior to salmon spawning in both years (Fig. 5a, 5b). This suggests that the lower reach is experiencing spring carryover from previous MDN exposure. Previous studies have shown that over-wintering fish retain MDN signatures into the spring (MacAvoy et al. 1998, Doucett et al. 1999) and likely represent isotope dilution between a MDN-derived diet in the fall to a stream-derived diet throughout the winter (Fry and Arnold 1982). However, our study illustrates that short-lived macroinvertebrates (e.g. grazers and collector-gathers) are acquiring a MDN signature, in absence of spawning salmon, after hatching in lower Fish Creek in the spring. In other words, primary consumers are incorporating marine nutrients retained in the freshwater food web.

Although many factors could affect the isotope values of food web components throughout the year, the conspicuous annual migration of salmon seems an important candidate for explaining the elevated values in the lower relative to upper Fish Creek. The range in % MDN incorporation in the lower reach during pre-spawning periods for all macroinvertebrates in 2000 and 2001 was 4-56 and 4-37 % for carbon and nitrogen, respectively (Table 2). Interestingly, the estimates of the legacy effect determined using

carbon isotope values differed from those determined using nitrogen isotope values (Fig. 5). MDN incorporation estimates are derived using a comparison between an upstream and downstream consumer (Equation 2). If factors other than MDN input influence isotope values between consumers from upper and lower Fish Creek this would lead to error and perhaps account for the disparity between estimates based on carbon and nitrogen isotope values. For example, in the case of carbon, upstream consumers are more likely to assimilate ^{13}C-depleted terrestrial CPOM than are downstream organisms. Thus, one could argue that carbon isotope values of consumers in upper Fish Creek might not provide an accurate basis for determining estimates of % MDN in our mass balance equations. However, the Salmon Creek data set demonstrated that groups of macroinvertebrates had similar carbon isotope values above and below a barrier (neither exposed to MDN) sampled during August 2001. An alternative explanation for differences in %MDN incorporation between estimates of carbon and nitrogen is that carbon has a longer residence time the Fish Creek food web than nitrogen. In addition, nitrogen is often a limiting nutrient in terrestrial and marine systems and is utilized more rapidly than carbon.

A legacy of MDN in streams could represent an important source of nutrients throughout the winter months and into the spring for young-of-the-year salmon. Consequently, salmon might not only affect the flow of energy and nutrients through the stream food web, but may also be a crucial factor in their own production and restoration (Kline et al. 1990, Bilby et al. 1996, Garman and Macko 1998). If MDN is indeed retained from the previous year's subsidy, then differences in salmon escapements would affect the % MDN legacy during the next spring. However, before management practices

are implemented to enhance smolt production based on the 'positive feedback theory' of salmon escapements, a more rigorous and systematic examination of the relationship between salmon escapements and the MDN legacy needs to be conducted. For example, the relationship between salmon escapements and smolt production might not be a linear relationship, but instead one with an asymptote representing the degree of MDN saturation in the stream food web. The delivery of the MDN subsidy to the macroinvertebrate food web appears to be substantial and remains throughout the year, whereby the macroinvertebrates can utilize the long-term resource the following year.

Freshwater Fishes

The quick and evident response of the macroinvertebrate food web to the MDN subsidy can be contrasted to that of resident freshwater fish, which may (1) have multiple exposures to MDN and (2) have multiple marine-derived food resources in Fish Creek. In Southeast Alaskan streams migratory fish can acquire energy from two marine sources: salmon-derived nutrients (SDN) and estuarine-derived nutrients (EDN). Like SDN, relative to terrestrial and freshwater nutrients, EDN is likely to have high isotope values because it is strongly influenced by marine nitrogen. Dolly Varden from lower Fish Creek appear to be a migratory fish, and therefore, would have access to both SDN and EDN. Thus their isotope values would not be a simple function of SDN. Instead the values would depend on the amount of time the fish foraged in the stream, estuary, and marine environments, the success of foraging in these environments, the trophic level of their prey, and their subsequent growth rates in each environment (Hansson et al. 1997).

Presumably, Dolly Varden enter Fish Creek when it is most physiologically beneficial, i.e. with the arrival of the MDN subsidy. It has been well documented that Dolly Varden feed on salmon eggs during the upstream migration of spawning salmon (Fechhelm et al. 1997). Therefore, there should be a pulse of SDN in the Dolly Varden diet that coincides with the time salmon spawn. Dolly Varden migrate out to saltwater sometime after their second, third, or fourth year in the stream (Reed 1967). Despite these complicating factors, Dolly Varden isotope values still appear to be influenced by the annual pulse of SDN. There are two lines of isotopic evidence suggesting that Dolly Varden migrate into the stream during the pulse of SDN, incorporate SDN, and then migrate out to saltwater as they age. First, larger Dolly Varden $\delta^{15}N$ values are more enriched in ^{15}N than smaller individuals. Freshwater fish that migrate and experience a new diet that is more enriched in ^{15}N show a specific patter in their $\delta^{15}N$ values. As the fish ages, or fork length increases, $\delta^{15}N$ values increase (Doucett et al. 1999b). Therefore, higher $\delta^{15}N$ values for larger Dolly Varden would indicate these fish are consuming a diet more enriched in ^{15}N. The diet could be richer in EDN or SDN and/or they are feeding at a higher trophic level in the estuary or stream. Second, this is most likely occurring in the stream because the greatest $\delta^{15}N$ and $\delta^{13}C$ observed for Dolly Varden in Fish Creek occur shortly after spawning. However, this data could be explained by either an increase in MDN or a shift to a higher trophic level non-MDN diet. Dolly Varden represent a highly migratory fish, which complicates any interpretation of results when evaluating the importance of SDN. Therefore, investigating a fish species that does not have multiple sources of MDN (i.e. non-migratory fish) would prove valuable in assessing the importance of SDN to freshwater fishes.

Coastrange sculpin are resident fish in Fish Creek and are not believed to be migratory (Reed 1967). Sculpin are effective predators of salmon eggs, salmon fry, and benthic and drifting invertebrates (Reed 1967, McLarney 1967, Foote and Brown 1998). Sculpin can derive the majority of their dietary requirements from salmon eggs, which are a very labile food resource with high lipid content (McLarney 1967). To demonstrate the high lipid content of the eggs we compared the isotope value of lipid to non-lipid extracted eggs. Two samples of coho salmon (*O. Keta*) eggs were separated into two aliquots, one set was not lipid-extracted and the other was lipid-extracted. The lipid extracted aliquot was nearly 2 ‰ more enriched in ^{13}C compared to the non-lipid extracted sub-sample (i.e. -18.6 ± 0.1 and -20.2 ± 0.5, respectively), indicating a large loss of ^{13}C-depleted lipids during extraction. $\delta^{15}N$ and $\delta^{13}C$ values of sculpin exhibit an increase from pre- to post-spawning periods and could represent a shift from a stream-derived diet to one influenced by MDN. Large differences were exhibited between sculpin carbon and nitrogen MDN incorporation, with MDN C % incorporation being nearly a magnitude greater. Sculpin live for many years, thus, their muscle tissue samples represent a time-integrated diet. Thus, it would be difficult to observe an evident response in isotope values to a seasonal MDN subsidy.

In contrast to sculpin, salmon fry are young fish that are more actively growing and, thus, their isotope values should respond more rapidly to a change in diet (Hesslein et al. 1993). Salmon fry will initially have a maternal SDN fingerprint when they first hatch in streams in early spring (ca. March). At this point the YOY salmon begin feeding on a stream-derived diet. Their diet may again be influenced by MDN when adult salmon return to spawn in the fall (Doucett et al. 1996). This change in diet would elicit a strong

response in their isotope values, but it would also be influenced by the age and growth rate of the fish. We estimated the stream-derived diet using stomach content data for lower Fish Creek YOY coho and associated isotope values in a mass balance model (Gould et al., 1997). Estimates of the resulting carbon and nitrogen isotopic values of the stream-derived diet are 1.5 ± 0.5 and -26.3 ± 1.0 ‰, respectively. In contrast, a salmon-derived diet would be 11.4 ‰ and -19.9 ‰ for nitrogen and carbon isotope values, respectively. The MDN-influenced stream diet, estimated using stomach contents of post-spawning YOY coho from lower Fish Creek and associated isotope values in a mass balance model are 8.5 and -23.2 ‰ for $\delta^{15}N$ and $\delta^{13}C$ values, respectively (Fig. 12). YOY coho isotope values increased after their first exposure to the MDN. At the beginning of the MDN subsidy, the $\delta^{15}N$ values of the YOY coho differ from the MDN-influenced stream diet by about 3 ‰. After the MDN subsidy, the difference between the YOY coho and the MDN-diet is about 1 ‰, at which point the values reach an asymptote (Fig. 12). Initially after hatching, the stream-derived diet could be used for metabolic requirements, but the majority of YOY growth could be derived from an MDN-influenced diet. Fantle et al. (1999) showed the difference between blue crab (*Callinectes sapidus*) $\delta^{15}N$ values and their diet, or the trophic fractionation, was a function of the protein quality of the diet. In other words, trophic fractionation varies with growth rate, which is a function of the amount of dietary protein (diet quality). Organisms that are rapidly growing, on a high quality diet, will fractionate dietary nitrogen less than slow growing organisms feeding on a lower quality diet. The trophic fractionation exhibit between the YOY coho and their estimated diet suggest that immediately before the MDN subsidy the trophic fractionation was greater than after the MDN subsidy, which is in accordance with the

higher quality diet of the direct consumption of MDN. Freshwater fishes in Fish Creek responded to the MDN subsidy. However, future work should make a greater effort to constrain confounding factors, including migratory behavior and multiple exposures to MDN before assessing the importance of the SDN subsidy to these stream consumers.

Longitudinal Trends

The influence of marine-derived nutrients varied longitudinally in Fish Creek for macroinvertebrates during and after spawning (Fig. 8). The longitudinal trend was documented by macroinvertebrates $\delta^{13}C$ and $\delta^{15}N$ values increasing downstream from the salmon barrier after spawning in 2000 and during and after spawning in 2001. To the best of our knowledge, this is the first time such spatial variation in MDN incorporation has been documented in streams. The longitudinal trend could be a result of (1) tissue turnover rates by stream consumers and/or (2) differences in marine-derived nutrient loading with distance downstream.

Previous stable isotope based diet switching experiments indicate that invertebrates have tissue turnover rates on the order of 30 days (e.g. Ostrom et al. 1997). We estimated tissue turnover based on the number of days over which isotope values increase from the stream-derived diet to the MDN-influenced diet. If a drifting macroinvertebrate enters lower Fish Creek from the upper reach, the time it takes their tissues to turnover and reflect the MDN-influenced diet provides an estimate of turnover rate. For example, during spawning, our grazer taxa exhibited rapid MDN incorporation of approximately of 30% after 12-14 days of the first arrival of salmon in Fish Creek (Fig. 11). Given enough time, macroinvertebrate tissues will approach isotopic

equilibrium, reflecting the MDN diet. However, many macroinvertebrates drift only a few meters (Rader 1997). Accordingly, since macroinvertebrates don't travel large distances longitudinally their isotopic signature should reflect the degree of the MDN in their immediate environment. If the degree of MDN base of the food web varies longitudinally then macroinvertebrates will mirror this trend. Initially MDN is deposited in streams as excretion and later as carcass deposition and decomposition. Salmon carcasses do decrease as you move upstream. However, the carcasses are heterogeneously dispersed in Fish Creek (Chaloner, unpublished data). Overtime the carcasses begin to decay, and produce a particulate pool of nutrients that will be entrained downstream. Therefore, the longitudinal trend could be a function of the accumulation of marine-derived nutrients downstream, in dissolved and particulate form, whereby the main mechanism for MDN retention is through the retention of remineralized MDN by the biofilm community. Carbon and nitrogen isotope values should be highest in areas where the most MDN is deposited. Nutrient dynamics in streams are tightly coupled with the physical movement of water (Newbold et al. 1983). An area downstream has had more exposure to marine-derived nutrients flowing by than an area further upstream. Biofilm located downstream, relative to upstream, are exposed to a higher concentration of dissolved and particulate marine-nutrients as a consequence of downstream flow of the salmon derived nutrient pool. Therefore, a trend of increasing isotope values in stream consumers downstream from the salmon barrier could be a function of streams being a longitudinally-dominated system where an organism downstream has a cumulative exposure to MDN.

Longitudinal isotopic variation in streams has major implications for evaluating the influence of salmon on stream ecosystems and associated management strategies. Depending upon the location of sampling, the estimated % MDN incorporations, using linear mixing models, could vary considerably and the % MDN incorporation could be at least 20 % different than estimates sampled from a downstream reach (Fig. 9). Accordingly, spatial sampling is crucial to assess the influence of MDN on the stream food webs.

Inter-Annual Data and Salmon Escapements

In addition to the spatial heterogeneity of MDN in streams, temporal variation in MDN can play a large role in the response of stream consumers. Lower Fish Creek consumers showed little difference in the % MDN incorporation during pre-spawning periods between 2000 and 2001 (Table 1, Fig. 10). Whereas, after spawning the macroinvertebrate carbon and nitrogen isotope values differed. In general, isotope values were greater in the samples collected in 2001 relative to 2000 (Table 1, Fig. 10). Initially, we examined if differences in salmon escapements could explain the observed trends. Using several different methods, the salmon escapement levels of Fish Creek appeared similar between 2000 and 2001 (Chaloner, unpublished data). In regards to MDN biomass, 2000 appears to have received more biomass than 2001 due to greater numbers of larger chum relative to pink salmon, but the difference is small (Chaloner, unpublished data). Thus, $\delta^{15}N$ values of stream consumers are not a simple proxy for salmon escapement levels. Instead, nitrogen isotope values can be influenced by many factors

including longitudinal variation in MDN accumulation, the spring carryover of MDN, and in general, nutrient cycling in streams.

The two-year database did reveal a rapid response to the MDN subsidy by primary consumers (Fig. 11). If the biofilm community transfers MDN to the macroinvertebrate food web via remineralization and utilization, then the scraper taxa will incorporate MDN prior to any other functional feeding group (e.g. predators, shredders). In Fish Creek, scrapers responded rapidly to the MDN pulse, with increases in $\delta^{13}C$ and $\delta^{15}N$ within weeks of the arrival of the first adult salmon. At the time we sampled during the spawning in 2001, only fresh salmon were present with no carcasses present. Furthermore, our sampling overlapped the peak of MDN-NH_4^+ attributed to excretion by the salmon spawning, which occurs only briefly when the salmon first arrive in the stream (N. Mitchell, MSc. 2002). Accordingly, a portion of the biofilm community selectively utilized by macroinvertebrates could be characterized by rapid incorporation of marine-derived dissolved inorganic nitrogen. Fish Creek data is in agreement with the conclusions of Matheisen et al. 1988 and Kline et al. 1991, 1993, i.e. autotrophic uptake of MDN. Bilby et al. 1996 argued primary production was limited by low light levels, cold temperatures, short day lengths, and frequent spate events after spawning (Bilby et al. 1996), and limited MDN incorporation via remineralization, therefore, MDN incorporation into the stream food web occurred by direct consumption. However, our results showing a rapid increase in isotope values associated with a peak in NH_4^+ suggests an important role for incorporation of dissolved inorganic nitrogen into the food web via the biofilm community.

Recently, Bilby et al. (2000) and Finney et al. (2001) suggested that $\delta^{15}N$ values of different food web components could be used to develop a historical perspective of salmon escapements. As we have demonstrated, nitrogen isotope values of macroinvertebrates vary longitudinally in Fish Creek. Secondly, % MDN incorporation varies temporally and would likely reflect the degree of retention and/or assimilation throughout the winter. The degree of ^{15}N-enrichment observed in one year would be a function of the previous years salmon escapements rather than just the current year. Pre-spawning macroinvertebrate $\delta^{13}C$ and $\delta^{15}N$ values were similar in 2000 and 2001 in lower Fish Creek (Fig. 10). As a result, we can not interpret differences in % MDN incorporation during a sampling period as a simple reflection of salmon biomass delivered to Fish Creek. Moreover, we discussed that depending on which organisms were sampled, the degree of incorporation may be function of tissue turnover rates, longevity, and migratory behavior. For example, in Fish Creek many grazer invertebrates responded rapidly to the MDN subsidy (Fig. 11), but predators, both Chloroperlidae and resident fishes showed pronounced response (Fig. 7).

Conclusion/Implications

I found that the stable isotope composition of primary consumers provided an understanding on how lower trophic levels are influenced by the delivery of marine nutrients and organic matter from spawning salmon. My research supports the results of previous studies, in that significant amount of MDN was incorporated into stream food webs, by both lower and higher trophic-level consumers. However, I also found

significant temporal and spatial variation, and results were not always as predicted from previous studies, especially with regard to the biofilm assemblage. Stable isotope analyses have great utility in the study of MDN in freshwater ecosystems, but there is still a need to better characterize the processes underlying the link between marine subsidies and isotopic enrichment of food web components, and how this is linked to other measures of ecosystem structure and function. This is especially critical if stable isotope techniques are to be explicitly used in the management of Pacific salmon fisheries.

Table 1: Food Web Component	$\delta^{15}N$ (‰)				$\delta^{13}C$ (‰)			
	2000		2001		2000		2001	
	FCU	FCL	FCU	FCL	FCU	FCL	FCU	FCL
Organic Matter Source								
Biofilm								
Pre-spawning	**3.9** 0.7(4)	**3.5** 2.7(5)			**-25.9** 1.9(3)	**-29.2** 0.8(5)		
Post-spawning	**2.7** 2.6(4)	**1.7** 3.4(4)			**-23.0** 3.9(4)	**-27.4** 3.8(3)		
Alder leaves	**2.2** 0.3(3)				**-28.7** 0.2(3)			
CPOM			**1.7** 1.6(2)	**-2.2** 0.1(2)			**-27.6** 0.4(2)	**-27.9** 0.4(2)
Macro-invertebrate								
Scrapers								
Cinygmula								
Pre-spawning	**1.6** 0.3(11)	**0.3** 0.3(7)	**1.6** 0.6(5)	**-0.5** 0.8(4)	**-26.1** 0.8(8)	**-30.3** 0.5(5)	**-26.1** 1.5(5)	**-29.8** 0.1(4)
During			**5.6** 0.6(5)	**-0.1** 0.2(3)			**-23.8** 0.5(5)	**-28.6** 0.5(3)
Post-spawning	**8.3** 0.5(5)	**0.2** 0.3(5)	**9.9** 1.4(5)	**0.4** 0.7(6)	**-22.3** 0.6(5)	**-30.6** 0.4(3)	**-21.5** 0.3(5)	**-28.5** 0.7(3)
Epeorus								
Pre-spawning	**0.8** (1)	**-0.1** 0.4(5)	**0.9** 0.2(5)	**-0.5** 0.8(4)	**-27.0** (1)	**-30.7** 0.6(3)	**-27.0** 0.4(5)	**-30.0** 0.2(4)
During			**5.3** 0.7(5)	**-0.1** 0.2(3)			**-26.4** 0.5(5)	**-29.9** 0.3(3)
Post-spawning	**5.3** 0.6(4)	**-1.0** 0.1(4)	**8.1** 0.5(5)	**0.4** 0.7(6)	**-25.0** 0.8(4)	**-31.6** 0.1(2)	**-25.0** 0.3(5)	**-28.5** 0.7(6)
Drunella								
Pre-spawning	**3.4** 0.6(2)	**1.4** 0.4(3)	**3.4** 0.2(2)	**1.1** 0.4(5)	**-25.4** 0.9(3)	**-30.0** 0.1(2)	**-26.0** (1)	**-29.1** 0.6(5)
During			**3.9** (1)	**0.4** 0.5(7)			**-25.6** (1)	**-27.9** 0.8(7)
Post-spawning	**5.2** (1)	**0.0** 0.4(5)	**7.4** 0.5(8)	**-0.1** 0.6(4)	**-30.3** (1)	**-30.5** 0.4(3)	**-24.6** 1.1(8)	**-28.4** 0.4(4)
†Small			**1.1** 0.3(4)	**0.3** 0.9(4)			**-25.3** 0.8(4)	**-28.0** 1.9(4)
†Large			**3.4** 0.3(2)	**1.1** 0.4(5)			**-25.1** 1.3(2)	**-29.1** 0.6(5)
Collector-Gathers								
Baetidae								
Pre-spawning	**1.5** 0.2(6)	**0.6** 0.3(8)	**1.7** 0.3(4)	**0.5** 0.2(3)	**-26.7** 0.3(6)	**-30.3** 0.7(6)	**-24.2** 0.6(4)	**-28.5** 0.3(3)

During			**5.5** (1)	**0.4** 0.2(5)			**-25.3** (1)	**-27.5** 0.4(5)
Post-spawning		**-0.1** (1)	**8.0** (1)	**0.0** (1)		**-21.2** 0.6(2)	**-23.2** (1)	**-27.6** (1)
Paraleptophelbidae								
Pre-spawning			**8.3** (1)				**-22.1** (1)	
Post-spawning			**7.1** (1)				**-24.4** (1)	
Shredders								
Zapada								
Pre-spawning								
During			**5.5** (1)	**-0.3** (1)			**-36.4** (1)	**-27.8** (1)
Post-spawning	**5.5** (1)	**-0.3** (1)	**6.6** 0.5(5)	**0.3** 0.9(5)	**-36.4** (1)	**-27.8** (1)	**-24.5** 0.5(5)	**-27.3** 0.2(5)
Predators								
Chloroperlidae								
Pre-spawning	**5.8** 2.9(8)	**1.8** 0.7(2)	**6.8** 1.8(4)	**5.6** (1)	**-23.9** 2.2(8)	**-26.5** (1)	**-23.0** 1.6(4)	**-24.3** (1)
During			**7.4** 0.6(5)	**2.5** 1.7(4)			**-22.2** 0.3(5)	**-25.5** 0.8(4)
Post-spawning	**11.1** (1)		**9.9** 0.6(6)	**3.6** 0.9(5)	**-20.3** (1)		**-21.3** 0.3(6)	**-25.3** 0.8(5)
Rhyacophilidae								
Pre-spawning		**1.6** (1)				**-29.1** (1)		
Post-spawning		**2.2** (1)	**6.6** 0.5(5)	**0.3** 0.9(5)		**-26.0** (1)	**-24.5** 1.1(5)	**-27.3** 0.2(5)
Perlodidae								
Pre-spawning				**3.2** 0.1(4)				**-29.3** 0.3(4)
During				**2.9** (1)				**-29.6** (1)
Post-spawning				**1.9** 0.6(4)				**-27.1** 0.1(4)
Freshwater Fish								
YOY coho								
Pre-spawning				**11.4** 2.2(3)				**-20.3** 1.0(3)
Post-spawning				**12.9** 0.7(7)				**-19.5** 0.3(7)
Coho Parr								
Pre-spawning				**11.4** 1.7(2)				**-21.3** 0.1(2)
Post-spawning				**12.7** 0.5(3)				**-19.8** 0.4(3)
Sculpin								
Pre-spawning				**10.6** 0.6(8)				**-20.3** 1.6(8)
Post-spawning				**11.8** 0.9(7)				**-18.3** 1.2(7)
Dolly Varden								

Pre-spawning	**11.8** 0.2(3)	**5.8** 0.2(3)	**6.1** 0.4(3)	**-22.7** 2.1(3)	**-27.1** 0.6(3)	**-26.0** 1.2(3)
Post-spawning	**13.3** 0.4(5)	**6.3** 1.5(5)	**5.9** 0.2(6)	**-19.6** 0.5(5)	**-26.6** 1.9(5)	**-26.5** 0.9(6)
Adult Salmon, *Fresh*						
Fish Cr. Females						
Chum	**11.2** 0.3(6)			**-19.4** 0.5(6)		
Pink	**11.5** 0.5(3)			**-20.2** 0.6(3)		
DIPAC Females						
Chum	**11.2** 0.4(3)			**-19.4** 0.6(3)		
Pink	**11.7** 0.2(3)			**-20.3** 0.4(3)		
Adult Salmon, *Dead*						
Fish Cr. Females						
Chum and Pink	**11.6** 0.4(5)			**-19.5** 0.5(5)		

Table 2:

Food Web Component	2000		2001	
	% MDN C	% MDN N	% MDN C	% MDN N
Macroinvertebrates				
Scrapers (1)				
Cinygmula				
Pre-spawning	37-39	10	34-35	12-13
During	-	-	49-52	36-38
Post-spawning	71-74	56-58	73-77	65-69
Epeorus				
Pre-spawning	22-23	4	24-25	10-11
During	-	-	32-33	36-38
Post-spawning	52-54	40-42	40-42	54-57
Drunella				
Pre-spawning	37-38	15-16	35-37	7
During	-	-	25-27	24-25
Post-spawning	-	-	41-43	50-53
Collector-Gathers (1)				
Baetidae				
Pre-spawning	45-48	7-8	34-36	8
During	-	-	26-28	35-37
Post-spawning	-	-	51-54	54-57
Shredders (1)				
Zapada				
Pre-spawning	-	-	-	-
During	-	-	14-15	19-20
Post-spawning	NA	38-40	33-35	44-46
Predators (2)				
Chloroperlidae				
Pre-spawning	46-56	32-37	21-25	23-26
During	-	-	44-50	31-34
Post-spawning	54-67	36-41	55-63	43-48
Freshwater Fish [1]				
YOY Coho (2)				
Pre- vs. Post-	-	-	34-57	22-28
Sculpin (2)				
Pre- vs. Post-	-	-	83-100	16-19
Dolly Varden (2)				
Pre-spawning	50-56	49-55	-	-
Post-spawning	80-91	59-67	-	-
Lower pre- vs. post-	62-80	23-30		

Table 3:

Food Web Component	$\delta^{15}N$		$\delta^{13}C$	
	Above Barrier	Below Barrier	Above Barrier	Below Barrier
Scrapers				
Cinygmula	0.5 0.3(3)	1.1 0.1(3)	-29.1 0.1(3)	-29.6 0.7(3)
Epeorus	0.0 0.6(3)	0.3 0.2(3)	-29.7 0.2(3)	-30.4 0.7(3)
Collector-gather				
Baetidae	0.6 0.1(3)	0.8 0.2(3)	-28.9 0.1(3)	-29.4 0.0(3)
Predator				
Chloroperlidae	3.3 (1)	3.0 (1)	-27.5 (1)	-27.5 (1)

Figure 1

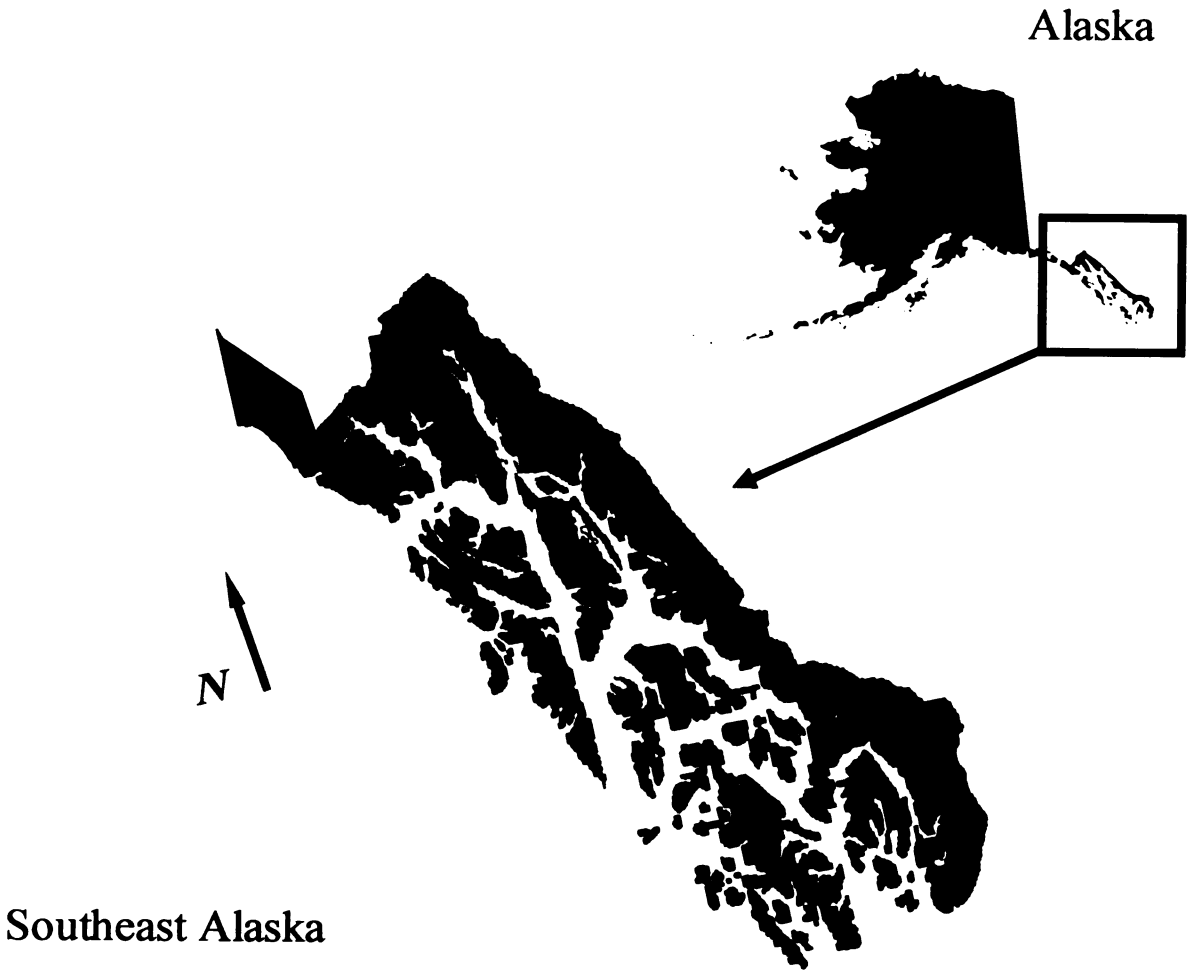


Figure 2

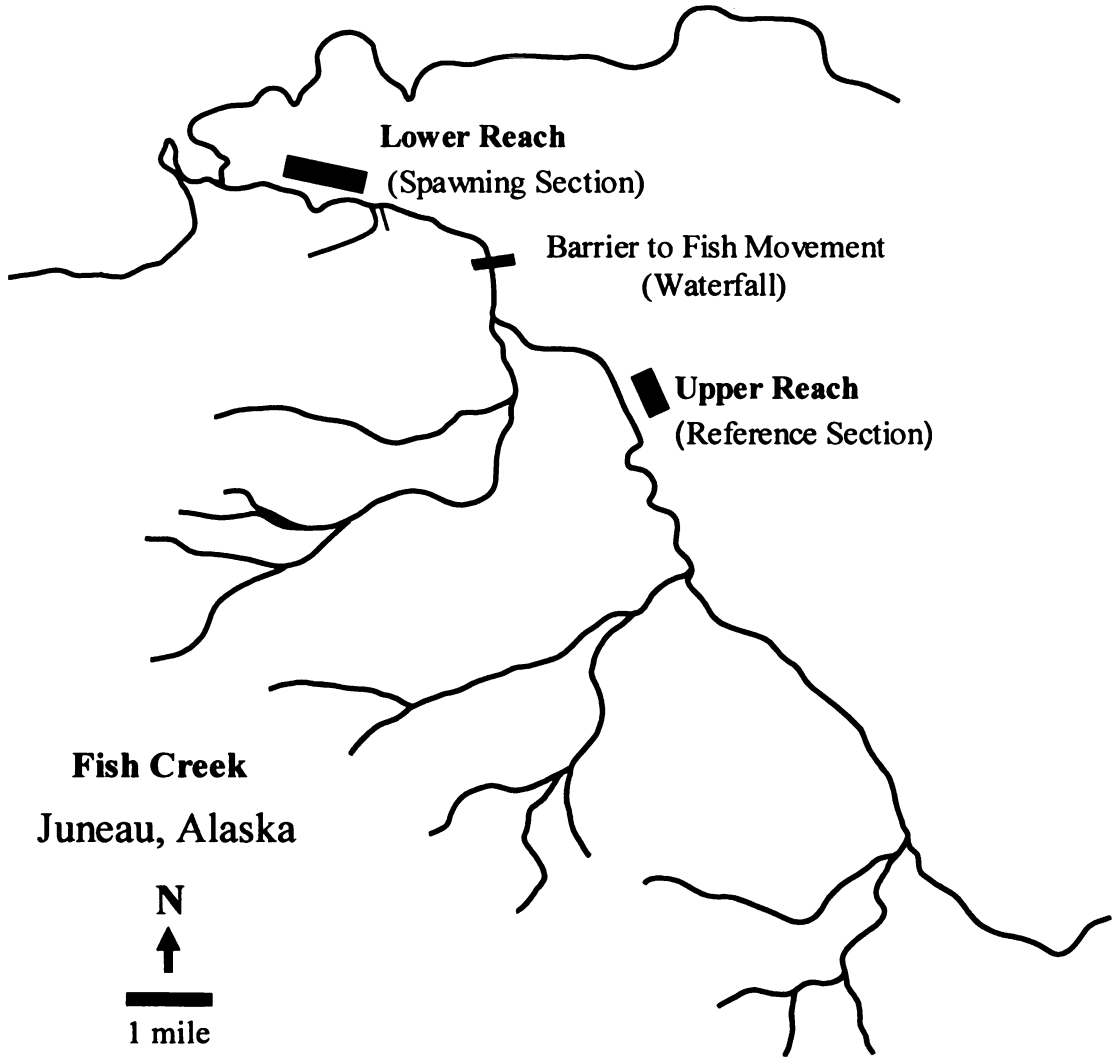

Lower Reach
(Spawning Section)
Barrier to Fish Movement
(Waterfall)
Upper Reach
(Reference Section)
Fish Creek
Juneau, Alaska
N
1 mile

Figure 3

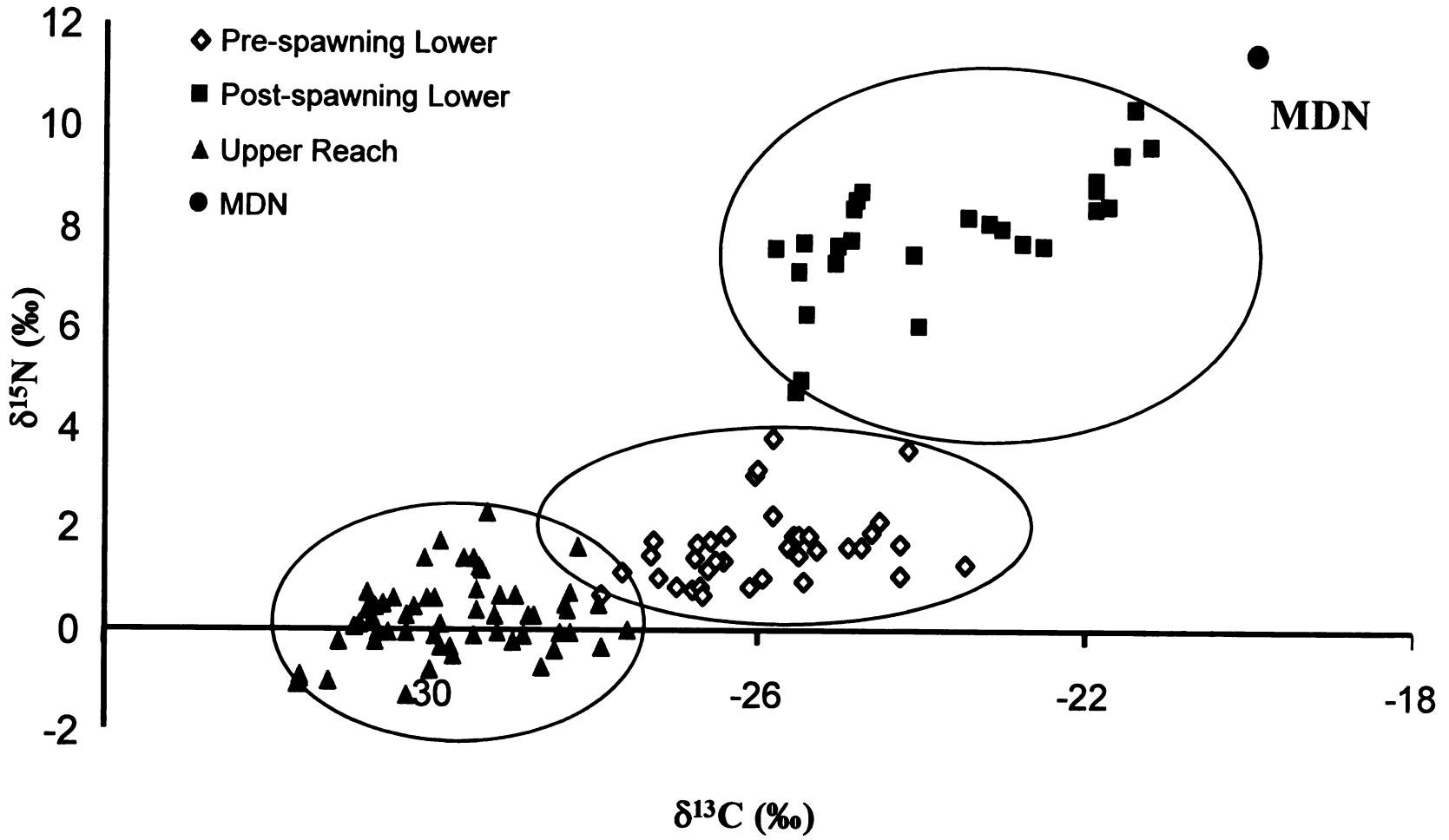

Pre-spawning Lower
Post-spawning Lower
Upper Reach
MDN
MDN
12
10
8
6
4
2
0
-2
δ15N (‰)
-30
-26
-22
-18
δ13C (‰)

Figure 4

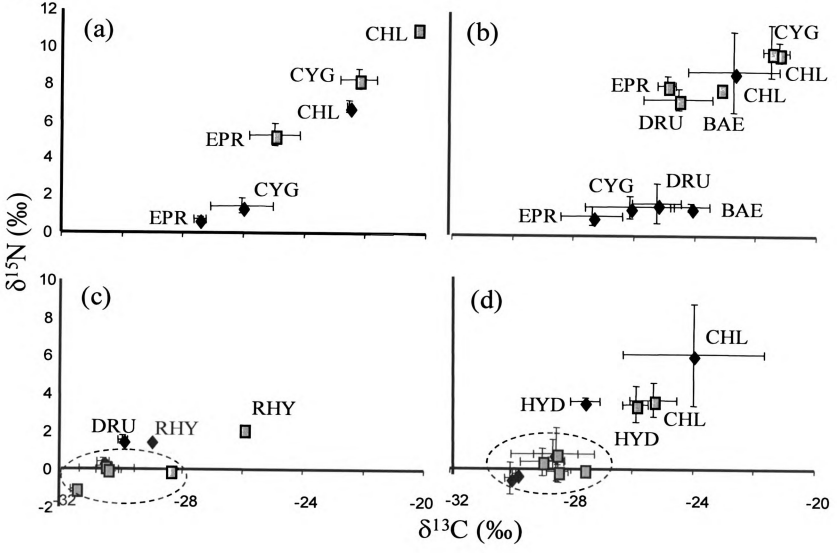

(a)
CHL
CYG
CHL
EPR
CYG
EPR
(b)
CYG
CHL
EPR
CHL
DRU
BAE
CYG
DRU
EPR
BAE
(c)
RHY
DRU
RHY
(d)
CHL
HYD
CHL
HYD
δ15N (‰)
δ13C (‰)
12
10
8
6
4
2
0
-2
-32
-28
-24
-20

Figure 5a

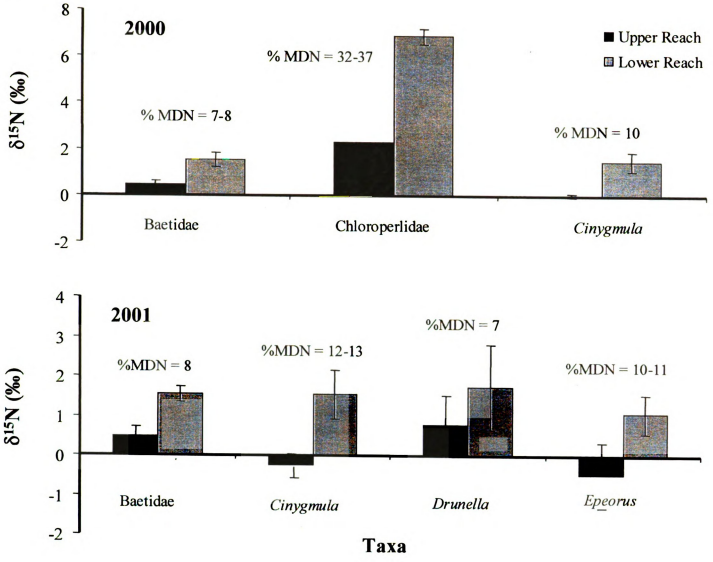

2000
Upper Reach
Lower Reach
% MDN = 32-37
% MDN = 7-8
% MDN = 10
δ15N (‰)
8
6
4
2
0
-2
Baetidae
Chloroperlidae
Cinygmula
2001
%MDN = 8
%MDN = 12-13
%MDN = 7
%MDN = 10-11
4
3
2
1
0
-1
-2
Baetidae
Cinygmula
Drunella
Epeorus
Taxa

Figure 5b

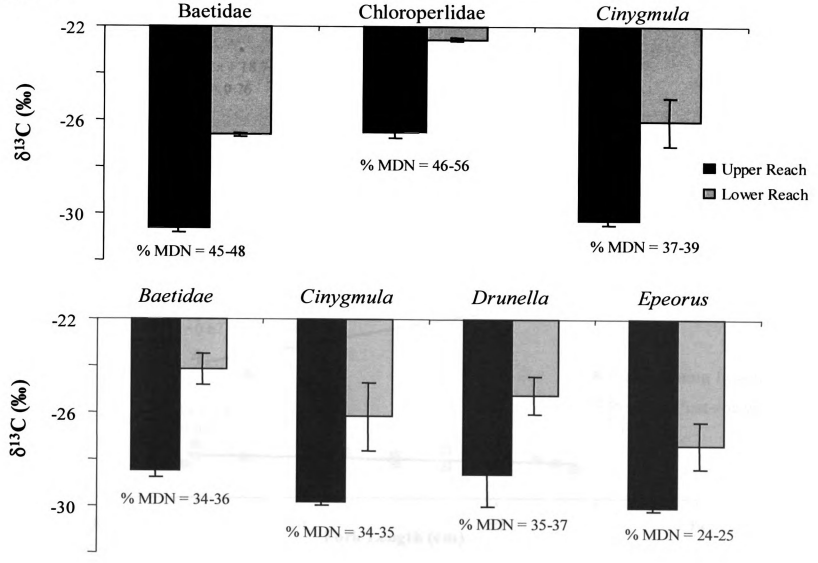

Baetidae
Chloroperlidae
Cinygmula
-22
-26
-30
δ13C (‰)
Upper Reach
Lower Reach
% MDN = 45-48
% MDN = 46-56
% MDN = 37-39
Baetidae
Cinygmula
Drunella
Epeorus
-22
-26
-30
δ13C (‰)
% MDN = 34-36
% MDN = 34-35
% MDN = 35-37
% MDN = 24-25

Figure 6

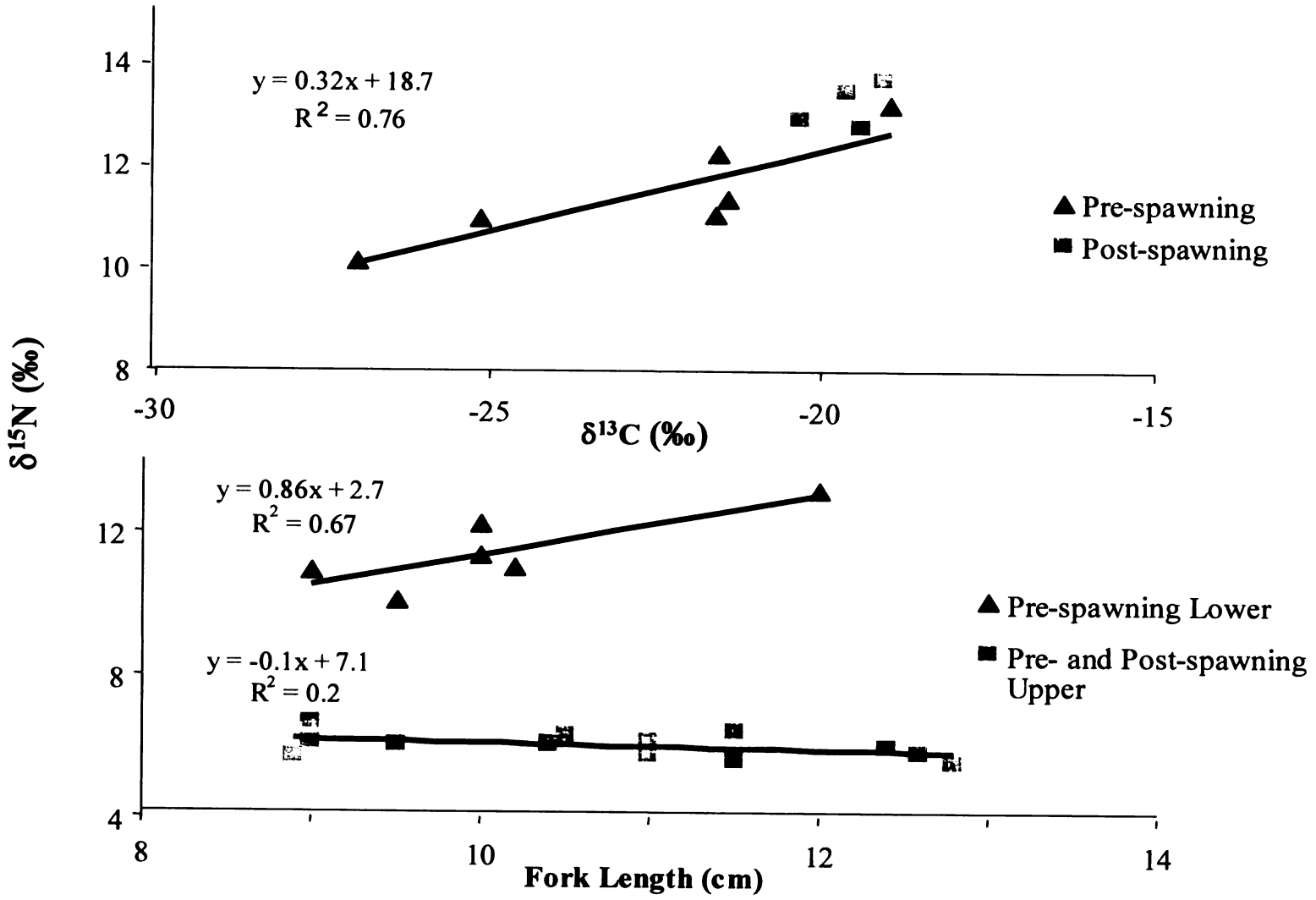

y = 0.32x + 18.7
R 2 = 0.76
Pre-spawning
Post-spawning
δ15N (‰)
δ13C (‰)
y = 0.86x + 2.7
R2 = 0.67
y = -0.1x + 7.1
R2 = 0.2
Pre-spawning Lower
Pre- and Post-spawning Upper
Fork Length (cm)

Figure 7

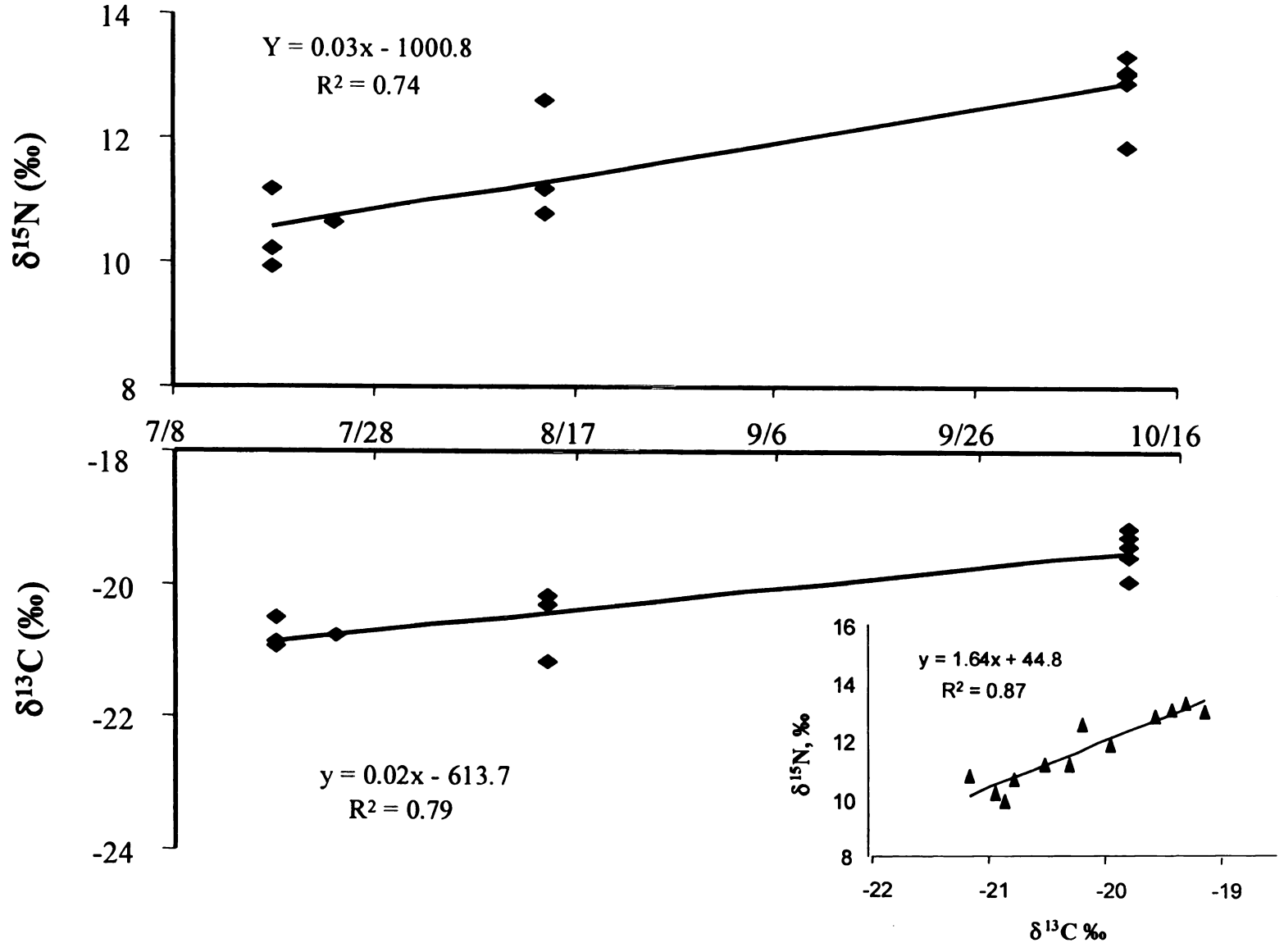

Y = 0.03x - 1000.8
R² = 0.74
δ15N (‰)
14
12
10
8
7/8
7/28
8/17
9/6
9/26
10/16
δ13C (‰)
-18
-20
-22
-24
y = 0.02x - 613.7
R² = 0.79
y = 1.64x + 44.8
R² = 0.87
δ15N, ‰
16
14
12
10
8
-22
-21
-20
-19
δ13C ‰

Figure 8

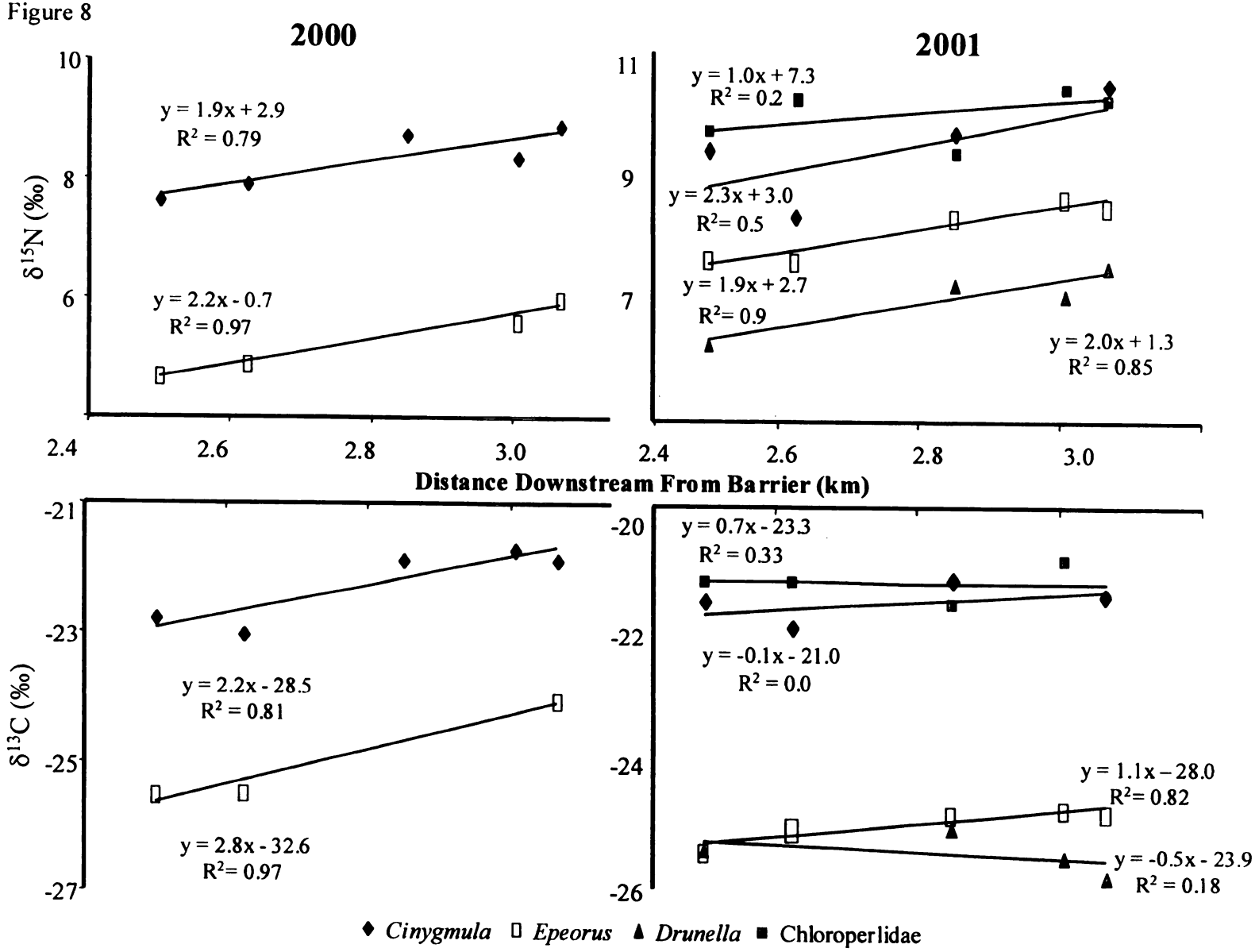

2000
2001
y = 1.9x + 2.9
R² = 0.79
y = 2.2x - 0.7
R² = 0.97
y = 1.0x + 7.3
R² = 0.2
y = 2.3x + 3.0
R²= 0.5
y = 1.9x + 2.7
R²= 0.9
y = 2.0x + 1.3
R² = 0.85
δ15N (‰)
Distance Downstream From Barrier (km)
y = 2.2x - 28.5
R² = 0.81
y = 2.8x - 32.6
R²= 0.97
y = 0.7x - 23.3
R² = 0.33
y = -0.1x - 21.0
R² = 0.0
y = 1.1x – 28.0
R²= 0.82
y = -0.5x - 23.9
R² = 0.18
δ13C (‰)
♦ Cinygmula □ Epeorus ▲ Drunella ■ Chloroperlidae

Figure 9

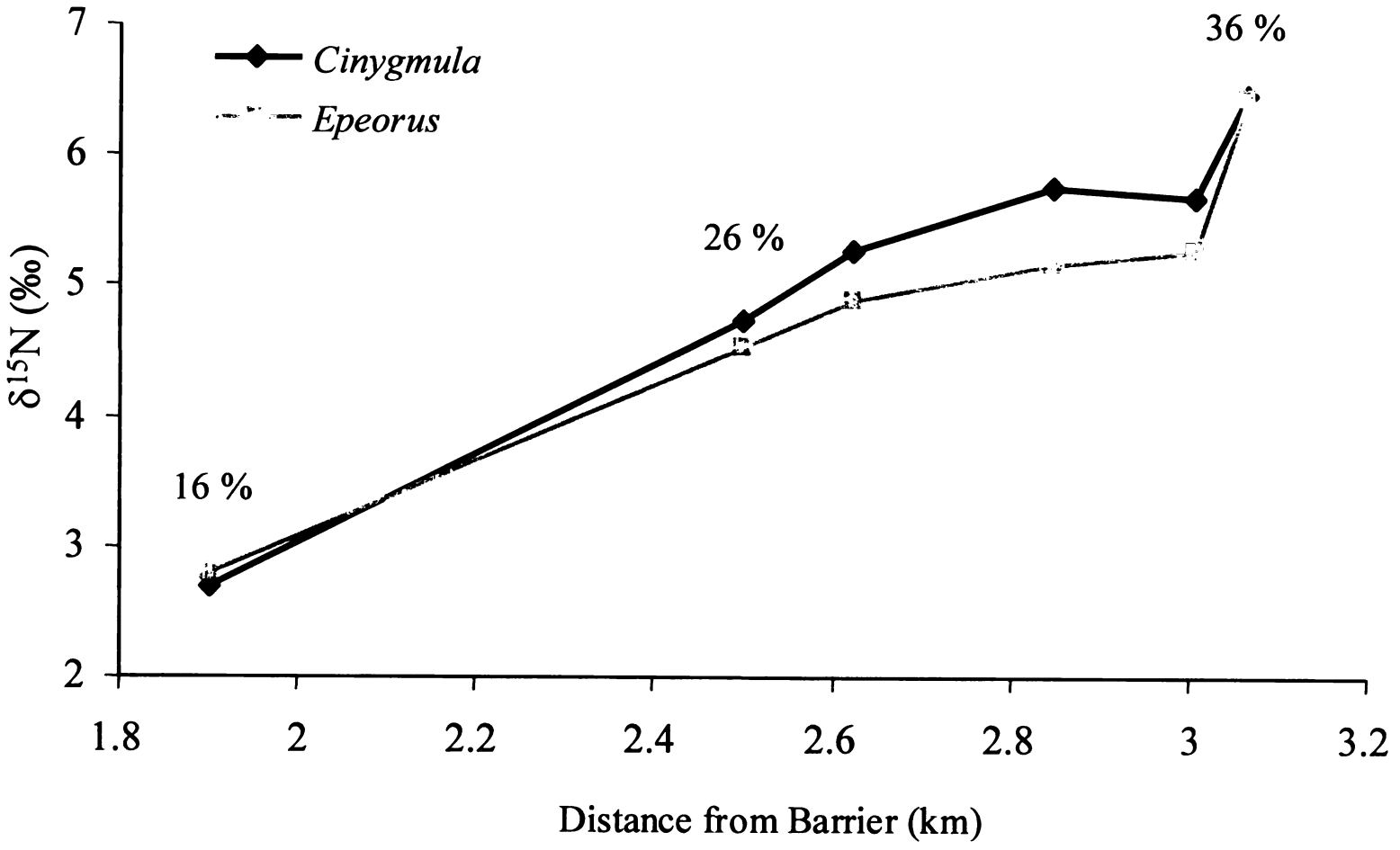

Cinygmula
Epeorus
36 %
26 %
16 %
δ15N (‰)
Distance from Barrier (km)
7
6
5
4
3
2
1.8
2
2.2
2.4
2.6
2.8
3
3.2

Figure 10

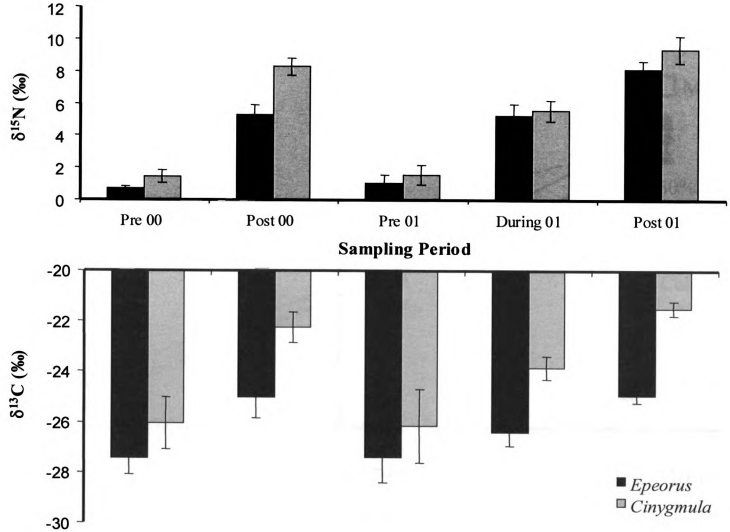

12
10
8
6
4
2
0
δ15N (‰)
Pre 00
Post 00
Pre 01
During 01
Post 01
Sampling Period
-20
-22
-24
-26
-28
-30
δ13C (‰)
Epeorus
Cinygmula

Figure 11

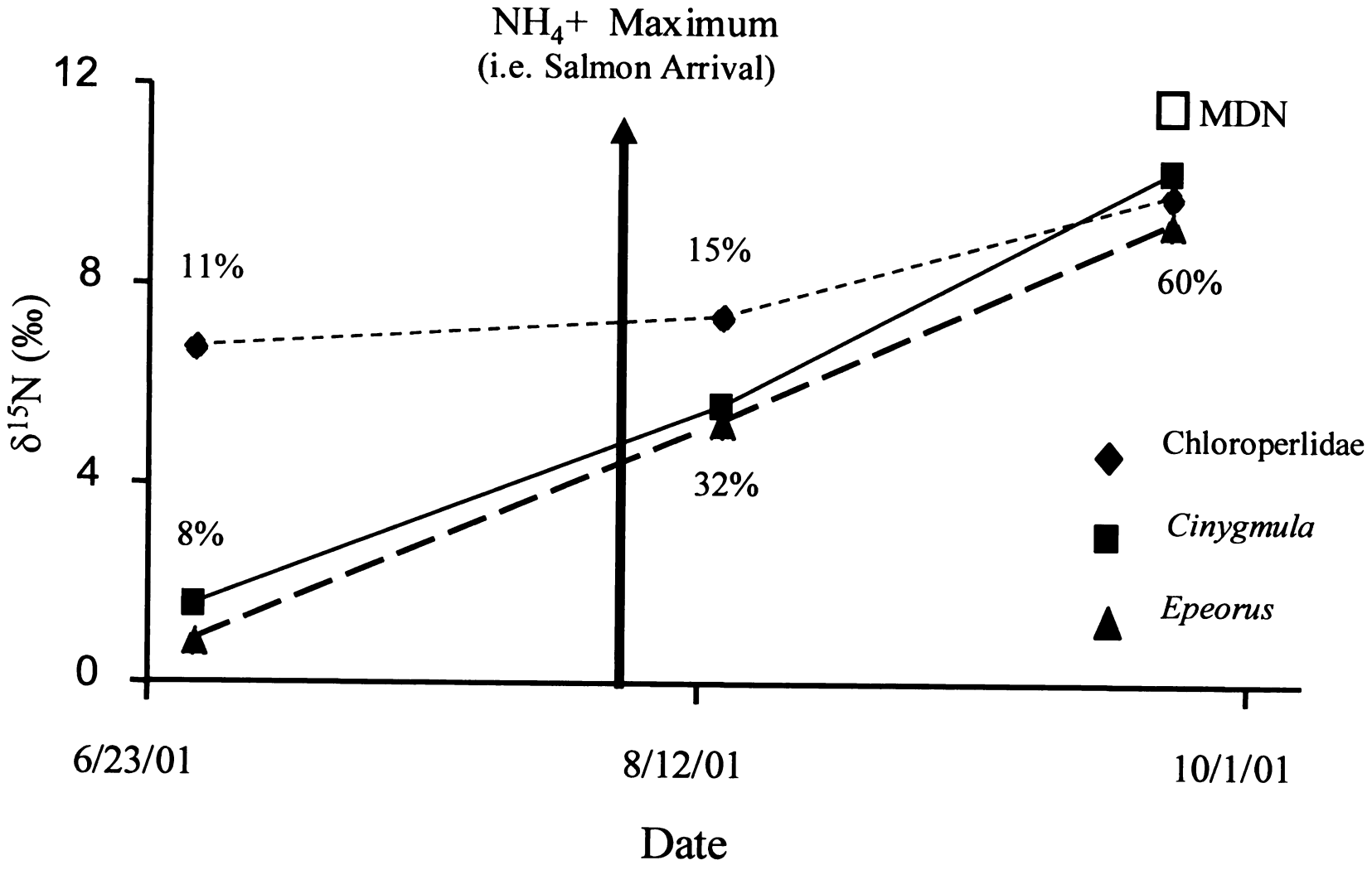
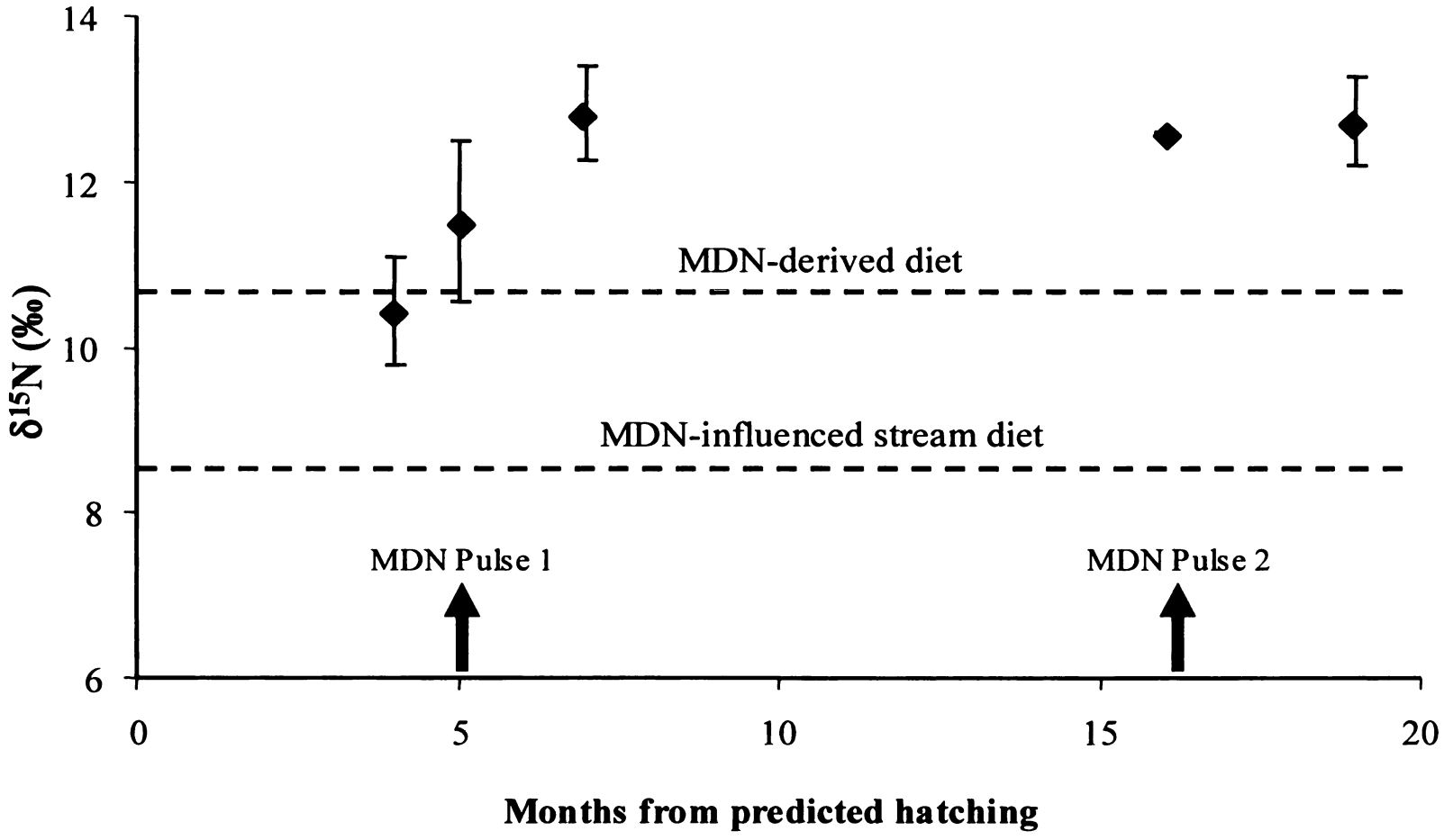

NH4+ Maximum
(i.e. Salmon Arrival)
MDN
12
8
4
0
δ15N (‰)
11%
15%
60%
8%
32%
Chloroperlidae
Cinygmula
Epeorus
6/23/01
8/12/01
10/1/01
Date

Figure 12


14
12
10
8
6
δ15N (‰)
MDN-derived diet
MDN-influenced stream diet
MDN Pulse 1
MDN Pulse 2
0
5
10
15
20
Months from predicted hatching

LITERATURE CITED

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