Tracing of Fish Farm Effects on Sediment and Food Web of Rufus Woods Lake, Columbia River

2009 Results

Prepared For:

Pacific Aquaculture Inc.
Nespelem, Washington

and

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Natural Resources Department
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Nespelem, Washington

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Principal Investigator: Jack Rensel Ph. D.[1]

[1/] Research Scientist
Rensel Associates Aquatic Sciences
4209 234th Street N.E.
Arlington, WA 98223

Field Assistance: SCUBA Underwater Recovery
Mr. Archie Dennis

EXECUTIVE SUMMARY

Fish farming of sterile steelhead trout in net pens has been practiced since 1989 in Rufus Woods Lake, a mid-Columbia River reservoir downstream of Grand Coulee Dam and the large Lake Roosevelt storage reservoir (Shallenberger 2009). Before pens were first placed, I was asked to examine water quality and potential impacts of the first operation. This led to a report quantifying the dissolved and total nutrient flux of the river throughout Washington State and estimated effects of different levels of fish farm production on the system through water and nutrient budget analyses (Rensel 1989). It was clear at that time that Columbia River and even downstream locations were highly impoverished with respect to the important macronutrients nitrogen and phosphorus. This results from nutrient trapping in the large, upstream Canadian and American storage reservoirs in this river system. This process, common in rivers with numerous or extensive storage reservoirs, traps particulate nutrients, prevent remineralization and curtails "nutrient spiraling" (i.e., flux downstream of nutrients from dissolved to particulate and inorganic to organic, both repeating). Chlorophyll *a* concentrations in mid Columbia River reservoirs drop to very low concentrations and dissolved inorganic phosphorus becomes immeasurably low throughout most of the year. Even nutrient additions from the eutrophic Snake and Yakima Rivers have little effect on the mainstem Columbia River as the total volumes of these rivers are very small compared to the mainstem. The nutrient poor nature of the mid Columbia River is evident to the most casual observer in terms of the very high water transparency after peak run off in June and even throughout the winter. Lack of appropriate aquatic food web support for migrating juvenile fish in the river and the estuary is an important factor limiting wild salmon and steelhead production in the lower river (ISG 1996), although little dedicated study has focused on this aspect of the mid Columbia River. As salmon and steelhead runs have declined in the Columbia River basin, only a tiny fraction (~6%) of the marine derived nutrients once transported by fish carcasses now enters the basin (Gresh et al. 2000) and none are available in Rufus Woods Lake.

In recent years there has been some limited efforts to examine lake bottom (benthic and epibenthic) food web status of the mid Columbia River. Relatively high rates of primary production of periphyton (algae such as benthic diatoms, cyanobacteria, heterotrophic microbes, and detritus attached to submerged surfaces) were reported in Parametrix et al. 2000 for Rocky Reach Reservoir but no such studies have been conducted in Rufus Woods Lake or the next downstream reservoir, Lake Pateros. This productivity is littoral based (nearshore) and related to the normally high clarity of the water in most of the growing season, relatively high water temperatures and the high rate of flow that supplies adequate nutrient flux despite the low ambient concentrations. Periphyton are typically a keystone component of the benthic and epibenthic food web of temperate rivers and reservoirs but poorly studied or understood in the mid Columbia River. Periphyton may be more important than macrophytes in the system in terms

of providing the base of the benthic food web, but a few species of periphyton are considered noxious or at least undesirable.

The purpose of this project was to study the feasibility of stable isotope analysis (herein: SIA) tracing methodology to estimate the extent of net pen fish farm effects on the epibenthic food web and sediments of Rufus Woods Lake. There has been virtually no quantitative study of the benthic and epibenthic food web of this unusual reservoir, which is narrower and deeper than other Columbia River impoundments and subject to considerable bank erosion and sloughing.

Initial stable isotope work in RWL sponsored by Columbia River Fish Farms was conducted in late summer of year 2000, but was limited to single sampling locations upstream and immediately downstream of fish farm Site 1, near Nespelem. That initial work guided me in selecting appropriate indicator species for the 2009 sampling and for the first time we also collected lake-bottom sediments by SCUBA diving for analysis of carbon and nitrogen and their stable isotopes. Stable isotopes of nitrogen and carbon are naturally occurring isotopes, identical to normal atoms except for an extra neutron, but are often preferentially retained or eliminated by different food web organisms. In some circumstances these properties allows stable isotope assays to serve as food web tracers and to distinguish anthropogenic nutrient discharge effects, particularly lower in the food web where fewer food sources may be utilized by various organisms. This report includes an extensive educational primer on the subject of stable isotope analysis.

The study reported here was conducted in late summer of 2009 and included diver-collected core samples for total nitrogen and total carbon as well as stable isotopes ^{15}N and ^{13}C. Total N and C increased very slightly statistically insignificantly immediately downstream of the fish farm but declined to upstream reference area concentrations by about 575' downstream of the primary fish farm site. Relatively high levels of both were found in the Chief Joseph Pool, but this is to be expected as the percent fines of silt and clay were much higher in the pool than in the coarse sediments in the upper reaches of RWL and it is well known that increased N and C are inversely correlated with sediment grain size. Stable isotope analysis of sediments indicated that enrichment of ^{13}C occurred to at least 1,300 feet downstream of the net pens at Site 1 but by one mile downstream was not statistically different from the reference area. No enrichment of sediment 15N was found, but rather a curious inverse pattern seen for carbon stable isotope ^{13}C. These data suggest that assimilation of the fish fecal and fish feed discharge are occurring within the cited distances and are not accumulating or being transmitted further downstream. The study design was biased to sampling exactly within the expected trajectory of dissolved and solid waste matter from the pens and as there is little horizontal mixing within such distances, the results overstate the probable effects of the net pens considerably, resulting in a highly conservative estimate.

Results of infauna analysis indicate distinct patterns of enrichment of stable isotope in Rufus Woods Lake for sediments, snails, crayfish and sculpins, each with their own unique signature. The nutrient enrichment effects of the fish farm vary spatially from a few hundred feet downstream to possibly a mile or more for these species. The data indicate that waste nutrients are being assimilated by different organisms and with some minor additional sampling, the percent sustenance being acquired by each type of organism at different points downstream may be estimated. While the patterns of enrichment or depletion of 13C and 15N in relation to the net pens are reasonable, some are curiously different. For example, snail 15N is enriched downstream of the net pens but 13C is depleted. Possible reasons for this are advanced in this report. For crayfish, a different result indicates enrichment of 13C but depletion of 15N downstream of the pens. Sculpins (small, epibenthic or demersal fish) showed enrichment of both and prior work showed that they actually consume waste feces and ostensibly waste feed. These data show that the wastes from the fish farm are being utilized in the system and not simply being accumulated downstream where they may have a biological oxygen demand from bacterial respiration. In a system that is generally considered nutrient starved, this may be viewed as a beneficial effect of the net pen operation. But all water bodies have a carrying capacity for nutrient discharge for differing reasons and there is an eventual need for further sampling to validate the reference results and fill in some of the blanks for the downstream locations beyond a mile and before the Chief Joseph pool.

As more is learned, the practical use of these data will become more apparent. Not included in this study were estimates of relative abundance of the target organisms that would indicate if the fish farms are enabling increased productivity, or just a shift in dietary basis. Additional food web studies are commencing in summer 2010, funded by Bonneville Power Authority to Colville Confederated Tribes and their contractors that may provide data to further test the enhancement hypothesis. These studies include estimates of periphyton growth rates and species diversity which are key indicators of water quality. Use of stable isotope methodology is proposed to further characterize the effects of the net pens in terms of spatial effects and possible perturbations of the biotic community. The net pen operations in Rufus Woods Lake offer an exciting opportunity to test the effects of large quantities of marine derived nitrogen and carbon in a system now entirely cut off from the introduction of ocean derived nutrients that was once common in the river. The present level of fish production does not exceed that of the past several decades and should the carrying capacity be exceeded, the effects will be short term and reversible but monitoring is necessary to detect such changes, as discussed herein.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

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Data interpretation and studies represent the views of Jack Rensel, Ph.D. of Rensel Associates Aquatic Sciences not Pacific Aquaculture or the Colville Confederated Tribes or any other groups or organizations.

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INTRODUCTION

The purpose of this project was to study the feasibility of stable isotope analysis (herein: SIA) tracing methodology to estimate the extend and degree of net pen fish farm effects on the epibenthic food web and sediments of Rufus Wood Lake (RWL). A complete review of the history of fish farming is beyond the scope of this report, but a few facts are set out for reviewers less familiar the history of fish farming in RWL. Rufus Wood Lake is a 51 mile long, narrow and relatively deep reservoir or the mid Columbia River in the eastern side of Washington State (Fig. 1). Net pen fish farming in Rufus Wood Lake began in 1989 with the introduction of a small number of cages on the Douglas County side of the river with a company known as Stolt Sea Farm (Rensel 1989, Shallenberger 2009). Eventually the project moved to the Colville Confederated Tribes (herein: CCT) Reservation side of the river near Nespelem and was acquired by a small, Washington State company known as Columbia River Fish Farms (CRFF). The company later established a second net pen site (Site 2) upstream of the initial site (Site 1).

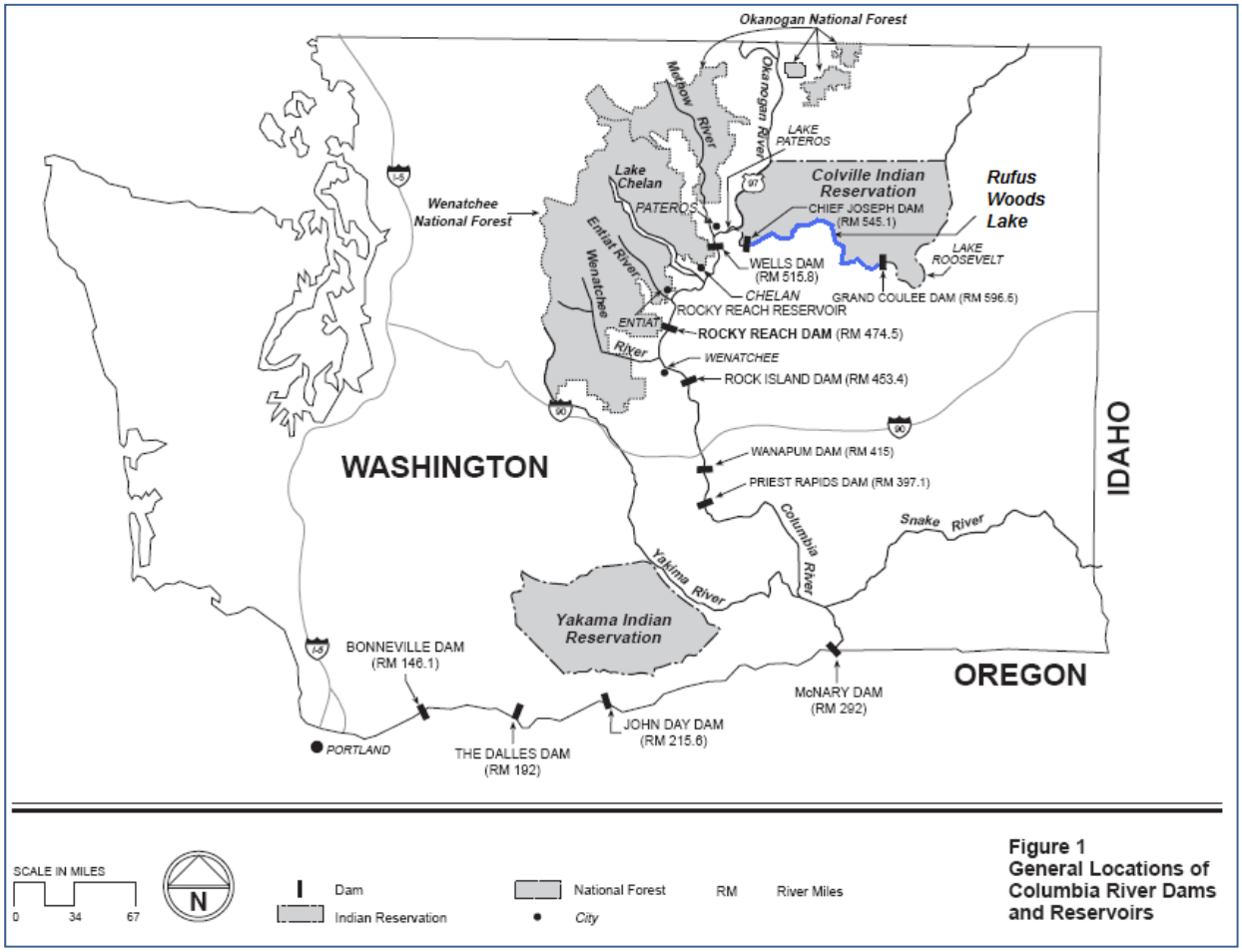


Figure 1. Vicinity Map and general locations of Columbia River dams and reservoirs.

Presently and since late 2008 the fish farm operation discussed herein has been owned and completely renovated by Pacific Seafoods, an Oregon-based corporations and one of the largest seafood processors and distributors in the United States. A separate company, Chief Joseph Orchards, operates another net pen facility downstream of both of the CRFF sites. Cumulatively the three sites have the capacity to produce about 6 million pounds of fish annually but production has been lower in recent years. In all cases, only triploid (sterile) rainbow trout (AKA steelhead, *Oncorhynchus mykiss*) are reared in the cages, mostly for commercial harvest but some are released for Tribal subsistence and general sport fishery enhancement.

RWL has a very short average water retention time of about 2.5 days and is one of several mid Columbia River "run-of-the-river" reservoirs designed to pass water released from the upstream storage reservoirs including Lake Roosevelt. Water column conditions in FWL vary little horizontally or vertically and there is little time for phytoplankton (primary) or zooplankton (secondary) productivity to occur in the lake. RWL is substantially deeper and has a steeper bottom slope than any of the other middle Columbia River reservoirs which limits rooted aquatic or epiphytic plant production. Thus biological production and washout from upstream Lake Roosevelt has a profound effect on the water column in RWL but for the present study we are more concerned with RWL's benthic production that has never previously been assessed. One fisheries authority who studied most aspects of the lake in relatively great detail considered the fish stock status to be "extremely low" and the potential for enhancement of the fishery resource as "extremely limited" (Stober 1977). In part, these assessments were made on fish abundance estimates, but probably also stomach fullness measurements that indicated the majority of the fish gillnetted, including walleye and kokanee, had empty stomachs. Such estimates were admittedly biased by time delay after catch of up to 24 hours and regurgitation, which netted walleye are known to do. Nevertheless, Dr. Stober's work constituted the most complete spatial assessment of water quality, plankton and fish abundance studies ever done in the reservoir. Fast flushing of the reservoir's water column means there is less chemical coupling between the benthic layer and the water column in terms of nutrient flux that would occur in a more slowly flushed lake but for grazing fish, the linkage cannot be ignored if we are to understand food web productivity and the possible beneficial or adverse effects of net pen culture discharge of macronutrients.

Rensel (1989) first prepared an analysis of the water column effects of proposed net pen culture in the reservoir, focusing on dissolved and total N and P concentrations with regard to phytoplankton growth. Rensel (1993) then completed a more detailed review of all available and relevant data and some information from Lake Roosevelt in an initial assessment of the water column effects of fish farming in RWL to conclude that the system was oligotrophic (nutrient poor), rich in oxygen and that nutrient additions from fish farming would be beneficial in supporting downstream primary productivity. He also concluded that there was little macrophyte productivity because of

the rapid drop off in depth in most locations in RWL and the frequent sloughing of river banks that made for unsuitable macrophyte attachment substrate. He noted there was little data or information on benthic conditions in the lake.

Over several years subsequent to the initial Rensel (1989, 1993) reports, the same author collected extensive water column nutrient data as the Department of Ecology samples were not being analyzed with sufficiently low detection limits that were alternatively available at the University of Washington Routine Chemistry Laboratory. A series of annual reports and presentations to limnological meetings and other venues were prepared that illustrated the continued impoverishment of the water column nutrient flux in the system. The Cominco Ltd. fertilizer plant at Trail British Columbia changed its processing and discharge methods in the late 1980s which resulted in a major reduction of nitrogen and phosphorus discharge into the river and reduction in algae. The change resulted in reduction of the nuisance macrophyte *Cladophora* sp., a green filamentous alga that had been forming large mats in Lake Roosevelt previously (Welch et al. 1992). The alga is still present in Lake Roosevelt (and all of the Columbia River) but at much reduced levels. Total phosphorus concentrations in the water column declined concurrently with the changes and in downstream reservoirs, a factor that never was properly addressed in Columbia River salmon survival work as the system went from eutrophic to oligotrophic (nutrient poor). In recent years, dissolve orthophosphate (aka soluble reactive phosphorus) is undetectable for an average of 6 months out of the year and at other times is just barely detectable in RWL.

The team of eleven independent Columbia River scientists charged with assessing research gaps and needs in the system has noted that the quantity and quality of salmon prey in the lower river was not adequate and that this shortfall may have been causing poor estuarine and early marine survival of salmonids related to poor food web productivity (ISG 1996). Despite these low levels of productivity, in extensive water column secondary production (zooplankton and insects) studies of Rocky Reach Reservoir (Fig. 1, the second reservoir downstream of RWL), we found a variety of important fish prey such as cladoceran zooplankton and a wide variety of other zooplankton and insects, but summer average density of only 1,888 m^{-3} (Parametrix et al. 2000, researched and written mostly by Rensel Associates). That was far lower than mesotrophic or eutrophic regional lakes such as Lake Osoyoos, in the Okanogan River drainage, that averaged 20,000 to 80,000 m^{-3} over a four year study period (Rensel 1997, 1998).

A companion study to our water column work in Rocky Reach Reservoir by Dr. Mike Faulter, University of Idaho, of periphyton in the same lake found that standing stock levels of attached benthic algae (ABA, such as periphyton) were unexpectedly large (Parametrix et al. 2000). Typical of most such studies, the authors used clean, marked cobble placed in the littoral zone and recovered after a few weeks to estimate standing stock concentrations of ABA. No study of periphyton on macrophytes was attempted. Although no further work in the mid Columbia River

has been performed, a conceptual model of productivity may be that light is plentiful for primary productivity and nutrient flux (not concentration) is sufficient along the reservoir bottoms to fuel photosynthesis and higher levels of production. In simpler terms, the lake bottom was relatively much richer than the water column in terms of potential primary and secondary (attached algae and invertebrate production). If this is the case, will additional enrichment from fish farms increase this production and result in transfer of nutrients up the food web to fish? If so, will the enhancement be "desirable" fish species or less desirable species such as suckers that are quite prolific further downstream in Columbia River reservoirs? These are unanswered questions but this report lays the groundwork for understanding these possible links. The actual extent of this difference and the lake-bottom food web complexity and richness remains to be seen, in studies to be commenced this summer (2010) for the CCT by our team including Science System Applications (modelers from Los Angeles) and EcoAnalysts Inc. (riverine food web and taxonomy experts).

To begin understand the linkages and complexities of the food web in RWL, Rensel (2000) first began an assessment of background and aquaculture affected stable isotope content of flora and fauna in RWL. The focus was to assess the biota to find potential indicators of fish farm enrichment (or eutrophication, herein defined as excessive enrichment beyond the carrying capacity of the lake). Data from the initial assessment are referenced herein but research funds were limited at the time and only minor follow up was allowed. Regional stable isotope laboratories were not processing sediment samples at that time, so the study was limited to plants and animals. These initial data indicated the tentative usefulness of the technique, but were limited in number and spatial distribution (upstream and downstream or under the cages) and limited to plants and animals. There had been no other stable isotope studies in Lake Roosevelt of Rufus Woods Lake at that time, so the data were only reported and not considered preliminary in nature. Now, in the year 2010, laboratory analysis for SIA is very affordable ($20/sample) and the available literature is more replete with guidance and comparative studies that were not available ten years ago.

Use of stable isotope analyses is not a panacea, there are serious limitations and complications to the methodology, particularly when an organism has two or more food sources or has seasonal and spatial fluctuations in food sources. As you would expect, sessile organism, for example those that reside on the river bottom are potentially the best indicators for fish farm effect as they are either in the waste plume of the fish farms or not. Mobile wild fish, on the other hand, such as suckers or even worse, walleye, are not good candidates for stable isotope tracking in RWL as they may be eating many food items that are only sometimes affected by food web enrichment that leaves a stable isotope signature. The initial year 2000 studies demonstrated this clearly.

In this context it is important to understand that RWL is formed by Chief Joseph Dam, a U.S. Army Corps of Engineers structure that impounds the lake. The most pertinent feature of the dam for this study is the fact that it blocks anadromous fish passage, thus there is no marine derived nitrogen (herein MDN) in Rufus Wood Lake except that which comes from the highly enriched fish feed. MDN has a highly different ratio of normal to heavy stable nitrogen isotope content, thus the basis of the technique.

This report begins with a presentation of the sampling locations and timings, and includes methods used in this study, an introduction on stable isotope analysis for biological aquatic studies, a section describing the hypotheses advanced, presentation of sediment total carbon and total nitrogen results, stable isotope results by species and a stable isotope summary section that attempts to draw together the results into a global view presented in bulleted form. A final chapter addresses future directions to be considered. If interested, the author has more materials to distribute to interested parties to further explain the SIA technique.

SAMPLING LOCATIONS, POSITIONING AND TIMING

Sampling locations were selected beginning immediate adjacent to the downstream (west end) of Site 1 Net Pens and continued downstream to one mile plus a sample from the Chief Joseph Dam pool (Table 1, Figs. 2 and 3). An upstream reference area was selected just above the confluence of the Nespelem River and upstream of all existing net pen sites.

Table 1. Sampling design spatial layout in relation to Site 1 net pens.

Station Name	**Station Code**	**Latitude** deg-min-sec	**Longitude** deg-min-sec	**Depth** at station	**Sediment Type** (in addition to cobble stations A-F)
Site 1 pens 0' downstream	A	N48 08 19.0	W119 05 57.0	55.8'	O' pea gravel + minimal fines, some patches of whitish sludge laying on sediment surface and sampled separately
200' downstream	B	N48 08 19.7	W119 05 59.8	60.0'	Mostly coarse sand
575' downstream	C	N48 08 20.9	W119 06 05.1	58.0'	Coarse sand
1,300' downstream	D	N48 08 24.9	W119 06 16.6	60.9'	Medium sand
5,280' downstream	E	N48 08 54.4	W119 07 03.9	59.9'	Fine Sand
Upstream Reference*	F	N48 07 37.9	W119 02 39.7	54.0'	Fine Sand
Chief Joseph Pool**	G	N48 0 50.98	W119 36 5.78	10'	Fine silt and clay

*Upstream of confluence of Nespelem River with Rufus Wood Lake
** At State Park on west shore, end of boat dock

Sampling was conducted before the older net pen cages were completely replaced with new cages. Pacific Aquaculture was in the process of thinning out the density of remaining fish and involved in other facility renovation activities. At the same time, revised regulations and permits were drafted, reviewed and adopted by the CCT to more effectively monitor and regulate fish farm operation.

Samples were collected on the 11th and 12th of August 2009 in Rufus Wood Lake. The weather was warm but overcast with some rain showers. All samples were collected at similar depths to the Site 1 fish farm depth, about 55 to 60 feet depth. Water currents at all these stations are very strong, often exceeding 50 cm/s and possibly 100 cm/s at high flows.

Positioning was accomplished through the use of a WAAS enabled, Garmin GPSMAP 188C, with recorded accuracy (95% probability) of about 2 m during the survey. The boat was anchored securely before position readings were saved, often with two anchors. SCUBA divers descended a weighted line beneath the anchored boat to collect samples to be placed in plastic jars of mesh dive bags. Reference samples were collected upstream of all the existing net pen system and the Nespelem River. Downstream samples were collected at various locations over a 1 mile distance as indicated herein and near the State Park in the Chief Joseph Dam pool near the boat ramp.

Figure 2. Fish farm Site 1 (lower left, prior to Pacific Aquaculture renovation) and nearby downstream sampling locations used in August 2009.

Figure 3. Region of Rufus Woods Lake about 10 miles downstream of Grand Coulee Dam showing Site 1 and Site 2 net pen locations and stable isotope sampling locations including upstream reference area above the confluence of the Nespelem River and Coyote Creek, dry at the time of sampling. Stations downstream of Site 1 pens are A = 0', B = 200', C= 1300', D = 5,280' (1 mile).

METHODS

Samples were collected in every case by diver and a video record was recorded of conditions in the immediate vicinity. Diving was conducted by Archie Dennis with Jack Rensel manning the boat and inspecting the bottom at each station through the use of a drop video camera. Two cm cores were used for sediment collection, capped with built-in lids and laboratory stoppers then placed in a diver's mesh bag for delivery to anchored support vessel above. Larger rocks were removed one at a time and brought to the surface for removal of snails, periphyton or other attached biota. Snails were so small that they were collected and processed whole. Crayfish were collected where found, typically around cobble or boulders, and placed in the diver's mesh bag for total length measurement and processing in the support boat. The fleshy abdomen tissues, without the carapace or pleopods (swimming legs) were collected. Prickly sculpins were collected by trapping with a mesh bag or use of a slurp gun and dorsal or mid dorsal to ventral muscle tissue were collected after total length measurement. Smaller specimens may have included some bone or backbone. All samples were place in double-labeled whirl pac bags and placed immediately in iced coolers for freezing later the same day.

Samples were further processed by homogenization and freeze drying at the University of Idaho Stable Isotope laboratory using standard methods. Sediment samples were individually inspected to remove wood, tree bark or pebbles before grinding into a homogenate Samples are packed into tin capsules and combusted in a CE instruments NC 2500 Elemental Analyzer. The combustion gases are entrained in a helium flow. The sample is diluted in a Finnigan MAT Conflo II and drawn into a Finnigan MAT Delta plus IRMS for analysis. Three or more replicates of two internal standards are used to bracket the samples and provide a two-point normalization of the data. At least three replicates of a known QC standard are run with the samples to test the resulting calibration. All standards have been calibrated to 3 NIST or IAEA standard reference materials. Sample runs are repeated if the standard deviation of the standards is greater than 0.1‰ for C or 0.2‰ for N. This generally happens only when temperature control in the lab is inadequate, the filament is about to burn out, or a new mass calibration or tuning is needed.

Data was analyzed first by plotting of spatial and species or sediment relationships, then analyzed by one way analysis of variance with Tukey's post hoc multiple comparison with alpha of 0.05. Pearson correlations were computed and correlation coefficients r or coefficients of determination r^2 calculated with probability of a type 2 error (alpha) displayed. Statistix 9.0 for windows was used. In all cases error bars shown in plots are standard deviation, which are greater than standard error ranges, allowing the reader to quickly likely statistical differences by simple inspection of overlap or not.

Data from Lake Roosevelt (Black et al. 2003) are included occasionally in this report for comparison to upstream reference data from RWL.

STABLE ISOTOPE PRIMER

Here I provide a brief overview of the methodology, which appears on the surface complex, but is conceptually simple. However, the applications can be complex because of multiple food sources for tested animals, as explained below. This primer is designed to give reviewers the key concepts, and I borrow freely, but in my own words and images, from Brian Fry's (2006) volume "Stable Isotope Ecology", written by a world's leader in the field. The volume could be renamed "Stable Isotope Ecology for Idiots" as it clearly sets out the principals and problems in a very readable and humorous fashion. The following is a synthesis of that material, amended for the present context:

Elements exist in stable and some in unstable (radioactive) forms. Most elements of biological interest (including carbon, hydrogen, oxygen, nitrogen and sulfur) have two or more stable isotopes. Among stable isotopes, the most useful as biological tracers are the heavy isotopes of carbon and nitrogen, the focus of this work. C and N are found in the earth, the atmosphere, and all living things (carbon as the carbon skeleton of organic matter and nitrogen as protein for example). Each has a heavy isotope (^{13}C and ^{15}N) with a natural abundance of only 1% or less and a light isotope (^{12}C and ^{14}N) that makes up all of the remainder. Do not confuse these with radioactive isotopes such as carbon 14 (typically indicated as ^{14}C) used in dating artifacts and materials or for spiking (labeling) of primary productivity algal experiments as a means to measure rates of photosynthesis, for example. Stable isotopes are perfectly harmless.

Biologists often use carbon and nitrogen isotope tracing to estimate what organisms consume and where they fit in the food chain or food web. Isotopes of the same element take part in the same chemical reactions, but the lighter isotope acts just a wee bit faster or slower, this is the key to understanding the process, known as "fractionation". Physical processes such as evaporation discriminate against heavy isotopes; and enzymatic discrimination and differences in kinetic characteristics and equilibria can result in reaction products that are isotopically heavier or lighter than their precursor materials. When assimilating C and N from their food, consumer organisms preferentially respire the light C isotope (^{12}C) and preferentially excrete the light N isotope (^{14}N). As a result, consumers are usually enriched with heavier isotopes in relation to their food. This is the basis of the methodology.

An example of how this works is shown diagrammatically in the cartoon Figure 4.

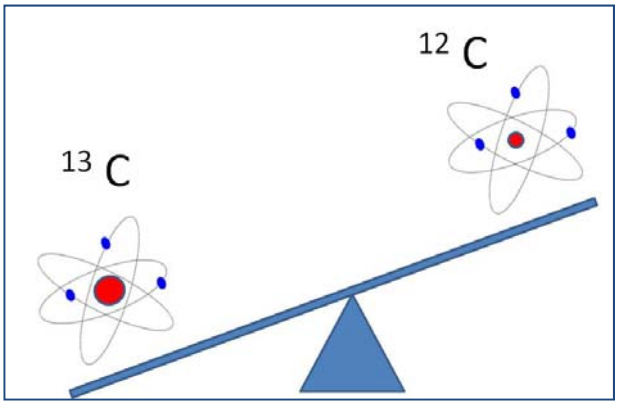


Figure 4. Cartoon of heavy ^{13}Carbon with one more proton than light ^{12}Carbon isotope, with red circle representing neutron mass of each and the teeter totter illustrating the relative weights (after Fry 2006, but drawn from scratch).

So what does this mean in practical terms? It means that the difference between the light and heavy isotope values can be used to measure and sometimes trace the flow of C and N from a source to the environment, in the sediments, and in food web to a plant producer or animal consumer.

If there are multiple sources of nutrient or food, and we have enough information, we can create a "mixing model" to estimate the contribution of elements C and N to an organism's body from a source as discussed below. But often this is very difficult or impossible without extensive research or ends up being too complex to be of practical use. Often the best use of stable isotope methodology is lowest in the food web. Higher up there are complications and a pertinent example for RWL is walleye, a non native fish in the Columbia River but prized by sports fishers. Overman and Parrish (2001) studied Walleye in Lake Champlain, Vermont, to determining relationships between isotopic composition and diet, location of capture, length, weight, and age. Age and location were significant influences on isotopic composition and without tracking these factors. The authors concluded that the risk of making faulty inferences of trophic position and food web interactions based on $\delta^{15}N$ values may be increased when age is unknown or not considered quantitatively.

So stable isotope studies are not cookbook science, every situation is unique and must be studied on its own and sometimes the results simply do not make sense for a variety of known or unknown reasons. So in food web investigations, we rely on the stable isotope methodology but often simple observations such as gut content or feeding ecology studies.

Isotope ratios are reported in "delta" (δ) notation that is defined as the "per mil (parts per thousand or 1/10 of a percent, the same units used in tax levies) deviation from the recognized isotope standard, atmospheric N_2 for $^{15}N/^{14}N$ and Peedee Belemnite (a limestone found in S. Carolina) for $^{13}C/^{12}C$ ratios. Herein I use the common notation for these units of "‰". These are internationally accepted standards that all laboratories use. So if there is no enrichment of heavy marine derived N (known as "MDN") for example, the δ values are low (for nitrogen). Values for C are often negative, only because it is measured relative to the standard, which can be confusing at first. Nitrogen isotopes are often more reliable indicators or predictors of the trophic level that an organism occupies in the food web because of the large ^{15}N enrichment from one trophic level to another (Owens 1987, Peterson and Fry 1987). But sometimes C isotope measurements are useful too, as in fish farms where most of the particulate waste has C but very little N (that is dissolved instead). We pay for both of them in the analysis and let the chips fall where they may! A helpful guideline is:

- Do not get hung up on the absolute values of these measurements, as you might be when observing basic water quality results. We are more concerned with the observed sample differences among locations in relationship to anthropogenic (e.g., pollution) or natural (e.g., riverine tributary) perturbations. In the present case, we are particularly interested in any results showing spatial trends downstream of the fish farms that indicates a reasonable trend.

It is important to emphasize that $\delta^{15}N$ and $\delta^{13}C$ isotopes do not pass through the food web intact, except at the beginning in plant primary production (photosynthesis). As atmospheric N is fixed in primary production, there is no fractionation in that process, hence the $\delta^{15}N$ values are low (from 0 to 4 or so) for phytoplankton and other forms of algae (e.g., periphyton in freshwater).

Each time the element is digested and egested by a higher consumer, it is fractionated again; and by measuring the ratio of the either set of isotopes, i.e., "normal" or light isotopes of C and N, biologists may be able to detect who ate what. In the present case, we start with a known ratio of both carbon and nitrogen, heavy to light isotope ratios in the feed, which is heavily influenced from fish meal and fish oil and other products that sometimes creates a distinctive ratio (or not, it depends on local conditions), that in some environments can be distinguished from other food sources. Most fish waste N is excreted as ammonium and a little urea and about ½ of consumed C respired as carbon dioxide, and roughly equal amounts are retained as fish tissue or egested as feces. The C and N compounds and molecules released are then subject to uptake by primary producers (in the case of dissolved C and N) or consumers, who are further up the food web. The fractionation occurs again so if the tracing system is working properly we expect an enrichment or positive change in δ15N and δ13C each time the element goes through a trophic level of the food web, so:

- The higher the δ value, the greater the amount of heavy isotope. The lower the δ value, the lower the amount of heavy isotope, or as Fry (2006) says "higher heavier, lower lighter."

For carbon, the result values are reported as negative, and the less negative ones represent more marine origin isotope, i.e., they are enriched. The negative delta-C values result from the way delta notation is calculated ((Rsample/Rstandard -1)*1000, where R is the ratio of ^{13}C to ^{12}C). It just means that almost all samples have less ^{13}C than the PeeDee belemnite standard which is an unfortunate condition as the results are usually negative, but the same idea applies as with N, higher values indicate enrichment, so another quasi-rule:

- If there is a single food source flowing one step up a food web, except from macronutrient to plant, one may anticipate a fractionation result difference of ~ +3.4 $^{0}/_{00}$ (range 3 to 4) for nitrogen and +0.5 $^{0}/_{00}$ (range 0.1 to 1.0) for carbon stable isotopes (DeNiro and Epstein 1978, DeNiro and Epstein 1981, Peterson and Fry 1987). Results are never exact, so +/- 1$^{0}/_{00}$ in both cases is considered reasonable and some species, such as marine mussels, have less whole-body nitrogen fractionation, in the range of 1.2 to 2.5 $^{0}/_{00}$ (Hill 2007).

For example, an increased $\delta^{15}N$ of 3.5 $^{0}/_{00}$ for nitrogen , e.g., from -18 to -14.5 $\delta^{15}N$ would constitute a single food web trophic level enrichment, as if a fish ate a insect larvae that was in turn feeding on periphyton or biofouling algae. Atmospheric N is isotopically lighter than plant tissues, and soil δ15N values tend to be higher still, suggesting that microbes discriminate against the light isotope during decomposition. Non-nitrogen-fixing plants, which derive their entire N from the soil N pool, can thus

be expected to be isotopically heavier than nitrogen-fixing plants, which derive some of their N directly from the atmosphere including the blue-green algae (cyanobacterial) group.

Biologist are able to determine if one or two sources of feed are involved in a food web study by preparing "dual isotope plots" which are little more that a scatter diagram with $\delta^{15}N$ on one axis and $\delta^{13}C$ on the other axis. Vertical and horizontal differences in distance in these plots can represent trophic level jumps, if the sources are initially different in profile. If not, sorry, the system doesn't work so well. If there are three or more sources, then complex mixing models are required, as are measurements for all the food sources likely contributing to the target organism that is the study focus (e.g., see Fig. 5). In this manner we could distinguish 5 sources if we had 4 actual sources characterized by baseline measures, with only one unknown remaining. These models are not, however, simple but there are published models and freely available spreadsheet models to accomplish this task (see EPA website: http://www.epa.gov/wed/pages/models/stableIsotopes/isoconc/isoconc1_01.htm)

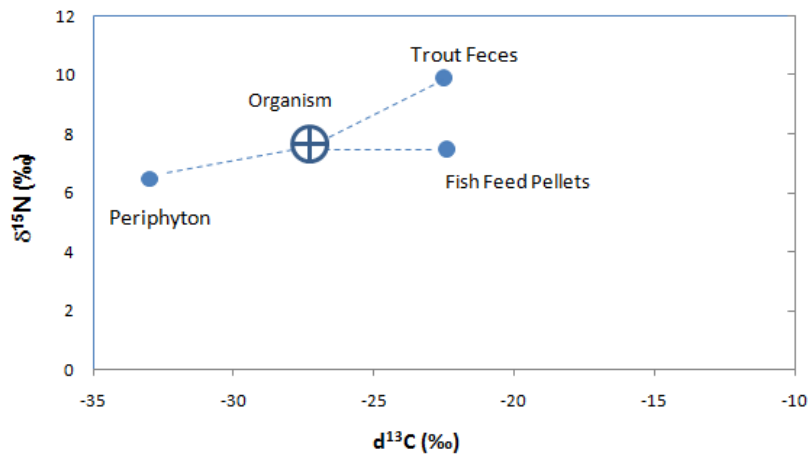


Figure 5. Dual isotope plot showing how three differing food sources for a hypothetical grazer and how multiple food sources can create a complex mixing model, decipherable only through extensive sampling and numerical modeling.

It has also been my observation (and that of others) that δ15N tracer studies work better in general in freshwater than marine waters. This is so because marine derived nitrogen (MDN) has a different isotope mixture on N than freshwater derived nitrogen. And in oligotrophic environments, such as Columbia River upstream of Chief Joseph Dam where no anadromous fish pass, the unique MDN signature should provide a powerful tracer. In marine waters where there are many sources of MDN, we have to hope the fish feed MDN is different enough from background MDN consumed by the study target organism to allow us to differentiate an effect, if it occurs. In other words, it is much tougher and we need to be lucky.

If you are confused by the above, this primer has not served its purpose, but a quick search on line will yield a PDF copy of the Fry (2006) volume which really is very readable. Reading individual scientific papers will usually only confuse you more, unless you read Fry's (2006) volume or a similar volume to

grasp the basics. So now we present the stable isotope results of the project to date, beginning with hypotheses.

HYPOTHESES AND APPROACH

This work was designed to look for spatial differences in stable isotope enrichment among invertebrates and sediments as a measure of how fish farm wastes are incorporated into the food web or are deposited on the lake bottom. The primary null hypothesis is that there is no enrichment effect of the fish farm on the benthic or epibenthic (in bottom or immediately above bottom) food web of the river. This is determined simply by comparing results from upstream of the pens to downstream, the latter at several locations appropriately spaced. If we see significant statistical trends by regression analysis or other parametric analyses, we reject the null hypothesis.

Secondly, we examine the amount of variation between source of N and C from the pens compared to content of enriched marine derived N to investigate the possibility of using such data to prepare a "mixing model" that will guide us on quantifying the relative amount of the food web components that originate from the fish farm.

This work does not compare the relative abundance of benthic or epibenthic species near the farm versus at remote locations or upstream. To some degree it is not feasible as the coarse nature of most of the RWL bottom precludes grab sampling and there is probably soft bottom lake infauna (e.g., oligochaetes and chironomids) except in the downstream Chief Joseph Dam Forebay and pool. However, no prior study of this has been done and in 2010 CCT has commissioned a study of the lake that will begin to examine the secondary trophic level of productivity.

SEDIMENT TOTAL CARBON, TOTAL NITROGEN RESULTS

An analysis of sediment total carbon and total nitrogen is included by default in most stable isotope analyses. These data are reviewed here, focusing on detection of spatial effects of the net pens. Importantly, the percent carbon (and probably nitrogen) is usually directly correlated with the background amount of fines (silt and clay) in sediments. This means you cannot simply measure sediment carbon and draw inference about effects of some potential source, like fish net pens. In this study, samples collected by core were too small to process for sediment grain size analyses, but I did attempt to note of the relative amount of fines in samples at each station. Surprisingly, fines appeared to increased with distance from the farm, not the inverse that would have been expected if sedimentation was a major issue at the farm site (Table 1). Sediment at the reference station upstream appeared to be similar to the 1 mile downstream station, i.e., fine sand. Table 2, Figures 6 and 7 indicate the results, differences vs. reference stations and variance estimates.

Table 2. Mean and standard deviation of total nitrogen and total carbon from lake bottom surficial (2 cm deep) sediments with differences between reference upstream location and other stations. Statistical differences (alpha = 0.05) indicated with an asterisk.

Location	Mean % Total N in Sediment	Mean % Total C In Sediment
Reference	0.04	0.26
0'	0.07	0.43
200'	0.06	0.37
575'	0.04	0.30
1300'	0.02	0.14
5280'	0.02	0.14
C.J. Pool	**0.30***	**2.48***
Differences: reference vs. other stations		
	Total N	**Total C**
0'	+0.03	+ 0.17
200'	+0.03	+0.11
575'	+0.00	+0.04
1300'	-0.02	-0.11
5280'	-0.02	-0.11
C.J. Pool	+0.26	+2.22
Standard deviation of above means		
Reference	0.00	0.02
0'	0.08	0.34
200'	0.03	0.20
575'	0.01	0.12
1300'	0.01	0.04
5280'	0.00	0.03
C.J. Pool	0.03	0.33

Given the estimated distribution of sediment grain size observed (Table 1), in an unperturbed system one would expect to see the highest levels of C and N at the reference station and farther downstream from the net pens at the 1 mile distance for example. Except for the Chief Joseph pool, highest total C and N concentrations were immediately adjacent to the pens (0' station) and declined gradually downstream, but curiously to less than the reference area. Sediment C and N values at the each station were highly correlated with each other with a coefficient of 0.997 (out of a possible 1.0 scale). A one way analysis of variance and post hoc test indicated no statically significant difference among stations, except higher concentration of both C and N at the Chief Joseph Pool station. Again, that was expected. There were typically 3 or 4 replicate samples per station, but the within station variance was sufficient to cause the hypothesis of no significant difference to be accepted. Likely there were true differences, but due to the nature of the sediments, with variable amounts of coarse sands that would affect the analysis, no differences could be detected. Adding more replicate samples usually improves the resolution of such tests, but again, sediments with this much pea gravel and rock are usually not sampled for sediment chemistry because the results are often not repeatable. In Puget Sound, the

Dept. of Ecology requires five replicates per station which was effective in resolving prior statistical problems when only three samples were collected. In many cases of soft bottoms in lakes, however, only three samples are needed due to the homogenous nature of the sediments.

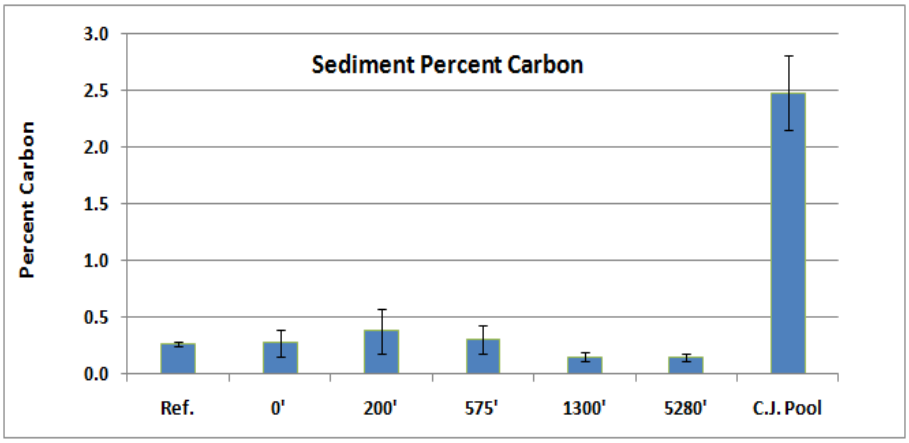


Figure 6. Sediment percent carbon up and downstream of net pens in Rufus Woods Lake during August 2009.

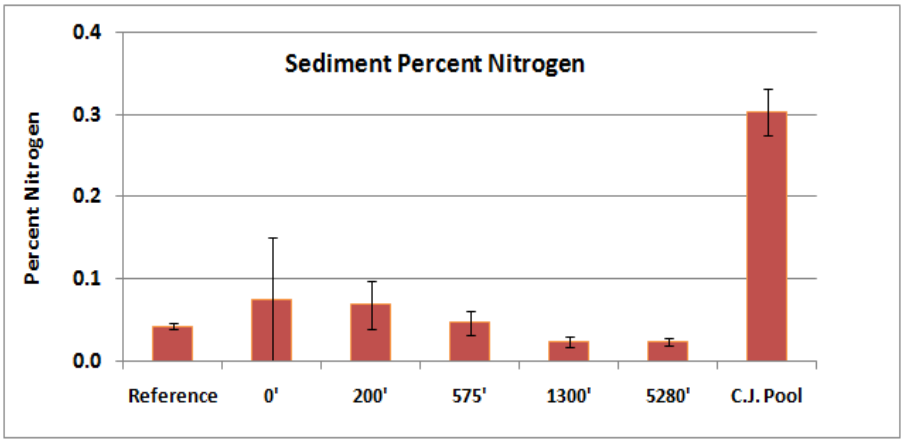


Figure 7. Sediment percent nitrogen up and downstream of net pens in Rufus Woods Lake during August 2009

Collectively, these total C and N data indicate a probable effect of the pens that is limited in spatial extent to about 400 to 600 feet downstream. This cannot be proved statistically, but the stable isotope methodology, discussed next, provides a different and more useful assessment of actual food web effects.

Note in the preceding that I am discussing total carbon, not total organic carbon (TOC), the screening metric used in Puget Sound net pen regulations. There is a difference but in freshwater sediments the two measures are more similar than in the ocean, where there is a large repository of carbonate shell material from shellfish and plankton. The reader should therefore know that the values reported here are likely been a little less than would have occurred if only TOC had been reported.

Finally, the levels of carbon observed at stations downstream of the pens were NOT relatively high compared to the levels of total organic carbon seen around net pens in marine waters of Puget Sound. In those marine waters, total organic carbon is naturally about 0.5% in sandy sediments and increases to 3.0% or more in silts and clays. Net pens often increase the levels a few tenths of a percent within about 30 to 50 meters, but not further away where the carbon is being assimilated at a rate proportionate to the supply rate.

Given these results, it may be feasible to hand cores sample sediments by diver in the future if there is a need for some sort of benthic impact assessment. I would recommend that both total organic carbon and total carbon be analyzed concurrently and that such a system not be used until a non bias sampling location system can be devised.

Sediment Stable Isotope Results

When considering the net pen effect with stable isotopes we are looking for a positive signal, i.e., a shift in high levels of heavy stable isotope. Figure 9 and Table 3, for carbon isotope content shows a very tidy relationship, lowest at the reference station and highest (least negative) at the 0' sampling location nearest the pens. The degree of effect between pens and reference was surprisingly strong, as discussed below and tapered off for samples taken in the Chief Joseph pool but were still enhanced. It is not possible to say if this is due to fish farming, without further study and looking at a few intermediate stations. More likely it is due to physical current differences that result in differing sediment grain size distributions (i.e., sand versus silts and clays).

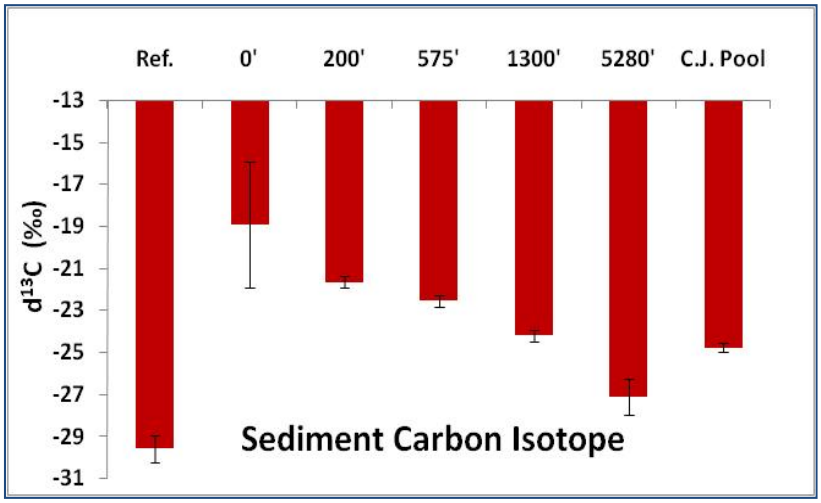


Figure 8. $\delta^{13}C$ results for sediments up and downstream of net pens in Rufus Woods Lake in August 2009.

Nitrogen stable isotope results shown in Figure 9 and Table 3 indicate the nearly complete opposite of carbon isotope image, with highest values at the reference area and 1 mile downstream but Chief Joseph Pool being the lowest. The correlation coefficient between ^{13}C and ^{15}N for these sediments was not extremely strong, as it was for total N and C, but was -0.41. The pattern for ^{15}N in sediments is at

first view inexplicable but the replicated data is real and it may represent that the bacterial or other sediment organism assemblages are preferentially using lighter ^{14}N, rather than heavier ^{15}N. Whatever the cause, the patterns are not easily discerned and potentially as useful as they are for ^{13}C.

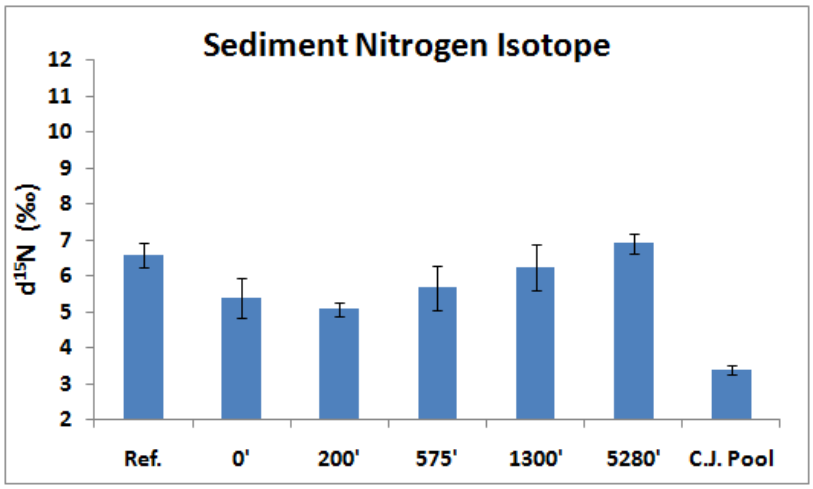


Figure 9. $\delta^{15}N$ results for sediments up and downstream of net pens in Rufus Woods Lake in August 2009.

Table 3. (right) Mean and standard deviation of nitrogen and carbon stable isotope results from lake bottom surficial (2 cm deep) sediments with differences between reference upstream location and other stations.

Location	Mean δ15N Sediment	Mean δ13C Sediment
Reference	6.6	-29.6
0'	5.4	-18.9
200'	5.1	-21.6
575'	5.7	-22.5
1300'	6.2	-24.2
5280'	6.9	-27.1
C.J. Pool	3.4	-24.7
Differences: reference vs. other stations		
0'	1.2	-10.7
200'	1.5	-8.0
575'	0.9	-7.1
1300'	0.4	-5.4
5280'	-0.3	-2.5
C.J. Pool	3.2	-4.9
Standard deviation of above means		
0'	-1.2	10.7
200'	-1.5	7.9
575'	-0.9	7.1
1300'	-0.3	5.4
5280'	0.3	2.5
C.J. Pool	-3.2	4.8

Statistical analyses here involves a “multiple comparison” (a test done after an analysis of variance) and those data are shown in Tables 4 and 5.

Table 4. Table of significant differences among sediment carbon stable isotope results (Tukey's Multiple Comparison Test, a = 0.05). Example: A group includes the first two locations, B group the 2nd thru 5th, C group the 4th through the 6th, group D the 6th and 7th.

Sampling Location	Mean δ13C Sediment (high to low)	Statistically Homogeneous Groups
0'	-18.9 (most)	A
200'	-21.6	AB
575'	-22.5	B
1300'	-24.2	BC
C.J. Pool	-24.7	BC
5280'	-27.1	CD
Reference	-29.6 (least)	D

Table 5. (below) Table of significant differences among sediment nitrogen stable isotope results (Tukey's Multiple Comparison Test, a = 0.05).

Sampling Location	Mean δ15N Sediment (high to low)	Statistically Homogeneous Groups
5280'	6.9 (most)	A
Reference	6.6	AB
1300'	6.2	ABC
575'	5.7	BCD
0'	5.4	CD
200'	5.1	D
C.J. Pool	3.4 (least)	E

In the statistical difference table for carbon (Table 4) that there were four groups of differences (A thru D) among locations with the 0 and 200 foot distant stations grouped as the locations with the most concentrated effect, but the 200' station not statistically different than three of the downstream stations including the Chief Joseph Pool. The mile downstream location was next, followed last by the reference area with the most negative, minimal concentration of $\delta^{13}C$. It is difficult to draw final conclusions from this without sampling in between one mile downstream and the pool, but based on statistical significance, it appears there is a signal to the 1300' downstream location that is statistically greater than the reference location. By 1 mile downstream, that difference had disappeared. However, Chief Joseph Pool was grouped mid way between the 1300' and 1 mile downstream stations. This is possible as the surficial sediments of this pool are apparently composed of silts and mineral clays and probably, in my experience diving there, relatively thick (meters, not centimeters). There could be electrostatic binding of the clays in this region, as I have seen such deposits in areas of the

upper pool that have relatively strong current flows but no apparent resuspension or saltation of the deposited sediments.

From the introductory primer above, the reader may recall a difference of stable isotope enrichment of ~+3.4 $^{0/}_{00}$ (range 3 to 4) for nitrogen and +0.5 $^{0/}_{00}$ (range 0.1 to 1.0) for carbon are significant changes experienced in a single trophic level fractionation. Table 3 indicates that the differences for both ^{15}N and ^{13}C between reference and downstream are very large relative to single trophic level enrichment steps, The table shows a maximum difference of 1.2 and 10.7 ‰, respectively, versus 0.5 and 3.5‰ expected, as explained in the SIA primer in this report's introduction. These differences between reference and treatment are so large, compared to what was expected, that they would represent several food web trophic level shifts, or indeed many. This is presently inexplicable but was replicated and statistically valid. There are at least two probable factors involved in this distribution, 1) a normal enrichment of C and N with distance downstream of a dam tailrace in any of the reservoirs as the current velocity decreases and sediment become finer and 2) the near-field enrichment of wastes from the pens. Consider that the pens are a relatively large source of C and N and that unlike a marine fish farm, with variable and often completely opposite tidal currents, the benthic effects of the RWL pens are expected to accrue only downstream and in a relatively narrow plume, based on the physical principals of advection and diffusion of solid and dissolved wastes.

FISH FOOD-TROUT FLESH-TROUT FECES-BOTTOM SLUDGE SIA CONNECTIONS

This section examines the probable connections between ^{13}C and ^{15}N pathways related to fish food to fish flesh, fish feces and bottom sludge adjacent to the farm site in August 2009. Recall that Pacific Aquaculture had recently taken over operations of the farm from a former owner who was apparently neglecting proper management, so this represented an opportunity to evaluate worst case conditions. Normally there is little or no bottom sludge but at the time of sampling the bottom immediately adjacent to the downstream Site 1 cages had a several cm thick coating of fluffy, whitish sludge that did not appear to be a bacterial mat, as occurs in marine water fish pen sites when overfeeding or carrying capacity is exceeded. It appeared to be composed at least in part of some decaying fish tissue, rich in fish oils that are essential in the diet.

The hypothesis associated with these data is as follows: The vast majority of fish feed is ingested by the cultured trout (>95%, or the operation would not be economically possible) and during metabolism fractionation of the amount of heavier C and N isotope occurs the flesh is enriched in ^{13}C and ^{15}N stable isotope. Other studies have shown these enrichment steps for cultured salmonids to be in line with normal one level trophic step of about 0.5 ‰ for ^{13}C and about 3.5 ‰ for ^{15}N. This is what was measured in the present study (Figs. 10 and 11), although fish fecal samples were limited.

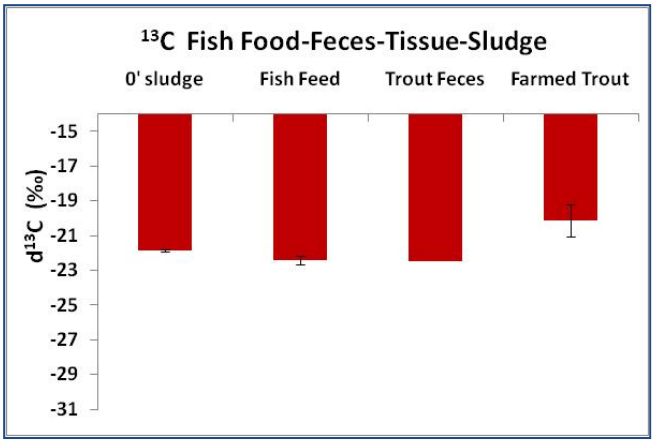


Figure 10. ^{13}C fish food, fish feces, fish tissue and bottom sludge immediately adjacent to Site 1 farm site in August 2009 sampling.

Fish feed to fish tissue ^{13}C samples indicated here show and unexpectedly large difference of 2.2‰, nearly double what would be expected. Sludge was not differentiated between fish feed or fish feces.

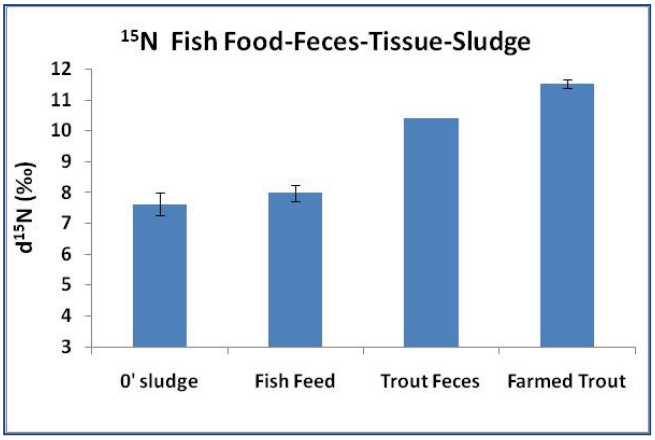


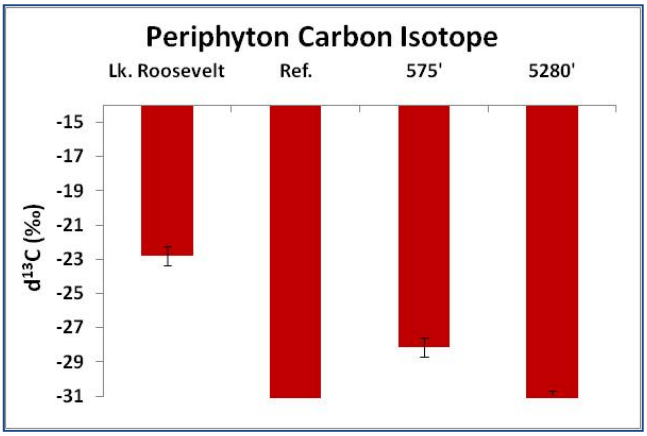
Figure 11. ^{15}N fish food, fish feces, fish tissue and bottom sludge immediately adjacent to Site 1 farm site in August 2009 sampling.

Fish feed to fish tissue ^{15}N samples indicate an enrichment of 3.9 ‰, well within the range of expected change. In this case sludge had the same profile as waste fish feed, but sample size of trout feces was limited. It is not clear why trout feces was enriched in ^{15}N, perhaps the urine (not measured) that is most of the excretion was depleted in the heavier isotope.

Periphyton and Phytoplankton

Periphyton samples were scraped from diver recovered cobble from RWL, but because of the depth (>50' deep), the samples may not represent mostly autotrophic (photosynthetic) colonizing organisms but rather heterotrophs that use ambient sources of nutrients. This sampling was intentional, as we wanted to focus on the depth of the fish farm and know from prior drogue studies and basic hydrology that lateral mixing downstream of pens is minor. Only a few stations were sampled and are compared to results from Lake Roosevelt (Black et al. 2003) samples that were scraped from colonizing submerged reed canary grass in the Hawk Creek and from Seven Bays area from substrate suspended at a depth between 1 and 2 m in the open water (Figures 12 and 13).

Figure 12. ^{13}C periphyton results from the August 2009 sampling and prior results of Black et al. (2003) from Lake Roosevelt.



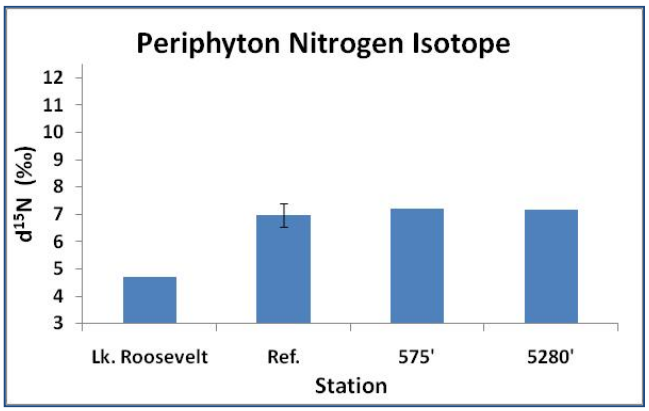


Figure 13 ^{15}N periphyton results from the August 2009 sampling and prior results of Black et al. (2003) from Lake Roosevelt.

These data suggest carbon isotope enrichment downstream of the pens, but not nitrogen which may be reasonable given the C concentrated in waste feces and feed, versus the nitrogen content of dissolved fish wastes. The data, however, are based on only a few samples as there was not much periphyton to be sampled in most cases.

In some habitats, such as nearshore algal beds, periphyton is considered the base of the C and N for benthic food webs. As discussed later in this report, it would be useful to have shallow and deep samples to compare and categorize conditions in Rufus Woods Lake to help sort out the remaining unknowns with regard to fish farm effects.

INVERTEBRATE AND WILD FISH STABLE ISOTOPE RESULTS

Results for various invertebrates and sculpins are presented in this section in addition to prior sampling results in year 2000 (Rensel 2001).

SNAILS

As in the prior study in the late summer and fall of year 2000, we found common, small black snails (taxonomy not determined yet, apparently native species) most abundant on small cobble that is common throughout all the sampled areas except Chief Joseph pool. They are considered herbivores, feeding on periphyton (that actually includes small plants and animals) but there are probably other species of snails in RWL too. In year 2000 I previously found that the same snail ^{13}C results from reference to immediately downstream of the pens were not explicable as enrichment declined. In 2009, the highest ^{13}C enrichment was at the reference location and increasing gradually downstream (Fig. 14) which fits the previously observed pattern.

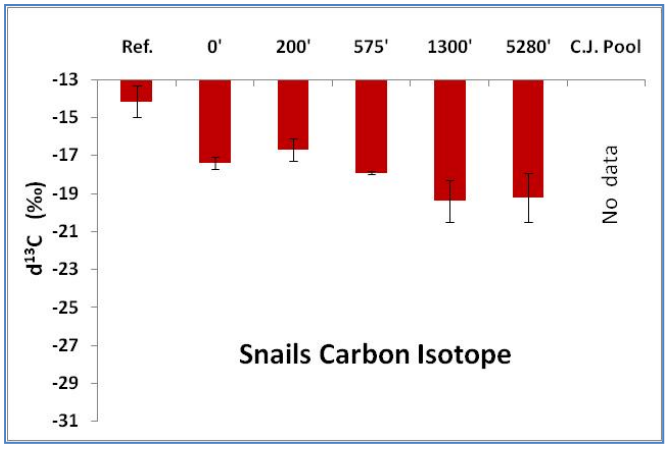


Figure 14. Snail carbon SIA results by location

Table 6. Statistical results for snail ^{13}C SIA

Sampling Location	Mean δ13C Snails (high to low)	Statistically Homogeneous Groups
Reference	-14.2	A
0'	-17.4	B
200'	-21.6	C
575'	-22.5	C
1300'	-24.2	D
5280'	-27.1	E
RWL Ref 2000	-19.3	
RWL 0' 2000	-22.7	

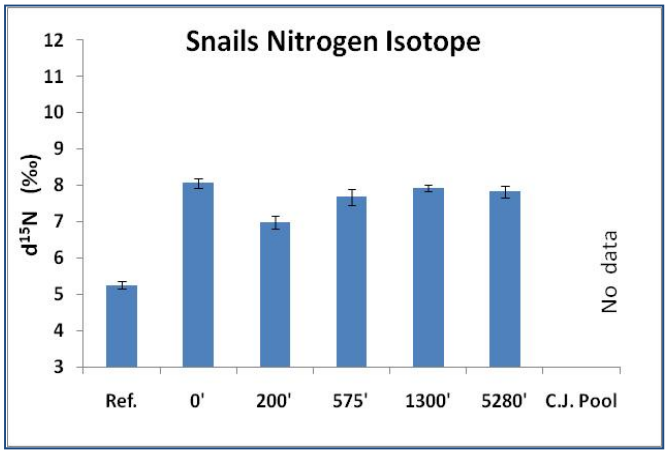


Figure 15. Snail nitrogen SIA results by location.

Table 7 Statistical results for snail nitrogen SIA.

Sampling Location	Mean δ15N Snails (high to low)	Statistically Homogeneous Groups
0'	7.6	A
5280'	7.3	A
200'	6.5	ABC
1300'	6.2	BC
575'	5.7	CD
Reference	4.8	D
RWL Ref 2000	4.3	
RWL 0' 2000	7.8	

Statistics for the more extensive data of 2009 (Table 6) suggest that Reference and 0' downstream of pen stations were significantly different , as were the combined 200' and 575' stations versus 1300' and 1 mile downstream stations.

These distinctive results beg the question: would ^{13}C SI enrichment levels increase if sampled further downstream? Or is this result a function of varying physical flows that could affect sedimentation and sediment grain size, as discussed above (i.e., apparent finer sediments at further downstream sampling station)? These results, when compared to periphyton results above (Fig. 12), have very different ^{13}C content which implies that the snails are not consuming the periphyton that was measured. But this seems unlikely as snails in lakes usually eat periphyton and mussels usually eat phytoplankton and seston, each with different SIA results (e.g., Post 2002). The uncertainty here points to the need for a few additional samples at additional downstream locations.

The ^{15}N snail results (Fig. 14, Table 7) were strongly indicative of a single trophic level increase downstream of the pens versus the reference location, with an enrichment of 2.8 ‰, very close to the average increase of 3.5 ‰ found for most organisms. The results were also comparable to prior measurements in year 2000 with a definite enrichment of about one entire tropic level, with only a minor difference of 0.4‰ and 0.2 ‰ between reference and downstream stations, respectively, in year 2000 versus 2009. This stability of result lends credence to the use of snails and ^{15}N isotope results as a possible indicator of net pen effects in RWL. However, there was no apparent decline in downstream enrichment or linear pattern as seen with other species or sediments. Again, this could be because the effects extend farther than a mile downstream, but without additional samples, it is not possible to speculate.

These curious trends for snails discussed above can be viewed in a dual isotope plot to further illustrate the differences (Fig. 16). Note in this figure that the reference location (noted as "ref" in the figure) is enriched in terms of ^{13}C but depleted with respect to ^{15}N.

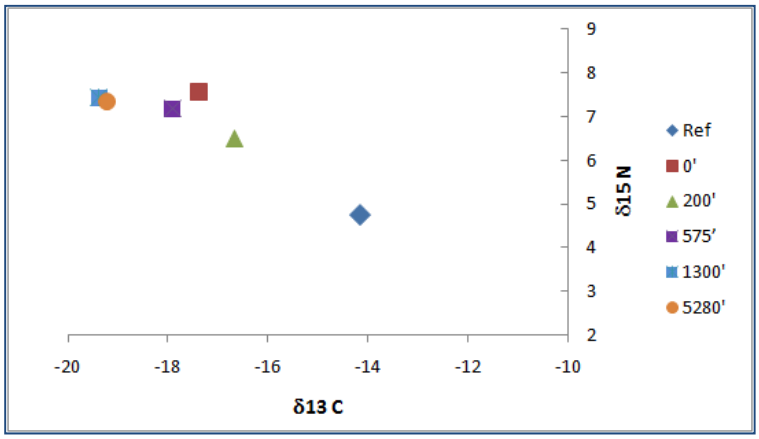


Figure 16. Dual isotope plot for RWL snail ^{15}N and ^{13}C.

Morgan et al. (2006) found a similar trend for another snail species (*Cerithidea californica)* in a tidal creek in California with an inverse relationship between ^{13}C enrichment and distance from a polluted creek adding nitrate. No mechanism to explain the reduction of ^{13}C near the creek was offered. As I observed in Rensel (2001), nitrogen SIA seems to be a simpler direct indicator for some species of interest in RWL. However, the strong spatial pattern observed here for both C and N and in year 2000 indicates that the depleted ^{13}C concentrations near the farm for snails may be a consistent and useful metric, despite the fact that it is the inverse of the ^{15}N enrichment that was documented. A few additional samples downstream of any of the fish farms would help clarify these enigmatic results. Recent studies show that different types of algae have different nitrogen isotope fractionation rates, resulting in differing snail stable isotope profiles (Han et al. 2010). Thus the enrichment from the fish farm may enhance a different type of periphyton, resulting in the shifts we have seen for snails. As shown later in the summary dual isotope plot (Figure 23), the SIA pattern of the snails is so much less than trout feed and feces, it is not likely that they are feeding on these sources at all.

This is not a trivial matter as snails are a keystone component of the epibenthic community in RWL and apparently an important prey species for sterile rainbow trout released into the lake to enhance the sport fishery.

CRAYFISH

Carbon isotope results of the native signal crayfish (*Pacifasticus leniusculus*) tissue shows a significant statistical effect at all sampled locations downstream of the pens, compared to the reference location (Fig.16). Note the large increase of ~3 ‰ from reference to 575' downstream of the cages, which was statistically significant (Table 8), with other stations overlapping statistically and not significantly different from the reference station crayfish that was very well sampled with an N of 6.

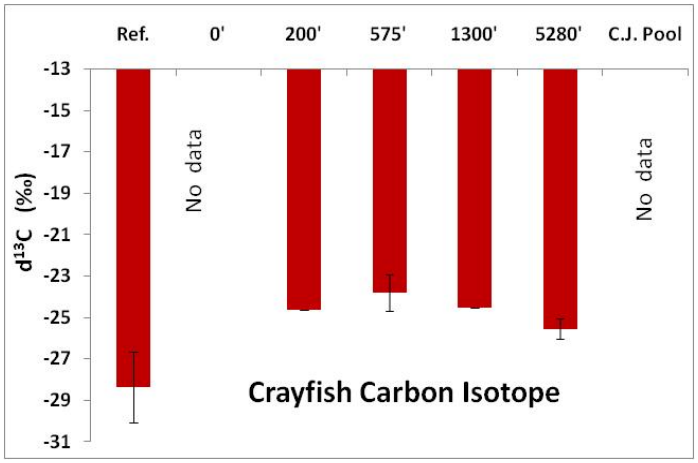


Figure 17. Crayfish carbon SIA results by location.

As crayfish are known to prey on small aquatic snails, the major (i.e., more than 0.5 $^{0}/_{00}$) enrichment of crayfish ^{13}C downstream of the pens is probably due to a multiple trophic level effect, i.e., nutrient flux from the pens, to periphyton, then snails and finally crayfish. To support this notion, compare Figure

12 (periphyton) and Figure 17 (crayfish) that show consistent ^{13}C enrichment between results of the two locations and among differing stations. The comparison is limited, however, due to a lack of periphyton data from the relatively deep stations sampled by diver. This is discussed in the later chapter, *Future Direction*.

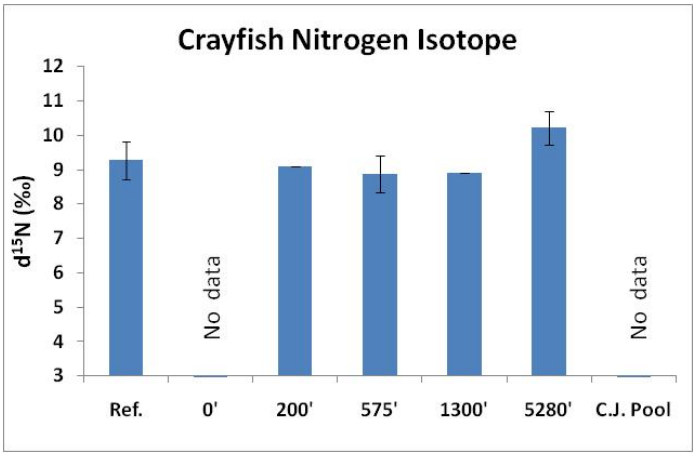


Figure 18. Crayfish nitrogen SIA results by location.

The ^{15}N crayfish results (Fig. 18) indicate some possible statistical effects (Table 8) but illustrate depletion, not unlike the pattern seen for ^{15}N sediment (Figure 9), with lowest nominal levels downstream of the pens to 1300' downstream, but the statistical differences are few, just reference vs. 575'.

For crayfish, a different numbers of replicates at each station may have affected this analysis, ranging from 1 only at 200' and 1300' to six at the reference location. None were found at the 0' location, possibly due to sedimentation from the prior ownerships operation that has since been reduced or eliminated by improvements in operation and facilities. No non-native, exotic crayfish such as the northern crayfish (*Orconectes virilis*) were found, possibly due to the depth of sampling (~ 50 feet below surface level). They previously have been reported from near Coyote Creek downstream of the Pacific Aquaculture pens (Larson et al. 2010).

Table 8. Statistical results for crayfish carbon SIA.

Sampling Location	Mean d13C	Statistical Grouping	Mean d15N	Statistical Grouping
575'	-23.8	A	9.7	A
1300'	-24.5	AB	8.8	AB
200'	-24.7	AB	8.6	AB
5280'	-25.5	AB	8.4	AB
Reference	-28.4	B	8.4	B
Ref. yr. 2000	-25.9	September	8.0	September
Ref. yr. 2000	-29.1	November	6.8	November

SCULPINS

Previously we have observed numerous prickly sculpins (*Cottus asper*), residing under and downstream of the trout net pens while SCUBA diving (Rensel 2002). Upon closer examination, we noticed that they appeared to be ingesting waste feces moving across the lake bottom with the current. As in most things thought novel, I found that others had previously seen sculpins feeding on which also has been documented elsewhere in the literature. These are among the larger of the sculpins that occur in the Pacific NW, and they prefer fish, fish larvae such as sticklebacks, insects and invertebrates as prey (Wydoski and Whitney 1979). Consumption of fish farm wastes is not considered a problem because the fish cultured in this facility have typically been in fine health and use of antibiotics or chemicals is extremely rare with this operation and sculpins are reported to consume waste feces of wild fish too. Results of sculpin SIA indicated major difference in ^{13}C, much more than would have been expected for a single trophic level, which is not explicable with existing data but nevertheless showed a consistent and expected spatial pattern (Fig. 19) with least enrichment at the reference area and highest at the 0' downstream station and not diminishing by one mile downstream (Table 9).

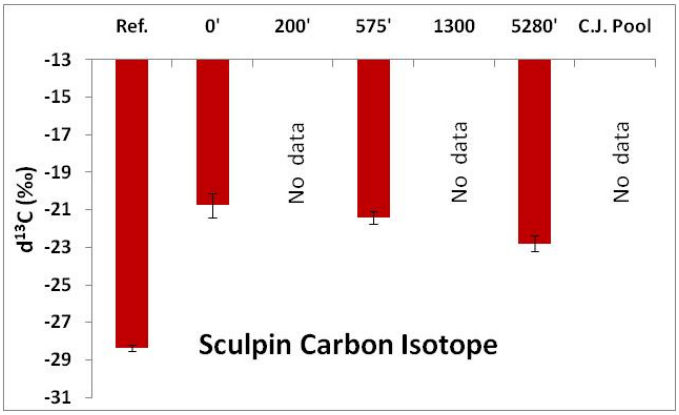


Figure 19. Sculpin carbon SIA results by location

Table 9. Statistical results for sculpin ^{14}C SIA.

Sampling Location	Mean ^{13}C Sculpin (high to low)	Statistically Homogeneous Groups
0'	-20.8	A
575'	-21.4	A
5280'	-22.8	A
Reference	-28.4	B

Table 10. Statistical results for sculpin ^{15}N SIA.

Sampling Location	Mean ^{15}N Sculpin (high to low)	Statistically Homogeneous Groups
0'	10.6	A
575'	10.4	AB
5280'	9.7	BC
Reference	9.3	C
Ref. yr. 2000	8.1	September
Ref yr. 2000	9.8	November

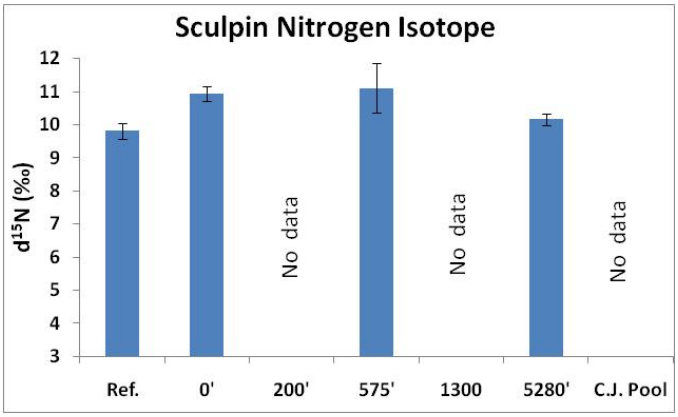


Figure 20. Sculpin nitrogen SIA results by location.

Significantly more nitrogen isotope enrichment was observed for at 0' downstream location than at the reference station (Fig. 20) but the effect was spatially more muted. Nitrogen isotope enrichment showed the expected pattern higher to lower with distance downstream of the pens but there were statistical overlaps (Table 10).

STABLE ISOTOPE RESULTS AMONG SPECIES AT SINGLE STATIONS

Figures 21 and 22 from 575' downstream of pens and the reference area, respectively, are shown to illustrate possible trophic links among sediments and species at each location. At the 575' downstream location, there is a steady increase in 15N from sediments to sculpins, but none of the steps approach the 3.4‰ average increase often found in other studies. This suggests a more complicated food web with multiple food sources.

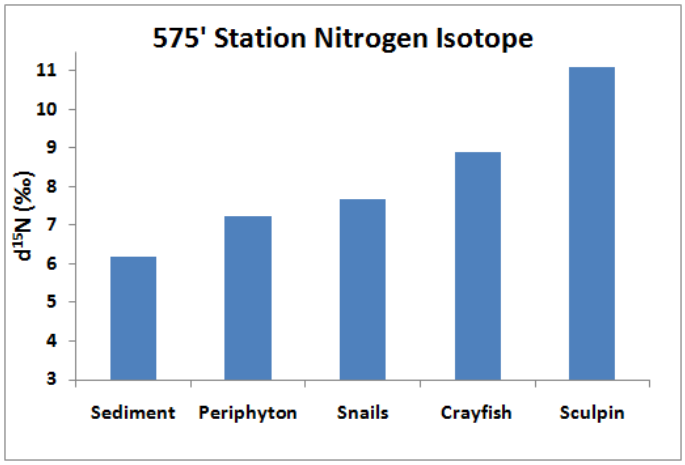


Figure 21. 15N results for 4 species and sediments at station 575' downstream of the pens.

For the reference area (Fig. 22), the relationships are quite different and indicate a more normal step increase from periphyton to crayfish, with the missing component probably aquatic insect larvae. Secondary production is a primary goal of the Bonneville Power Authority funded food web study commencing in 2010.

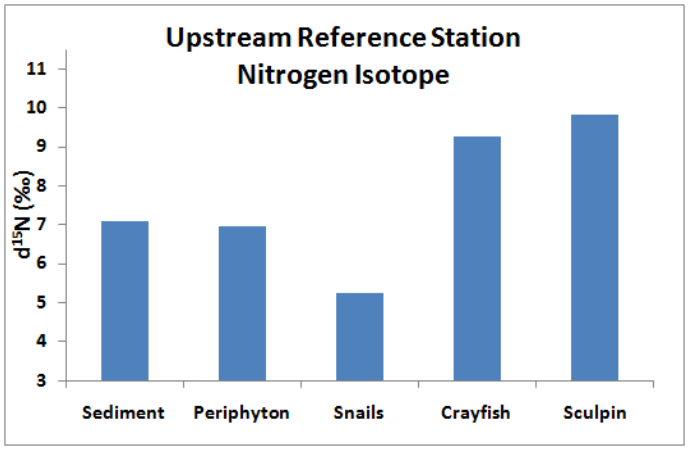


Figure 22. 15N results for four species and sediments at station at the reference area upstream of all net pens.

STABLE ISOTOPE SUMMARY

The above data show that there are pathways of isotope enrichment and depletion downstream from the pens but the challenge is to integrate the information and understand how the system is operating. In particular, the observed depletion of the snail tissue for ^{13}C is of interest. One method to do this is a dual isotope plot that allows us to see some of the key data for both ^{13}C and ^{15}N results as shown in Figure 20. The results of the figure are summarized below in bullet format.

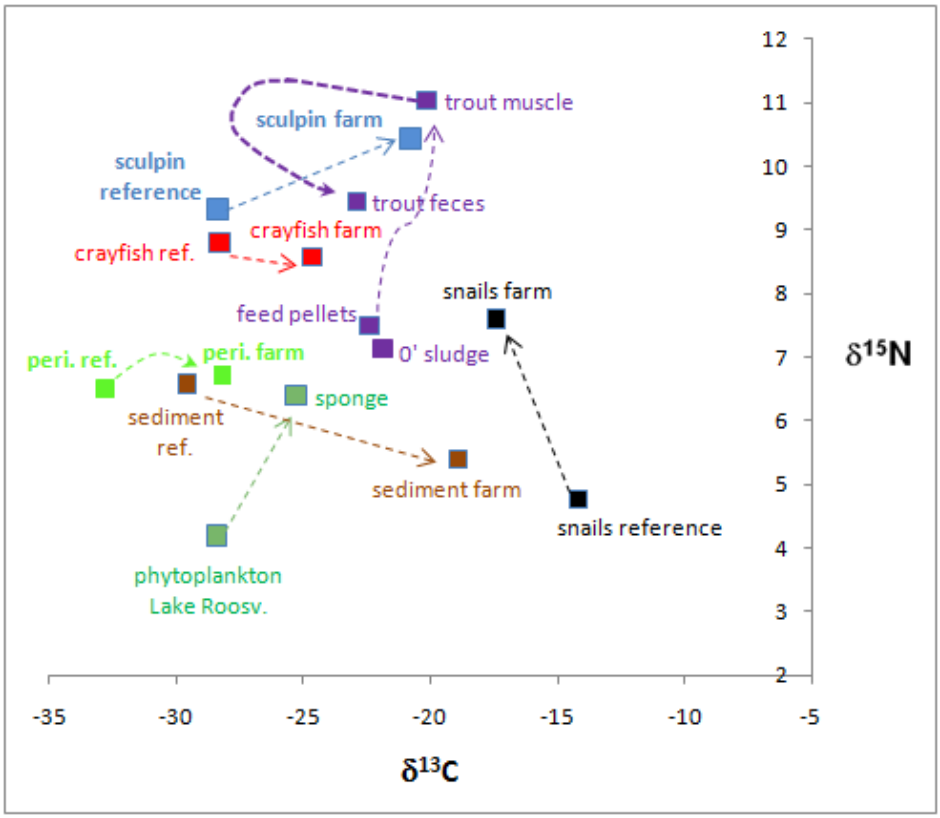


Figure 23. Summary dual isotope plot showing relationships among food web components of Rufus Woods Lake and net pen effects. All data from RWL except phytoplankton from Lake Roosevelt study (Black et al. 2003)

Key:

Ref. = reference station upstream of farms

peri. = periphyton

0' sludge = at 0' downstream station

- The most surprising result was the near "flat line" response of enrichment or depletion of ^{15}N for periphyton or crayfish, as compared between reference and fish farm areas and shown by the dashed-line bright green and red lines in the figure above. This indicates no likely impact on sediment ^{15}N of farm operation, but an expected ^{13}C effect.

- Sediments and sculpin ^{15}N response was muted, the former depleted, the latter enriched as shown by brown and black arrows, respectively, in Figure 20. This suggests multiple sources of nitrogen besides fish farm sources, i.e., a mixing model is needed with more food web components to fully understand the relationships. Conversely for sediments and sculpins, ^{13}C appears to have a larger and hence more detectable shift, which is inexplicable presently, but repeatable, as shown by low variance among replicate samples. I would have expected a large shift for ^{15}N, as previously explained, and the fish pens are a significant source of marine derived and ^{15}N enriched nitrogen via the fish oil and meal in the feed. I can find no other studies showing this result for any taxa.

- Snails exhibited a major enrichment of ^{15}N downstream of the pens vs. the reference samples, but ^{13}C was depleted, the only such contrary case in this study. It is a fairly safe assumption that snails in this area feed on the periphyton, but noticed the huge gap between in both ^{13}C for snails and periphyton at either reference or farm locations shown in figure 20. Again, we do not have to fully understand these results to use them in the future for detecting spatial effects of fish farms.

- Sculpins had expected enrichment in both ^{13}C and ^{15}N although the former was very large and the latter was ~1/3 of a normal trophic step. Clearly they are eating other N derived sources as the observed shift was overly enriched for C but minimal for N. Possibly this can be explained by the fact that waste N from trout is mostly dissolved in the water column but most waste C is tied up in solids such as the feces or waste feed and these fish are more positively linked to the benthic food web than the pelagic realm.

- The reference to farm shift of ^{13}C varied from large for several species (e.g., snails +3.2‰) to extremely large (+10.7‰) for sediments for inexplicable reasons. All were increases except for depletion for snails, also an inexplicable result, but one cited in the literature in at least one other case as discussed above.

- Fish feed to net pen trout flesh resulted in the expected ~ 0.5 ‰ ^{13}C and 3.5 ‰ ^{15}N enrichment.

- Phytoplankton (from Lake Roosevelt, Black et al. 2003) was relatively low in terms of 15N but similar in ^{13}C compared to reference sediments. Compared to reference periphyton, it was lower than both. These differences are pronounced and may allow differentiation of benthic vs. water column food webs in the future, but only to the extent they are mutually exclusive or a simple mixture of two or three components. An unknown species of sponge is often observed on boulders in the lake, but I did not have reference-area sponge for this study.

- A thin layer of sludge found on the bottom nearest the most downstream pen (0' station) was highly similar to feed, suggesting some prior farm ownership overfeeding as the isotopic mixture was nearly identical (see purple markers and arrows in Fig. 20). The fish farm had undergone recent ownership change when acquired by Pacific Aquaculture and the prior owner had neglected the operation according to former employees, CCT officials and the results of a review by Rensel and Forster (2009). Normally substantial amounts of bottom sludge do not accumulate at this site, but if it does, it would be in August or September during the annual low river flow periods so our sample timing was appropriate.

- "Periphyton" was not enriched in ^{13}C like in Lake Roosevelt (not shown here, ^{13}C = -22.7, ^{15}N = 4.2 or much lower and right in Fig. 20). But there appeared to be a possible downstream enrichment at 575' downstream of the net pens, although sample numbers were limited.

- No aquatic insect data were available for this study, but should be addressed as a possible

missing component and link between periphyton and higher organisms such as crayfish and sculpins.

- Greater or lesser than a single trophic step difference among samples is not a problem with use of the methodology as long as the observed effects are interannually repeatable. As previously shown, year 2000 results were similar to highly similar to year 2009 results in every case.

FUTURE DIRECTION

The results of this study are encouraging in that that a relatively inexpensive tool ($20 per sample including preparation costs) is available to demonstrate food effects of the net pens over spatial scales needed to understand net pen effects. The question posed here was…. What is the fate of waste solids from net pens in Rufus Woods Lake on sediments and benthic epifauna? We do not have a complete answer to that question, but we are now able to evaluate the spatial effect more quantitatively that ever before. The reader is asked to keep in mind that these effects represent maximum influences, as the sampling was directly downstream. Had we sampled the other side of the river in the littoral or sublittoral zone, we probably would have seen no effect in all cases.

This report demonstrates fairly conclusively that waste solids are not going entirely "to waste" in terms of unused bottom deposition or bacterial oxygen demand but rather are being incorporated into the food web. With the initiation of BPA funded food web studies in the summer of 2010, there will be the opportunity to measure standing stock and production rates of invertebrates and at least one station each can be upstream and downstream of the net pens.

Sampling of sediments yielded useful results in this study but remains a problem for quantitative assessments. Although reasonable results showing a logical spatial relationship were achieved, few of the bottom sediment samples were free of pebbles or pea gravel that had to be removed from the sample before grinding and analysis, resulting in volumetric bias. Such hard substrate bottoms are not usually monitored by grab sampling or cores due to this difficulty that may result in biased outcomes. One way to deal with this would be to install "sediment traps" can be installed on the bottom to measure rates of sedimentation over short time periods. Concentrated salt solution or formalin can be used to preserve the settling sediments during collection. However, I have never believed sediment traps are especially useful as fish farm waste solids that fall to the bottom are resuspended, sorted and rapidly transported by currents in a system like RWL. Another option would be to install inexpensive periphyton collection units, often just a glass slide in a holder that is placed on the bottom inside a protective cage while periphyton accumulates. In either case, the contents of the cups or biofouling on the slides can be scraped, measured for chlorophyll content and stable isotope analysis. This could be done at a number of stations downstream for a short period in the summer to more provide a methodical representation of the effects of the fish farms on the base of the epibenthic food web.

An easily achievable future goal would be to collect enough data to estimate the percentage contribution of the net pens to invertebrates such as snails and demersal fish such as sculpins over a series of stations downstream. This can be done by calculating the trophic level of each consumer and by estimating the proportion of pelagic or benthic carbon utilized by each taxa through plotting a line with a slope of 3 through the primary consumer for both benthic and pelagic components of the food web in a dual isotope $^{13}C/^{15}N$ plot (DeNiro and Epstein 1981). This was not done for the present study as representative shallow water periphyton was not measured and deep water periphyton samples were probably not autotrophic (photosynthetic based). As the focus was the bottom, no phytoplankton data were collected but used from the prior study in Lake Roosevelt. Preparing trophic level estimates and percent contribution estimates for the farm is a logical next step and not difficult, if the additional samples are collected, and some of this will be done in the Bonneville Power Authority funded food web study.

In terms of spatial analysis, I have indicated the need for additional samples further downstream of the fish farm, some of which can be collected as part of the BPA sponsored study. There is also a need to collect additional upstream-reference area samples to be sure that all upstream conditions are similar and not anomalous at the one reference area used herein. Sampling in the Chief Joseph pool could also be done along the littoral zone, to compare to upstream reference areas. In particular collection of periphyton on macrophytes may be useful to detect or reject the notion that fish farm effects are registered that far downstream.

The probable efficacy of using stable isotope analysis in Rufus Woods Lake has been demonstrated, based on this brief study, the offshoot of a prior study first conducted 10 years ago. The goal of this study was to begin to understand the spatial effects of the net pens and how waste trout feces or feed pellets contribute to the lake's ecology, specifically the near bottom and bottom organisms. This goal was achieved in particular with regard to the spatial effects of the chosen epibenthic indicators organisms and sediments.

If further sampling is done, it should first focus on phytoplankton vs. periphyton vs. fish feed and feces stable isotope signatures. If sufficiently different, which appears to be the case, this methodology should present an excellent tool for future understanding and management of net pens in Rufus Woods Lake. In the past, the only tools I have had to judge conditions were water chemistry (nutrients, dissolved oxygen, etc.). All water bodies have carrying capacity for aquaculture development and effects. The challenge is to understand the biological system well enough to make rationale scientific estimates of limits that managers can apply to protect the ecosystem while allowing a reasonable and sustainable level of production that does not endanger wild fish or invertebrates. If the right balance is achieved, and periodic monitoring conducted to detect possible biological shifts, both may benefit.

Periodic monitoring of biological conditions or surrogate indicators may lead to regulatory tools to inexpensively but carefully manage the lake system. It took many years of baseline sampling of net pen effects in Puget Sound to achieve a rational regulatory system, but without the annual monitoring, establishment of sediment impact zones and methods for routine monitoring would have not been possible.

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DATA APPENDICES

Station Code	Location	Type	Trophic level	Type Code	Qualitative Sediment Grain Size
		Snails	2	1	(without cobble overlay)
A	0' downstream of Site 1	Sculpin	2	2	coarse sand with pea gravel with fluff
B	200' downstream of Site 1	Sediment	1	3	coarse sand
C	575' downstream of Site 1	Crayfish	2	4	coarse sand
D	1300' downstream of Site 1	Trout		5	coarse sand with pea gravel
E	5280' downstream of Site 1	Periphyton	1	6	medium sand
F	Chief Joseph Pool, State park	Fish Feed		7	silt/clay
G	Upstream Reference	Bottom Sludge	1	8	medium sand
H	Other	Sponge	1	9	

Sample No.	Station Code	Type	Date Collected	$d^{15}N$ (‰)	N%	$d^{13}C$ (‰)	C%
1A	A	1	11-Aug-09	7.72	2.35	-17.09	23.13
1B	A	1	11-Aug-09	7.49	2.43	-17.38	23.38
1C	A	1	11-Aug-09	7.48	2.59	-17.71	24.08
7	A	2	11-Aug-09	10.58	12.67	-19.92	46.65
13A	A	2	12-Aug-09	10.24	12.84	-20.63	45.14
22A	A	2	12-Aug-09	10.69	13.73	-21.31	47.26
23	A	2	12-Aug-09	10.25	13.41	-21.18	47.51
2	A	3	11-Aug-09	5.63	0.02	-15.68	0.20
3	A	3	11-Aug-09	5.85	0.08	-20.72	0.41
4	A	3	11-Aug-09	4.58	0.18	-22.09	0.92
5	A	3	11-Aug-09	5.48	0.02	-17.21	0.20
26	A	8	12-Aug-09	7.52	3.67	-21.93	32.86
51	A	8	12-Aug-09	7.05	3.30	-21.86	28.10
52	A	8	12-Aug-09	6.81	3.43	-21.81	27.83
49	A	9	12-Aug-09	6.42	7.24	-25.31	34.50
48	B	1	11-Aug-09	6.66	2.21	-16.41	20.51
6B	B	1	11-Aug-09	6.30	2.00	-16.26	19.66
6C	B	1	11-Aug-09	6.48	2.44	-17.34	21.66
46	B	3	11-Aug-09	5.09	0.11	-21.55	0.67
9	B	3	11-Aug-09	4.91	0.06	-21.72	0.32
10	B	3	11-Aug-09	5.35	0.06	-21.33	0.28
11	B	3	11-Aug-09	4.95	0.05	-21.97	0.23
47	B	4	11-Aug-09	8.58	13.82	-24.65	46.91
14	C	1	11-Aug-09	7.02	2.72	-17.96	21.30
13B	C	1	11-Aug-09	7.34	2.78	-17.86	22.02
6A	C	2	11-Aug-09	9.48	13.10	-21.15	46.93
33	C	2	12-Aug-09	10.85	13.22	-21.47	45.93
53	C	2	12-Aug-09	11.07	13.13	-21.20	45.84
63	C	2	12-Aug-09	11.02	13.35	-21.90	44.85
50	C	3	11-Aug-09	5.90		-22.84	0.40
15	C	3	11-Aug-09	6.29	0.03	-22.58	0.16
16	C	3	11-Aug-09	5.71	0.06	-22.16	0.41
17	C	3	11-Aug-09	4.80	0.05	-22.55	0.24
12	C	4	11-Aug-09	8.60	14.37	-23.07	46.58

Sample No.	Station Code	Type	Date Collected	$d^{15}N$ (‰)	N%	$d^{13}C$ (‰)	C%
28	C	4	12-Aug-09	8.02	14.27	-23.86	46.31
29	C	4	12-Aug-09	7.87	13.98	-23.25	46.24
60	C	4	12-Aug-09	9.02	13.43	-25.03	46.56
8	C	6	11-Aug-09	7.01	0.15	-27.76	1.20
21	C	6	11-Aug-09	6.43	0.21	-28.55	1.28
18	D	1	11-Aug-09	7.49	2.99	-18.11	20.04
22B	D	1	11-Aug-09	7.48	3.54	-20.10	22.47
22C	D	1	11-Aug-09	7.34	3.19	-19.94	21.45
19	D	3	11-Aug-09	5.78	0.03	-24.36	0.20
24	D	3	11-Aug-09	5.95	0.02	-23.88	0.12
25	D	3	11-Aug-09	6.99	0.02	-24.36	0.12
20	D	4	11-Aug-09	8.40	14.05	-24.52	45.91
62	E	1	11-Aug-09	7.36	3.15	-20.21	20.07
27B	E	1	11-Aug-09	7.47	2.30	-17.77	17.93
27C	E	1	11-Aug-09	7.16	2.87	-19.66	18.56
61	E	2	11-Aug-09	9.73	13.24	-22.30	45.60
54	E	2	12-Aug-09	9.49	13.38	-23.30	45.79
55	E	2	12-Aug-09	9.89	12.96	-22.97	44.62
56	E	2	12-Aug-09	9.54	13.20	-22.63	45.28
58	E	3	11-Aug-09	6.57	0.02	-26.90	0.14
59	E	3	11-Aug-09	6.81	0.02	-26.42	0.14
30	E	3	11-Aug-09	7.09	0.02	-28.36	0.14
31	E	3	11-Aug-09	7.15	0.03	-26.71	0.20
57	E	4	12-Aug-09	9.38	12.76	-25.21	45.41
27A	E	4	12-Aug-09	10.06	13.66	-25.88	45.52
32	E	6	11-Aug-09	6.67	0.44	-31.28	2.75
68	F	3	12-Aug-09	3.49	0.27	-24.92	2.14
69	F	3	12-Aug-09	3.46	0.32	-24.52	2.49
70	F	3	12-Aug-09	3.25	0.32	-24.85	2.81
44a	G	1	12-Aug-09	4.68	1.67	-13.22	16.88
44b	G	1	12-Aug-09	4.72	2.15	-14.78	19.25
44c	G	1	12-Aug-09	4.87	2.29	-14.48	20.11
64	G	2	12-Aug-09	9.46	13.78	-20.93	46.63
65	G	2	12-Aug-09	9.04	13.58	-20.61	46.33
66	G	2	12-Aug-09	9.45	13.06	-20.65	46.18
36	G	3	12-Aug-09	6.82	0.05	-30.39	0.28
37	G	3	12-Aug-09	6.59	0.04	-29.31	0.25
38	G	3	12-Aug-09	6.81	0.04	-28.93	0.28
39	G	3	12-Aug-09	6.08	0.04	-29.72	0.25
34	G	4	12-Aug-09	8.89	13.66	-28.43	46.39
35	G	4	12-Aug-09	9.20	13.60	-29.52	46.86
40	G	4	12-Aug-09	8.28	14.46	-28.80	45.65
43	G	4	12-Aug-09	9.21	13.40	-29.73	45.77
45	G	4	12-Aug-09	9.17	13.40	-28.64	46.16
67	G	4	12-Aug-09	7.90	14.65	-25.01	45.05
41	G	6	12-Aug-09	6.47	0.17	-32.96	1.03
42	G	9	12-Aug-09	5.48	5.64	-29.70	24.46
71	H	5	12-Aug-09	11.16	8.54	-21.08	60.16

Sample No.	Station Code	Type	Date Collected	$d^{15}N$ (‰)	N%	$d^{13}C$ (‰)	C%
72	H	5	12-Aug-09	10.94	1.18	-20.40	6.52
73	H	5	12-Aug-09	10.87	12.59	-18.86	52.02
74	H	5	12-Aug-09	11.15	10.45	-20.22	56.96
75	H	7	12-Aug-09	7.17	8.58	-22.13	50.19
76	H	7	12-Aug-09	7.61	7.72	-22.57	48.51
77	H	7	12-Aug-09	7.67	7.70	-22.49	49.35