

The impact of dam removal on marine-derived nitrogen in tree-rings in the Elwha River watershed, Washington

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1 CONTENTS

2	Project Summary	1
3	Project Description.....	2
3.1	Background and Rational	2
3.2	Experimental Plan.....	9
3.3	Methods.....	11
3.3.1	Benthic invertebrates	11
3.3.2	Macroalgae	11
3.3.3	Freshwater fish	11
3.3.4	Foliar and Incremental Core samples.....	11
3.3.5	Sample Preparation.....	11
3.3.6	Analysis	12
3.4	Broader Impacts	12
4	References	13

2 PROJECT SUMMARY

Over the last 15 years there have been many efforts to determine whether marine-derived nitrogen (MDN) measured in riparian tree rings can serve as a useful record of historical salmon abundance in Pacific Northwest rivers. To date these investigations have led to mixed results, with only one study showing a convincing correlation between $\delta^{15}\text{N}$ and salmon escapement data. Such studies are hindered by a poor understanding of the time scale over which MDN is incorporated into tree rings, and of the extent to which nitrogen is cycled internally within trees.

The ongoing removal of two ~100-year-old dams on the Elwha River in Washington state provides an opportunity to study the effects of a sudden return of salmon on MDN sequestration in tree rings. We propose a 5-year study to collect high temporal resolution data on the $\delta^{15}\text{N}$ in a

variety of organisms at four points along the river. By measuring $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and C:N ratios in increment cores from Sitka spruce, we plan to observe the migration of MDN through the ecosystem in real-time, shedding light on open questions about the utility of tree rings as a tool for studying salmon returns.

3 PROJECT DESCRIPTION

3.1 BACKGROUND AND RATIONAL

Anadromous salmonids provide an important annual pulse of nutrients to Pacific Northwest rivers. As the fish return to the river to spawn each year, they transport marine-derived nitrogen (MDN), carbon and phosphorus to the upper reaches and smaller tributaries. After spawning, the salmon carcasses decompose and release these nutrients into the environment. Many northwest streams, including the Elwha River, are oligotrophic and nitrogen-limited (J. J. Duda et al. 2011). In these watersheds, the 130g of nitrogen contained in the average salmon affects the entire ecosystem. The released nitrogen is metabolized by salmon fry, other freshwater fish, benthic macro-invertebrates, phytoplankton, riparian plants, and (higher up the trophic level) bears and other predators. 30 - 70% of nitrogen in aquatic organisms in these rivers comes from salmonid MDN.

The provenance of nitrogen in these organisms can be identified by its isotopic signature. Marine nitrogen is enriched in ^{15}N by about +10-15‰ compared to terrestrial nitrogen. Since nitrogen is usually the limiting nutrient, another indicator of marine nitrogen uptake in organisms is lower-than-expected C:N ratios.

Riparian trees also benefit from the pulse of nutrients during salmon returns. There are several pathways for MDN to become available to trees. Summer floods wash salmon carcasses onto the floodplains, where they decompose and allow nutrients to enter the soil. Piscivores are also a significant vector. Bears may transport MDN a significant distance from the stream via urine, feces, and partially eaten salmonid carcasses (Hilderbrand et al. 1999). In total, riparian plants along spawning sites receive ~27% of their annual nitrogen from MDN (Helfield and Naiman 2001).

Much work has been done to investigate the uptake of MDN by riparian trees. Uptake can be quantified in several ways: foliar $\delta^{15}\text{N}$, tree ring $\delta^{15}\text{N}$, tree ring C:N, and indexed tree ring widths (used as a proxy of MDN uptake). Helfield and Naiman (2001) found that riparian trees along salmon-spawning rivers in Alaska grew three times faster than trees along non-spawning rivers. They measured the $\delta^{15}\text{N}$ in foliage from riparian Sitka spruce and Douglas firs, finding that that trees adjacent to spawning streams had 2-5‰ higher $\delta^{15}\text{N}$ compared to trees near non-spawning streams (Figure 1: From Helfield and Maiman, 2001). Other studies have examined the $\delta^{15}\text{N}$ content of foliage along a transect perpendicular to spawning rivers. Hilderbrand et al. (1999) found that foliar $\delta^{15}\text{N}$ was highest in white spruce closest to the rivers and tapered off with

increasing distance, though it was still elevated as far as 1 km away due to MDN dispersion by bears.

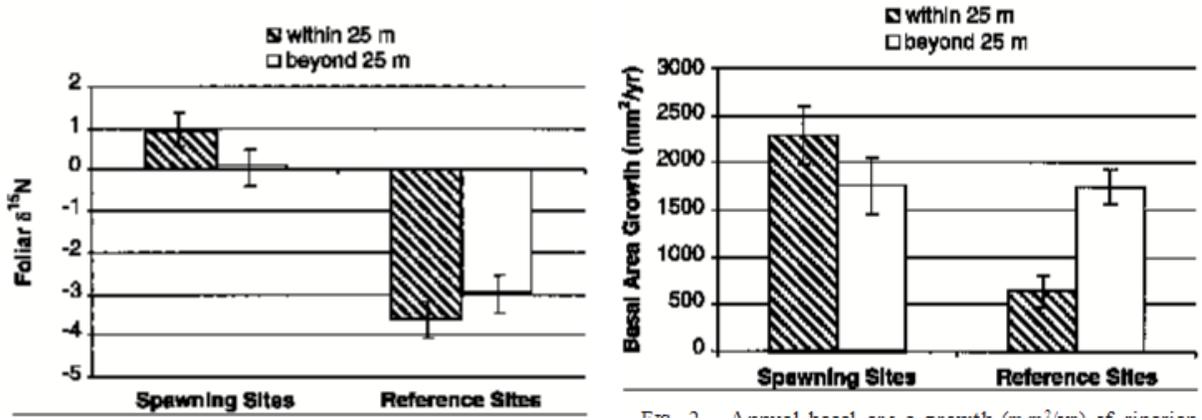


Figure 1: From Helfield and Maiman, 2001

These studies suggest a positive feedback loop between salmon returns and riparian plant growth. In return for the additional nutrients provided by salmon, riparian trees provide ecosystem services to rivers – shade, temperature regulation, nutrient return in the form of litterfall, and a source of large woody debris – which benefit both spawning salmon and salmon fry.

Over the last ten years, an increasing number of studies have focused on tree rings in order to explore the tantalizing possibility of using MDN in trees as a record of historical salmon abundance. If such a link could be established, it would have profound consequences for our understanding of how salmon returns have been affected by human activity over the last 200 years. Many old growth trees in the Pacific Northwest are over 100 years old, and their tree ring records might allow us to examine salmon abundances stretching well into the early 19th century. This would be a boon to conservationists, as historical salmon return data is often sparse and limited to large rivers in years after dam construction (a common method of quantifying salmon returns is by counting fish traversing fish ladders and weirs).

As intriguing as the tree-ring—salmon link may be, studies so far have yielded mixed results. Three such studies are of interest here. Drake et. al. (2002) measured Sitka spruce ring widths in several Alaska watersheds, and found a good correlation between ring widths and historical salmon catch for some watersheds (Figure 2: From Drake et. al., 2002). Their study suggested that tree ring widths could be a good proxy for MDN uptake by trees, but there was no attempt to directly measure structural nitrogen in trees.

In another study, Kiernen and Johnson (2009) attempted to correlate 20 years of historical salmon escapement data (fish/km) in northern California rivers with three different tree-ring factors from coastal redwoods and Douglas firs: $\delta^{15}\text{N}$, structural nitrogen concentration, and

indexed ring width. Of these factors, they found the strongest correlation ($p < 0.001$) between escapement and $\delta^{15}\text{N}$, with a lesser correlation ($p = 0.002$) between escapement and indexed ring width (Figure 3: From Kiernen and Johnson, 2009). For most watersheds, they found the best correlation when they assumed that the time lag between salmon return and tree-ring data was one year. Using $\delta^{15}\text{N}$ and ring width as predictive factors, they were then able to predict salmon escapement as far back as 1945.

Although this was a promising result, Kiernen and Johnson noted several problems with this method. In some localities, intra-site variation in $\delta^{15}\text{N}$ for a given year was as high as 5-7‰, overwhelming the variation due to salmon escapement. At one watershed, the correlation was best when the authors assumed a seven-year time lag, suggesting that the tree rings were recording more of a moving average of MDN accumulation in the environment rather than recording the individual pulses. The residence time of MDN in the riparian environment is a question that needs to be addressed.

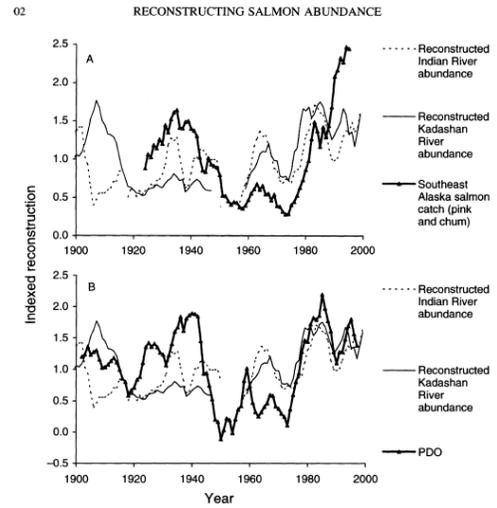


Figure 2: From Drake et. al., 2002

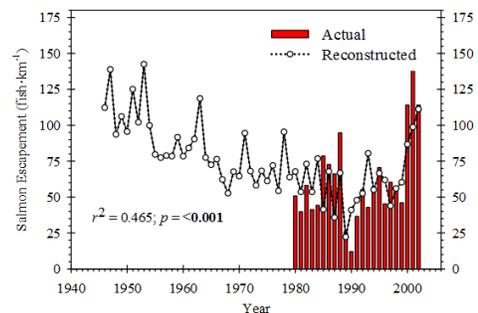
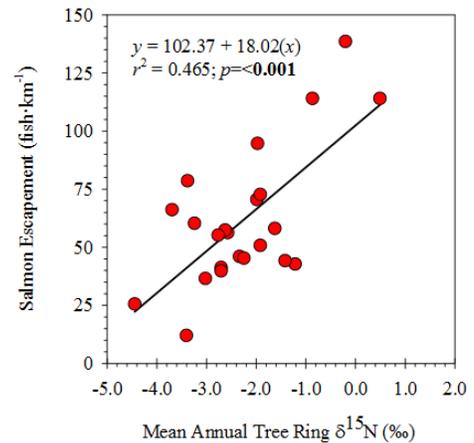


Figure 3: From Kiernen and Johnson, 2009

In contrast to Kiernen and Johnson, Drake et. al. (2011) studied Ponderosa pines along two central Oregon rivers and found that tree-ring $\delta^{15}\text{N}$ was not a reliable indicator of historical salmon abundance. A key piece of evidence were the trees along the Metolius River, where salmon disappeared after the construction of a dam in 1920. The pre-1920 $\delta^{15}\text{N}$ signals from the Deschutes River, which has never had salmon, were unexpectedly *higher* than those from the Metolius, which did have salmon at that time (Figure 4: Drake et. al., 2011). Moreover, one of the trees from the Metolius showed higher $\delta^{15}\text{N}$ *after* 1920 compared to before 1920. If such a dramatic and sudden change in salmon returns does not appear in the tree-ring record, it casts serious doubt on the utility of tree-rings to estimate historical salmon abundance.

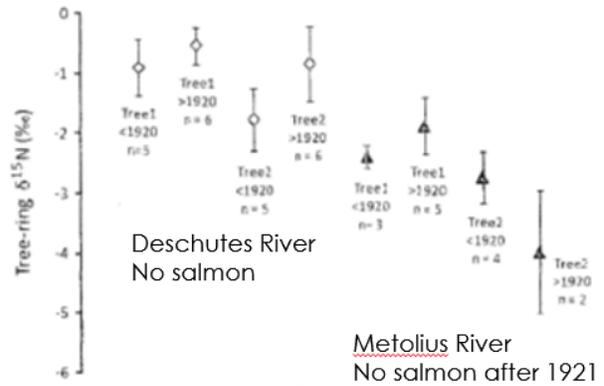


Figure 4: Drake et. al., 2011

In a second experiment, the authors added a spike of radio-labeled $^{15}\text{NH}_4^+$ to soil near trees, representing the equivalent of one salmon at the base of each tree. They found that the nitrogen did not appear in woody xylem until the following year, but because the tree rings were very small, they had to average several years' worth of rings and were not able to elucidate the signal in high resolution. Still, they determined that the maximum signal that could be expected from salmon MDN is 0.08-0.43‰, well within the range of natural intra-site variation. In a third experiment in another watershed, a $^{15}\text{NH}_4^+$ spike to soil was detected in 5 years' worth of Western hemlock tree rings, suggesting that internal transport within trees may blur the signal from any individual year.

This study highlighted the confounding issue of internal cycling of nitrogen within trees. On long time-scales, nutrients are transported across ring boundaries from sapwood to heartwood, blurring the signal from each year's returns. On short time-scales, dissolved nitrogen is transported by sap within the woody xylem of trees.

It is therefore still an open question to what extent MDN in tree rings preserve a record of annual salmon returns along a river. Part of the problem is that the temporal scale of MDN enrichment in the riparian environment has been poorly-studied (Helfield and Naiman 2001). It is not known why in some watersheds the $\delta^{15}\text{N}$ in riparian foliage and tree rings fluctuates annually in proportion to that year's salmon escapement, while in other it appears to represent a more muted "rolling average" over a longer time scale. Nor is it known how long it takes for variations in MDN in a river to show up in tree-rings; that is, whether the $\delta^{15}\text{N}$ composition of a tree ring represents the nitrogen taken up in that year of growth. The answer likely has to do with the residence time of MDN in the riparian environment and internal transport within trees. It may in fact take years for changes in riverine MDN to show up at higher trophic levels (Gillikin 2012).

There is a need for more high temporal resolution studies of nitrogen in tree rings to answer the following open questions:

- Does the $\delta^{15}\text{N}$ content of a tree ring represent the N taken up in that year of growth?
- How long does it take for changes in salmon nutrient loading to show up in tree rings?
- Will the $\delta^{15}\text{N}$ in tree rings respond to a sudden, large-scale change in salmon escapement?
- Are the annual MDN variations from salmon too small to be resolved, given other processes that fractionate N, and given internal N cycling in trees?

As discussed above, Kiernan and Johnson compared $\delta^{15}\text{N}$ data to historical salmon escapement data. Drake et. al. examined the effects of a sudden extirpation of salmon and the effects of artificial ammonium spikes to stream water and soil. However, no study has yet been done to determine, in high temporal resolution, the effects of the sudden return of salmon to streams that had previously been inaccessible.

The ongoing removal of two dams on the Elwha River provides just this opportunity. The Elwha is a 72-km long river that empties into the Strait of Juan de Fuca just west of Port Angeles, Washington (Figure 5). In 1913 and 1927, two small hydropower dams were constructed on the river, forming two reservoirs and cutting off the upper reaches of the river from annual salmon migration. In 1992, Congress passed the Elwha River Ecosystem and Fisheries Restoration Act which authorized the National Park Service to restore the salmon runs along the Elwha. After years of study, NPS decided that removal of both dams was the best option. In 2012, the lower Elwha dam was finally removed and both reservoirs were drained, resulting in a massive and ongoing mobilization of sediment toward the river mouth, where a new delta is forming. The upper Glines Canyon dam is scheduled to be completely removed by the end of summer 2013.



Figure 5

The Elwha is an ideal river to study because its watershed is undeveloped in the upper reaches and only lightly developed in the lower reaches. 80% of the river lies within Olympic National Park, which yields two benefits: 1) there is little anthropogenic nutrient input to the watershed, and 2) there are many old-growth trees older than 100 years, potentially allowing us to see the effects of both the historical salmon extirpation *and* the contemporary salmon return. Finally, because the watershed is close to the Pacific Ocean and receives precipitation coming from the

west, the effects of atmospheric $\text{NO}_3^-/\text{NH}_4^+$ from anthropogenic inputs is minimized.

Before the dams were constructed, the Elwha is known to have had large salmon runs for its size (Duda et. al. 2008). An estimated 400,000 salmonids – including all 5 species of Pacific salmon and 3 species of trout – returned annually to the river in the pre-dam era. Currently only 4,000 salmonids return to the 5 miles of spawning ground beneath the Elwha dam (Figure 6). After Glines Canyon dam is removed in 2013, the National Park Service expects salmon to return to the upstream portions of the river as soon as 2014 (Figure 7). In 15 to 20 years, as many as 200,000 salmonids are predicted to return.

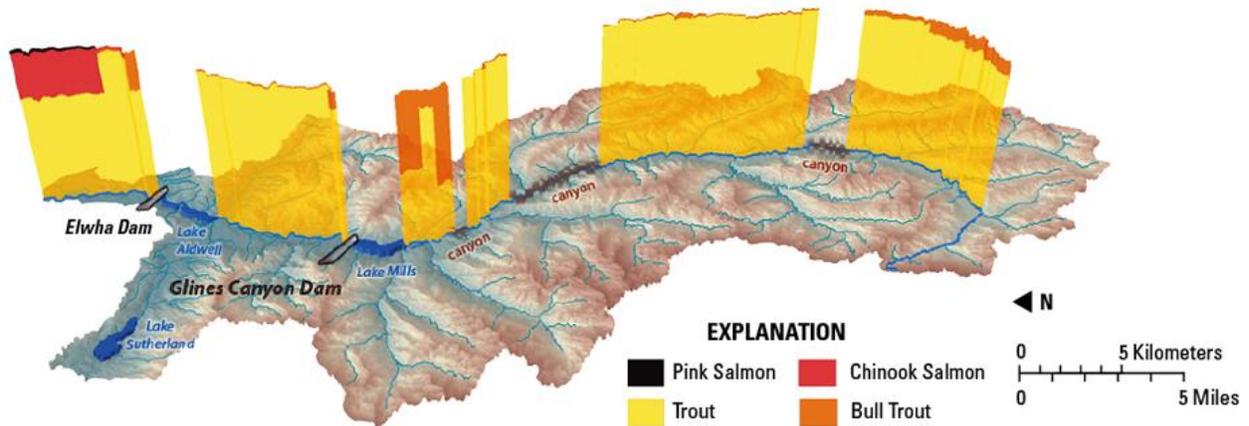


Figure 6

Aside from being a model watershed in terms of historical salmon-spawning and lack of urbanization, we have excellent baseline data on the $\delta^{15}\text{N}$ of aquatic organisms in the Elwha. Prior to dam removal, the river was extensively studied by the U.S. Geological Survey in partnership with the National Park Service, the Environmental Protection Agency and the National Fish and Wildlife Service. An entire issue of Northwest Science was dedicated to baseline studies on the Elwha (ibid.).

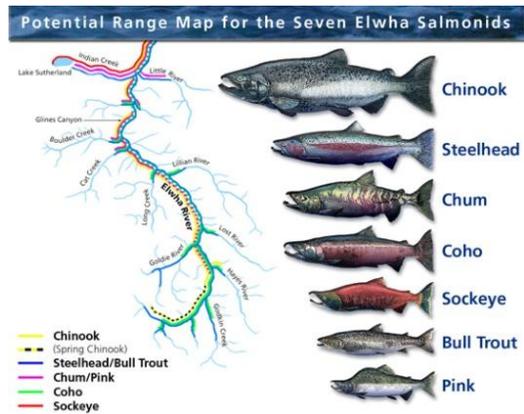


Figure 7

Duda et. al. (2011) characterized the $\delta^{15}\text{N}$ content of fish, benthic insects, and algae in three sections of the Elwha River above, below, and between the two dams. They found that the $\delta^{15}\text{N}$ values were highest in the lower reaches of the Elwha, which are accessible to salmon, but lower in the upper inaccessible reaches (Figure 8: From Duda et. al. (2011)). They did not collect any increment cores.

The Elwha represents a unique opportunity to study the effects of salmon return on a river with a known pre-salmon $\delta^{15}\text{N}$ baseline. With the both dams soon to be removed and the anticipated return of salmon to the middle and upper reaches of the river by spring 2014, we propose to investigate the rate of MDN transport to the upper river and in particular the effect of this sudden change on tree-ring MDN. This study will analyze the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of aquatic organisms, riparian foliage, and riparian tree rings in four locations along the river over the 5-year period from 2014-2018. The goal is to obtain snapshots of MDN as it moves through the environment from river to trees.

The specific questions this study will address are:

1. Will we see a salmon return signal at all over the next 5 years, looking at these three factors?
 - a. Indexed tree ring widths
 - b. C:N ratios in tree rings
 - c. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in tree rings, foliage, benthic invertebrates, macroalgae, fish
2. If so, what is the time scale in which these effects will appear?
3. Do any old growth trees in the watershed record the extirpation of salmon after the construction of the dams, and if so, can these be used as a historical pre-dam baseline for salmon abundance in the Elwha?

We hypothesize that:

1. *Over 5 years, $\delta^{15}\text{N}$ should become more equally distributed along the river compared to Duda et. al. (2011). The $\delta^{15}\text{N}$ values in the middle and upper reaches of the Elwha begin rising after the first return.*
2. *The $\delta^{15}\text{N}$ signal should increase first in aquatic organisms, then in foliage, and finally in tree rings. $\delta^{15}\text{N}$ in benthic invertebrates, fish, and phytoplankton should show increases just weeks to months after the first salmon return.*
3. *Rings in old-growth trees should show a dramatic decrease in $\delta^{15}\text{N}$ after 1913, when the first dam was built. Rings in all trees should show a more gradual increase in $\delta^{15}\text{N}$ over the next 5 years.*

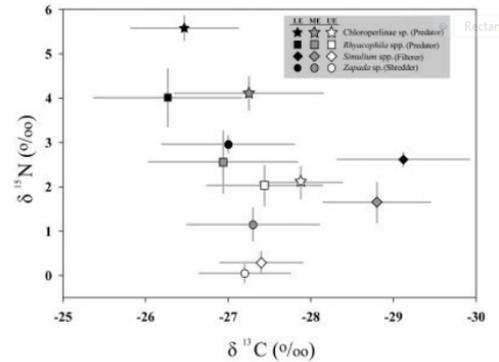


Figure 3. Plots of mean (SD) $\delta^{15}\text{N}$ (ordinate) and $\delta^{13}\text{C}$ (abscissa) values for four benthic macroinvertebrate taxa collected above (UE), between (ME) and below (LE) two dams in the Elwha River during 2005 and 2006 (combined)

Figure 8: From Duda et. al. (2011)

The National Park Service plans to monitor and quantify the salmon returns after the demolition of Glines Canyon dam, so there will be good data on salmon abundance to compare with the tree ring record. This study will be the first time that such a large change in salmon abundance is studied contemporaneously, and in high temporal resolution.

This will also be an opportunity to study the confounding effects of internal transport within trees. We will take cores from the same trees over the course of a 5-year study, analyzing the same rings multiple times to determine if there are changes over time in the $\delta^{15}\text{N}$ and C:N values.

When interpreting the $\delta^{15}\text{N}$ data, it is important to take into consideration other environmental processes that fractionate nitrogen, chief among them denitrification. We will account for denitrification in two ways. First, we will measure $\delta^{18}\text{O}$ in addition to $\delta^{15}\text{N}$. When plotted on a $\delta^{15}\text{N}$ vs. $\delta^{18}\text{O}$ chart, denitrification products and residuals tend to fall on a denitrification line, with the residuals becoming enriched in heavier ^{15}N and ^{18}O (Kendall and Doctor 2010). Samples that show fractionations in both nitrogen and oxygen lying along such a line are evidence for denitrification. Second, we will follow the example of Helfield and Naiman (2001) by sampling trees at two distances from the river, 5m and 25m. They and other authors have shown that net denitrification potential is highest in valley floors in Pacific Northwest mountains, with denitrification decreasing with distance from the stream (e.g., Nadelhoffer and Fry 1994).

There are also fractionations that occur from one trophic level to the next (Figure 9). The magnitude of these fractionations is still poorly understood and it is difficult to account for them. However, by sampling organisms at multiple trophic levels (macroalgae, benthic invertebrates, freshwater fish, trees), we hope to observe these effects. Trophic fractionation will affect the composition of nitrogen delivered to trees farther from the stream. The bulk of MDN entering the soil close to rivers will come directly from salmon carcasses, while farther from the stream it will come from the feces of bears and other high trophic level predators.

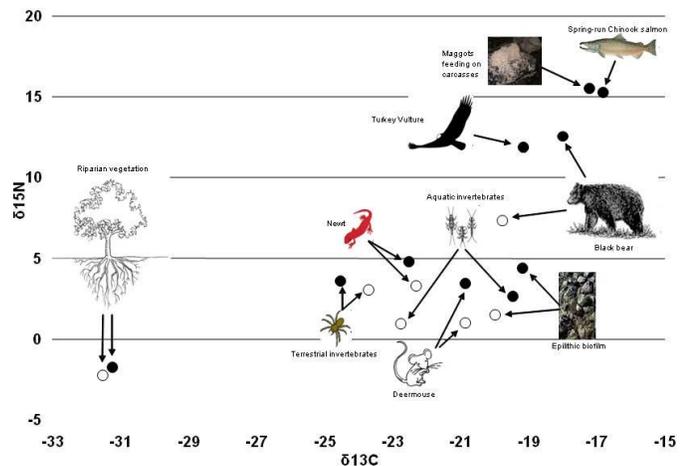


Figure 9

3.2 EXPERIMENTAL PLAN

We propose a 5-year study (2014-2018) with annual sampling taking place in the late spring before the salmon runs begin. We have identified 4 sampling localities designed to be representative of each portion of the river:

1. The *lower* locality is 2 miles downstream of the Elwha dam site. This represents the portion of the river that has remained accessible to all 8 species of salmonids.
2. The *middle* locality is 2 miles downstream of Glines Canyon dam. This represents the portion of the river between the two dams that was inaccessible prior to the Elwha Dam removal in 2012, and where chinook salmon, pink salmon, steelhead trout, and possibly coho salmon are expected to return.
3. The *upper* locality is ten miles upstream of Glines Canyon dam. This represents the portion of the river above both dams where chinook salmon, pink salmon, steelhead trout, and possibly coho salmon are expected to return.
4. The *headwaters* locality is 30 km upstream of Glines Canyon dam between Hayes River and Godkin Creek, where only spring chinook salmon, coho salmon, and steelhead trout are expected to return.

Sampling laterally along the river will allow us to test our Hypothesis #1: *Over 5 years, $\delta^{15}\text{N}$ should become more equally distributed along the river.* We will have to account for the different relative abundances of the 8 salmonid species at each locality when correlating escapement with $\delta^{15}\text{N}$. Fortunately, the National Park Service intends to collect data on both abundance and species of salmonid returns along the entire river.

In addition to these 4 Elwha River locations, we will sample one *reference* locality along the Quinault River to the south to serve as a control. The Quinault River is a salmonid spawning river which drains into the Pacific Ocean and has never had any dams (though there is one natural lake).

At each locality, we will collect 5 types of samples:

1. Benthic invertebrates
2. Freshwater fish (rainbow trout)
3. Macroalgae (filamentous green algae)
4. Foliage from riparian Douglas firs (5m and 25m from river channel)
5. Increment core samples from Douglas firs (5m and 25m from river channel)

The invertebrate, fish, and macroalgae samples will allow us to gauge the uptake of MDN within the river at a variety of trophic levels. Comparing isotopic data across trophic levels is important because a variety of nitrogen fractionation effects have been observed as MDN moves through the food web. These samples will also allow us to directly compare our $\delta^{15}\text{N}$ data with that of Duda et. al. (2011), which will serve as a baseline.

We have chosen Douglas fir for our foliar and core samples because 1) it is abundant in Olympic National Park, and 2) past studies, particular the work of Helfield and Naiman (2001), have shown it to be a good species for ^{15}N dendrochronological work. In particular, it has a low sapwood:heartwood ratio, low radial permeability, and low moisture content, all of which minimize the amount of internal nitrogen cycling.

By sampling both aquatic organisms and riparian trees at two distances from the river, we hope to better understand how MDN moves through the ecosystem. These samples will allow us to test our Hypothesis #2: *The $\delta^{15}\text{N}$ signal should increase first in aquatic organisms, then in foliage, and finally in tree rings.*

3.3 METHODS

3.3.1 Benthic invertebrates

Benthic invertebrates will be sampled from within the river using a slack sampler. Because sampling in the upper reaches of the Elwha requires a multi-day backpacking trip, the invertebrates will be preserved in ethanol.

In the lab, four taxa will be selected for analysis: Zapada, Rhyacophila, Simulium, and Chloroperlinae. These are the same taxa used by Duda *et. al.* and will permit comparison with their data.

3.3.2 Macroalgae

We will collect macroalgae, in the form of filamentous green algae, from rocks within the river. These will be preserved in ethanol as well.

3.3.3 Freshwater fish

Rainbow trout are the dominant freshwater fish in the Elwha. We will sample them by net or by angling with artificial flies.

3.3.4 Foliar and Incremental Core samples

At each sampling locality, we will select six Douglas fir trees, three at 5 m from the river bank and three at 25 m. Only mature canopy trees will be selected to avoid the effects of canopy competition. At each tree, we will collect foliar samples from low branches and four orthogonal 5mm increment cores at breast height.

3.3.5 Sample Preparation

We will prepare the invertebrate and macroalgae samples using the same methodology as Duda *et. al.*: rinse with DI water, freeze dry to desiccate, and pulverize with a ball-mill grinder. For the trout, we will take a small dorsal plug sample, desiccate, and grind. Foliage samples will be dried at 60°C for 48 hours and then ground.

The incremental cores will be analyzed using standard dendrochronological techniques (e.g., Stokes and Smiley 1968): sanding and polishing each specimen, measuring the width of each tree ring, and cutting the cores into individual ring sections. The four orthogonal cores from each tree will be grouped together for analysis. Drake *et. al.* (2011) demonstrated the importance of removing tree sap from incremental core samples to avoid contamination by soluble nitrogen, which is mobile in the xylem of certain tree species and hence tends to obliterate the tree ring

record. They developed a process of oxidative extraction to remove the sap, and we will do the same with our samples.

3.3.6 Analysis

Each sample will be analyzed for the C:N ratio using a CHN analyzer and for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ using an isotope ratio mass spectrometer. Both instruments are available at the NOAA Northwest Fisheries Science Center in Seattle.

3.4 BROADER IMPACTS

The Elwha River is currently the focus of several long-term studies involving the collaboration of multiple agencies and organizations:

- National Park Service
- US Geological Survey
- Environmental Protection Agency
- US Fish and Wildlife Service
- Washington Department of Fish and Wildlife
- Lower Elwha Klallam Tribal Government
- NOAA
- Western Washington University

Our study will benefit from the data collected by these organizations and from the understanding we gain over the next decade of how the river responds to dam removal. Since there will be a significant field component to this project, we plan to involve graduate students from Western Washington University, high school students from Port Angeles high school, and local volunteers from the City of Port Angeles and the Klallam reservation in the collection of samples.

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