Memo

DATE: **3/12/10**

TO: **Tony Grover, Bill Maslen**

From: **Yakama Nation (John Jorgensen)**

RE: **ISRP Response Report**

At the Council's June 17, 2009 request, the ISRP began a review of the Yakama Nation's Columbia River Fish Accord proposal titled Upper Columbia Nutrient Supplementation (2008-471-00). The project is intended to assess and characterize nutrient availability, and if needed the project proponents will perform controlled experimental addition of limiting nutrients to enhance natural production of anadromous salmonids and their supporting ecological functions and limnological conditions in rivers in the Methow Subbasin.

On July 10, 2009, the ISRP released a preliminary report requesting a response to nine specific items (ISRP 2009-27). On October 26, 2009, the Council submitted the Yakama Nation's response documents which included point-by point responses to ISRP review comments and an updated project proposal that incorporated those responses.

On December 2, the ISRP provided their second review (ISRP 2009-50), which had some items that were confusing to the project sponsors and seemed to ratchet the experimental approach up beyond what was requested and inferred in the ISRP's preliminary review (July 10, 2009; ISRP 2009-27). The ISRP said the confusion in the December review stemmed from attributing some elements from a similar nutrient proposal by the Shoshone Bannocks to the YN proposal that the ISRP was reviewing concurrently. The ISRP attempted to correct these mistakes in an updated memo that was posted February 19, 2010 (ISRP 2009-50 Update). After the second ISRP review in December 2009, project sponsors prepared: 1) a formal response report detailing issues resulting from both ISRP reviews, and 2) a brief project summary for the ISRP.

To resolve these ongoing issues, a subsequent conference call was held on February 22, 2010, including ISRP members Eric Loudenslager, Pete Bisson, Bob Bilby, coordinator Erik Merrill and John Jorgensen from the Yakama Nation, and Paul Anders, an independent consultant subcontracted by the YN to discuss the Yakama's Upper Columbia nutrient enhancement proposal, response, and ISRP review. Mark Fritsch, Council staff, joined as well. Roy Beaty, BPA, and Steve Parker, YN, were invited but couldn't participate.

To inform the conference call discussion, John Jorgensen sent the project’s draft response document to the ISRP before receiving the ISRP's updated memo on Friday, February 19. After the conference call, the group agreed that the ISRP would circulate summary notes to the teleconference participants, John will update his response document and proposal, and the ISRP will review those documents with a quick turnaround. A decision needs to be made soon for the YN to contract with BPA to begin gathering baseline data such as water quality information. The ISRP members’ comments at the teleconference were shared with the full ISRP, who will consider the response and reach consensus on a final finding.

Thus, along with this cover letter, project proponents submit, as requested: 1) an updated response report addressing the nine ISRP issues brought up during both reviews, including new responses to issues discussed on the February 22, 2010 conference call; and 2) an updated final project proposal narrative for final review by the ISRP.

Accord Project Sponsors ISRP Response Report

Date: 3-10-10

Project Number	2008-471-00
Proposer	Yakama Nation Fisheries Resource Management
Project Title & Brief Description	Upper Columbia Nutrient Supplementation
Contact Name	John Jorgensen
Contact email	john@mid-columbia-coho.net

ISRP Review History:

Original Narrative submission date: 6-15-09
Date ISRP Review comments were received: On July 10, 2009, the ISRP released a preliminary report requesting a response on nine specific items (ISRP 2009-27

ISRP Review results: *[Check appropriate box]*

☐ Meets scientific criteria.

☐ Meets scientific criteria (qualified).

☐ Response requested - meets scientific criteria (qualified).

☒ Response requested – does not meet scientific criteria.

Response to ISRP Summary: [*Please check appropriate box and respond below in: Response to ISRP Comments]*

☒ The narrative will be revised and resubmitted by (concurrently).
☒ A response to ISRP comments is provided in this document.

[*Your response should include 1) areas of agreement with ISRP comments, i.e., additional information and/or any changes in the project scope of work and, 2) areas of disagreement, i.e., state why you believe the project is based on sound scientific principles, benefits fish and wildlife, and has a clearly defined objective and outcome with provisions for monitoring and evaluation of results, and provide additional information which supports your perspective].*

Response to ISRP Comments:

Please respond to each ISRP comment. ISRP Comments are provided in *italics*, followed by project sponsor responses in plain font.

Background

At the Council's June 17, 2009 request the ISRP began a review of the Yakama Nation's Columbia River Fish Accord proposal titled Upper Columbia Nutrient Supplementation (2008-471-00). The project is intended to assess and characterize nutrient availability, and if needed the project proponents will perform controlled experimental addition of limiting nutrients to enhance natural production of anadromous salmonids and their supporting ecological functions and limnological conditions in rivers in the Methow Subbasin.

On July 10, 2009, the ISRP released a preliminary report requesting a response on nine specific items (ISRP 2009-27). On October 26, 2009, the Council submitted the Yakama Nation's response documents which included point-by point responses to our review comments and an updated project proposal that incorporates the responses. We organize our review around the nine items in our preliminary review.

ISRP Recommendation

Does Not Meet Scientific Criteria

Overall Comments

The increasing popularity of trophic system enhancement as a method for increasing salmon production in the Columbia Basin indicates the need for careful assessment of the technique's effectiveness. Unfortunately, the study described in this proposal is unlikely to improve our understanding of this technique. Many of the issues that were raised in the initial set of ISRP comments were not adequately addressed in the response and would need to be addressed for this project to be technically justified.

Project Proponent Response Report for the Upper Columbia Nutrient Supplementation Project (2008-471-00)

This project response report is organized into two major sections:

- I. Project proponent's response to the general ISRP comment on the conference call regarding the need for the project to ensure measurable responses to nutrient addition; and
- II. Responses to ISRP's nine review issues identified and developed in their two reviews, including updates on issues discussed in the 2/22/10 conference call.

Section I. Project proponent response to the general ISRP comment on the conference call regarding the need for the project to ensure measurable responses to nutrient addition.

ISRP: *A measurable response - The ISRP asked, in sum, is there a reasonable capability to measure a response from the treatment?*

Project sponsor response: Yes, the probability is very high that this project will measure biological responses from experimental nutrient addition. The probability is also very high that biologically and statistically significant biological responses (treatment effects) will be measured across most if not all trophic levels, given sufficient treatment and response durations. Based on decades of empirical nutrient addition studies around the world, it is also expected that the magnitude of treatment effect responses, across metrics, will decline progressively up the trophic levels (i.e. from the bottom up), consistent with energy transfer efficiency laws of ecology.

This predicted high probability of detecting biologically and statistically significant treatment effects is supported by several lines of logic and evidence:

- First, the design of this project benefits from several decades of empirical nutrient addition studies that have individually and collectively refined sampling designs statistical power, and the application of appropriate statistical tests to measure responses to nutrient addition with high levels of sensitivity.
- Secondly, the proposed study design was reviewed and contributed to by numerous collaborators and input from nutrient addition experts from the U.S. and Canada.
- Finally, biologically and statistically significant treatment effects are also expected due to the magnitude and scale (whole river) of treatment. Most nutrient addition studies occur in river or lake systems that have been culturally denitrified by various mechanisms to their current, pre-treatment, ultra-oligotrophic condition. As such, ambient nutrient concentrations (especially phosphorus in rivers) often exist at or below detection limits of most labs (e.g. SRP < 0.02 micrograms/L). Thus, in-river target nutrient concentrations of lower mesotrophic or natural oligotrophic ranges are typically two or three orders of magnitude greater than current conditions in these culturally denutrified systems. Because the magnitude of the treatment is matched with the equally large reduction in pre-treatment nutrient availability, biological and statistically significant treatment effects are expected.

ISRP: *The teleconference discussion focused on the primary question: is the study design sufficient to provide enough statistical power to detect a response to system enrichment, if one should occur?*

Project sponsor response: Yes. Again, many years of experience in other ongoing nutrient addition programs and input from nutrification scientists in the U.S. and Canada contributed to the sampling regime, statistical analyses, and positive results that will be implemented in this project. Rigorous sample size, power analysis, multivariate analyses, and Analysis of Variance (ANOVA) and Principal Components Analysis (PCA) tests will be performed using data from each trophic level or community to assess nutrient addition effects. For example, applying this identical approach in the Kootenai River provided a 90% probability of detecting down to a 10% change in individual fish condition factor (http://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P115021)

For this study, a minimum of two and preferably three years of statistically adequate pre-treatment biomonitoring are required to produce a reasonable baseline condition for the Twisp River. Water quality sample size analysis of data collected by the Yakama nation was completed using two years of pilot project data from the Twisp River (2008, 2009). This analysis already facilitated modification of the sampling regime that included increasing replications at the same 6 Twisp River sites by sampling two transects at three positions each (See updated sampling design figure and associated text in the proposal for more details).These same sample size, power, and multivariate analyses will also be performed for chlorophyll, primary production, and invertebrate and fish metrics.

Empirical data from the first year will be used to provide data for the sample size determination needed to ensure an adequately rigorous sampling design for subsequent pre- and post-treatment years. Multivariate techniques such as Principal Components Analysis (PCA) will also be performed to reduce the dimension of biological community data and to determine which taxonomic groups and biological metrics are contributing significantly to observed variation. Data will be selected to represent taxonomic orders and biological or ecological metrics that are common across dates and sites.

ANOVA will be performed annually using data from each and all years during pre- and post-treatment periods to investigate the average algal/periphyton and macroinvertebrate abundance, biomass, and richness, to test for site or time effects on these metrics, and to assess effects of experimental nutrient addition. Transformation of response variables will occur when necessary. All summaries, tests, and graphics will be performed using the SAS package and other software.

All analyses will be done using annual and among-year data as appropriate before and after experimental nutrient addition to determine and characterize treatment effects in terms of water quality, nutrient availability and composition, and all relevant response variables in the algal/periphyton, invertebrate, and fish communities.

Please refer to the statistical analysis section of the project proposal for more details.

ISRP: *The ISRP suggested that the YN attempt to use published information to estimate the magnitude of the response they might expect and use the data collected to date to run some rough power analyses to determine if they have the power to discern a response of this size.*

Project sponsor response: Pertinent issues of sample size analysis and their use to improve the project sensitivity are addressed in the previous comment. These are elaborated below from

Statistical Consulting Services, (Clarkston WA), a subcontractor to this project, followed by a summary of information indicating types and magnitudes of treatment effects in all trophic levels.

Sample size analysis - Three pieces of information are required for sample size determination. The first is an estimate of the population variance. This can be estimated from sample statistics such as the data at hand or prior knowledge. The second piece needed is the desired precision (bound on the error of estimation) for future estimates of the population mean. This should be determined by the researcher(s) and may be specified as either a relative value, such as a percentage of the mean, or an absolute value measured in the units of the specified variables. The final piece of information necessary is the level of confidence (confidence coefficient) desired in the final estimate. Typically, this value is set to a number between 0.9 to 0.99 (e.g. 90 to 99% confidence coefficient). As might be expected, higher levels of confidence will result in larger estimated sample sizes.

The formulation for calculating sample size can be derived from a confidence interval constructed for the population mean and is given by (Cochran, 1977):

$n = (z*s/d)2$ (1)

where n is the estimated sample size, s is the sample standard deviation, d is the desired precision, and z is a tabulated critical value related to the level of confidence and is specified as a quantile of the standard Normal distribution. Given the nature of this value, it should be noted that the above procedure assumes normally distributed data. Failure to meet this assumption will result in inflated (biased) variance estimates, which will in turn produce sample size estimates that may be inaccurate. To meet the assumption of Normality, a transformation of the data may be required prior to sample size estimation.

It should be noted that for all sample size calculations, the resulting sample size values are preliminary, as the calculations are based on available data. Furthermore, the actual precision of mean estimates may vary by the timing or location of sampling. Therefore, sample size estimates should be used cautiously for setting policy regarding future sampling protocols.

Power Analysis – Statistical power refers to the probability of not making an error in a statistical analysis. There are, however, two types of error that can be made during hypotheses testing. The first, or Type I Error occurs if a null hypothesis is rejected when it is actually true. The probability of this type of error, denoted by α is controlled by setting the confidence level of the test to $1 - \alpha$. The second type of error, Type II Error, occurs when the null hypothesis is not rejected when it is actually false. The probability of this error is related to the statistical power of the test through the relationship: power = 1 – ß (Hocking, 1985). The goal of experimental and sampling designs should be to minimize both types of error, thereby maximizing the statistical power, 1 – ß. Computations for the power of a given test are carried out by examining the significance of the statistical test while varying the assumed sample size and simultaneously holding the variability and confidence level constant. Following these computations, plots of the computed power versus sample size, i.e. power curves, can be made to investigate the effect of sample size on the power of a given hypothesis.

Site-specific sample size determination results will be provided by SCS for the trophic level responses at a given confidence coefficient (95%) and relative precision level (10% of the specified response mean). Response variables included are the TN/TP ratio for water quality, Total Chlorophyll Accrual Rate (mg/m^2/30d) for algae, abundance (#/m^2) for macroinvertebrates and catch per effort (#/hr) for fish, respectively. Macroinvertebrate analyses may concentrate on the aggregated total abundances over all taxa groups, while the fish analyses may need to utilize data specific to mountain whitefish (*Prosopium williamsoni*) if permitting if sampling adequate numbers of listed juvenile salmonids is not possible. These same response variables are used in presenting the power analyses results, pertinent to a specific pre-determined contrast, namely control vs. treatment (nutrient addition).

Summary of empirical biologically and statistically significant treatment responses across trophic levels

An exhaustive review of biological responses by trophic levels would confirm the ability of well designed studies to detect and quantify the magnitude of biological responses to nutrient addition. However, such a task is well beyond the scope of this response to the ISPR and the time resources of project proponents. Suffice it to say that a large number of published studies have report detectable and statistically testable biological responses to nutrient. Quantified responses have been reported for periphyton (Perrin et al. 1987; Ambrose et al. 2004; Cak 2005), benthic communities (Biggs et al. 2000; Charloner et al. 2000), salmonids (Hyatt and Stockner 1985; Stockner and MacIssac 1996; Bilby et al. 1998; Hudson et al. 2000; Heintz et al. 2004), riparian vegetation (Reimchen et al 2002; Bilby et al 2003; Edmonds et al. 2006), and ecological responses among trophic levels (Cederholm et al 1999; Gende et al. 2002; Naimam et al. 2002; Stockner 2003 and references therein; Ward and Slaney 2003; Pearsons et al. 2007; Kohler et al. 2008, and others). A partial list of publications addressing biological responses is provided as Appendix 1 of this response and will continued to be used to further develop and refine this project.

In addition, because the ongoing Kootenai River nutrient addition project is simultaneously quantifying biological responses to fertilization within and across all trophic levels, results from that study (BPA Project 199404900) by trophic level are highlighted below to support similar design elements of the YN project questioned by the ISRP.

Water quality response to nutrient addition - Large increases in biologically available forms of nutrients following nutrient addition are typically not observed due to the large biological demand in ultraoligotrophic systems. Furthermore, repressed water column nutrient concentration values downstream from nutrient addition compared to upstream controls are often observed in these studies, resulting from stimulating biological uptake that exceeds the amount of added nutrients. Nitrogen stripping could also occur in following phosphorus additions in rivers, resulting from phosphorus-stimulated biological uptake that also consumes N downstream form nutrient addition (Figure 1) often during alter summer.

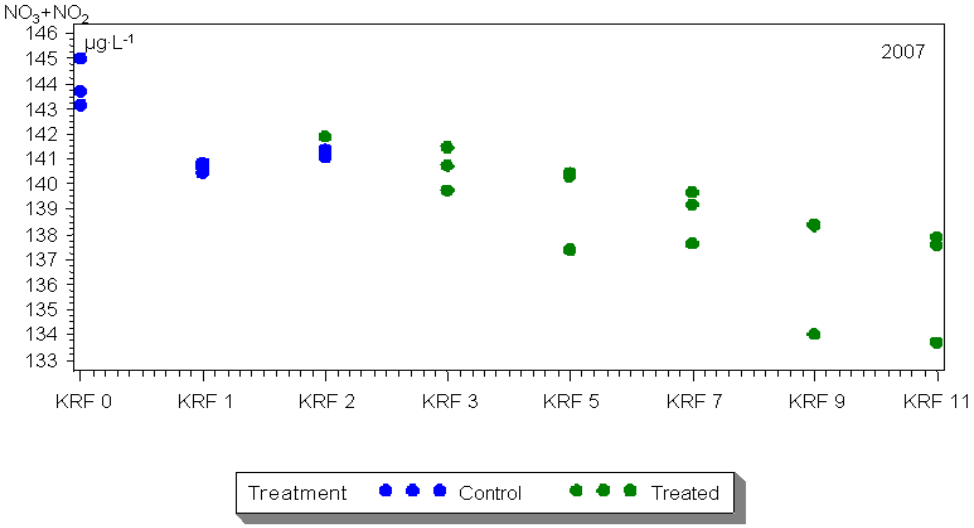


Figure 1. Mean NO_3+NO_2 concentrations (µg·L^{-1}) in the mainstem Kootenai River at the eight monitoring sites and the injection site from June through October, 2006-2008. Nutrient addition occurs at the right bank of KRF-2; All sites > KRF2 are downstream treatment sites; Figure 18 in Hoyle et al. In Prep.)

As a general rule, biological response times to nutrient addition are typically hours to days for water column responses, days to weeks for algae, weeks to months for invertebrates, and months to years for fish, depending on metrics).

Primary production - During years of nutrient addition, chlorophyll accrual rates (µg·cm^{-2}·day^{-1}) were significantly higher in the treatment sites than in the reference sites during all years (Figures 2-4).

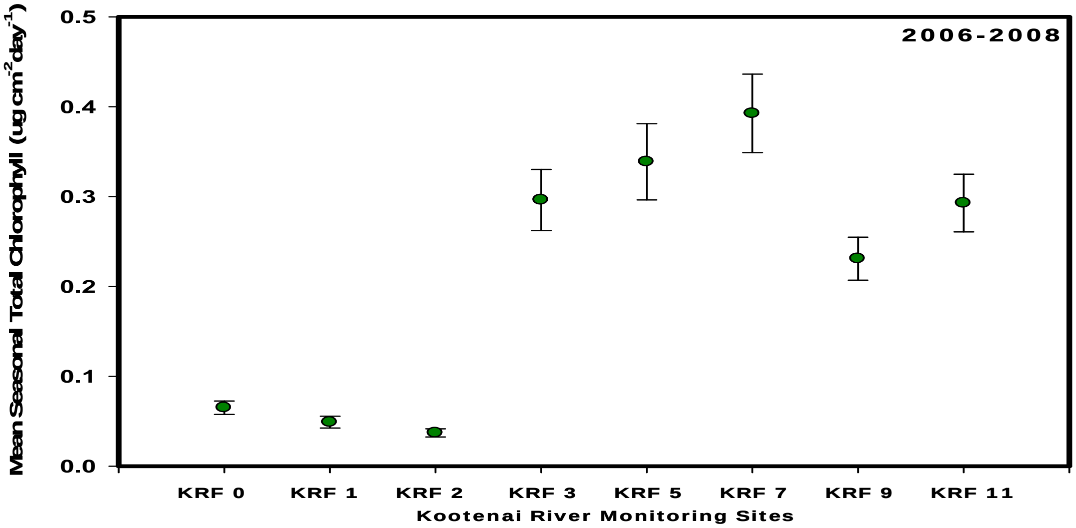


Figure 2. Mean algal biomass (µg·cm^{-2}·day^{-1}), measured as total chlorophyll, at the nutrient monitoring sites in the Kootenai River, Idaho, 2006-2008. (KRF0-KRF2 are control sites; $\geq$ KRF3 are treatment sites; Figure 38 In Hoyle et al. In prep).

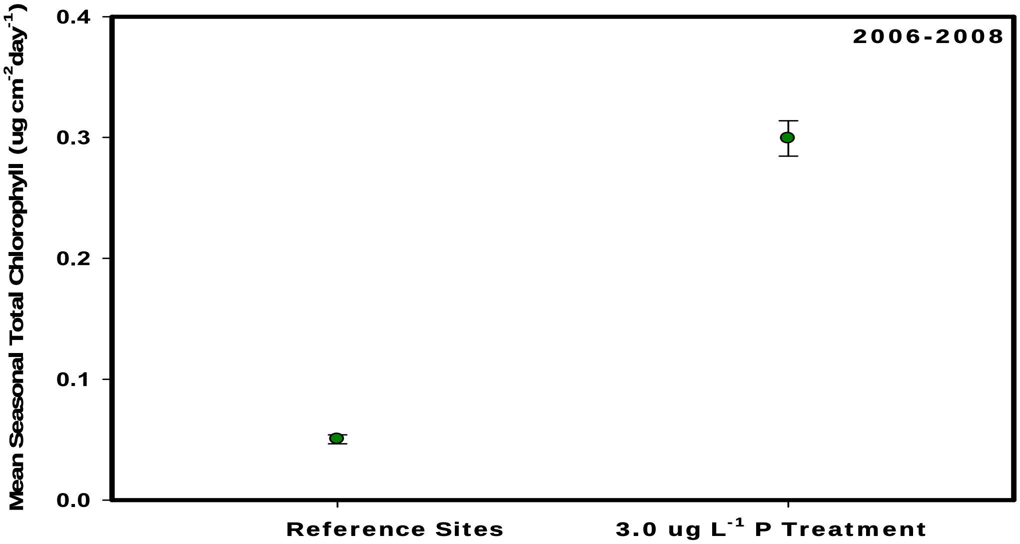


Figure 3. Mean algal biomass (µg·cm^{-2}·day^{-1}), measured as total chlorophyll, at the reference and 3.0 µg·L^{-1} P treatment sites in the Kootenai River, Idaho, 2006-2008. (Figure 39 in Hoyle et al. In prep)

Mean seasonal chlorophyll accrual rates at most sites during most treatment years were also significantly higher ($p < 0.05$) at the treatment sites than the control sites from 2006 through 2008 (Figure 4; Hoyle et al. In Prep.).

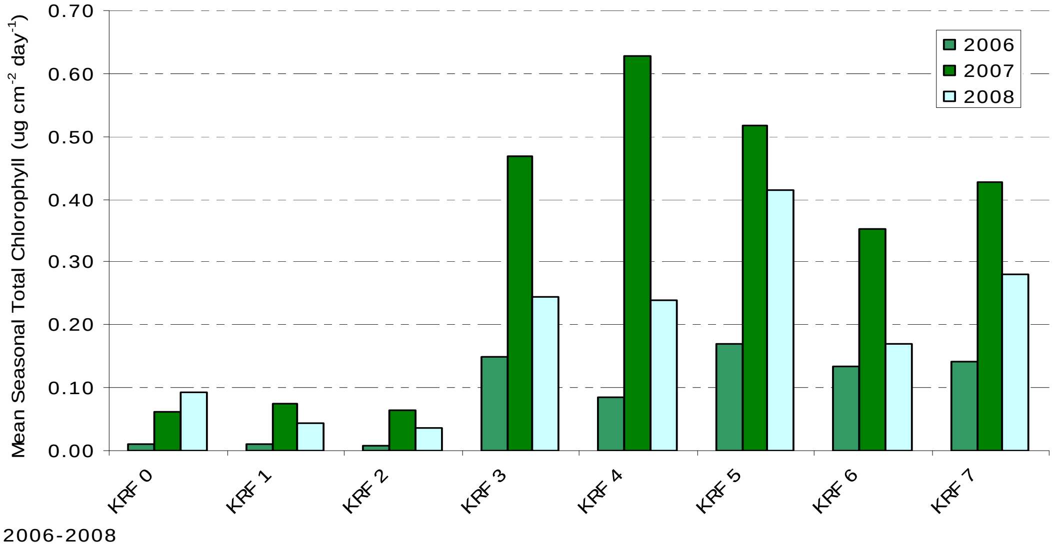


Figure 4. Mean algal biomass (µg·cm^{-2}·day^{-1}), measured as total chlorophyll, at the nutrient monitoring sites in the Kootenai River, Idaho, 2006-2008. (Figure 46 in Hoyle et al. In prep.)

Consistently, total algal accrual rates were significantly higher during post-treatment years than during pre-treatment years, often more than doubling in response to treatments (Figure 5).

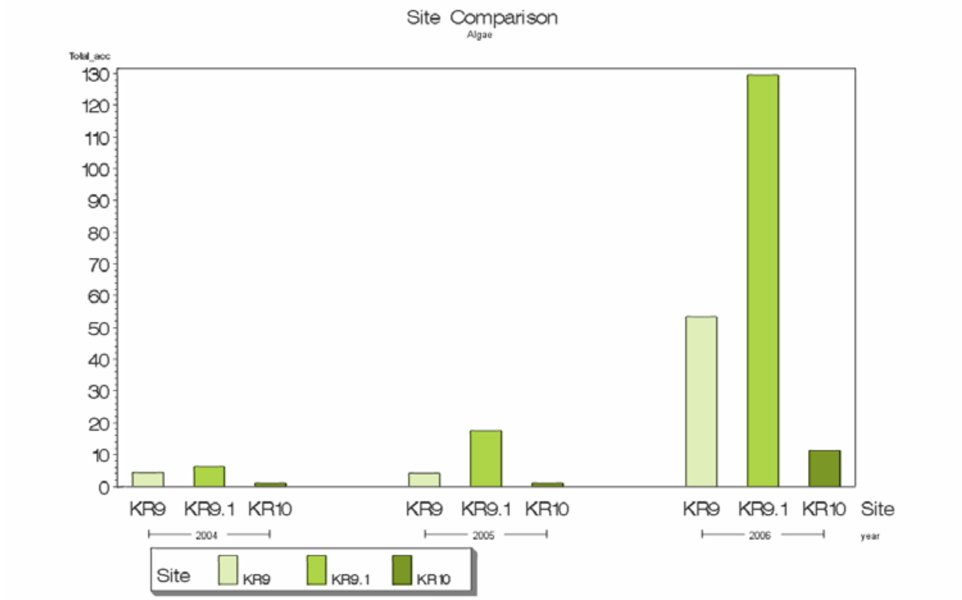


Figure 5. Total chlorophyll accrual at KR-9, KR-9.1, and KR-10 from 2004 through 2006. KR 10 is an upstream reference site, KR-9.1 is the nutrient addition site, and KR-9 is a treatment site about 5 km downstream from KR-9.1 (Holderman et al. 2009).

Algal community composition responses – A critical, desirable algal community response to nutrient addition is an increase in the edible green algae (Chlorophyta) and diatom (Bacilianophyta) communities, and a decrease in inedible bluegreens (Cyanobacteria). This is exactly the response observed following fertilization in the Kootenai River, as illustrated by 2007 response data; diatom representation increased over 25% whereas representation of bluegreens dropped by nearly 75% in when comparing treated and control sites (Figure 6).

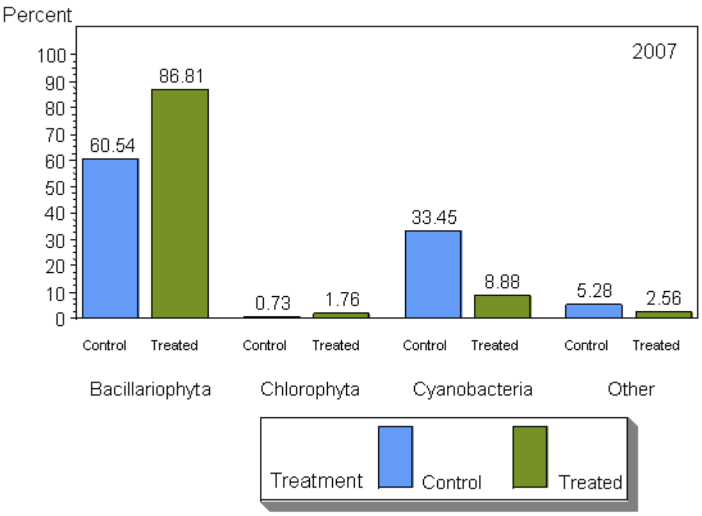


Figure 6. Percent algal cell density (cells·mm^{-2}) of the four main taxa groups in the treatments (KRF 3 through 11) and controls (KRF 0, 1, and 2) in the Kootenai River, 2007 (Figure 49 in Hoyle et al. In prep).

Macroinvertebrate response to fertilization - Nutrient addition also had a significant effect (*p<0.0001*) on invertebrate abundance, biomass, and richness at KR-9 and KR-9.1 (two treatment sites) combined (Table 1). Mean abundance and biomass both increased significantly following nutrient addition, whereas mean richness also exhibited a significant increase, nearly doubling from 20 to 35 taxa (Table 2).

Table 1. ANOVA results for pre and post-addition aggregated average abundance, biomass, and richness at sites KR-9 and KR-9.1 combined for all years sampled. (Table 1 from Holderman et al. 2009)

Response	Source	DF	Type III SS	Mean Square	F Value	Pr > F
Log(Abundance)	Nutrient Addition	1	54.69	54.69	37.30	<.0001
Log(Biomass)	Nutrient Addition	1	41.14	41.14	27.27	<.0001
Richness	Nutrient Addition	1	7794.25	7794.25	110.75	<.0001

Table 2. Least square means and standard error values for response variables (abundance, biomass and richness) in analysis of variance for pre- and post-fertilization years. (Table 2 from Holderman et al. 2009)

Response	Fert	LSMEAN	S.E.
Log(Abundance)	Pre	6.90	0.12
	Post	8.33	0.20
Log(Biomass)	Pre	-0.52	0.12
	Post	0.72	0.20
Richness	Pre	20.05	0.91
	Post	34.93	1.08

Inter-trophic comparisons – Although not statistically tested to date, comparisons of chironomid abundance and total chlorophyll (a + b) accrual rates at the fertilization site on the Kootenai River (KR-9.1) showed similar, dramatic increases from 2004 through 2006, with larger changes in both metrics between 2005 and 2006 consistent with increased inorganic fertilizer treatments (Figure 7). Frequency of chironomids in the KR-9.1 samples increased by nearly two orders of magnitude from about 50 to nearly 900 between 2004 and 2006.

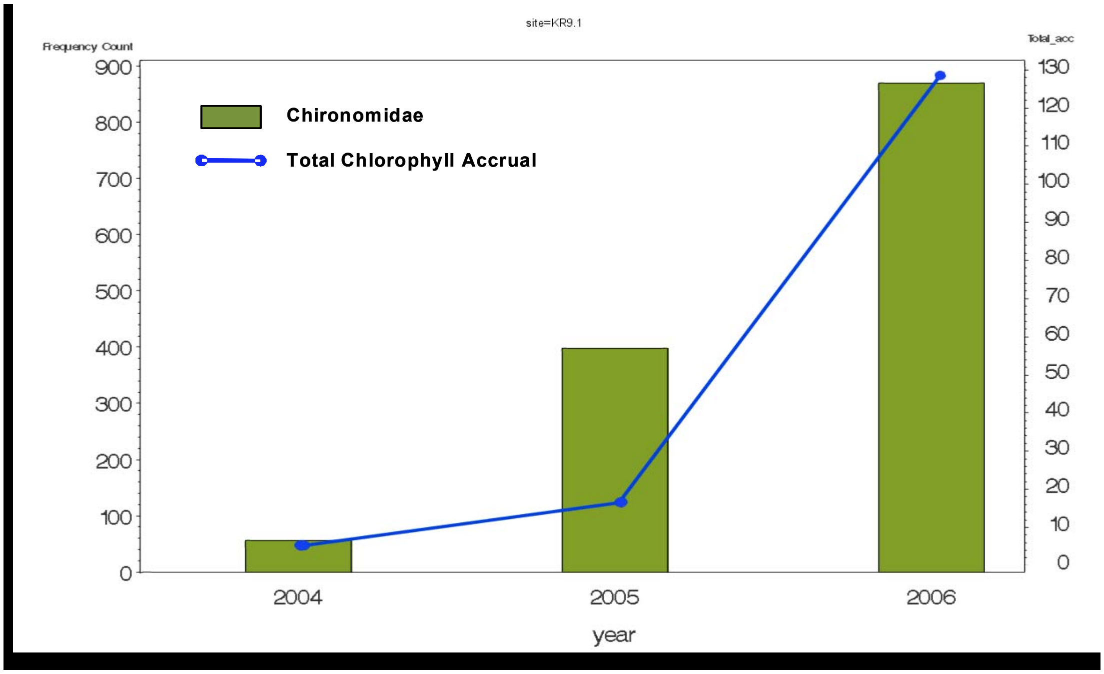


Figure 7. *Chironomidae* abundance and total chlorophyll accrual at the nutrient addition site (Figure 16 in Holderman et al. 2009).

Pre- and post-fertilization representation of various chironomids taxa in stomach samples of mountain whitefish I the Kootenai River increased and changed significantly more than tripled in the nutrient addition zone from < 300 in 2004 to > 900 in 2006 (Figure

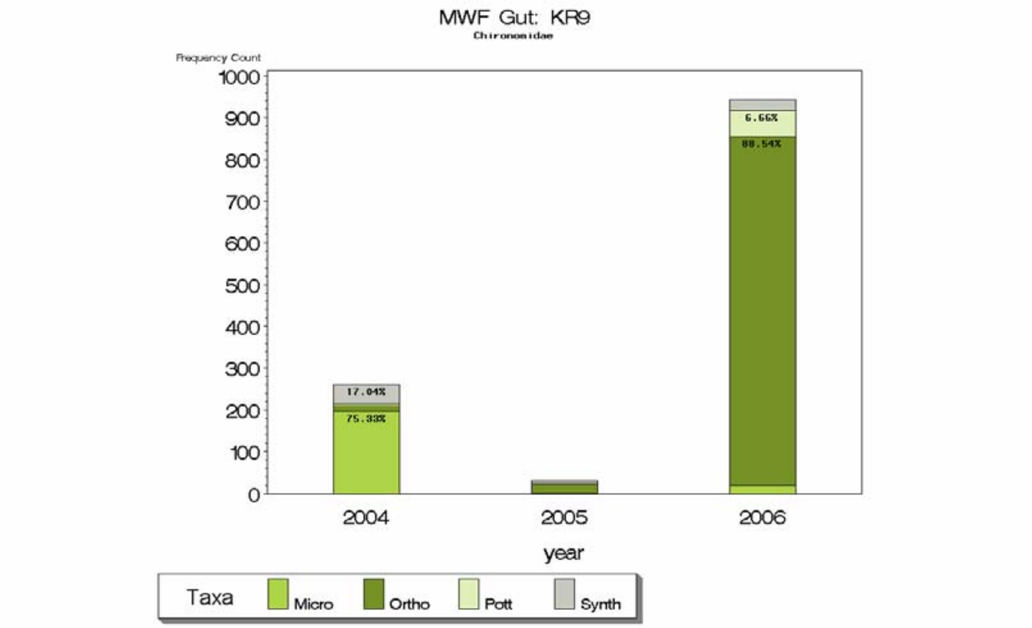


Figure 8. Composition of major chironomid taxa in mountain whitefish stomach content samples at KR-9, 2004 through 2006. (Figure 18 in Holderman et al. 2009)

Fish response to nutrient addition – Numerous studies have reported significant increases in salmonid production in nutrient-depleted lakes and streams in British Columbia, Idaho, and Alaska following nutrient additions (Stockner et al. 2000; Ashley et al. 1999; Johnston et al. 1999; Korman and Walters 1999; Budy et al. 1998; Kyle et al. 1997; Stockner and MacIssac 1996; Stockner 1987; Hyatt and Stockner 1985). Investigations into the effects of phosphorous and nitrogen additions have revealed increases in invertebrate biomass (Quamme and Slaney 2003) and periphyton accrual following nutrient addition (Perrin and Richardson 1997), which in turn support increased fish biomass in rivers (Slaney et al 2003; Deegan et al. 1997; Deegan and Peterson 1992; Warren et al. 1964) and lakes (Ashley et al 2003; Hyatt and Stockner 1985; LeBrasseur et al. 1978).

Kootenai River - Using seven years of Kootenai River whitefish length frequency data collected collaboratively by the KTOI and the IDFG, Shafii et al. (In Prep.) performed a statistical mixture model analysis indicating significant increases in length at age distribution for Age-0 and Age-1 mountain whitefish occurred following nutrient addition (Figure 9).

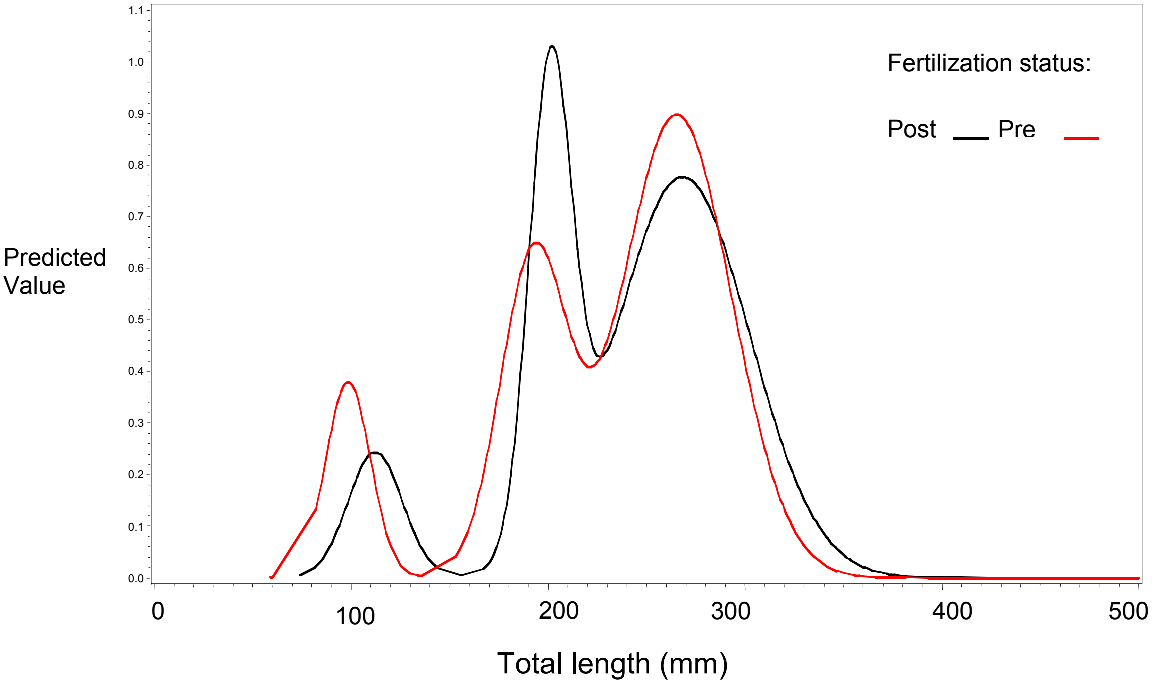


Figure 9. Significant increase in size distribution of mountain whitefish (*Propospium williamsoni*) following nutrient addition in the Kootenai River (From Shafii et al. *Accepted*).

Appendix 1: Selected references regarding biological responses to nutrient addition

Ambrose, Heather E. 2003. Periphyton response to increased light and salmon carcass enhancement in six northern California streams. MS Thesis Humboldt State University.

Ambrose, Heather E., Wilzbach, Margaret A., Cummins, Kenneth W. 2004. Periphyton response to increased light and salmon carcass introduction in northern California streams. Journal of the North American Benthological Society 23(4): 701-712.

Ashley, K.I. and P.A. Slaney. 1997. Accelerating recovery of stream, river and pond productivity by low-level nutrient replacement (Chapter 13). In: Fish Habitat Rehabilitation Procedures. P.A. Slaney and D. Zaldokas (eds.). Province of B.C., Ministry of Environment, Lands and Parks, and Ministry of Forests. Watershed Restoration Technical Circular No. 9:341 p.

Biggs, Barry J.F., Steven N. Francoeur, Alexander D. Huryn, Roger Young, Christopher J. Arbuckle, and Colin R. Townsend. 2000. Trophic cascades in streams: effects of nutrient enrichment on autotrophic and consumer benthic communities under two different fish predation regimes. Can. J. Fish. Aquat. Sci. 57:1380-1394.

Bilby R.E., Beach E.W., Fransen B.R., Walter J.K. & Bisson P.A. 2003. Transfer of nutrients from spawning salmon to riparian vegetation in western Washington. Transactions of the American Fisheries Society, 132, 733–745.

Bilby, R.E., B.R. Fransen, P.A. Bisson and J.K. Walter. 1998. Response of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*O. mykiss*) to the addition of salmon carcasses to two streams in southwestern Washington, U.S.A. Can. J. Fish. Aquat. Sci. 55:1909-1919.

Cak, Anthony D. 2005. The influence of spawning salmon on nutrient dynamics and epilithon growth in southeastern Alaska streams and estuaries. Thesis Notre Dame, Indiana.

Cederholm C.J., Kunze M.D., Murota T. & Sibatani A. (1999) Pacific salmon carcasses: essential contributions of nutrients and energy for aquatic and terrestrial ecosystems. Fisheries, 24(10):6–15.

Chaloner, D.T., and M.S. Wipfli. 2002. Influence of decomposing Pacific salmon carcasses on macroinvertebrate growth and standing stock in southeastern Alaska streams. Journal of the North American Benthological Society 21:430-442.

Chaloner, D.T., G.A. Lamberti, A.D. Cak, J.P. Hudson, J.L. Lessard, R.W. Merritt, P.H. Ostrom, J.L. Tank, and M.S. Wipfli. 2003. Do salmon carcasses and pellets of processed salmon carcasses have the same effects on streams? NABS Bulletin 20(1): 180. (Chaloner Abstract #2)

Chaloner, D.T., G.A. Lamberti, M.S. Wipfli, R.W. Merritt, P.H. Ostrom, N.L. Mitchell, J.L. Lessard, B.S. Graham, B.E. Wright, and J.P. Hudson. 2001. Ecological consequences of salmon enrichment for streams: where do marine-derived nutrients go? NABS Bulletin 18(1):184.

Chaloner, D.T., M.S. Wipfli, G.A. Lamberti, R.W. Merritt. 2001. Influence of enrichment by Pacific salmon on macroinvertebrate growth and standing stock in southeast Alaska streams. International Conference: Restoring Nutrients to Salmonid Ecosystems, American Fisheries Society North Pacific International Chapter, Eugene, OR, April. (AFS abstract)

Chaloner, D.T., M.S. Wipfli, J.P. Hudson, J.P. Caouette, and K.M. Martin. 2000. Carcass-invertebrate interactions: implications for the influence of Pacific salmon on ecosystem productivity. Special Session: Wanted dead or alive: role of fish in benthic food webs.

North American Benthological Society annual conference. Keystone, CO, May. (NABS abstract) (Chaloner Abstract #1)

Chaloner, Dominic T., Kristine M. Martin, Mark S. Wipfli, Peggy H. Ostrom, and Gary A. Lamberti. 2003. Marine carbon and nitrogen in southeastern Alaska stream food webs: evidence from artificial and natural streams. CJFAS 59:1257-65.

Claeson, S.M., J.L. Li, J.E. Compton, and P.A. Bisson. 2006. Response of nutrients, biofilm, and benthic insects to salmon carcass addition. Canadian Journal of Fisheries and Aquatic Sciences 63:1230-1241.

Compton, Jana E., Christian P. Andersen, Donald L. Phillips, J Renée Brooks, Mark G. Johnson, M Robbins Church, William E. Hogsett, Michael A. Cairns, Paul T. Rygiewicz, Brenda C. McComb, Courtney D. Shaff. Ecological and water quality consequences of nutrient addition for salmon restoration in the Pacific Northwest. Frontiers in Ecology and the Environment, February, 4(1):18-26.

Craig, J.D.C. and H. Wright. 2003. East cost Vancouver Island salmon carcass retention/distribution program – seed project Summary Report. For Ministry of water, Land and Air Protection Nanaimo, B.C. and Habitat Conservation Trust Fund, Victoria, B.C. (project #2978125).

Dept of Fisheries & Oceans. Guidelines for in-stream placement of hatchery salmon carcasses for nutrient enrichment.

E. L. Greda, M. D. Kunze, B. G. Marcot, J. F. Palmisano, R. W. Plotnikoff, W. G. Pearcy, C. A. Simenstad, and P. C. Trotter. 2001. Pacific salmon and wildlife – ecological contexts, relationships, and implications for management. Pages 628-684 in D. H. Johnson and T. A. O'Neil, editors. Wildlife-habitat relationships in Oregon and Washington. Oregon State University Press, Corvallis, Oregon.

Edmonds, Robert L. and Kerri Mikkelsen. 2006. Influence of salmon carcass placement in red alder riparian areas on stream chemistry in lowland western Washington. NAJFM 26:551-558.

Cederholm, C. J., D. H. Johnson, R. E. Bilby, L. G. Dominguez, A. M. Garrett, W. H. Graeber,

Fish First. Nutrient enhancement, salmonid habitat restoration. How-to-guide for Washington State. Woodland, WA 98674.

Gende, Scott M., Richard T. Edwards, Mary F. Willson, and Mark S. Wipfli. 2002. Pacific salmon in aquatic and terrestrial ecosystems. BioScience 52(10:917-928.

Gresh, T., J. Lichatowich and P. Schoonmaker. 2000. An estimation of historic and currrent levels of salmon production in the northeast Pacific ecosystem: Evidence of a nutrient deficit in the freshwater systems of the Pacific Northwest. Fisheries 25(1):15-21.

Groot and Margolis.(eds),1991. Pacific Salmon Life Histories. UBC Press, 564 p.

Hansen, Loreta. 2003. Ash River instream fertilization 2001. B.C. Ministry of Water, Land and Air Protection, Fisheries Section, Nanaimo.

Hansen, Loreta. 2003. Stream enrichment of Vancouver Island's upper Salmon River including Grilse Creek, Memekay River, and Paterson Creek, 2003.

Heintz, Ron A., Bonita D. Nelson, John Hudson, Marie Larsen, and Larry Holland. 2004. Marine subsidies in freshwater: effects of salmon carcasses on lipid class and fatty acid composition of juvenile coho salmon. TAFS 133:559-567.

Hicks, Brendan, Mark S. Wipfli, Dirk W. Lang, and Maria E. Lang. 2005. Marine-derived nitrogen and carbon in freshwater-riparian food webs of the Copper River Delta, southcentral Alaska. Oecologia 144:558-569.

Hogbom, Lars, Hans-Orjan Nohrstedt, and Sten Nordlund. 2001. Nitrogen fertilization effects on stream water cadmium concentration. J. Environ. Qual. 30:189-193.

Hudson, J.P., Wipfli, M.S., J.P. Caouette, and R.A. Heintz. 2000/1999. Salmon carcasses increase growth and lipid content of stream-dwelling salmonids in southeast Alaska. American Fisheries Society Alaska Chapter meeting, Fairbanks, AK, November, 2000, and American Fisheries Society Alaska.

Hudson, M.S. Wipfli, N.L. Mitchell, J.P. Caouette, R.A. Heintz, D.T. Chaloner, B.S. Graham, C.L. Crenshaw, J.L. Lessard, G.A. Lamberti, R.W. Merritt, P.O. Ostrom, and J.L. Tank. 2002. Salmon carcasses vs. fertilizer: stream food web responses to organic and inorganic nutrient enrichment in Alaska.

Hyatt, K. D., and J. G. Stockner. 1985. Responses of sockeye salmon (Oncorhynchus nerka) to

Kohler Andre E., Rugenski, Amanda, Taki, Doug. 2008. Stream food web response to a salmon carcass analogue addition in two central Idaho, U.S.A. streams Freshwater Biology 53:446-460.

Martin, A.E., M.S. Wipfli, R. Spangler. 2006. Freshwater community development in response to salmon carcass additions to newly created fish habitats in Alaska. North American Benthological Society annual meeting. Anchorage, AK, 4-8 June. (NABS abstract)

Martin, A.M., M.S. Wipfli, and R. Spangler. 2006. Aquatic community development in response to salmon carcass and salmon analog enrichment in newly-restored fish habitat, Resurrection Creek, Alaska. American Fisheries Society Alaska Chapter annual meeting, Fairbanks, AK, 14-16 November. (AFS abstract)

Merz, Joseph E. and Peter B. Moyle. 2006. Salmon, wildlife and wine: marine-derived nutrients in human-dominated ecosystems of central California. Ecological Applications 16(3):999-1009.

Naiman, R. J., R. E. Bilby, D. E. Schindler, and J. M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems (2002)5:399-417.

Nelson, B.D., R.A. Heintz, M.S. Wipfli, and J.P. Hudson. 2001. Effect of supplementation with marine-derived nutrients on the lipid class and fatty acid composition of juvenile coho salmon. American Fisheries Society Alaska Chapter meeting, Sitka, AK, November. (AFS abstract)

Oregon Department of Fish and Wildlife. Nov 2000. ODFW fish health guidelines for use of salmon and steelhead carcasses for nutrient enrichment. 2 p.

Ostrom, P.H., M.S. Wipfli, R.A. Heintz, J.P. Hudson, M.L. Larsen, D.T. Chaloner, and R.W. Merritt. 2001. Interpreting stream ecosystem responses to marine-derived nutrients using stable isotope and fatty acid analyses. North American Benthological Society annual meeting, LaCrosse, WI, May. (NABS abstract)

Pearsons T.N., Johnson C.L., Schmuck M.R., Webster, D.D. & Bilby R.E. (2007a). Do salmon carcass analogues reproduce food pathways provided by salmon carcasses and impact the growth and abundance of salmonids? North American Journal of Fisheries Management, in press.

Pearsons, T. R., D. D. Roley, and C. L. Johnson. 2007. Development of a carcass analog for nutrient restoration in streams. Fisheries 32(3):114-124.

Perrin, Chris J., Max L. Bothwell and Pat A. Slaney. 1987. Experimental enrichment of a coastal stream in British Columbia: Effects of organic and inorganic additions on autotrophic periphyton production. Fish. Aquat. Sci. 96, 486 p.

Reimchen, T.E., D. Mathewson, M.D. Hocking, J. Moran, and D. Harris. 2002. Isotopic evidence for enrichment of salmon-derived nutrients in vegetation, soil, and insects in riparian zones in coastal British Columbia. Transactions of the American Fisheries Society XX:000-000.

Roni, Phil, Karrie Hanson, Tim Beechie, George Pess, Michael Pollock, and Devin M. Bartley. 2005. Habitat rehabilitation for inland fisheries. FAO Fisheries Technical Paper 484.

Roni, Philip, Timothy J. Beechie, Robert E. Bilby, Frank E. Leonetti, Michael M. Pollock, and George R. Pess. 2002. A review of stream restoration techniques and a hierarchical strategy for prioritizing restoration in Pacific Northwest watersheds. N.A.J.F.M. 22:1-20.

Sanderson, B., and P. Kiffney. 2003. Assessment of three alternative methods of nutrient enhancement (salmon carcass analogs, nutrient pellets, and carcasses) on biological communities in Columbia River tributaries. 2001-2003 Technical Report, Project No. 200105500, BPA Report DOE/BP-00007621-2. 20p.

Shively, D. 2001. The role and benefits of salmon carcass supplementation – selected research findings and quotes. Nov. 2001. 6 p.

Slaney, P.A. and K.I. Ashley. Case studies of whole-stream fertilization in British Columbia. B.C.'s Watershed Restoration Technical Bulletin.

Slavik, K., B.J. Peterson, L.A. Deegan, W.B. Bowden, A.E. Hershey, and J. E. Hobbie. 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. Ecology 85(4):939-954.

Stockner, J. 2003 (ed.) Nutrients in salmonid ecosystems: sustaining production and biodiversity. AFS symposium series No. 34, Bethesda, Maryland, 286p.

Stockner, J.G. 1987. Lake fertilization: The enrichment cycle and lake sockeye salmon (Oncorhynchus nerka) production, p. 198-215. In: H.D. Smith, L. Margolis and C.C. Wood (eds.). Sockeye salmon (Oncorhynchus nerka) population biology and future management. Can. Spec. Publ.

Stockner, J.G. and K. I. Ashley. 2001. Salmon Nutrients: closing the circle. P. 1-17. In: J. G. Stockner,

Stockner , J. 2003 (ed). Nutrients in Salmonid Ecosystems: Sustaining Production and Biodiversity, Am. Fish. Soc. Symposium 34, Bethesda. Pp. 59-69.

Stockner, J.G., and MacIsaac, E.A. 1996. British Columbia lake enrichment programme: two decades of habitat enhancement for sockeye salmon. Reg. Rivers: Res. and Management. 12: 547-561.

Stockner, John PhD, Ken Ashley PhD. The role of nutrients in rebuilding lake and river salmonid populations. UBC Fisheries Centre & Eco-Logic Ltd.

Thomas, S. A., T. V. Royer, G. W. Minshall, and E. Snyder. 2003. Assessing the historic contribution of marine derived nutrients to Idaho streams. Pages 41-55 in J. G. Stockner, editor. Nutrients to salmonid ecosystems: sustaining production and biodiversity. American Fisheries Society, Symposium 34, Bethesda, Maryland.

U.S. Forest Service (a). East Fork Lewis River nutrient enhancement project. Mount St. Helens National Volcanic Monument Gifford Pinchot National Forest, Skamania County, Washington.

U.S. Forest Service (b). USGS Watershed nutrient enhancement research project. Mr. Adams Ranger District Gifford Pinchot National Forest, Skamania County, Washington.

Ward, B.R., D.J.F. McCubbing, P.A. Slaney. 2003. Stream restoration for anadromous salmonids by the addition of habitat and nutrients.

Washington Department of Fish and Wildlife. Protocols and guidelines for distributing salmonid carcasses, salmon carcass analogs, and delayed release fertilizers to enhance stream productivity in Washington State.

Watkinson, Stephen. 2000. Life After Death: The Importance of Salmon Carcasses to British Columbia's Watersheds. Arctic 53(1):92-99.

Wilzbach, M. A., B. C. Harvey, J. L. White, and R. J. Nakamoto. 2005. Effects of riparian canopy opening and salmon carcass addition on the abundance and growth of resident salmonids. Canadian Journal of Fisheries and Aquatic Sciences 62:58-67.

Wipfli, M.S., Hudson, J.P. & Caouette, J.P. (2004) Restoring productivity of salmon-based food webs: contrasting effects of salmon carcass and salmon carcass analogue additions on stream-resident salmonids. Transactions of the American Fisheries Society 133:1440–1454.

Wipfli, M.S., J. Hudson, & J. Caouette. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. Can. J. Fish. Aquat. Sci. 55: 1503-1511.

Wipfli, M.S., J.P. Hudson, D.T. Chaloner and J.P. Caouette. 1999. Influence of salmon spawner densities on stream productivity in Southeast Alaska. Can. J. Aquat. Sci. 56:1600-1611.

Wipfli, M.S., J.P. Hudson, J.P. Caouette, and D.T. Chaloner. 2003. Marine subsidies in freshwater ecosystems: salmon carcasses increase growth rates of stream-resident salmonids. Trans. Am. Fish. Soc. 132:371-381.

Wright, H. 2004. East Coast Vancouver Island salmon carcass program implementation.

Wright, Harlan. 2006. Nutrient enrichment of Vancouver Island's Cluxewe River, 2006. B.C. Conservation Foundation Nanaimo, B.C.

Yanai, Seigi, Kaori Kochi. 2005. Effects of salmon carcasses on experimental stream ecosystems in Hokkaido, Japan. Ecological Research 20(4):471-480.

Appendix 2: Influence of inorganic nutrient additions on periphyton and benthic macroinvertebrates

Mesocosm experiments - Implementation of nutrient enrichment programs requires understanding of local limnological conditions and how N:P ratios and nutrient concentrations influence the composition and productivity of algal, invertebrate, and fish species. To assess the potential response of the Kootenai River to nutrient additions, a mesocosm study was carried out to analyze the effects of nitrogen and phosphorous addition on primary and secondary production, and on juvenile Kootenai River white sturgeon in controlled, replicated experiments (Hoyle 2003; Quamme and Slaney 2003; Oliver 1998).

Numerous nutrient addition experiments utilizing various kinds of mesocosms show that P or N have an enriching effect on epiphytic communities and benthic macroinvertebrate assemblages (see Johnson et al. 1990 for references). As an example, Quamme (2003) found that P addition in an experimental mesocosm fed by a third-order coastal stream on Vancouver Island stimulated the aquatic food web by increasing the periphytic community (chlorophyll a and peak algal biomass), which in turn increased populations of some benthic invertebrate taxa. Invertebrate taxa shown to increase by Quamme (2003) included the baetid (Ephemeroptera: Baetidae) mayflies (adult and nymphs), nemourid & perlodid (Plecoptera: Nemouridae and Perlodidae) stoneflies, and hydroptilid & polycentripodid (Tricoptera: Hydroptilidae and Polycentipodidae) caddisflies. Moreover, both nymphal and adult populations of these families (except for the polyentripodids) increased in response to P addition. Responses to nutrient addition were nonlinear as most of these families reached an asymptote at 2.5-10 ug P/L. All these families but especially the baetid mayflies have been shown to be important food items for juvenile salmonids (Johnson et al. 1990).

Free-flowing streams and rivers - In a tundra stream Peterson et al. (1985) found the inorganic P additions transformed in-stream conditions from heterotrophic to autotrophic conditions, which in turn increased the size of blackfly (Diptera: Simuliidae) and *Orthocladius* (Diptera: Chironomidae) larvae. Johnson et al (1990) found that nutrient addition greatly increased the standing crop of periphyton and benthic invertebrates downstream from nutrient addition sites relative to control sites in a free flowing lower order mountain stream in British Columbia. In this study, additions of organic nutrients increased Simuliid (Diptera: Simuliidae) and chironomid (Diptera: Chironomidae) larvae while additions of inorganic nutrients increased chironomid larvae and hydropsychid caddisfly nymphs. Moreover, size of salmonid fry increased during fertilization, suggesting that fry were food limited prior to fertilization and benefited from nutrient additions (Johnson et al 1990). Bourassa and Canttaneo (1998) found that in mountain rivers of Quebec, algae and scrapers were positively correlated with river P concentrations.

In some rivers affects of nutrient addition on macroinvertebrates may be mediated by physical factors. For example, Bowman et al. (2007) found that scrapers (e.g. snails and mayfly fauna) were positively correlated with algal biomass in oligotrophic free flowing rivers of Alberta, Canada, but that algae was only weakly correlated with anthropogenic sources of P and only when light were optimal (i.e. not limited). These authors concluded that correlations of P and algal biomass were weak because P concentrations are a poor indicator of P availability. They also found that physical factors (discharge, turbidity, temperature, light, and accumulation period) more strongly influenced primary producers (periphyton) than chemical (nutrients) factors.

References

Bowman, M.F., Chambers, P.A., and Schindler, D.W. 2007. Constraints on benthic algal response to nutrient addition in oligotrophic mountain rivers. River Research and Applications 23: 858-876.

Bourassa, N. and Canttaneo, A. 1998. Control of periphyton biomass in Laurentian streams (Quebec). Journal of the North American Benthological Society 17: 420-429.

Merrit, R.W. and Cummins, K.W. (1996). Aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Quamme, D.L. 2003. The relationship between nutrient concentration and stream insect abundance. In (Ed. J. Stockner) Nutrients in Salmonid ecosystems: sustaining production and biodiversity. American Fisheries Society, Bethesda, Maryland.

Johnson, N.T., Perrin, C.J., Slaney, P.R., and Ward, B.R. 1990. Increased juvenile salmonid growth by whole-river fertilization. Canadian Journal of Fisheries and Aquatic Sciences 47: 862-872.

Section II. Responses to ISRP's nine review issues identified and developed in their two reviews.

NOTE: The following text was prepared during fall of 2009 in response to both ISRP reviews, and was updated following the February 22, 2010 conference call with ISRP members, Council staff, and YN project personnel and collaborators. Closure or agreement with the ISRP on project review issues is indicated where appropriate at the beginning of each project sponsor response following ISRP comments. A combination of updated and previously drafted responses address the ISRP questions listed below.

In general, project proponents addressed the ISRP review comments in one of three ways- Project proponents either: 1) agreed; 2) disagreed, or 3) identified and clarified a misunderstanding.

1) *Agreement:* Each ISRP issue of agreement was identified, and responses, where needed, are provided in this Response Report and were incorporated into the appropriate sections(s) of the project proposal narrative.
2) *Disagreement:* Each ISRP issue of disagreement was also identified, and responses, where needed, are provided in this Response Report and were incorporated into the appropriate section(s) of the project proposal narrative.
3) *Misunderstanding:* Issues of misunderstanding between the ISRP and project proponents were a bit more complicated than the review items assigned to the above two categories.
    a. A series of misunderstandings appeared to have resulted from the reviewers erroneously applying comments from the Sho-Ban's Salmon River Nutrient Enhancement Project (2008-904-00) to this Upper Columbia Nutrient Supplementation project (2008-471-00) submitted by the Yakama Nation. These items were individually identified as inappropriate for the Yakama Nation project and appropriate for the Sho-Ban's proposal. In such instances, further responses were not always needed. However, due to the similarity of issues and activities between the two nutrient enhancement proposals, some of the ISRP review items, while identified by review of the Sho-Ban's nutrient proposal may have been appropriate for the Upper Columbia proposal. In these cases, responses are provided in this Response Report and were incorporated into the appropriate section(s) of the project proposal narrative as needed.
    b. A second type of misunderstanding appeared to result from misinterpretation by the reviewers, ambiguity in the proposal text, or from some combination of both. These misunderstandings are also identified and clarified in this Response Report, and where needed, responses were incorporated into the appropriate section(s) of the project proposal narrative.

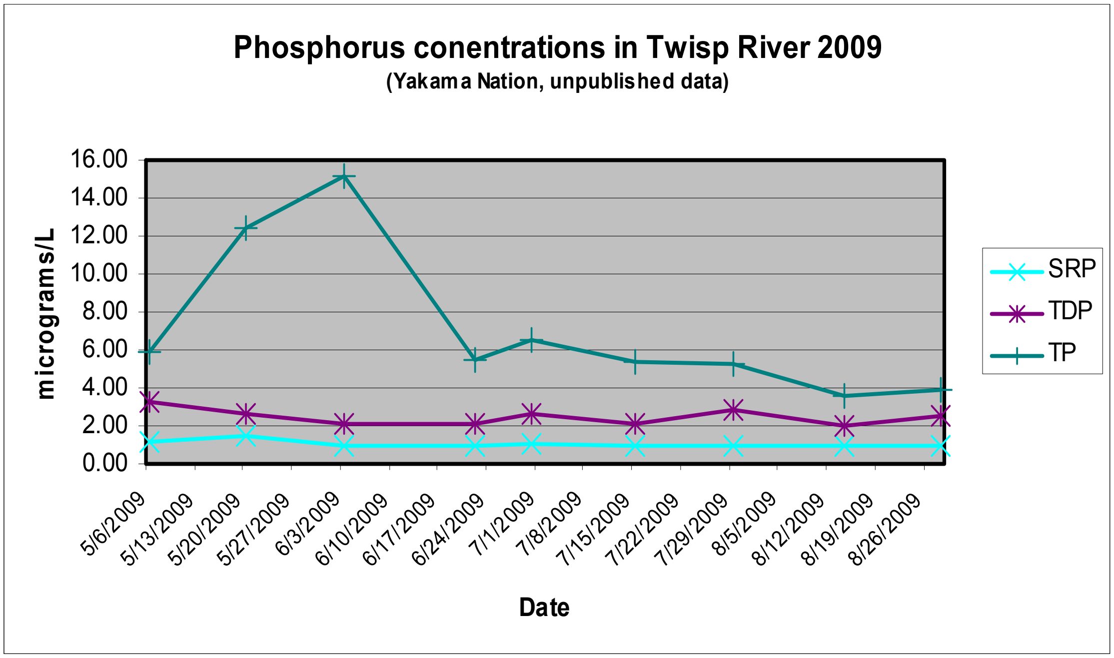
ISRP Specific Comments on Nine Items Raised in Preliminary Review

ISRP Preliminary Comment 1: *Provide more detail on the process that will be used to determine nutrient limitation. How will the information on nutrient concentration, trophic processes, etc. be used to determine whether there is a nutrient deficiency and, if so, what element is constraining production? Consider the use of nutrient diffusing substrates to augment this portion of the study. Additional background information on current carcass abundance in the system also would be useful.*

ISRP Final Comments: *The discussion on this issue was considerably expanded in the revised proposal and some of the ISRP concerns were addressed. The addition of the nutrient diffusing substrate (NDS) experiments will provide a more definitive indication of the nutrients limiting primary production at the study reaches. We were puzzled that existing water quality data from the Methow watershed was not included in the proposal. Information of this type is likely available and would provide some indication of the current nutrient status of the study sites.*

Updated Project sponsor response: This issue was partially resolved during the 2/22/10 conference call when: “The ISRP concluded during the 2/2/10 conference call that: The outcome of the NDS experiments will provide the information required to make an informed decision about the most appropriate method”.

Previous Project sponsor response: Project sponsors were surprised that the ISRP reviewers identified this request in their second rather than first review due to its broad or general nature. Nonetheless, preliminary data collections from a Yakama Tribal pilot project on the Twisp River that measured water quality parameters during the past two years were summarized, and a subset of these data (soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP) and total phosphorus (TP) from 2009) are provided below, as requested, initially suggesting possible nutrient (P) limitation.



Preliminary phosphorus concentration data in the Twisp River during 2008 and 2009 consistently indicated an oligotrophic to ultraoligotrophic condition (< 12 ug/L; Carlson 1977) , with SRP and SRP and TDP often existing at or below detection levels (< 1-2 micrograms/L) and TP existing in the oligotrophic range (< 10 micrograms/L), with occasional freshet pulses into the lower Mesotrophic range (10-20 micrograms/L: Carlson 1977; http://dipin.kent.edu/tsi.htm).

Carlson, R.E. 1977. A trophic state index for lakes. Limnology and Oceanography. 22:361-369.

Water quality data from the Twisp River collected during 2008 and 2009, included: site, date, river channel, discharge, temperature, and the water quality responses: TOC (Total Organic Carbon), NH_4, $NO_2 + NO_3$, SRP (Soluble Reactive Phosphorous), TDP (Total Dissolved Phosphorous), TN (Total Nitrogen), and TP (Total Phosphorus). In addition SCS computed two relevant ratios: TN/TP and DIN/TDP = $(NO_2+NO_3 + NH_4)/TDP$. A total of 438 observations were recorded for 3 replications in each of 6 sites (TR1 through TR6). Sample timings were approximately biweekly from early May to late October in both years.

Sample size analysis were also performed on these data by a private consulting firm (Statistical Consulting Service, Clarkston, WA.) as a subcontract to the pilot project, The sample size report is appended to this response report, providing additional quantitative, statistical rigor to project design described in the Response report.

ISRP: *Comparison of current nutrient concentrations with the Redfield ratio could provide a preliminary indication of what nutrients are likely to be limiting and might indicate the extent to which nutrient limitation varies spatially within the study watersheds. These data should prove useful in designing the NDS experiments.*

Updated Project sponsor response: This issue was resolved during the 2/22/10 conference call when: “The ISRP concluded during the 2/2/10 conference call that: The outcome of the NDS experiments will provide the information required to make an informed decision about the most appropriate method”.

Previous Project sponsor response: We agree with the ISRP regarding the value and utility of these data. Not only will empirical N:P ratio data (TN:TP; DIN:TDP) generated by this project be useful for designing the NDS experiments, but the results of the NDS experiments will also provide useful empirical data to characterize nutrient availability/limitation, and whether the systems are N-, P-, or co-limited. Along with these N:P ratios we will present the magnitudes of the nutrient concentration values. The magnitude of these values are in and of themselves valuable general indicators of nutrient availability or fertility level, which is critical to evaluating nutrient dynamics, food web dynamics, and the production and avoidance of desirable and undesirable algal taxa respectively. Project proponents are aware that nutrient imbalance at any fertility level can affect algal taxa composition, and that imbalances at higher fertility levels may be more ecologically and aesthetically problematic.

ISRP: *However, a larger question remains regarding the value of identifying the nutrients limiting primary production to the overall experiment. Understanding that a specific stream* *reach may be N or P limited, or co-limited by N and P, is informative to this experiment only if the treatments to be applied will be customized for the identified nutrient deficiency.*

> **Project sponsor response:** We agree with the ISRP regarding the need to align prescriptive nutrient treatment(s) with the specific nature of nutrient limitation(s). This can be achieved in several ways, depending on the type of nutrient assessment method chosen. Applications of liquid inorganic fertilizers (N and/or P) continue to be successfully used to address this issue in nutrient addition programs in the U.S. and Canada (e.g. Arrow Lakes, Kootenay Lake B.C., Dworshak Reservoir, Kootenai River, Idaho, USA; project sponsors can provide additional details/studies if desired). Furthermore, compositional analysis of nutrients in salmon carcasses and/or SCAs has been or can be performed to address this issue, pending the nature of potential nutrient limitation in study area waters.

ISRP: *Regardless of the deficiency identified, however, it appears that the same treatment will be applied in this study; addition of salmon carcass analogs (SCA).*

> **Updated Project sponsor response:** This issue was resolved during the 2/22/10 conference and was addressed in the ISRP's February 19, 2010 memo (ISRP 2009-50 Update).
>
> **Previous project sponsor response:** Project sponsors never proposed that SCAs were going to be exclusively used regardless of the nutrient deficiency identified.

ISRP: *The real question of relevance to this study is whether or not primary production will respond to the nutrients released from the SCA. In order to relate the results of the NDS experiments to the SCA treatment, it would be very important to understand the N and P content of the SCA and the rate at which they release these nutrients. There is no mention in the proposal that this information need will be addressed.*

> **Updated Project sponsor response:** This issue was resolved during the 2/22/10 conference and was resolved in the ISRP's February 19, 2010 memo (ISRP 2009-50 Update).
>
> **Previous project sponsor response:** Project sponsors never proposed that SCAs were going to be exclusively used regardless of the nutrient deficiency identified. Again, the use of different nutrient application treatments and defining the chemical composition of each will be provide as needed, as described above.

ISRP: *Finally, the very detailed assessment of nutrient limitation associated with this study clearly implies that the project proponents believe that the primary mechanism by which the SCA will impact trophic system dynamics is by bottom-up enrichment caused by increased nutrient availability. However, as noted in the original comments from the ISRP, SCA can be incorporated into the trophic system of a river via two different pathways. Stimulation of primary production by the nutrients released from decomposing SCAs is one possible mechanism. However, existing research on SCA has clearly established that fish will directly consume this material. Therefore, the SCA have the potential to impact trophic productivity even at sites where no clear nutrient limitation to primary production can be identified. Direct consumption was not adequately addressed in this proposal.*

Updated Project sponsor response: This issue was resolved during the 2/22/10 conference and was resolved in the ISRP's February 19, 2010 memo (ISRP 2009-50 Update).

Previous project sponsor response: Project sponsors never proposed that SCAs were going to be exclusively used regardless of the nutrient deficiency identified. If liquid inorganic supplementation were to be implemented, it would distinguish whether SCA responses were governed by bottom-up or top-down responses in the food web, as nutrient additions could only affect the system through bottom-up pathways.

Additionally, if SCAs are used, direct consumption of SCA material by target fish species will be assessed through stomach content sampling and analysis, to the degree possible under ESA direct and incidental take. Such sampling difficulties are presumably appreciated by the ISRP, in terms of practical difficulties and expense associated with obtaining these data. Our narrative currently includes ways to evaluate SCA ingestion and ways to evaluate other nutrient supplementation methods.

Finally, stable isotope analyses could also be used to evaluate the relative contribution of direct uptake versus indirect utilization of marine derived nutrients, in the event that salmon carcasses or SCA are used as nutrient supplements.

ISRP Preliminary Comment 2: *Consider enhancing the methods to be used for measuring primary production. At a minimum, total periphyton biomass should be measured along with the measure of chlorophyll content. A measure of whole-system metabolism would considerably improve this aspect of the study.*

ISRP Final Comments: *Several issues related to this comment were not adequately addressed. Periphyton biomass determination was added to the study. However, sufficient detail was not provided on the methods to be used in chlorophyll, biomass, and algal assemblage determinations to enable a thorough evaluation.*

Project sponsor response: Standard methods of estimating chlorophyll biomass (mg/m^2) and accrual rate (mg/m^2/day or month) for chlorophyll a, b, and total were presented in the proposal narrative, and algal assemblage determinations will be assigned to order to identify potential taxonomic compositional responses following experimental nutrient addition treatments. (Algal taxa were grouped as Cyanophyta (blue-greens), Chlorophyta (greens), Bacillariophyta (diatoms), and others. Methods for estimating periphyton biomass were described in project sponsor responses, along with the clarification that whole system metabolism experiments were not proposed because they are beyond the scope and cost of this project.

ISRP: *The response document included mention that measures of whole-system metabolism would be included in the study. The ISRP feels that this measure would provide a valuable indication of alterations in trophic system function with the addition of SCA as it enables the determination of both primary production and community respiration. An increase in trophic productivity would be reflected in increases in either or both these system attributes. However, the methods to be used in conducting the whole-system metabolism assessment were not included* *in the revised proposal. This technique is sophisticated and requires specialized equipment, so a detailed discussion of how this aspect of the study will be accomplished should be included.*

> **Updated Project sponsor response:** This issue was resolved during the 2/22/10 conference call: "The ISRP indicated that whole-system metabolism measures provide an estimate of primary production rates, which the proposed measures of algal biomass and chlorophyll do not provide, and that some consideration might be given to less expensive methods of measuring production and respiration (e.g., propane evasion) at some point. However, the ISRP did not indicate that the omission of this technique would represent a fatal flaw in the project. Whole-system metabolism would be a nice complement to the work but not essential to their primary objectives".
>
> **Previous Project sponsor response:** Project sponsors never proposed whole system metabolism experiments because they are beyond the project scope of this project and are too costly: "Such apparatus [whole system metabolism apparatus] is beyond the scope and cost of this study, which focuses on characterizing baseline ecological conditions, assessing nutrient availability, and measuring treatment responses among trophic levels if experimental nutrient supplementation is warranted and implemented".
>
> Thus, as previously identified in our second project narrative the project will not include whole system metabolism experiments to prohibitive cost and scale.

ISRP Preliminary Comment 3: *The invertebrate sampling protocols are not fully described and in some cases appear to be inappropriate to answer the questions being asked. Indicate how the Hess samples will be processed and approximately how many samples will be taken, given the significant costs inevitably associated with sample processing. Why is there no measure of invertebrate density and biomass included? How will the information on invertebrate community composition be related to nutrient status and productivity? Fully describe how the Hess samples and kick-net samples will complement each other.*

ISRP Final Comments: *The description of the methods for invertebrates has been expanded considerably in the section entitled "Methods by work element and trophic level" and some of the clarifications requested in the original ISRP review have been addressed. This section implies that biomass, density and an estimate of invertebrate production will be incorporated into the study. However, no details on how production will be estimated are provided and the methods to be used in measuring biomass appear incomplete (for example no mention of the manner by which biomass estimates will be corrected for loss of weight caused by storage in ethanol was provided). Biomass, density and production are likely to be more closely allied with alterations in trophic productivity than invertebrate taxonomic composition, and therefore may be more useful in assessing impacts on food availability for fish. However, the section of the proposal beginning on page 28 that discusses data analysis indicates that the only invertebrate metrics that will be considered are abundance and taxonomy, conflicting with the information provided in the earlier section of the proposal. A comprehensive discussion of the methods that will be used to estimate secondary productivity and the statistical procedures that will be used to assess response of biomass and production to the addition of SCA should have been included in the revised proposal.*

Updated Project sponsor response: Biomass will be used to calculate secondary production of these macroinvertebrates. Because of the complexity of the sample data (i.e. multivoltine taxa), we propose to use a noncohort technique, the Size-Frequency Method, for estimating secondary production (see Benke and Huryn 2007). In this method, the average density N (No./m^2) and biomass of invertebrate specimens is measured for a finite number of size classes, per taxon of interest, over the sample year. Size classes will be determined per taxon in the lab and from the literature, and measurements and weights will be taken in the laboratory. Production (P) is then calculated by multiplying ΔN (i.e. changes in density between size classes) by $\hat{W}$ (i.e. mean individual biomass between size classes), and subsequently summing the products (i.e. $\Delta N \times \hat{W}$) by size class after first multiplying the products per size class by the number of size classes. This later step is done to fulfill the assumption that the total number of size classes is equal to the number of cohorts per year. With these data P/B values can be calculated for any desired time period, providing information on biomass turnover rates in the study area. Naturally, some macroinvertebrate taxa (e.g. some mayflies and midges, etc.) have very short life cycles, and sampling on a monthly basis may yield biased or inaccurate estimates when calculating secondary production for these taxa. On the other hand, most temperate macroinvertebrates are univoltine (Merrit and Cummins 1996), and sampling six sites extending over an elevational gradient will help to capture representative size classes for the multivoltine taxa.

We are aware of the potential biases associated with using preserved specimens in biomass and secondary production estimates (Leuven et al. 1985). Nevertheless, we feel there are few options to using ethanol-preserved specimens given the toxic and carcinogenic nature of formalin. We also believe that use of ethanol will not bias our results substantially for two reasons. First, we are only interested in relative comparisons of biomass or secondary production between the pre- and post-treatment periods. Biomass losses due to storage in preservatives will generally stabilize during the first 20 to 60 days of storage (Leuven et al. 1985, p. 157), hence any biases due to the preservative should stabilize after this time period, allowing relative comparisons of faunal attributes. In addition, our sampling regime should averaging effect on preservative-related biases given the number sampling locations and time periods during the pre- and post-treatment periods.

In addition to enumeration, taxonomic analysis and biomass determination community attributes will be analyzed and reported. Invertebrate community attributes will include structural or functional guild analyses, taxonomic and temporal and spatial analyses of other ecological metrics (e.g. diversity, richness, and others).

Previous project sponsor response: Secondary production is a measure of biomass, or energy, of the macroinvertebrate community through time (e.g. g/m^2/time), whereas biomass is only a snapshot of production in time (e.g. g/m^2) (Benke and Huryn 2007). Secondary production takes into account the constantly changing life stage distribution (i.e. phenology) of invertebrate species within the community being measured, and hence the changing amount of biomass present at any given time. For this reason scientists often calculate secondary production to quantify energy flow or transfer within food webs (Benke and Huryn 2007).

Sampling multiple times throughout the season as proposed will enable calculation of growth rates for dominant taxa (perhaps EPT) and secondary production. More specifically, selected invertebrate taxa from each sample date will be placed in drying ovens, dried for approximately 12 hours, weighed, dried for several more hours, and weighed again to ensure that the invertebrates are at a stable dry weight.

Subsequently, biomass weights will be used to calculate secondary production:

$$P = g * B$$

where P = production, g = growth rate and B = mean population biomass for any two consecutive dates (Benke and Huryn 2007); here g is calculated as:

$$g = \ln(W_{t+\Delta t}/W_t)/\Delta t$$

where W_t = *average mass of an individual at time t,* $W_{t+\Delta t}$ = *average mass of an individual at time* $t+\Delta t$, *and* Δt = time period interval (Benke and Huryn 2007).

Once P is known, production to biomass ratios (*P*/*B)* will be calculated, providing a rate of biomass accumulation for any specified unit of time (e.g. week, daily, etc.) during the study. Biomass, P, and P/B ratios provide a standardized, quantitative method for comparing benthic invertebrate baseline conditions and invertebrate response(s) to nutrient addition. This will be valuable for comparisons of pre- and post-nutrient addition conditions, and for comparisons of treatment effects in river reaches relative to the control and nutrient addition sites.

In response to the ISRP comment regarding invertebrate specimen shrinkage in ETOH, we are using ETOH because it is much less of an irritant to lab workers than formalin. However, to minimize any bias associated with use of ETOH, benthic invertebrate specimens will be delivered to the lab as quickly after field collections as possible (1-3 months), and subsequently dried in drying ovens as rapidly after species identifications as feasible. Moreover, any meaningful bias introduced by storage in ETOH will be equally shared among samples because all specimens will be collected and preserved in ETOH in an identical manner.

ISRP: *A rationale for including 250 macroinvertebrates in each sample should be provided. Some research suggests that larger sample sizes would be more appropriate as some rare, larger bodied taxa may be omitted with small sample sizes.*

Updated Project sponsor response: Benthic macroinvertebrates will be sampled monthly at right bank, mid-channel, and left bank positions at each of the six standard Twisp River sites from April through November as flow condition dictate using a standard Hess sampler.

Transects will be placed perpendicular to stream flow at each sampling site on cobble substrate within riffle and run habitats. On each transect the macroinvertebrates will be collected from the center of the channel and midpoint between the stream bank and center on both sides of the river (i.e. total of three collection sites/transect). At each of the three sampling positions on each transect, the benthos will be randomly sampled three times with Hess samplers and their contents pooled into a single sample container, such that 3 samples based on macroinvertebrates of nine Hess samples are taken from each site. However, during the spring months the center of the stream might be inaccessible due to high flows, in which case only two collection positions (right bank and left bank) would be sampled at each site. Sampling will be carried out monthly from April through November for a maximum of 144 samples per year. Sampling along a transect, as in the proposed study, will enable us to capture the known variability of invertebrate assemblages associated with depth gradients running from stream edge to stream center (see Merrit and Cummins 1996, p. 21).

All collected benthic invertebrates will be stored in 90% ethanol and delivered to Invertebrate Ecology Inc. (Moscow, ID.) for processing. At the lab, a minimum of 500 organisms will be randomly sub sampled using a tray delineated into a grid. Subsequently, the macroinvertebrates will be sorted from substrate, identified by expert taxonomists to the finest level of taxonomic resolution, i.e. primarily genus and species level, and enumerated. To reduce processing time and thus costs, Chironomidae (midge flies) will only be identified to the family or subfamily level. Identification of the invertebrates to the genus and family levels will allow us to evaluate the response of specific taxa to nutrient addition. Lab personnel will also perform a large-rare sort of macroinvertebrates after the sort and enumerated.

Previous project sponsor response: In response to the ISRP review comment regarding sample size (i.e. rationale for 250 specimens per sample), our sampling method will not compromise our ability to detect large or rare species, because the contents of each Hess Sampler will be full-sorted in the lab and not sub-sampled. Analysis of benthic invertebrate composition from our pre-treatment pilot project on the Twisp River indicated that the proposed sampling method yields a robust fauna complete with both rare and large species (data available upon request) even though the average number of benthic invertebrates is < 250/sample. Thus, at current (pre-treatment) levels of nutrient availability, invertebrate sample analysis represents counts of entire samples, not subsamples, thereby reducing potential subsampling biases on taxonomic composition and specimen abundance. However, subsampling will be considered during post-treatment years if significant increases in invertebrate abundance or density occur.

ISRP: Biomass, density and production are likely to be more closely allied with alterations in trophic productivity than invertebrate taxonomic composition…

Although this general ISRP comment may be true in some systems and in some cases, Principal Component Analysis (PCA) of up to 19 invertebrate response metrics to multiple years of experimental nutrient addition in the Kootenai River clearly and consistently identified taxonomic diversity and richness metrics the most prominent responses (Holderman et al. 2009).

Holderman, C., P. Anders, B. Shafii and G. Lester. 2009. Characterization of the Kootenai River aquatic macroinvertebrate community before and after experimental nutrient addition, 2003-2006. Report to Kootenai Tribe of Idaho and Bonneville Power Administration. 94 pp.

ISRP Preliminary Comment 4: *More fully describe the methods to be used in evaluating juvenile fish populations. Will density and biomass be measured? If so, how will these population attributes be measured?*

ISRP Final Comments: *The protocols to be used for juvenile fish populations remain incomplete in the revised proposal. However, parr is the life-stage of the fish that is likely to be most influenced by any changes caused by SCA addition. Therefore, assessment of juvenile salmon population levels, biomass, and growth rates are key to understanding the mechanisms by which SCA addition affects the fish.*

Updated Project sponsor response: Because the main objective of this project is to increase the production and condition of juvenile anadromous salmon species in project waters, a series of standard, diagnostic fish metrics will be evaluated before and after nutrient addition. These metrics include:

1. Annual escapement/run size
2. Redd counts
3. Relative abundance
4. Individual fish length, weight, biological condition factor,
5. Growth rates
6. Gut fullness
7. Diet composition
8. Annual smolt production
9. Number of juveniles per redd
10. Egg to immigrant survival

The project will also report fish abundance and density in the sampling areas between the two transects at each site. Abundance and density data will be estimated from a combination of repeated snorkel surveys and electrofishing efforts in the 100 m reaches between the two transects at each of the six sites as permits approve such activities. For further details see project narrative.

Annual escapement, measured as adult abundance provides a measure of the number of potential spawners available to seed habitat for natural spawning. Because there is no collection weir on the Twisp River, annual counts of upstream migrating spawners corrected for the Twisp River fraction of the run will be used as a general annual index of run size. Redd counts provide a standard annual estimate or index of the amount of spawning in a surveyed river reach. Relative abundance is a standard comparative indicator of fish community health and river productivity, whereas individual fish length, weight, and condition factor are standard, comparative indicators of fish community health and river productivity. Fish growth rates are another valuable, comparable fish productivity and coarse scale fish health metric, because fish growth directly reflects the amount of food consumed in a river, which is a function of its availability. Gut fullness and diet composition reflect the amount and type of food eaten by fish, and describing food availability as it relates to fish health. Annual smolt production is a valuable annual comparative metric and is one of the benchmarks of program success. The number of juveniles per redd and egg to emigrant survival rates provide standard, comparable estimates of annual smolt production.

> Finally, stable isotope analysis and bioenergetics modeling are proposed as a direct way to track, evaluate, and estimate effects of nutrient routing through the trophic levels before and after experimental nutrient addition. Stable isotope analysis and bioenergetics modeling can tell us about the sources of nutrients that produce fish food (algae and invertebrates), and whether and how these food sources and energy pathways change after adding nutrients to a river. This is the best way to determine if experimentally added nutrients are traveling through desired food web pathways across the trophic levels as edible algae to edible invertebrates, to fish food to ultimately increase growth, condition, and survival of juvenile anadromous salmon.
>
> **Previous Project sponsor response:** The narrative described use of summer snorkel surveys to estimate parr rearing densities and trap or seine sampling to determine fish size and growth rates. Additional details were added to better define sampling efforts. Use of stomach content data will help confirm consumption of the invertebrate food base by juvenile anadromous salmonids, and whether this changes following nutrient supplementation. However, such efforts are time consuming and expensive to conduct. Eliminating this analysis would still allow us to draw conclusions on potential effects of nutrient supplementation from analysis of fish density, abundance, and growth data. If SCA were to be used as nutrient source, stomach content analysis would confirm and characterize direct consumption of carcass material by juvenile salmon.

ISRP: *The response of the project proponents indicated that they were not sure that they could obtain permission to sample parr because of the ESA status of the Chinook and steelhead populations in the Methow drainage. It should have been possible to determine the feasibility of obtaining an ESA permit for electrofishing, stomach sampling, and PIT tagging prior to developing this section of the proposal. The question of ESA permit aside, the proponents also note that "Adding these in-stream measures will significantly increase costs for the proposed work and may not be possible with funds available." This statement seems to indicate that even if sampling approval is possible, this work may not occur. The ISRP believes these data would be among the most relevant for assessing the value of SCA for increasing productivity of listed stocks of salmon and steelhead. For this reason, we would encourage the project proponents to consider economizing on some of the other study elements (nutrient limitation determination, benthic macroinvertebrate sampling, or organic matter transport measurements) and shifting resources to juvenile fish sampling.*

ISRP: *The extent to which the SCA are consumed by juvenile salmon, steelhead and resident fishes should receive additional attention. As noted above, distinguishing between the effects of direct consumption versus bottom-up, trophic enhancement on fish growth rate will be important. Measures of juvenile fish density and growth rates over time coupled with stomach samples would enable this determination. It would be of critical importance to collect stomach samples at study sites at the time the SCAs are present in the stream. However, the proposal indicates that the proponents will be relying on stomach samples collected as part of another study. The degree to which these other projects have been coordinated with this effort was not discussed in the proposal.*

> **Project sponsor response:** Project sponsors contacted permitting agencies and inquired about the probability of obtaining section 10 permits. Given the 9 month application

process, there are still uncertainties about our ability to collect certain invasive fish metrics. The revised narrative includes stomach content sampling and analysis to address the issue of direct consumption by fish and the need to separate those potential effects on the study in the event SCA supplementation is used. But again, the ultimate effectiveness of nutrient supplementation will be based on realized production effects (increased summer growth, parr rearing density, or smolt abundance or condition) regardless of the source of nutrients (inorganic liquid fertilizer(s), carcasses or SCA).

ISRP: *The response mentioned the use of stable-isotope analysis in the study but provides no specific information as to how this method would be employed, simply indicating that this part of the study is still being developed. With an appropriate sampling schedule, stable isotope analysis could be used to determine the relative effect on fish growth of direct consumption of SCA versus bottom-up effects on trophic productivity. It has been established that a relatively small fin clip will provide sufficient tissue for analysis. Ideally, this technique would be used in conjunction with stomach samples. But if ESA permitting prohibits gastric lavage, stable isotopes analysis may provide a viable option for determining fish diet. If this restriction on sampling does occur, the stable isotope samples may become critical to understanding how SCA influence system productivity. Therefore, a full description of this method should have been included.*

Updated Project sponsor response: This issue was discussed on the 2/22/10 conference call and was agreed upon as an ongoing component of the study. The ISRP stated that: "The YN are talking with Beth Sanderson about this. The ISRP said tracking nitrogen through the system is especially useful if they are using carcasses and carcass analogs".

Previous Project sponsor response: As noted, stable isotope analyses could be used to evaluate the relative contribution of direct uptake versus indirect utilization of marine derived nutrients, in the event that salmon carcasses or SCA are used as nutrient supplement. This approach is non-lethal and would require only a subsample of the population to provide usable results. Methods and design for applying stable isotope work are being developed.

ISRP Preliminary Comment 5: *Describe how adult abundance and smolt production will be measured at the Methow study sites. Without this information, determining the effect of nutrient addition on the productivity on salmon and steelhead will be either very difficult or impossible.*

ISRP Final Comments: *The methods to be used for enumerating returning adults and emigrating smolts appear to be appropriate. The project proponents propose to use smolts/redd as an indicator of the effect of enhanced trophic production on smolt production. This metric is appropriate providing that a sufficient proportion of the river upstream of the trap will be treated with SCA to ensure that most, or all, of the juvenile fish above the screw trap have access to SCA. A power analysis of the smolts-per-spawner values would provide an indication of the size of change that could be detected following SCA addition.*

Updated Project sponsor response: This issue was resolved during the 2/22/10 conference call because all work in the Methow River was postponed for inclusion in future proposals.

Previous Project sponsor response: No further response needed for ISRP 5.

ISRP Preliminary Comment 6: *Describe how potential density-dependent effects of fish population response to food limitation will be addressed. How will the effects of water temperature, flow, and changes in other habitat attributes be accounted for when assessing the responses to nutrient addition?*

ISRP Final Comments: *It might be possible to infer that SCA addition improved growth and productivity of fish if all other things were relatively constant, but it is highly unlikely that other things will remain constant over the duration of the study. Year-to-year changes in habitat attributes like temperature and flow are very common and can have substantial effects on the performance of juvenile salmon. The density of fry and parr at the study sites also is likely to vary among years and changes in density could have a considerable impact on fish response to alterations in trophic productivity. Our comments on these issues were intended to encourage the project proponents to develop methods for addressing this variability in their study design. However, the responses provided did not address this concern.*

Updated Project sponsor response: We agree with the ISRP regarding the ability of non-treatment habitat and environmental variables to confound interpretation of results in multi-year pre-and post-treatment studies like this one. We also agree with the ISRP regarding the importance of accounting for inter-annual variability of parr density and the factors that may affect it. This variability will be addressed both qualitatively and quantitatively.

In terms of qualitative assessment, project personnel spend considerable time in the field involved in monitoring for this and other projects, and maintain communication with a network of other regional and local researchers. Thus, exposure to and familiarity with small and large scale habitat alterations, disturbances, and other stochastic environmental events such as floods, fires, spills, and large erosional events will help evaluate the degree to which such events might alter or confound data and their interpretation from this study.

Quantitative assessment of effects from above non-treatment variables and temperature, water year and flow aspects, and previously mentioned stochastic environmental effects (when data are available) on inter-annual variability in parr density, condition, and other fish metrics will involve various multivariate statistical approaches. Such approaches include means testing and correlative analysis, ANOVA, MANOVA, PCA, and other methods described below. Data collected by this project will also be provided for use in climate variability modeling being developed by the NOAA and the USGS. These agencies are jointly sponsoring a demonstration project in the Columbia River Basin with the aim of developing a practical integrated approach to organizing and collecting information about climate variability and change to support decision making at both regional and local scales. They intend to focus initial development of this approach through a pilot application looking at the impacts of climate variability and change and other factors on water availability and water management options in the Methow. Annual fish run size, condition, and distribution data and other available quantitative habitat and environmental data from the watershed and from the Twisp River specifically will be reviewed and evaluated to address this important issue.

> **Previous Project sponsor response**: The value of this multi-year project is in the opportunity to investigate the effectiveness of nutrient supplementation under a range of environmental conditions. Provided sufficient information, environmental variation can be controlled for statistically to indicate if underlying patterns are related to experimental manipulations (nutrient additions) or existing environmental variability. As noted in the proposal:
>
> "The primary analytical method will be multivariate analysis of variance (MANOVA, PROC GLM, SAS Institute Inc., Carey, NC) in which response variables (juvenile densities, growth rates, smolt-spawner ratios) will be related to environmental and productivity variables. Canonical variate analysis (PROC CANCOR) will be used to identify which response variables were associated with differences when they occur. Single variable analysis of variance (ANOVA) will be used to better define relationship between important fish production variables and environmental data".
>
> Similar to analyses to assess effects of nutrients on productivity, a multi-year study such as this provides a multivariate approach to evaluating the effectiveness of nutrient supplementation on salmon production. Differences in fish abundance and growth associated with nutrient supplementation will be tested using analysis of covariance (ANCOVA), using adult escapement (nutrient source) as the covariate, and multivariate analyses (MANOVA) to determine if multiple responses are occurring. We will also use stepwise multiple regression to evaluate what independent variables are best associated with the variability in production metrics. Independent variables to be included in the analyses include: year, amount of nutrient added and nutrients added adjusted by flow to account for dilution, mean flow and temperature during winter, spring and summer, and spawner abundance. Best fit model(s) will be determined using Akaike Information Criteria (AIC). This approach will help control for environmental conditions while investigating for potential effects of nutrient supplementation on fish production.

ISRP: *The project proponents provided the following paragraph in response to our concerns about density-dependent effects:*

> *"Regarding density-dependent growth regulation, if food is/becomes limiting we would expect to see a response manifested as lower fish condition, length, weight, and smolt production per spawner, or possibly reduced numbers of outmigrants. Conversely, if nutrient augmentation increases food availability (relative to empirical pre-treatment values), we would expect to see some level of increase in mean fish length, weight, condition, production rates. If food is not limiting smolt production, then little response to nutrient augmentation should be observed relative to fish condition and production rate over time."*

This response does not address the ISRP concern that parr growth rate response to enhancement of trophic productivity could be greatly modified by density-dependent effects. There are several density-mediated scenarios that could complicate the interpretation of growth rates. Very high densities may result in limited or no response to SCA addition because, even with augmented food availability, food could still be sufficiently scarce that nearly all consumption by the fish is used for metabolic maintenance; little growth actually occurs. At low population levels, food may not be limiting growth, even if trophic production is low. Under these conditions, additional food availability due to SCA addition may not be reflected in any increase in growth. As fry and *parr density is likely to change from year to year, an accurate estimate of density each year of the study will be key to interpreting any changes observed in growth rates. The project proponents suggest that they can adequately account for the effect of variable environmental factors like temperature and flow, by monitoring outmigration timing:*

> *"Regarding density-independent regulation, flow and temperature can directly affect system productivity, habitat suitability, and therefore fish growth and condition. Some of these responses occur in predictable a manner. One means to address effects of environmental condition is to monitor outmigration timing. Presumably, unsuitable conditions, such as low flows and high temperatures, would prompt early emigration of juvenile salmonids from rearing areas. By continuously operating screw traps at the mouths of the Methow and Twisp rivers throughout the outmigration season, and at any additional new locations, we will document outmigration patterns and events, such as premature emigration of parr and pre-smolt stages, along with the standard suite of fish performance metrics described above, and relate that to environmental conditions."*

ISRP: *This response does not address the concern. If conditions at a study site became so inhospitable that emigration occurs, it would reveal very little or nothing about the effect of trophic enhancement on these fish, other than to suggest that the study location was inappropriate for enhancement. Rather the ISRP comment was raising the issue that any responses in fish growth rate following SCA addition may be modified by environmental conditions like flow, water temperature, etc. and these conditions will vary temporally and spatially. Therefore, the effect these habitat attributes may have on salmon parr needs to be considered in interpreting any responses associated with SCA addition.*

> **Updated Project sponsor response:** See the above updated comment regarding the project's qualitative and quantitative assessments of non-treatment, density-dependent, and density-independent variable effects as they relate to data interpretation for the project.
>
> **Previous Project sponsor response:** Although we agree with the general premise described here by the ISRP that causes of response behaviors in fish metrics may be affected differently at opposite ends of the density continuum, it is not possible within the scope and resources available to this project to provide experimental designs to test such assumptions. However, we do agree that it is critical to consider possible effects of these habitat attributes in concert with density measures when interpreting any changes in fish metric values or trends, especially after experimental nutrient addition treatments, to: 1) best ascertain cause and effect, and 2) separate potentially confounding effects of habitat or environmental conditions, density dependent effects, and effects of nutrient addition on fish response metrics values and trends.

ISRP Preliminary Comment 7: *Consider the application of a bioenergetics model to identify appropriate hypotheses and design experiments.*

ISRP Final Comments: *The project proponents should consider using an available bioenergetics model rather than developing one (e.g., Fish Bioenergetics 3.0 http://limnology.wisc.edu/research/bioenergetics/bioenergetics.html). Using an existing model* *and paramaterizing and/or modifying as required for this study could substantially reduce the amount of effort required to accomplish this part of the study.*

> **Updated Project sponsor response:** This issue was resolved during the 2/22/10 conference call. The ISRP stated: "The YN plans to use their field data to populate a bioenergetics model as another method of exploring the interaction of various factors influencing the fish before and after system enrichment. They will examine existing models and develop their approach over time". No additional response is needed.
>
> **Previous Project sponsor response:** We appreciate this suggestion from the ISRP and have modified the project budget to allow for the informed development of useful modeling scenarios, involving experts in the field to identify appropriate hypotheses and design experiments as the budget allows consistent with project objectives and within the scope of the project. This work could fit well within a graduate study program.

ISRP Preliminary Comment 8: *Include a more detailed description of the adaptive management process that will be used in moving this study forward.*

ISRP Final Comments: *A section has been added to the revised proposal entitled "Adaptive Management Framework." However, this section really provides only a brief discussion of the experimental design of the study, not a description of how the results of this, and other nutrient-enrichment efforts in the basin, could be used to inform management decisions regarding the application of this method. However, this section is useful in that a timeline for the project is provided.*

> **Updated Project sponsor response:** This issue was resolved during the 2/22/10 conference call. This ISRP requested no further information on this issue from the YN.
>
> **Previous Project sponsor response:** Results of this work could inform management decisions in many important ways, including: 1) providing better informed decision points and criteria, and information regarding the pros and cons of different nutrient addition methods, with applications to at least two different rivers in the upper-mid Columbia basin (Twisp and Methow rivers); 2) adding to the empirical record of ecological responses to experimental nutrient addition; 3) feasibility and efficacy of nutrient assessment; and 4) the cost-effectiveness if nutrient supplementation as an ecosystem and salmon population restoration tool.

ISRP Preliminary Comment 9: *Describe how the evaluation will deal with the presence of and confounding effects of hatchery fish and the role of hatchery fish carcasses in the study design and evaluation, including the identification of their marine-derived nutrient contribution.*

ISRP Final Comments: *The ISRP comment that hatchery fish spawn in the area was misinterpreted. We were not suggesting that hatchery carcasses should be introduced as a treatment but that carcasses are present from returning hatchery fish (and the few wild fish that do return) and will add MDN into the system. If enough salmon return to the study reaches, the carcass material could stimulate a trophic response. The amount of carcass tissue deposited at the study reaches is also likely to vary considerably among years. Therefore, some process for controlling the amount of carcass tissue added to the study sites should be included in the study to minimize confounding effects from this source. At a minimum, the amount of carcass tissue deposited at each site each year of the study should be measured.*

Updated Project sponsor response: This issue was resolved during the 2/22/10 conference call: “The ISRP participants noted there is a supplementation program in the Twisp and wondered how they will account for the variability this will bring. The YN said outplanting varies year to year in late April and May. The outplanted fish are almost all placed in an acclimation pond near the downstream end of the Twisp River. 100,000 steelhead are stocked per year, and coho are stocked as well. They should be able to identify supplemented fish by an ad clip, size and fin condition”.

The ISRP then asked: *Will these fish interfere with the study?* It seems that most of the hatchery fish move out with the spring freshet, and those that residualize should be identifiable”. The ISRP requested no further information to resolve this question.

Previous Project sponsor response: Data from weekly spawning ground surveys and kelt counts conducted for spring chinook, summer chinook, coho, and steelhead by regional fish agencies in the study area waters will be used to quantify carcass loading and to determine if those kelt loads are large enough to expect any potential confounding effects on study results. If so, at a minimum, the amount of carcass tissue deposited at each site each year of the study will be measured and factored into the interpretation of study results.

Project Number	BPA 200847100
Proposer	John Jorgensen, Yakama Nation Fisheries
Short Description	This project will assess and characterize nutrient availability, and if needed will perform controlled experimental addition of limiting nutrients to enhance natural production of anadromous salmonids and their supporting ecological functions and limnological conditions in rivers in the Methow Subbasin.
Province(s)	Columbia Cascade
Subbasin(s)	Methow
Contact Name	John Jorgensen
Contact email	john@mid-columbia-coho.net

10.A Abstract

Pacific salmonid populations have declined dramatically across the Columbia River Basin. These population declines are often due to cumulative effects of multiple factors affecting production in freshwater and marine environments. An important result of these population declines is the concurrent nutrient, productivity, and ecosystem function losses associated with significantly reduced marine derived nutrient (MDN) loading rates from the loss of salmon carcasses. Anadromous salmon carcasses provide significant amounts of MDN, which historically provided the basis for primary productivity in stream systems, especially in the interior areas of the Columbia Basin that are naturally oligotrophic. Lower MDN loading from diminished salmon runs results in negative feedback through reduced juvenile rearing capacity for Pacific salmon systems. Recent research has indicated that MDN loading rates as low as 6 to 15% of historical levels currently exist among anadromous salmon spawning streams in the Pacific Northwest.

This project will quantify and evaluate nutrient status and availability in two watersheds of the Methow River Basin (Twisp and Methow rivers), under current conditions of diminished anadromous salmon runs. More specifically, this project will conduct a multi-trophic level sampling program to quantify and evaluate baseline water quality and nutrient availability, primary, secondary, and tertiary productivity rates including algal, periphyton, and benthic macroinvertebrate, and fish communities. An appropriate sampling scheme for each trophic level will be used at pre-determined sites. The goal is to develop a comprehensive pre- and any post-treatment biological assessment of experimental nutrient addition. Finally, this project provides the necessary adaptive management framework to determine if nutrient limitation and/or imbalance currently exist, and to generate empirically-based recommendations for restoring ecological processes needed to increase natural production of anadromous salmonids, with additional unquantified benefits to anadromous Pacific lamprey, resident fish, riparian ecosystems, and wildlife populations.

10.B Problem statement: technical and/or scientific background

Problem statement - The problem addressed by this project is the continued low level of natural production of anadromous Pacific salmonids (*Onchorynchus spp.*) in the Methow River Basin in North Central Washington (Upper Columbia Basin, Figures 1 and 2) and the potential relationship with diminished marine derived nutrients (MDN) inputs to the system. The Methow River historically supported multiple viable anadromous salmonid populations as well as Pacific Lamprey (*Lampetra tridentata*), resident trout, and numerous other fish and wildlife populations. Population abundance of these species has declined dramatically from historical levels. Numerous factors are associated with these declines, stemming from in- and out-of-basin sources of mortality. Although significant measures have been implemented to reverse this trend during recent decades, improvement in numbers of salmon returning to this region of the Columbia River Basin has been inadequate.

In fact, depressed natural production due to reduced MDN inputs is a chronic problem not only in the study area, but across the Columbia River Basin. The Upper Columbia Spring Chinook Salmon and Steelhead Recovery Plan calls for nutrient enhancement as a restoration strategy, but also points out the need for a better understanding of why, where, and how much nutrients may be needed (UCSRB 2007). A more holistic approach to understanding and resolving underlying conditions that limit productivity in our aquatic systems in general can be a critical step in salmon restoration. By characterizing nutrient availability, trophic status, and potential nutrient limitation related to reduced MDN levels in the Methow River Subbasin (Twisp and Methow rivers), it may be possible to specifically mitigate identified anthropogenic nutrient, productivity, and ecological function losses and contribute to increased natural productivity.

In addition to nutrient limitation, we understand that loss and deterioration of physical habitat may also limit natural production of salmonids to varying degrees in different parts of the study area (Methow Subbasin). Large efforts are underway to preserve, rehabilitate, and restore river processes and physical habitat conditions throughout the Methow Basin and the Upper Columbia (UCSRB 2007; NPPC 2004). Recovery criteria have been established and desired increases in natural production, if co-limited by habitat quantity, quality, and food availability, would require coordinated efforts~~,~~ to restore both nutrient availability and physical habitat. In this context we are currently pursuing collaborative efforts with local and regional researchers and managers. This integrated approach appears to provide the best chance of improving natural production in the study area by working to restore the biological and physical habitat conditions required for survival of early life history stages of salmonids.

Technical and Scientific Background/Justification

Factors limiting natural production of Pacific salmonids - Current low levels of natural production of anadromous Pacific salmonids in the Columbia River Basin and other west coast North American river systems are the cumulative result of multiple factors in the freshwater and marine environments. Reduced natural production in the freshwater environment can occur at various life stages and can be caused by physical and biological limitations. These can include degradation of spawning, incubation, and rearing habitats, effects of invasive species through competition and predation, passage restrictions to and from critical habitats, climate change, and nutrient limitation and resulting cascading trophic effects (NRC 1996; Ruckelshaus et al. 2002; Williams 2006). Mortality in the Columbia River, the estuary, and in marine environments can also occur at multiple life stages, and may be affected by physiological acclimation, competition, predation, harvest, passage and migration success, and other immediate or delayed artificial and natural factors (Ruckelshaus et al. 2002; Williams 2006). One estimate suggested that recent salmon escapement levels may provide as little as 6-7% of historical MDN inputs to salmon rivers in the Pacific Northwest (Gresh et al. 2000). Another analysis suggested that < 2% of historical marine-derived P is currently returning to the Snake River (Scheuerell et al. 2005), and that, under some circumstances, there could even be a net export of nutrients when adult escapement is extremely low (Moore and Schindler 2004).

Roles of marine-derived nutrients – Nutrient availability is central to natural productivity in aquatic systems in general, and for Pacific salmonids in particular (e.g. Gende et al. 2002; Naiman et al. 2002; Wipfli et al. 1999; Kohler et al. 2008). Historically, anadromous Pacific salmonids provided significant inputs of MDN to freshwater streams (Cederholm et al. 1999, 2001; Gresh et al. 2000), likely serving as a metabolic driver for interior systems otherwise characterized as oligotrophic or ultraoligotrophic (nutrient-poor). This nutrient input can affect ecosystem metabolism from the bottom up, enhancing biological productivity at all trophic levels (Wipfli et al. 1998).

Kline et al. (2007) reported two main pathways by which nutrients make their way from salmon carcasses to the environment: (1) the *direct pathway*, where salmon spawn and carcasses are directly consumed, by bears, birds, fish (young salmon and resident species), and stream invertebrates; and (2) the *remineralization pathway*, where nutrients are released back into the water by microbes during the decomposition of salmon carcasses. Increased nutrient availability from decomposing salmon carcasses, in the forms of N, P, and C, provides the basis for increased algal and periphyton production and microbial growth in streams (Bothwell 1989; Peterson et al. 1993; Yani and Kochi 2004). This in turn can enhance productivity and diversity of the invertebrate community and production of juvenile salmonid forage (Johnson et al. 1990; Mundie et al. 1991; Quamme and Slaney 2003; Yani and Kochi 2004; Holderman et al. 2009a, 2009b). In addition, carcasses can significantly increase substrate surface area available for microbial and invertebrate productivity and diversity. Increased secondary production can enhance in-stream growth, condition, and survival for juvenile resident and anadromous fish populations and may ultimately contribute to increased numbers of out-migrating salmonids and survival due to higher fitness (Peterson et al. 1993; O'Keefe and Edwards 2003).

Numerous studies suggest broad cycling of salmon-derived nutrients into multiple trophic levels in riparian and terrestrial ecosystems (Gende et al. 2002; Reimchen et al. 2003). MDN has been identified in the hyporheic zone and in riparian and adjacent terrestrial forest soils, vegetation, invertebrate, and vertebrate communities associated with Pacific salmonid ecosystems (Ben-David et al. 1997; Cederholm et al. 2000; Hildebrand et al. 1999a, 1999b; Bilby et al. 2003). The preponderance of evidence has made it clear that current discussions on restoration efforts must include the role of MDN in restoring salmon populations and the systems on which they rely (Peery et al. 2003; Stockner 2003, and references therein).

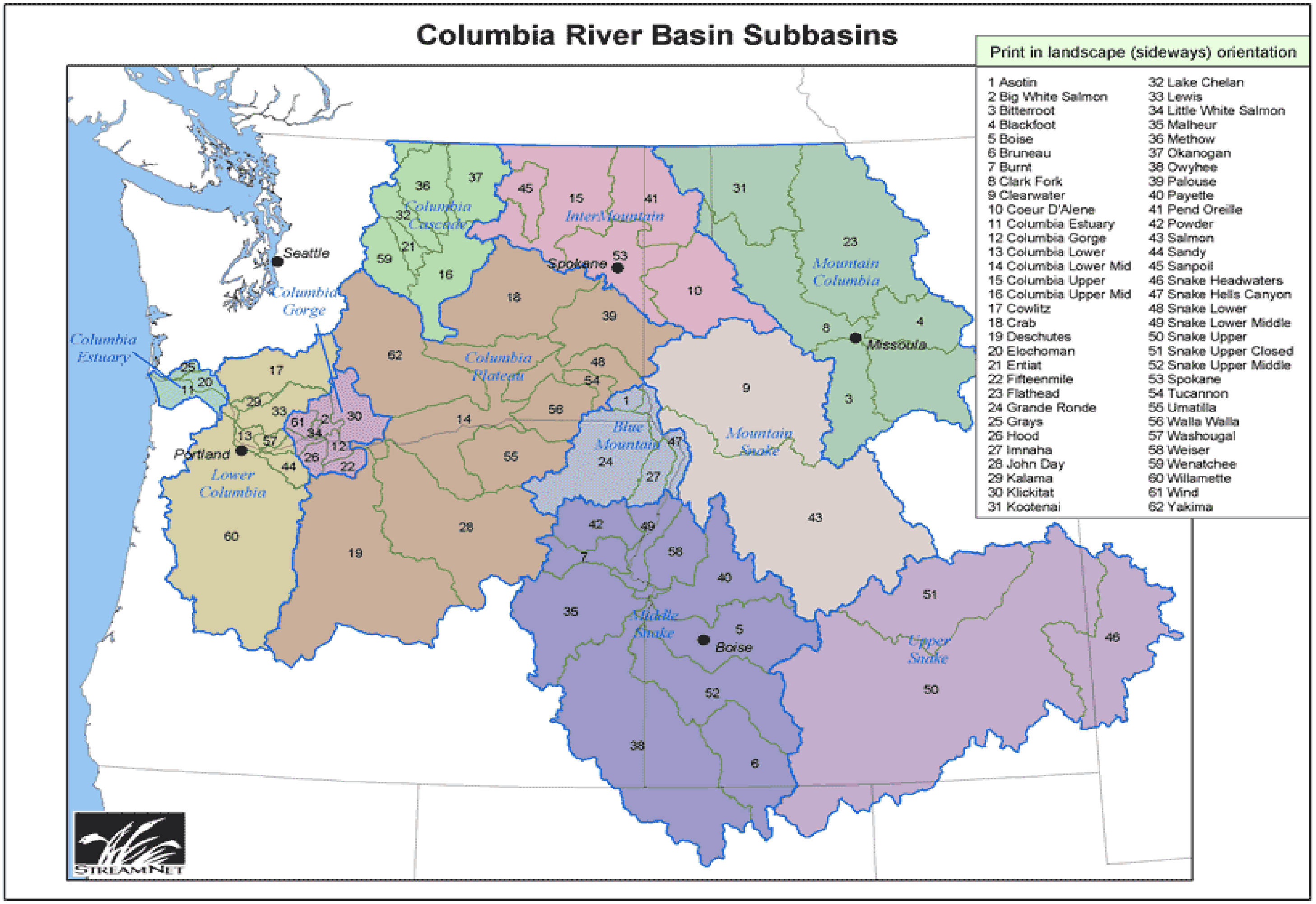


Figure 1. Columbia River Basin map showing all Subbasins, including the Methow River Subbasin (#36) in the upper (Northwest) corner of the Columbia Cascade Ecological Province, bounded on the north by the US-Canada border.

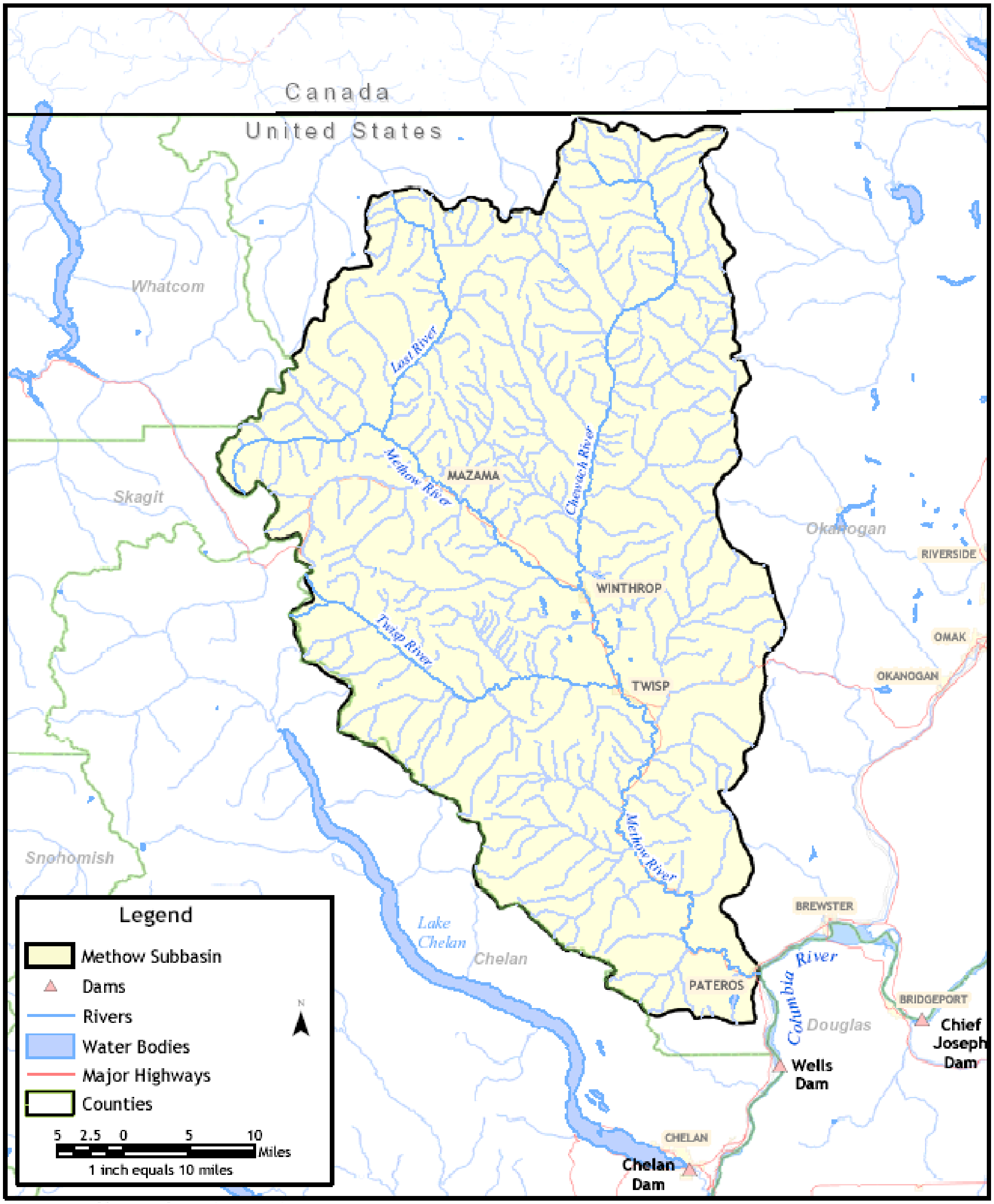


Figure 2. Map of the Methow River Subbasin (shaded) showing the Twisp and Methow rivers, which serve as study areas for this project.

Justification for proposal - This proposal directly addresses nutrient availability and its potential limitation for natural production of Pacific salmonids in the Methow River Subbasin, specifically in the Methow and Twisp rivers. We believe an assessment of nutrient availability and the potential to test experimental nutrient augmentation is justified for the following reasons:

(1) Salmon habitat in the Twisp and Methow rivers does not appear to be critically limiting, but it is acknowledged that physical habitat improvements may also be beneficial and needed in these systems to improve natural production and compensate for additional anthropogenic limitations downstream;

(2) Non-native species do not occur in significant numbers;

(3) Efforts to improve out-of-basin survival (hydrosystem passage) and instream production of salmon and steelhead (hatchery programs) have generated little improvement in the abundance and productivity of natural origin fish;

(4) Current MDN loading from anadromous salmonid carcasses is significantly reduced from historical levels; and

(5) The Twisp River has very low egg-emigrant survival rates (e.g. ~1% vs. ~12% in Chiwawa, Wenatchee basin; [see Hillman et al. 2007]), indicating a production bottleneck that could be addressed with experimental nutrient addition if nutrients are found to be limiting.

10. C Rationale and significance to regional programs

This section describes the relation of this proposed project to the: 1) objectives in the Columbia River Basin Accords, 2) objectives, strategies, and hypotheses identified in the Methow River Subbasin Plan, 3) objectives of the 2000 Fish and Wildlife Program (Program), and 4) the 2003 Mainstem Amendments. This section also describes applicable relationships between the proposed project and Biological Opinions, recovery plans, Habitat Conservation Plans, and other relevant regional or local plans.

10.C.1 Columbia River Basin Accords – As with Columbia River Basin Fish and Wildlife Program projects proposed in the past, the ISRP will conduct scientific review of proposed Columbia River Basin Fish Accords projects using criteria established by the Act. These criteria include whether projects:

1. Are based on sound scientific principles;
2. Benefit fish and wildlife;
3. Have a clearly defined objective and outcome;
4. Include provisions for monitoring and evaluation of results; and
5. Are consistent with the Council's Fish and Wildlife Program.

This proposed project is consistent with project requirements under the Columbia River Basin Accords by being based on sound scientific principles and by providing direct benefits to fish and wildlife populations within and beyond the project area, and increased nutrient and food availability within the immediate project areas (see Section 10.B, "Technical and scientific background/Justification"). This project also meets project requirements under the Accord by providing clearly defined objectives, outcomes, and monitoring and evaluation (see Sections 10.F.1."Biological /physical objectives, 10.F.4 "Metrics", and 10.G "Monitoring and Evaluation"), and by addressing goals and objectives of the Council's Fish and Wildlife Program (see Section 10.C.3. "Objectives of the 2000 Fish and Wildlife Program").

10.C.2 Objectives identified in the Methow River Subbasin Plan (Page numbers provided below refer to printed Subbasin Plan pages, not electronic page numbers)

This proposed project is justified by and directly addresses the following limiting factors, strategies, objectives, and hypotheses from the Methow Subbasin Plan:

- Limiting factor: Nutrient availability (Table 54, Page 300 of Methow Subbasin Plan)
- Salmon Carcasses (Table 54, Page 300 of Methow Subbasin Plan): (low abundance of salmon/steelhead and their nutrients contribution to stream ecology including benthic macroinvertebrates and fish growth)
- Management strategy (Table 54, Page 300 of Methow Subbasin Plan): Increase or maintain artificial production capacity at levels necessary to meet management needs, maintain new and existing acclimation sites, and support existing and new scatter plantings. Program is intended to support conservation, reestablishment of natural broodstock and interim harvest opportunities.

Section 5.5 (Subbasin Plan Assessment, Unit summaries; Page 301)

- Hypothesis 4 (Page 310) - Increasing food availability within the AU (assessment units)* will increase survival for spring Chinook, steelhead, and bull trout in the following life stages: a) fry colonization (spring Chinook, steelhead, and bull trout), and; b) rearing (spring Chinook, steelhead, and bull trout). Westslope cutthroat trout survival will increase for migration and overwintering.
- Objective 3 (Page 310) - Conduct productivity analysis (invertebrate sampling and organic/inorganic constituent sampling/analysis), and determine appropriate nutrient supplementation program.
- Objective 4 (Page 310) - Supplement nutrients as needed and determined from Objective 3 of Hypothesis 4 of this proposal. One example provided in the Methow Subbasin Plan was to: "Achieve 125 salmon carcasses/mile as an interim target, based on estimates of historic run size" (Mullan et al. 1992 distributed in areas of current spawning and rearing; WDFW unpublished data). However, no empirical linkage currently exists between the relevance of this 125 kelt/mile estimate and current nutrient availability in the proposed study area. (NOTE: For this project it is currently unclear whether or the degree to which project waters are nutrient limited, and/or unbalanced. Therefore, kelt addition is currently unwarranted due to this lack of quantification. However, if experimental nutrient addition is found to be warranted following baseline assessments described in this proposal, kelts, time-released nutrient briquettes (i.e. carcass analogues), or liquid inorganic fertilizer(s) will be reviewed and compared in terms of appropriateness for this project.
- Strategy 1 (Page 311) - Restore nutrients through salmon carcass or analogue distribution.

> *Note: Similar language was used for most of the Methow River Subbasin AUs (assessment units), indicating broad support for the need for increasing food availability. Repetitive language was avoided in the interest of brevity. Likewise, nutrient addition and studies to determine the appropriate locations, quantities, and methods of nutrient additions were also identified in the Salmon and Steelhead Recovery Plan.

10.C.3 Objectives of the 2000 Fish and Wildlife Program (Program)

The Program's goals, objectives, scientific foundation and actions are structured in a "framework", which is an organizational concept for fish and wildlife mitigation and recovery efforts that the Council introduced in the 1994-1995 version of the Program. The 2000 program, organized with the framework concept, is intended to bring together, as closely as possible, Endangered Species Act requirements, the broader requirements of the Northwest Power Act and the policies of the states and Indian tribes of the Columbia River Basin into a comprehensive program that has a solid scientific foundation. The Program also explicitly states the Northwest Power and Conservation Council's the (Council's) goals and links the Program to a specific set of objectives, describes the strategies to be employed, and establishes a scientific basis for the program. Thus, the program guides decision making and provides a reference point for evaluating success.

The Northwest Power Act directs the Council to develop a program to "protect, mitigate, and enhance" fish and wildlife of the Columbia River and its tributaries, including related spawning grounds and habitat affected by the development and operation of the federal hydrosystem. In support of this programmatic vision, the Council has stated four overarching biological objectives for this program:

- A Columbia River ecosystem that sustains an abundant, productive, and diverse community of fish and wildlife;
- Mitigation across the basin for the adverse effects to fish and wildlife caused by the development and operation of the hydrosystem;
- Sufficient populations of fish and wildlife for abundant opportunities for tribal trust and treaty right harvest and for non-tribal harvest; and
- Recovery of the fish and wildlife affected by the development and operation of the hydrosystem that are listed under the Endangered Species Act.

This proposed project addresses all four of the Council's above programmatic biological objectives. Nutrient assessment and potential enhancement in project watersheds will enable monitoring and restoration of ecological functions and process, promoting improved levels of biological productivity from the bottom up. The proposed project also directly assesses and if needed experimentally treats (mitigates) anthropogenic nutrient deficiency to counteract the adverse effects of development and operation of the hydrosystem and other downstream limitations. Ultimately this project is designed to address the Council's programmatic objectives of recovery and the provision and maintenance of sufficient fish and wildlife populations to support opportunities for tribal trust and treaty right harvest and for non-tribal harvest.

10.C.4 The Northwest Power and Conservation Council's 2003 Mainstem Amendment plan includes the following objectives relating to:

- the protection and enhancement of mainstem habitat, including spawning, rearing, resting and migration areas for salmon and steelhead and resident salmonids and other fish;
- system water management;
- passage spill at mainstem dams;
- adult and juvenile passage modifications at mainstem dams;
- juvenile fish transportation;
- adult survival during upstream migration through the mainstem;
- reservoir elevations and operational requirements to protect resident fish and wildlife;
- water quality conditions; and
- research, monitoring and evaluation.

This proposed project directly addresses three of above Mainstem Amendment objectives (the first and the last two), by enhancing spawning and rearing habitats for salmon, steelhead, resident salmonids, and other fishes identified as nutrient-limited. If experimental nutrient addition is deemed appropriate based on project bioassessment, it will improve water quality conditions, in terms of biological productivity. Furthermore, iterative, adaptive experimentation as part of the project design will generate valuable information, data, and protocol evaluations to inform future RM&E programs.

10.C.5. Applicable relationships to Biological Opinions, recovery plans, Habitat Conservation Plans, or other plans.

The Biological Strategy of the Upper Columbia River Technical Team (UCRTT 2008) lists "nutrient enhancement" as a critical uncertainty in the upper tributaries of the Methow Basin. As a recommendation, the UCRTT stated that: "An assessment is needed to determine the location and magnitude for potential nutrient enhancement projects "Within current and historic ranges, consistent within individual stream capacity and recovery objectives." These recommendations are consistent with our project goals.

The Methow Implementation Schedule (MIS) from the Upper Columbia Salmon Recovery Board (UCSRB document in review) lists "depleted nutrients" as a limiting factor in all reaches of the main-stem Methow and for most of its anadromous tributaries. Specific recommended actions include "fertilizer, carcass analog and carcass placement". Recommended timelines for specific actions include, for 2008-2010: "evaluate approach, identify appropriate methods and obtain permits and approval", for 2011-2013: "add nutrients" and for 2014-2017: "continue to add nutrients to make up the difference between annual escapement and needed abundance for recovery". This sequence of steps needed to get to the appropriate actions defined in the MIS is consistent with this project's proposed goals and timeline.

Data Gap Prioritization analysis (unpublished UCRTT 2008 document) stated: "Understand the need and magnitude of adding nutrients as part of an ESU wide plan to determine where, how, and how much nutrient supplementation is needed" as a Tier 1 data gap.

10.D Relationships to other projects

Methow Subbasin Projects - The Yakama Nation is a contributing member to the Methow Restoration Council, the basin's Watershed Action Team. Members of the MRC include WDFW, USGS, USFWS, USFS, BOR, DOE, Methow Conservancy, Washington Rivers Conservancy and Wild Fish Conservancy. Projects among the different groups include hatchery monitoring and evaluation programs, habitat restoration projects, flood plain protection, and habitat effectiveness monitoring.

Project personnel work collaboratively with the WDFW hatchery monitoring and evaluation program. The locations of their rotary screw traps provide valuable sampling sites and data for measuring condition factor population attributes of resident and anadromous fish in the study areas. Data collected at the traps, including, survival, egg to emigrant, and SAR rates will provide estimates of pre- and post- fertilization production. We are also pursuing collaborations with the Wild Fish Conservancy and DOE as part of a basin-wide water quality evaluation program.

USGS effectiveness monitoring – Initial discussions confirmed that BOR, USGS (Pat Connelly, Cook WA) and Dr. Colden Baxter (ISU, Pocatello) will be collaborating on evaluations of physical habitat improvements and operating instream PIT tag stations within the Methow Basin to assist in monitoring juvenile and adult production and addressing potential project treatment (experimental nutrient addition) effects. Collaborative discussions between key project personnel and these within-basin cooperators are ongoing and are undertaken to provide mutually beneficial monitoring, evaluation, and analytical outcomes among all parties.

Kootenai/y fertilization projects - Most key personnel (Drs. Anders, Ashley, Shafii, Smith, Ward, and Yassien) have been involved with many aspects of the Kootenay Lake and Kootenai River nutrient assessment and subsequent fertilization projects and their development since 1990. Interaction of key project personnel with those of other pioneering, long-term successful nutrient evaluation and addition projects in North America and elsewhere provide invaluable project design, implementation, monitoring, evaluation, and analytical attributes for this project. These scientific and management networks also provide logistical efficiencies required for successful long-term scientific and management collaborations.

British Columbia Projects – Several key project personnel (e.g. Drs. Ashley, Ward and Yassien) have also been instrumentally involved in the design, implementation, evaluation, and analysis of numerous successful nutrient evaluation and nutrient addition projects from conceptual design through implementation of experimental phases through implementations phases as ongoing management phases. Several examples of such project in B.C. involving key proposed project personnel include nutrient assessment and enhancement projects on the: Adams River, Mesilinka, and Keogh rivers, Big Silver Creek, and the Salmo and Chilliwack rivers.

10.E Project history (for ongoing projects)

Because this is a new project it is exempt from a response in this project history section.

10.F Biological/physical objectives, work elements, methods, and metrics

10.F.1 Biological/physical objectives

This project has five sequential, complementary objectives, to:

1) Determine whether nutrient availability and/or imbalance significantly limits natural production of salmonids in the Methow River Basin, in the Twisp River (Years 1-3);
2) Select nutrient supplementation form and design a treatment prescription
3) If significant nutrient limitation is confirmed by work funded under Objective 1, quantify changes in natural production of juvenile anadromous salmonids in response to experimental nutrient addition (Years 3-8);
4) Implement and evaluate ongoing nutrient management (Year 8 and beyond as needed); and
5) Determine if results can be successfully scaled up to larger geographic areas, and applied to other rivers in the Columbia Basin.

10.F.2 Work Elements

Several BPA work elements (WE) are needed to satisfy Objective 1:

WE-157	Collect/Generate/Validate Field and Lab Data
WE-160	Create/Manage/Maintain Database
WE-162	Analyze/Interpret Data
WE-132	Produce (Annual) Progress Report
WE-183	Produce Journal Article

An additional work element will be implemented under Objectives 2 and 3 if Objective 1 and 2 confirm significant nutrient limitation and show desirable ecological response to experimental nutrient addition respectively:

WE-44 Add Nutrients Instream

10.F.3 Methods

This section describes methods needed to successfully address each project work element. For more details regarding specific BPA project work elements see: www.efw.bpa.gov/contractors/statementsofwork.aspx

Adaptive management framework

This project is designed and proposed within an adaptive management (AM) framework to address inherent uncertainties associated with research, monitoring, and evaluation in complex, altered river systems. A short description of adaptive management and how this project will function within a hierarchical adaptive management framework is presented below, followed by detailed descriptions of methods by work element and trophic level.

Adaptive management is a valuable process of 'learning by doing' that involves much more than simple monitoring and response to unexpected management impacts (Walters 1986, 1997). It has been proposed that adaptive management should begin with a concerted effort to integrate existing interdisciplinary experience and scientific information into dynamic models that attempt to make predictions about the impacts of alternative policies (Holling 1978; Walters 1986; Van Winkle et al. 1997). This modeling step is intended to serve three functions: (1) provide problem clarification and enhanced communication among scientists, managers, and other stakeholders, (2) policy screening to eliminate likely unsuccessful options, and (3) identify key knowledge gaps". Typically, the design of management experiments (such as this project) is a key second step in the process of adaptive management, and a new set of management issues may arise regarding how to deal with the costs and risks of large-scale experimentation.

Two critical AM components include: (1) a direct feedback loop between science and management, and (2) the use of coordinated research, monitoring, and evaluation to guide and refine management (Halbert 1993; Figure 3). These features differentiate adaptive management from traditional trial-and-error or learn-as-you-go management (Hilborn 1992; Halbert 1993).

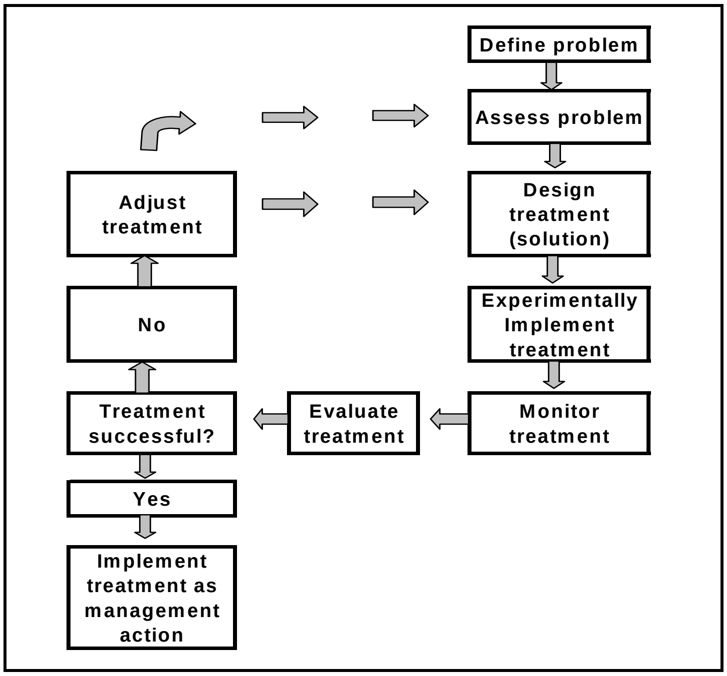


Figure 3. A generalized adaptive management model to be used in this project.

Within this general AM framework, the following sequence of iterative actions are provided below, and illustrated in Figure 4.

1) Design and implement a biomonitoring program with appropriate response variables for each trophic level (water quality, including nutrient availability), primary (algae/periphyton), secondary (macro invertebrates), and tertiary (fish) production;
2) Implement replicated trophic level sampling to compare empirical nutrient concentration with defined limiting values, and any reconstructed historical nutrient availability estimates;
3) Perform sample size and power analyses by trophic level to ensure adequate statistical rigor to detect treatment effects, and follow a defined logic path (Figure 4), including possible outcomes of treatments among intended, unintended target species or communities;
4) Assess nutrient limitation using analysis of empirical chemical, biological, and ecological metric data.
5) Repeat the above steps annually during 2-3 pre-treatment years to assess current trophic status.
6) Conclude nutrient status of the Twisp River.
7) Provide nutrient addition prescription if needed (detailed program of controlled addition of limiting nutrients).
8) Implement experimental nutrient addition for up to 5 years, along with annually repeated biomonitoring activities used during the pre-treatment years using similar sampling protocols and study sites as pre-treatment years.
9) Determine the success of the project's experimental treatment phase and determine whether nutrient addition should be recommended as a future ongoing management action.
10) Provide recommendations to resource managers as needed.

Within this hierarchical AM framework, this project has four sequential phases (Figure 4) presented below. A staggered implementation schedule is expected because work will begin in the Twisp River, with the possibility of expansion to other tributaries based on success in the Twisp.

1) Pre-treatment (diagnosis) Phase (Years 1-3) ; biomonitoring activities collect data to characterize the ecological baseline condition, including nutrient availability;
2) Decision Phase (Year 3) ; data from Phase 1 is analyzed to decide whether the study area rivers are nutrient deficient;
3) Treatment Phase (Years 3-9) ; experimental nutrient supplementation treatments are administered, monitored; and evaluated; and
4) Recommendation Phase (after year 8); based on performance and success of experimental treatments in Phase 3, recommendations are provided concerning whether nutrient addition should be considered as a future ongoing management action.

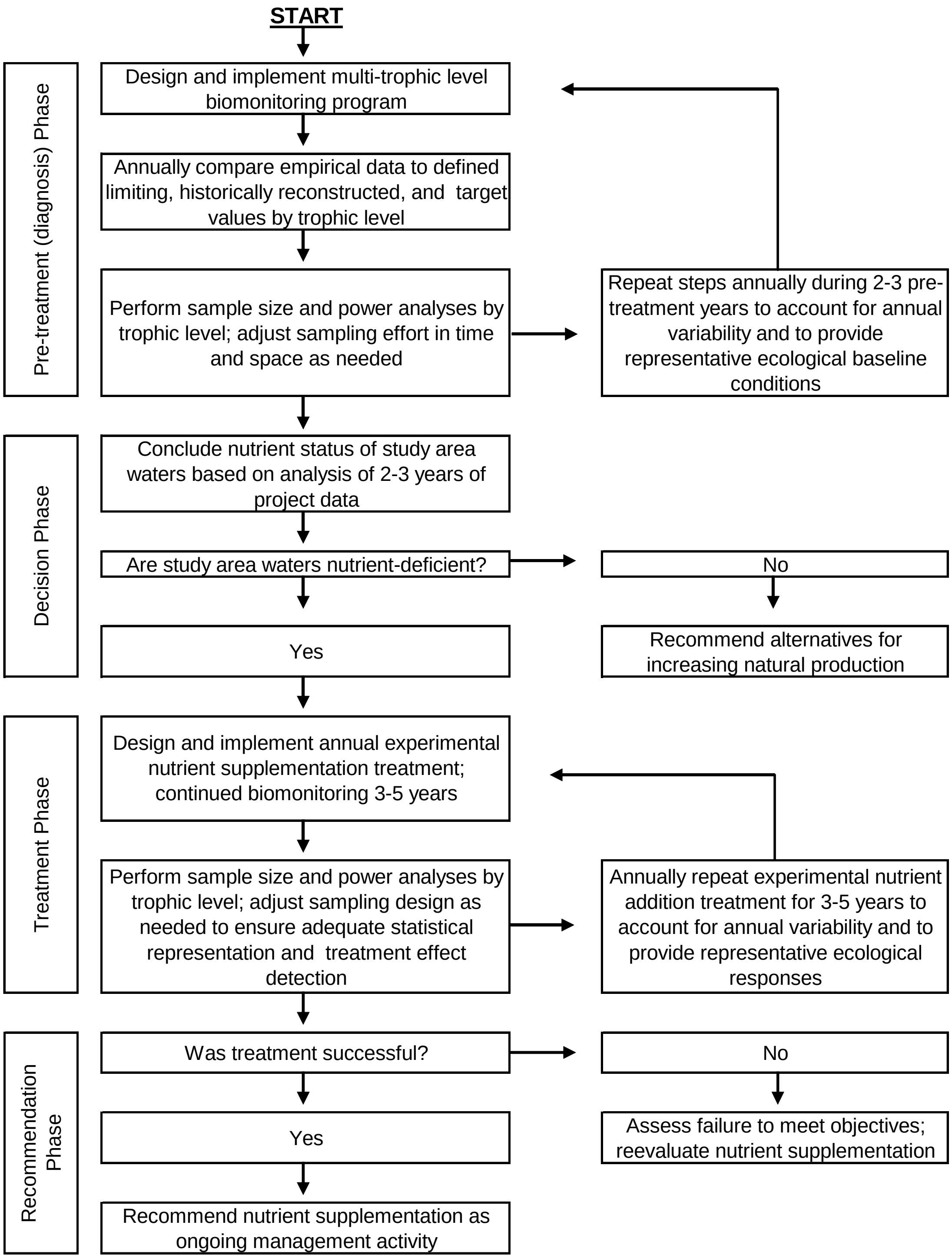


Figure 4. Adaptive project design and implementation flowchart.

Overall Project methods organization

Specific methods for all project aspects are described below. A suite of complementary data collection and analysis methods across trophic levels, along with collaborative efforts, collaborators, and annual implementation timing are provided in Appendix A. This project has sequential pre-treatment (diagnosis) and experimental treatment phases, both of which involve a standard rigorous biomonitoring component. The pre-treatment phase determines if study area waters are nutrient-deficient, to the degree that they limit natural production of anadromous salmonids in the study area. If they are, the subsequent treatment phase includes implementation, monitoring, and evaluation of an experimental nutrient supplementation prescription.

Approach to identifying nutrient limitation

Selecting and using the appropriate suite of metrics and methods to identify nutrient limitation in study area waters is critical to the success of this project. This aspect of the project is a prerequisite for determining if any of the study waters will warrant recommendations for experimental nutrient addition.

A combination of approaches will be used to assess nutrient availability and potential limitation in project streams, including nutrient diffuser experiments and empirical data collection and analyses of various chemical and biological metrics.

Nutrient diffuser experiments - Sanderson et al. (in press) have recently published an analysis of nutrient limitation in Idaho streams that used agar-based nutrient diffusing substrates to evaluate whether streams were limited by nitrogen, phosphorus, or some combination of both nutrients (co-limited). We will modify their protocols as needed in this study to characterize the nature of nutrient limitation and to subsequently evaluate how availability and limitation vary over time, as well as before and after experimental nutrient supplementation. Additional detailed methods for nutrient diffuser apparatus and protocols are provided below.

The following information further describing nutrient diffuser apparatus and experimental methods was summarized from Tank et al. (2007; Chapter 10, pgs. 215-216 in F. R. Hauer and G.A. Lamberti, eds., 2007: Methods in Stream Ecology):

Nutrient diffusing substrates (NDS) provide a fundamentally simple, cost-effective, yet informative means for determining whether primary production is nutrient limited, and if so, which specific nutrients (N,P, or both) may be limiting, as measured by periphyton or algal biomass and accrual. NDS are constructed using a series of small, sealed plastic cups or containers filled with nutrient-augmented agar and topped with an inorganic surface for periphyton growth, such as a glass disk, that provides the substrate for primary production. An array of cups is attached to an angle iron that can be securely staked into the substrate, where the replicated series of three nutrient treatments (N, P, combined N&P) and control cups is incubated in the river or stream for 18-20 days. Three nutrient diffusing substrate racks containing 32 randomized, replicated cups (8 for N, 8 for P, 8 for N+P, and 8 controls; no nutrients added) will be placed in each study river, in the upper, middle, and lower reach. (If resources are limiting, a single nutrient diffuser experiment could be performed exclusively in the downstream end of the farthest downstream river reach).

An example of a nutrient diffuser with four replicates (compared to our proposed 8) is pictured below, referred to as a "perihytometer" (http://nespal.cpes.peachnet.edu/images/carey%20figure%202.jpg).

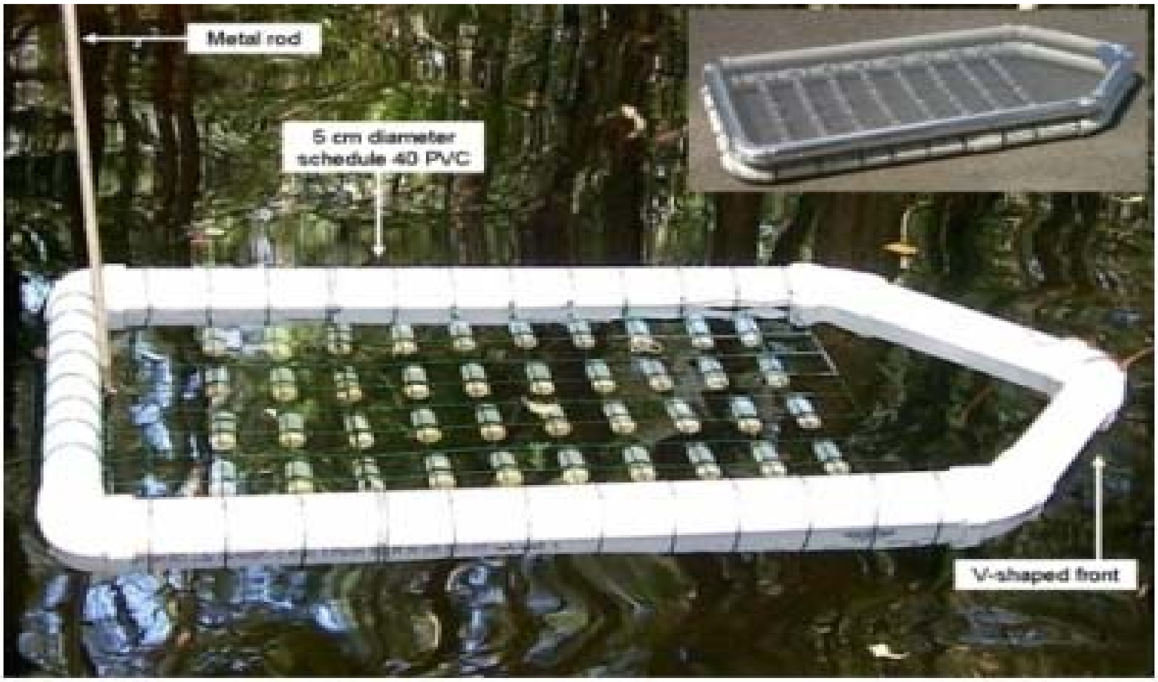


Figure 2. Photographs of periphytometer modified from Matlock et al. (1998) showing primary frame with forty 20 ml scintillation vials and inset showing an inverted picture of primary and secondary frames together; when deployed, the secondary frame lies beneath the primary frame.

Following this in-stream incubation period for periphyton growth, each glass disk will be removed from each cup with forceps and placed into individually labeled ziplock bags and stored on ice in a dark cooler for transfer to the lab where chlorophyll a biomass is estimated.

Statistical significance will be assessed assuming a two-factor (N, P) factorial arrangement and analyzed using analysis of variance (ANOVA) procedures. These tests will provide information on whether periphyton biomass was significantly affected by the single and combined N and P treatments, relative to the in-stream controls. Possible outcomes are presented in the following table.

N effect	P effect	N x P Interaction	Interpretation
*			Nitrogen limited
	*		Phosphorus limited
		*	N and P co-limited
*	*		N and P co-limited
*	*	*	N and P co-limited
*		*	Primary N-limited, secondary P-limited
	*	*	Primary P-limited, Secondary N-limited
			No limitation

* In each column indicates a significant N or P limitation in the two-factor *ANOVA* $(P< 0.05)$; * in the N x P column indicates a significant interaction between the two treatments indicates colimitation (N and P). No significant difference in algae biomass between treatments and controls indicates the absence of nutrient limitation. (Source: Tank et al. 2007; Page 216 *In*; Hauer and Lamberti 2007)

Use of empirical data to assess nutrient limitation – In addition to the nutrient diffusing substrate work described above, we will also use a series of empirical data analyses to assess nutrient status of study area waters. Historical escapement estimates, reconstructed historical nutrient availability, current nutrient ratios (e.g. N:P ratios), overall trophic status (e.g. ultraoligotrophy vs. mesotrophy), comparative primary and secondary productivity rates (algal/periphyton accrual) invertebrate taxonomic composition, as well as fish condition, abundance, and biomass information will be evaluated to assess potential nutrient limitation in project streams. Metrics representing these trophic levels and processes will be used to determine whether candidate study streams are nutrient limited, along with comparisons of current and historical (reconstructed) escapement scenarios. Analogous values in streams with production deemed to be healthy and productive will also be compared to values generated by this study.

As mentioned above, N:P ratios are typically used to determine whether systems are N-limited, P-limited or co-limited. These ratios can come from the NDS experiments and from empirical in-river water sampling data. The following information from Ashley and Stockner (2003) summarizes a standard method for assessing nutrient limitation: The Redfield ratio that is, the cellular atomic ration of C, N, and P in marine phytoplankton, provides a standard, useful benchmark for assessing nutrient limitation in aquatic systems, most commonly applied to N and P (Borchardt 1996). Rivers with atomic N:P ratios > 20:1 are considered P limiting, < 10:1 are considered N limited; at values between 10:1 and 20:1 the distinction is equivocal.

Methods by work element and trophic level

Due to the complexity of this proposal, and the importance of spatial and temporal alignment of sampling across trophic levels, project proponents provide the following short summary of the project below. More specific methods are then described by work element and ascending order of trophic levels.

To compensate for lost fisheries and the dramatic loss of nutrient input, the Yakama Nation's Upper Columbia Nutrient Supplementation Project is designed to quantify nutrient availability and biological production in the Twisp River (and subsequently in other rivers), quantify potential nutrient limitation of natural production of native anadromous salmonids, and experimentally add nutrients to increase natural production of salmon. To do so successfully requires an ecologically integrated approach to understand which nutrients are lacking and how they are routed through the food web from the water to juvenile salmonids.

This project assumes that a general food web in the Twisp River delivers nutrients from their elemental forms in water into primary production of algae that supports the benthic macroinvertebrate community, which then provides the food base for fish. Each of these trophic levels is linked, each relying on the lower supporting trophic levels, such that all are needed to successfully produce greater numbers of healthy, naturally produced salmon smolts to the outmigration phase.

Diagnosis and improvement of low smolt production requires simultaneous evaluation of nutrient availability and biological production within each trophic level. To accomplish this, the project will measure metrics in all trophic levels, including nutrient availability, chlorophyll biomass and accrual rate, algal community composition, and various aspects of the macroinvertebrate (aquatic insects) and fish community before and after experimental nutrient addition. The importance of each of these metrics is summarized in Table 1 and briefly described below.

Nutrients - Nine nutrient metrics characterizing the major nutrient sources and their biologically available forms will be systematically measured before and after experimental nutrient addition (total nitrogen (TN), and its components nitrite + nitrate (NO_2 + NO_3), and ammonia (NH_4), along with total phosphorus (TP) and its component parts total dissolved and soluble reactive phosphorus (TDP and SRP). Because the nitrogen to phosphorus ratio determines which kinds of algae will dominate the algal community (edible green algae and diatoms vs. inedible bluegreens), two N:P ratios will also be calculated (TN:TP, and DIN: TDP). In addition, nutrient diffuser experiments will be conducted to more accurately characterize whether study area waters are nitrogen-, phosphorus-, or co-limited. Nutrient diffuser experiments provide a series of fertilized substrates, some with N, some with P, and some with both N and P, to assess the nature of nutrient limitation. Results from this work will help identify the most appropriate experimental nutrient addition treatment.

Chlorophyll - Chlorophyll is vital for photosynthesis, which allows algae in rivers to convert sunlight into chemical energy, increasing algal biomass and production to feed invertebrates that feed fish. Separate chlorophyll biomass (g/m^2) and chlorophyll accrual estimates ($g/m^2/day$) will be calculated as a standard, comparable way of estimating primary production before and after experimental nutrient addition. These estimates will be calculated using standard chlorophyll samples (core punches) from submerged Styrofoam pads anchored to concrete tiles placed on the bottom of the river.

Algae taxonomy - Taxonomic composition of the algae community will also be assessed, at a relatively coarse level to address relevant ecological issues at a reasonable cost. Non-quantitative algae samples for taxonomic identification will be collected from scrapes of representative submerged rocks on the river bottom. Sampled algae will be identified as greens, diatoms, bluegreens, or other. This monitoring will ensure that an appropriate composition of algal taxa is maintained following nutrient addition. Ideally, nutrient addition should increase the presence of edible diatom and green algae and reduce toxic bluegreen algae. These algal community responses to nutrient addition should benefit the macroinvertebrate community, which feeds young salmon.

Macroinvertebrates - The project will also provide estimates for up to 19 invertebrate (aquatic insect) metrics, including abundance, biomass, production, diversity, and will characterize functional feeding guild representation before and after experimental nutrient addition. Abundance ($\#/m^2$) measures the number of organisms present, biomass (g/m^2) quantifies the amount of living tissue mass that is present at a given time per unit area, whereas invertebrate production will indicate the amount (weight or biomass) of invertebrates produced in a river over a given time period (g/m^2/month or year). Diversity measures will indicate how many invertebrate taxa (families, species, etc.) are present in the river, and will provide information about their relative abundances and representation within the invertebrate community. Assignment of sampled invertebrate taxa to functional groups (guilds) categorizes invertebrates by distinct feeding behaviors. Changes in the dominance or prevalence of certain invertebrate feeding guilds following nutrient addition will be compared with changes in the algae community to assess the routing of nutrients and energy from primary to secondary production, or from the algae to the invertebrate communities.

Fish – Because the main objective of this project is to increase the production and condition of juvenile anadromous salmon species in project waters, a series of standard, diagnostic fish metrics will be evaluated before and after nutrient addition. These metrics include:

1. Annual escapement/run size
2. Redd counts
3. Relative abundance and density
4. Individual fish length, weight and biological condition factor
5. Growth rates
6. Gut fullness
7. Diet composition
8. Annual smolt production
9. Smolt outmigration timing
10. Number of juveniles per red
11. Egg to emigrant survival

Annual escapement, measured as adult abundance provides a measure of the number of potential spawners available to seed habitat for natural spawning. Because there is no collection weir on lower portion of the Twisp River, annual counts of upstream migrating spawners corrected for the Twisp River fraction of the run will be used as a general annual index of run size. Redd counts provide a standard annual estimate or index of the amount of spawning in a surveyed river reach. Relative abundance is a standard comparative indicator of fish community health and river productivity, whereas individual fish length, weight, and condition factor are standard, comparative indicators of fish community health and river productivity. The project will also report fish density and biomass in the sampling areas between the two transects at each site. However, sampling limitation may preclude extrapolation of fish biomass and density estimates to the river scale. Fish growth rates are another valuable, comparable fish productivity and coarse scale fish health metric, because fish growth directly reflects the amount of food consumed in a river, which is a function of its availability. Gut fullness and diet composition reflect the amount and type of food eaten by fish, and describing food availability as it relates to fish health. Annual smolt production is a valuable annual comparative metric and is one of the benchmarks of program success. The number of juveniles per redd and egg to emigrant survival rates provide standard, comparable estimates of annual smolt production.

Finally, stable isotope analysis and bioenergetics modeling are proposed as a direct way to track, evaluate, and estimate effects of nutrient routing through the trophic levels before and after experimental nutrient addition. Stable isotope analysis and bioenergetics modeling can tell us about the sources of nutrients that produce fish food (algae and invertebrates), and whether and how these food sources and energy pathways change after adding nutrients to a river. This is the best way to determine if experimentally added nutrients are traveling through desired food web pathways across the trophic levels as edible algae to edible invertebrates, to fish food to ultimately increase growth, condition, and survival of juvenile anadromous salmon.

Trophic level	Metrics	Purpose of metrics	Sampling technique
Water quality/ nutrients	NH4	These standard nutrient metrics characterize nutrient availability and balance, pre- and post-treatment. The amount of these nutrients available determines that amount of biological productivity in rivers.	Sterile water bottles dipped in river
	NO2 + NO3		
	TN		
	TDP		
	TP		
	SRP		
	TDP		
	TN:SRP		
	DIN:TDP		
	Nutrient Diffusing Substrates	This experiment identifies if a river is nitrogen-, phosphorus-, or co-limited.	Replicated experimental containers with different amount of nutrients to quantify algae growth
Primary production	Chlorophyll biomass	Chlorophyll biomass and chlorophyll accrual estimates provide a standard way of estimating primary production.	Submerged tile samplers and standard Styrofoam area punch core
	Chlorophyll accrual		
	Algae taxonomy	Algal taxonomy (greens, diatom, bluegreens, other) provides algal community composition (proportion edible/inedible taxa) and assesses treatment effects on community composition.	Native substrate (rock) scrapings
Secondary production (Macroinvertebrates)	Abundance	Measures the number of organisms present	Hess sampler
	Biomass	Quantifies the amount of living tissue mass that is present in a population at a given time per unit area (e.g. g/m^2)	
	Diversity measures	Indicates how many invertebrate taxa (families, species, etc.) are present in a river, and provides information on their relative abundances and heterogeneity.	
	Functional Guilds	Categorizes invertebrates by groups (guilds) representing distinct feeding behaviors.	
	Invertebrate production	Indicates the amount (weight or biomass) of invertebrates produced in a river over a given time period (e.g. g/m^2/year).	

Trophic level	Life stage	Metrics	Purpose of metrics	Sampling technique

Fish	Spawners	Escapement	Standard index of annual run side	Fish counts at Wells Dam, Twisp River proportion
	Spawners	Redd counts	A standard estimate of the amount of salmon spawning in a surveyed river reach	Stream survey/redd counts, WDFW, YN, USFWS
	Parr, smolts	Relative abundance and density	Standard comparative indicators of fish community health and river productivity	Snorkel surveys and electro fishing as permitted
	Parr, smolts	Length, weight, condition factor	Standard comparable indicators of fish biological condition	Angling, electrofishing, screw trap
	Parr, smolts	Growth rates	Standard comparable fish productivity metric. The rate of fish growth directly reflects the amount of food consumed in a river	1) Angling and/or electrofishing with mark-recapture techniques in 100 m fish sampling zone between transects at each site; 2) subsequent recapture at downstream screw trap
	Parr, smolts	Gut fullness; diet composition	The amount and type of food eaten by fish describes food availability as it relates to fish health.	Lavage on fish captured by angling and/or electrofishing
	Smolts	Annual smolt production	A standard annual production metric	Expanded annual estimate from smolt trap data in lower Twisp
	Spawning adults and smolts	Juveniles/Redd	Standard comparable annual smolt production metric	Smolt enumeration at screw trap and Stream survey/redd counts, WDFW, YN, USFWS
	Spawning adults and smolts	Egg to emigrant survival	Standard, comparable annual smolt production metric	Fecundity estimates and screw trap data
	Invertebrates, parr, and smolts	Stable isotopes and bioenergetics modeling	Stable isotope analysis and bioenergetics modeling can tell us about the sources of nutrients that produced fish food and whether those sources change after adding nutrients to a river.	Stable isotope sampling from invertebrates and fish tissues

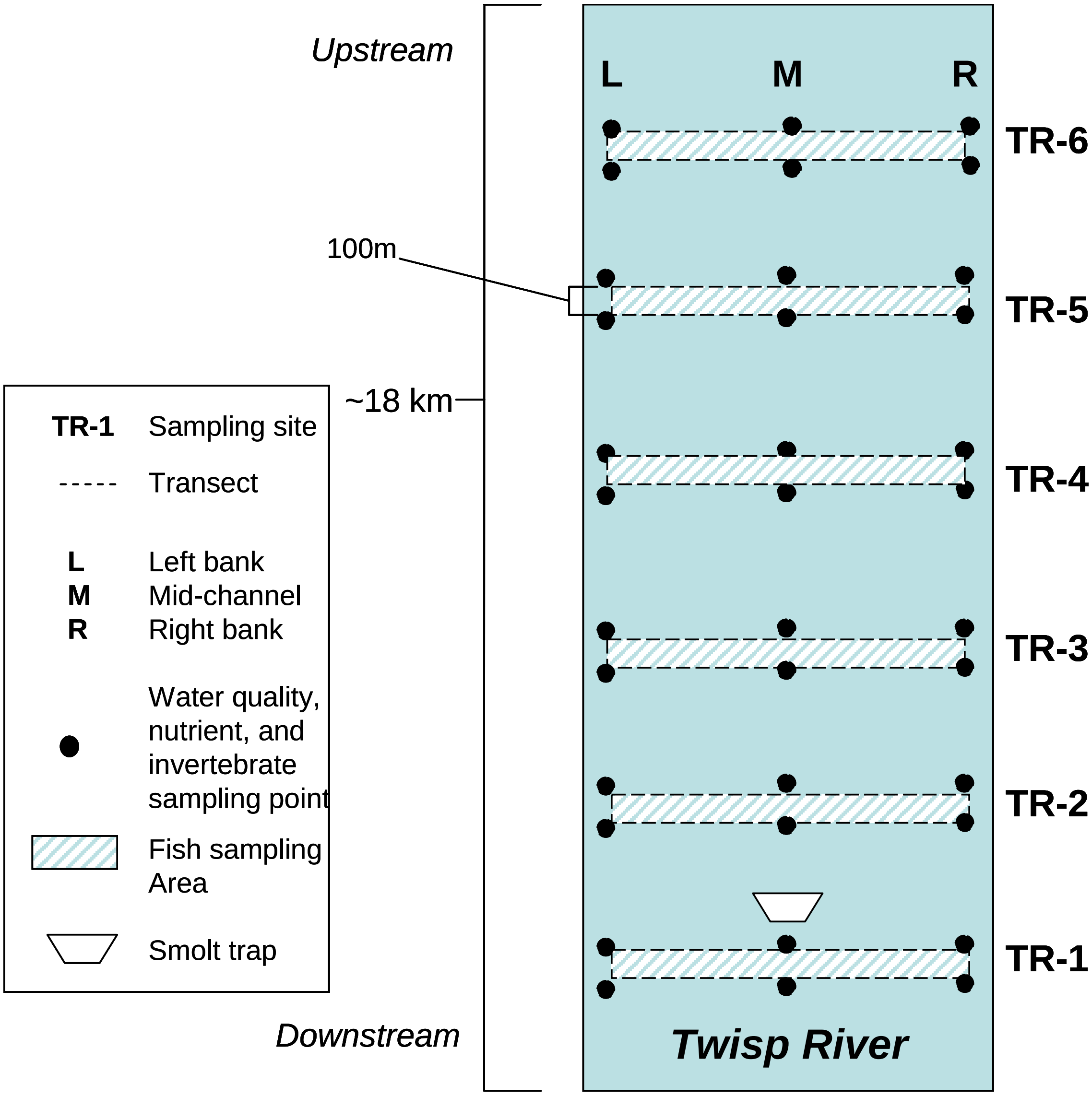


Figure 5. Schematic diagram of the Upper Columbia Nutrient Supplementation Program sampling design.

WE 157 - Collect/Generate/Validate Field and Lab Data - Current sites within the study area were chosen to represent habitat and biological conditions along the length of the Twisp River. Sampling at these sites will involve a minimum of three replicates, with adjustments made based on sample size and power analysis of empirical project data as needed. For example, if statistical power resulting from a given sampling regime is insufficient to separate nutrient addition treatment effects from background variability the spatial and temporal components of the sampling protocol will be assessed to determine what changes are necessary to observe such differences. These standard sites will be used for sampling water quality, estimating primary productivity, and characterizing the algal/periphyton and benthic macroinvertebrate communities.

A standardized multi-trophic level bio-assessment is proposed for all years of the 10-year study. Biomonitoring during the first three consecutive years will establish baseline conditions after Year 3. Continued implementation of this refined bio-assessment will be used to evaluate experimental nutrient addition if Objective 1 confirms significant nutrient limitation. Nutrient limitation and nutrient availability targets will be defined through collaborative regional group efforts supported and coordinated through this project, including development of decision pathways for evaluating nutrient addition options.

The assessment will include water quality, nutrient availability, Chlorophyll *a*, and total chlorophyll ($a + b$) concentrations and accrual rate, up to 19 aquatic benthic macroinvertebrate metrics. Benthic macroinvertebrate sampling will include stationary Hess samplers to quantify and characterize the invertebrate community occupying the substrates and the interstitial space in the underlying hyporheic zone. Stomach content sampling will occur throughout the sampling period spatially and temporally aligned with all other trophic level sampling. Fish will be sampled within the 100m reaches between both transects at each of the 6 sites. Due to the invasive nature of the procedure and existing sampling regulations, it may be necessary to consider stomach content analysis on exclusively non-threatened (unlisted under ESA) indicator fish species such as mountain whitefish (*Propospium williamsoni*), coho, and cutthroat trout. Such data are vital, however in order to assess diet item availability shifts and cascading trophic effects in response to experimental nutrient addition. Furthermore, any community data from other collaborative, sympatric projects will be incorporated into the ecological assessment.

More specific methodological information by trophic level is provided below:

Water Quality - Water samples will be collected monthly from the right bank, mid-channel, and left bank sections at each site to measure ambient nutrient concentrations as river conditions permit. Water quality sampling will occur from April through November (Appendix Table 1). As with all sampling in this project, sample size and power analyses will be performed as soon as adequate amounts of empirical data are collected to optimize sampling regimes based on sample representation and the associated empirical temporal and spatial variability. All samples will be collected in 250 mL bottles pre-rinsed with de-ionized water. All samples will be stored on ice and shipped to Aquatic Research Incorporated Laboratory in Seattle for analysis within 24 hours.

Water samples will be analyzed for soluble reactive phosphorous (SRP), total phosphorous (TP), total dissolved phosphorous (TDP), NO3+NO2, N:P ratios, and ammonia (NH_4 and total organic carbon (TOC). Minimum detection limits for TP and TDP will be 2 µg·L-1, 1 µg·L-1 SRP, 10 µg·L-1 for NO3+NO2, and 5 µg·L-1 for ammonia, and 0.25 mg/l for TOC.

Periphyton (Primary production) Analysis (algal accrual and Chlorophyll biomass) – Four standard chlorophyll metrics will be used to measure and characterize primary production at each site on each sampling date: 1) chlorophyll *a* biomass (mg/m^2); 2) chlorophyll *a* accrual rate (mg/m^2/30d); 3) total chlorophyll biomass (chlorophyll *a*+*b* (mg/m^2); and 4) total chlorophyll accrual rate (mg/m^2/30d). At each site, 4 to 6 algal accrual tiles will be deployed to assess primary productivity using these four metrics. Tile arrays will be run perpendicular to the riverbank to ensure consistent provision of monthly data across variation in river stage throughout the annual field season (April through November; Appendix Table 1).

Algae biomass - Total periphyton biomass will be calculated as an informative metric of primary productivity using a standard ash free dry weight procedure in the lab. Periphyton is a complex mixture of algae, cyanobacteria, heterotrophic microbes, and detritus attached to submerged substrates in most aquatic systems and provides additional information about primary production that algal studies alone do not. A standard ash free dry weight procedure will be used to estimate total periphyton biomass. Algae will be collected from standard punch cores sampled from Styrofoam blocks glued to the cement tiles monthly from April through November during each annual field season. Sample cores will be placed in Whirl-paks, stored in brown plastic bottles, and frozen at -20°C until delivery to the lab. Chlorophyll analysis will be performed by the University of Idaho Analytical Sciences Laboratory, Holm Research Center, (Moscow, ID) using the Winterman/DeMots method for extraction and analysis.

Algae taxonomy - Algal community composition reflects local nutrient availability, balance, and ecological river conditions. The periphyton taxonomy samples will be collected from natural substrates and preserved with Lugol's solution and 10% formalin. Algal taxa will be identified and taxonomically grouped as Cyanophyta (blue-greens), Chlorophyta (greens), or Bacillariophyta (diatoms), with further taxonomic identification carried out to genus where possible and beneficial. Dominant algal species and mean algal densities (#/ml) in periphyton taxonomic samples will also be calculated for each sample site and date.

Benthic macroinvertebrate taxonomy and secondary production - Benthic macroinvertebrates will be sampled monthly at right bank, mid-channel, and left bank positions on both transects at each of the six standard Twisp River sites from April through November as flow condition dictate using a standard Hess sampler.

Transects will be placed perpendicular to stream flow at each sampling site on cobble substrate within riffle and run habitats. On each transect the macroinvertebrates will be collected from the center of the channel and midpoint between the stream bank and center on both sides of the river (i.e. total of three collection sites/transect). At each of the three sampling positions on each transect, the benthos will be randomly sampled three times with Hess samplers and their contents pooled into a single sample container, such that 3 samples based on macroinvertebrates of nine Hess samples are taken from each site. However, during the spring months the center of the stream may be inaccessible due to high flows, in which case only two collection positions (right bank and left bank) would be sampled at each site. Sampling will be carried out monthly from April through November for a maximum of 144 samples per year. Sampling along a transect, as in the proposed study, will enable us to capture the known variability of invertebrate assemblages associated with depth gradients running from stream edge to stream center (see Merrit and Cummins 1996, p. 21).

All collected benthic invertebrates will be stored in 90% ethanol and delivered to Invertebrate Ecology Inc. (Moscow, ID.) for processing. At the lab, a minimum of 500 organisms will be randomly subsampled using a tray delineated into a grid. Subsequently, the macroinvertebrates will be sorted from substrate, identified by expert taxonomists to the finest level of taxonomic resolution, i.e. primarily genus and species level, and enumerated. To reduce processing time and thus costs, Chironomidae (midge flies) will only be identified to the family or subfamily level. Identification of the invertebrates to the genus and family levels will allow us to evaluate the response of specific taxa to nutrient addition. Lab personnel will also perform a large-rare sort of macroinvertebrates after the sort and enumerated.

In lotic habitats, Hess samplers provide a quantitative estimate of benthic macroinvertebrate density (Merrit and Cummins 1996, p. 13). In this study, mean density is simply calculated as the number of invertebrates captured/ area of the cylindrical Hess sampler that is pushed into the substrate. Hess samplers are designed to reduce escape of organisms and contamination from drift, two problems commonly associated with other aquatic invertebrate samplers, including a Surber sampler. Analysis of habitat proportion data from the Twisp River indicated that the study area is strongly dominated by lotic habitats (e.g. riffles and runs; approximately 76% of study area length) justifying the proposed sampling with a Hess sampler along fixed, transects at the 6 standard project sites.

Biomass (*B*) of invertebrate taxa groups and of all taxa combined will be measured directly using standard lab dry weight techniques, i.e. drying ovens (Benke 1996). Biomass of benthic invertebrates has been shown to be sensitive to nutrient addition, providing causal linkage for increased abundance of tertiary consumers (e.g. salmonid parr) after such treatments (Johnson et al. 1990).

Biomass will be used to calculate secondary production of these macroinvertebrates. Because of the complexity of the sample data (i.e. multivoltine taxa), we propose to use a noncohort technique, the Size-Frequency Method, for estimating secondary production (see Benke and Huryn 2007). In this method, the average density N (No./m^2) and biomass of invertebrate specimens is measured for a finite number of size classes, per taxon of interest, over the sample year. Size classes will be determined per taxon in the lab and from the literature, and measurements and weights will be taken in the laboratory. Production (P) is then calculated by multiplying ΔN (i.e. changes in density between size classes) by $\hat{W}$ (i.e. mean individual biomass between size classes), and subsequently summing the products (i.e. $\Delta N \times \hat{W}$) by size class after first multiplying the products per size class by the number of size classes. This later step is done to fulfill the assumption that the total number of size classes is equal to the number of cohorts per year. With these data, P/B values can be calculated for any desired time period, providing information on biomass turnover rates in the study area. Naturally, some macroinvertebrate taxa (e.g. some mayflies and midges, etc.) have very short life cycles, and sampling on a monthly basis may yield biased estimates when calculating secondary production for these taxa. On the other hand, most temperate macroinvertebrates are univoltine (Merrit and Cummins 1996), and sampling six sites extending over the proposed elevational and longitudinal gradients of the Twisp River will help to capture representative size classes for the multivoltine taxa.

We are aware of the potential biases associated with using preserved specimens in biomass and secondary production estimates (Leuven et al. 1985). Nevertheless, we feel there are few options to using ethanol-preserved specimens given the toxic and carcinogenic nature of formalin. We also believe that use of ethanol will not bias our results substantially for two reasons. First, we are only interested in relative comparisons of biomass or secondary production between the pre- and post-treatment periods. Biomass losses due to storage in preservatives will generally stabilize during the first 20 to 60 days of storage (Leuven et al. 1985, p. 157). Hence any biases due to the preservative should stabilize after this time period, allowing relative comparisons of faunal attributes. In addition, our sampling regime should provide an averaging effect on preservative-related biases given the number sampling locations and time periods during the pre- and post-treatment periods.

In addition to enumeration, taxonomic analysis and biomass determination community attributes will be analyzed and reported. Invertebrate community attributes will include structural or functional guild analyses, taxonomic and temporal and spatial analyses of other ecological metrics (e.g. diversity, richness, and others).

Fish metrics and sampling – Because the main objective of this project is to increase the production and condition of juvenile anadromous salmon species in project waters, a series of standard, diagnostic fish metrics will be evaluated before and after nutrient addition. These include:

1. Annual escapement/run size
2. Redd counts
3. Relative abundance and density
4. Individual fish length, weight, biological condition factor,
5. Growth rates
6. Gut fullness
7. Diet composition
8. Annual smolt production
9. Smolt outmigration timing
10. Number of juveniles per redd
11. Egg to emigrant survival

1. Escapement (Adult abundance) – Spawning anadromous salmonid abundance (escapement) will be estimated using a combination of direct counts and spawning ground surveys. Annual escapement, measured as adult abundance, provides a measure of the number of potential spawners available to seed habitat for natural spawning. Because there is no collection weir on lower Twisp River, annual counts of upstream migrating spawners corrected for the Twisp River fraction of the run will be used as a general annual index of run size.

Upstream passage counts – This project will integrate fish data currently being collected by WDFW to evaluate Chinook salmon and steelhead production within the Twisp River. Adult counts from all upstream (adult) passage facilities will be used to estimate adult abundance. These data are used to calculate smolt per redd, smolt per spawner, and recruit per spawner indices of production (See Snow et al. 2007; 2008).

2. Redd counts - Redd counts provide a standard annual estimate or index of the amount of spawning in a surveyed river reach. Spawning ground surveys (redd counts) performed by WDFW, YN, USFS, USFWS and USGS may also provide useful data for estimating escapement or adult abundance.

3. Relative abundance and density is a standard comparative indicator of fish community health and river productivity. Abundance and density measurements will be collected through snorkeling and electrofishing at the six 100 meter sections between existing transects. Although

these sections may not be representative of the entire study area, it will a rough estimate of abundance and density within our sampling areas and will serve as valuable annual and pre- and post-treatment indices to evaluate inter-annual variability and potential treatment effects.

4. Individual fish length, weight, and condition factor (e.g. Fulton's *K*) are standard, comparative indicators of fish community health and river productivity. Size of fish (including length-weight relationships) and biological condition factor at outmigration will be compared between and among years as well as within and among pre- and post-treatment years to assess biological condition of fish as a function of nutrient availability. Size and condition will be measured for multiple species at numerous locations throughout the study area.

5. Growth rates will be addressed using empirical data from marked fish that are recaptured downstream at outmigrant screw traps. Currently WDFW is tagging fish throughout the Twisp River, those fish are recaptured in the screw trap while migrating downstream. Project proponents are currently working with WDFW to increase their tagging effort. Capture efficiency is calculated daily. Growth rates will be compared between and among years and species as well as within an among pre- and post treatment years.

6. Gut fullness and 7) diet composition reflect the amount and type of food eaten by fish, and describing food availability as it relates to fish health. Stomach content sampling is one of the few ways to follow energy transfer from a secondary trophic level to a tertiary trophic level. An increase in invertebrate production may lead to increased fish condition and stomach sampling is a way to view that link. Consumption of carcass material would also be detected in the case of whole carcass or carcass analog treatment prescription. A proposed fish stomach content sampling scheme would sample fish within the 100 meter sections between transects at each site, this will allow us to spatially and temporally link trophic levels. All fish collected would be measured (TL), weighed, and possibly PIT tagged. Stomach contents from up to 20 fish per site per species (chinook, steelhead, possibly more from mountain whitefish) will be sampled using non-invasive lavage techniques. Sampling methods could include electroshocking, hook and line and or seining.

8. Annual smolt production - Annual smolt production is a valuable comparative metric and is one of the benchmarks of program success. The number of smolts produced per redd is an accepted, standard metric used to compare the relative productivity of Chinook and steelhead during freshwater rearing. This approach has been successfully used in the study area. WDFW will use a rotary screw trap data to estimate the number of spring Chinook salmon and summer steelhead smolts emigrating from the Twisp and Methow River basins. For example, 401 wild spring Chinook salmon smolts at the Methow River trap and 283 smolts at the Twisp River trap. A total of 180 and 333 wild steelhead emigrants were captured at the Methow and Twisp River traps, respectively. The number of these species captured each day was expanded by trap efficiency estimates derived from mark/recapture efficiency trials. Using this methodology, we estimate that a total of 33,710 wild spring Chinook salmon smolts emigrated from the Methow River, including 3,329 smolts emigrating from the Twisp River. An estimated 15,003 wild steelhead emigrated from the Methow River, including 3,312 fish from the Twisp River.

Using data gathered during spring Chinook salmon spawning ground surveys in 2005, we estimated that the number of emigrants produced from each 2005 brood spring Chinook salmon redd in the Twisp River (121) was 39.1% greater than the number of emigrants produced in the remainder of the Methow River basin (87). Steelhead in the Methow Basin and in the Twisp

River produced an estimated four and five emigrants from 2003 brood redds, respectively, although no estimate of age-1 emigration could be calculated for the Twisp River. Excluding Twisp River production, Methow Basin steelhead produced an estimated 4.1 emigrants per 2003 brood steelhead redd. While data for spring Chinook salmon for each trapping location were similar, we were unable to assess the relative contribution of naturally spawning hatchery fish to smolt production without similar data from non-supplemented reference populations.

WDFW will also measure smolt production in the study stream and how production responds to nutrient supplementation. It will be possible to calculate a density estimator for smolt production based on estimates of available rearing habitat (i.e. smolts per stream km or smolts per 100 m^2 of stream habitat). Smolt production will also be standardized on spawner numbers, as described above. Using estimated smolt production and mean fish weights, we will also estimate fish biomass/production which will be standardized by available habitat and spawner numbers. This study will determine whether data collected from outmigrants will be suitable to adequately assess all juvenile anadromous salmon performance and condition metrics. If analyses indicate that they are inadequate for this purpose, additional sampling will be adaptively implemented to ensure desired sensitivity of metrics and sampling (based on empirical sample size analysis).

To calculate total production and emigration estimates for species, we will apply the egg-to-smolt (immigrant) survival rates calculated for those redds upstream of trap to the estimated number of eggs deposited downstream of the trap. Total brood year smolt production estimates will be calculated by adding the estimated number of smolts produced downstream from the trap to the estimate of smolts produced upstream from the trap location.

9. Smolt outmigration timing – Although smolt outmigration timing is affected by in-river conditions such as water temperature and flow, it is an important response metric to ensure to avoid unintended consequence of nutrient addition treatments. Unsuitable conditions or increased food availability could affect smolt outmigration timing. Potential density-dependent and density independent relationships will be addressed by comparing total smolt production relative to spawner numbers (i.e. smolt to adult ratios), although multiple years of data would be required to reveal discernable patterns.

10. Number of juveniles per redd - The number of juveniles per redd and egg to emigrant survival rates provide standard, comparable estimates of annual smolt production.

11 Egg to emigrant survival - For spring Chinook salmon, egg deposition values used to calculate egg-to-emigrant survival will be derived from carcass surveys and hatchery broodstock sampling. For each brood examined, the number of redds deposited will be estimated by age and origin of the female spawning population within each basin as determined through spawning ground surveys. Each redd will then be multiplied by the mean fecundity values by age and origin determined through sampling of Methow Hatchery broodstock, and adjusted by the percent of eggs retained in the body cavity determined through spawning ground surveys. For summer steelhead, egg deposition values will be derived by multiplying the total number of redds in each basin by mean fecundity values by age and origin of the female steelhead population as determined through run composition and hatchery broodstock sampling at Wells Hatchery.

Implementation of such sampling efforts is dependent on take approval under ESA permits and adequate funding and resource allocation. Although measures of parr salmon and steelhead growth and in-stream biomass would also help judge the effects of nutrients on productivity, these measures are intermediate steps toward the critical measure of smolt production. Adding these in-stream measures will significantly increase costs for the proposed work and may not be possible with funds available.

Collectively, implementation of the fish sampling activities described above is expected to provide valuable data necessary to assess and compare annual fish production and performance attributes within and among pre- and post-treatment (fertilization) years. However, unlike proposed activities involving lower trophic level data collection and analysis in this study, fish data are currently being collected largely by regional collaborating agencies and are subject to ESA permitting and project budgeting approvals. If these data are found to be inadequate, unreliable, or unavailable during the study, we will evaluate and pursue options to collect needed fish metric data within this project's budget and scope of work.

Even with this information, adult (spawner) abundance and smolt production numbers are the result of a myriad of factors, many beyond the control and scope of this project. Furthermore, direct links between effects of nutrient addition and subsequent adult returns can be masked by many factors in the migration corridor, the estuary, and the marine environment, and during subsequent adult upstream spawning migrations. Therefore, results of this project are best evaluated within the freshwater rearing area until progeny produced in the study area migrate to the Mainstem Columbia River downstream.

Confounding factors - In terms of qualitative assessment of confounding factors, project personnel spend considerable time in the field involved in monitoring for this and other projects, and maintain communication with a network of other regional and local researchers. Thus, exposure to and familiarity with small and large scale habitat alterations, disturbances, and other stochastic environmental events such as floods, fires, spills, and large erosional events will help evaluate the degree to which such events might alter or confound data and their interpretation from this study.

Quantitative assessment of effects from above non-treatment variables and temperature, water year and flow aspects, and previously mentioned stochastic environmental effects (when data are available) on inter-annual variability in parr density, condition, and other fish metrics will involve various multivariate statistical approaches. Such approaches include means testing and correlative analysis, ANOVA, MANOVA, PCA, and other methods described below. Annual fish run size, condition, and distribution data and other available quantitative habitat and environmental data from the watershed and from the Twisp River specifically will be reviewed and evaluated to address this important issue.

Data collected by this project will also be provided for use in climate variability modeling being developed by the NOAA and the USGS. These agencies are jointly sponsoring a demonstration project in the Columbia River Basin with the aim of developing a practical integrated approach to organizing and collecting information about climate variability and change to support decision making at both regional and local scales. They intend to focus initial development of this approach through a pilot application looking at the impacts of climate variability and change and other factors on water availability and water management options in the Methow

Regarding density-dependent growth regulation, if food is/becomes limiting we would expect to see a response manifested as lower fish condition, length, weight, and smolt production per spawner, or possibly reduced numbers of outmigrants. Conversely, if nutrient augmentation increases food availability (relative to empirical pre-treatment values), we would expect to see some level of increase in mean fish length, weight, condition, production rates. If food is not limiting smolt production, then little response to nutrient augmentation should be observed relative to fish condition and production rate over time.

Regarding density-independent regulation, flow and temperature can directly affect system productivity, habitat suitability, and therefore fish growth and condition. Some of these responses occur in predictable a manner. One means to address effects of environmental condition is to monitor outmigration timing. Presumably, unsuitable conditions, such as low flows and high temperatures, would prompt early emigration of juvenile salmonids from rearing areas. By continuously operating screw traps at the mouth of the Twisp River throughout the outmigration season, and at any additional new locations, we will document outmigration patterns and events, such as premature emigration of parr and pre-smolt stages, along with the standard suite of fish performance metrics described above, and relate that to environmental conditions.

A final confounding factor when interpreting results of this project could be the presence of hatchery fish and the role of hatchery fish carcass outplanting in the study area. Although hatchery-produced juvenile anadromous salmon smolts (spring chinook and summer steelhead) are acclimated and released into project waters, most are released when they quickly exhibit outmigration, minimizing the degree and duration of ecological interaction with any naturally produced conspecifics. Stocking varies year to year in late April and May. The outplanted fish are almost all placed in an acclimation pond near the downstream end of the Twisp River. 100,000 steelhead are stocked per year, and coho are stocked as well. They should be able to identify supplemented fish by an ad clip, size and fin condition. Thus, this practice and the behavior of the released fish will minimize any confounding effects of project evaluation due to competition from releases of hatchery produced fish.

Regarding stocking of hatchery carcasses, up to 602 coho and 1,455 chinook carcasses were available during recent years for distribution throughout the basin, though currently they are distributed outside of study area. At this time, only carcasses from natural spawning (spring and summer Chinook, coho and steelhead) anadromous fish are found within the study area.

Bioenergetics modeling and isotope analysis - Bioenergetics modeling and stable isotope analysis are currently being developed for subsequent incorporation into this project, after the upcoming fiscal year, and will be proposed in more detail at that time.

Bioenergetics modeling could improve this project by developing a framework to: 1) estimate the extent to which increased food resources could increase juvenile salmonid food consumption, and 2) predict how individual fish growth may change with increased food resources. Applicable models would use metrics from both the abiotic (e.g. temperature, flow) and biotic environments (e.g. food availability) to: 1) predict juvenile salmonid consumption and growth, 2) determine whether and where productivity may be limiting fish production, and 3) assess how fish might respond to experimental nutrient addition.

The data we are proposing to collect (fish diet, stream temperature, fish body size and mass over time, etc.) would be useful in developing bioenergetics models that can help predict and explain ecological responses to experimental treatments as part of this study if the study streams are found to be nutrient deficient. This approach would involve assessing how food availability might change (both direct from carcasses and indirect pathways) via bottom up increases in periphyton and invertebrate abundance, biomass, and richness. Subsequently collected empirical data could be incorporated into the model for additional informed runs. The project could also consider using diet, temperature, and fish body size information to model and compare fish performance before and after experimental nutrient supplementation.

To supplement and inform the general bioenergetics approach described above, stable isotope monitoring can provide information about how carcass derived nutrients are incorporated into the food web and whether those nutrients may impact the growth and survival of juvenile salmon and steelhead. Data from stable isotopes would inform bioenergetics models by quantifying important trophic linkages.

WE 160 - Create/Manage/Maintain Database

Data quality issues are very important when conducting long-term multifaceted studies involving several teams of researchers. Without the use of a standardized protocol, independent data collection is often carried out by separate research efforts, all too commonly leading to inconsistencies, confusion, and errors throughout the larger project.

A database management system will be used to help avoid the aforementioned problems. The centralization of data into a common relational unit (i.e. a relational database) shifts the responsibility for data quality and maintenance from multiple individuals to a single database manager, thus allowing data quality issues to be assessed and resolved in a timely manner. The proven relational database system proposed also provides a convenient, efficient mechanism for standardizing data components, such as variable names and values uniformly across all segments of a project. This is particularly important when data are collected from a variety of locations, times, and by different personnel.

For the database user, the efficiency of database functions is maximized by using data formats based on familiar software products such as Excel or Quatro Pro. For the project manager, the database facilitates monitoring and evaluating data quality and data collection. Project and identified cooperating resource managers can track all aspects of data collection as they happen and can pinpoint areas that need attention.

In sum, the proposed relational online, secured database system will integrate all segments of a large, multidisciplinary ecological study into one organizational and functional unit at one location, while providing oversight and accessibility to the data collection process. The quality of all data collected is uniformly maintained and compatibility among research efforts is thus ensured. While the physical database would exist in a central location, access will not be physically limited. Database interfaces can be created to operate over the internet, allowing project members to access their data from virtually anywhere. These interfaces provide users with the ability to upload, download, edit, and search data remotely creating a dynamic system that is continually updated with the most recent information. At the same time, data are protected through user access restrictions. For example, researchers might be able to read any data, but only edit data from their own project. This accessibility could be set to any combination of read/write/edit abilities from an administrator capacity with full access to all data, to a highly restricted public access capability limited to general project information. Generation of customized summary reports, such as graphs or tables, will also be easily obtained through a web based interface. Using this type of feature, users can track trends over time or location, compare results from various disciplines and evaluate, for example, average responses. Exploration of data in this manner will help users define and clarify their research goals as well as provide a means of integrating the various disciplines of a larger research project.

In terms of data warehousing and archiving, project crews will collect data, produce and proof an Excel database. These data will be backed up electronically and in hard copy form, and will be archived separately on site and off site. These data (in spreadsheet form) will then be sent electronically to Statistical Consulting Services Inc (SCS). After receiving the data, SCS will back them up on and off site locally, and if necessary will repeat this process after any and all proofs and edits/modifications are completed. Data will then be uploaded onto the web-based relational database, which is housed on a dedicated machine.

Furthermore, construction and maintenance of a centralized database management system will be monitored and updated by a designated database manager to address data quality assurance and maximize efficiency in dissemination of information. Periodic upgrades and enhancements to this system will ensure availability of quality data in real time, and validity of statistical analyses and interpretations for which such data are will be utilized. Additionally, housing all databases for related basin projects in one central, accessible, protected location will allow for consistency and efficient use of data among projects.

We will incorporate all project data into the relational database as they are collected and become available. System enhancements may include full text data descriptions for all incorporated components, implementation of data availability matrix for every component of the project, implementation of various mapping formats including topographic, GIS, etc, addition of data censoring options for all trophic level data, restructuring and enhancement of graphic capabilities (line plots, bar plots, pie charts), incorporation of multi-trophic/multi-year plotting routines, and implementation of more advanced security features.

WE 162 - Analyze/Interpret Data

Data description - Numerous biological and ecological response variables or metrics will be evaluated by site and by year for all sites and periods of data availability. These are discussed in the next section (10.F.4 Metrics). Response variables will include:

Water Quality (Including nutrient availability and primary productivity/chlorophyll accrual rates): Water samples will be analyzed for soluble reactive phosphorous (SRP), total phosphorous (TP), total dissolved phosphorous (TDP), NO3+NO2, N:P ratios, and ammonia (NH_4 and total organic carbon (TOC). Minimum detection limits for TP and TDP will be 2 µg·L-1, 1 µg·L-1 SRP, 10 µg·L-1 for NO3+NO2, and 5 µg·L-1 for ammonia, and 0.25 mg/l for TOC.

Chlorophyll/Primary production: Chlorophyll a concentration (mg/m^2) and chlorophyll a accrual rate (mg/m^2/ 30 days), and total chlorophyll (chlorophyll a + b; mg/m^2) and total chlorophyll accrual (mg/m^2/ 30 days) will be calculated.

Algae/Periphyton: abundance, biomass, total richness (# of species), richness by taxa, taxa composition represent a standard suite of algae and periphyton metrics.

Benthic macroinvertebrates: Abundance, biomass, numerical and percent richness by feeding ecology functional group (e.g. filterer, gatherer, predator and scraper).

Fish datasets will include numbers of juveniles collected, collection method, collection sites, fish size (length, weight), condition (K), estimated age class, numbers marked (PIT-tagged), PIT tag codes, all recapture histories. PIT tag files will be loaded to the PTAGIS database. Recaptures of PIT tagged fish will be retrieved from PTAGIS. Adult datasets will include numbers of fish of each species, fish size, recaptures of marked fish, and index redd counts for study areas.

Annual salmon and steelhead outmigrant (smolting) data will also be available through the WDFW operated rotary smolt trap on the Twisp River. Fish data will be correlated with empirical project water quality, nutrient availability, primary and secondary productivity data and metrics to assess effects of the project and to characterize system effects on salmonid status and productivity

Juvenile fish data will include: approximate abundance of summer parr, growth rates, smolt abundances, estimated population size (from mark-recapture calculations), fish size, growth, and condition factor (K), growth.

Adult fish data: numbers returning to weirs, size, condition factor (K), growth rates, estimated survival (requires estimate of ocean and downstream harvest rates), redd and carcass counts in river study reaches.

Statistical analyses - Sample size, power analysis, multivariate analyses and Analysis of Variance tests will be performed using data from each trophic level or community to assess nutrient addition effects.

A minimum of two and preferably three years of statistically adequate pre-treatment biomonitoring are required to produce a reasonable baseline condition for the study rivers. Empirical data from the first year will be used to provide data for the sample size determination needed to ensure an adequately rigorous sampling design for subsequent pre- and post-treatment years. Multivariate techniques such as PCA will be performed to reduce the dimension of biological community data and to determine which taxonomic groups and metrics are contributing significantly to observed variation. Data will be selected to represent taxonomic orders and biological or ecological metrics that are common across dates and sites. ANOVA will be performed annually using data from each and all years to investigate the average algal/periphyton and macroinvertebrate abundance, biomass, and richness, to test for site or time effects on these metrics, and to assess effects of experimental nutrient addition. Transformation of response variables will be used when necessary. All summaries, tests, and graphics will be performed using the SAS package. These analyses will be done annually before and after experimental nutrient addition (if that is warranted and implemented) to determine and characterize treatment effects in terms of water quality, nutrient availability and composition, and all relevant response variables in the algal/periphyton, invertebrate, and fish communities.

We intend to characterize current productivity of fish communities in the Twisp River focusing on chinook salmon and steelhead, and if warranted, to test if productivity improves with experimental nutrient addition. Primary metrics of productivity include: (1) juvenile outmigrant abundance, a nominal measure of smolt production, as determined from catch-per-unit effort (numbers of fish collected per hour of trap operation), (2) estimated total outmigrant abundance (calculated from mark-recapture methods), (3) smolts per spawner and, for later years, (4) smolt-to-adult ratios (SARs). Secondary measures of productivity will include (5) summer parr (subyearlings) abundance indices (catch-per-unit-effort), (6 & 7) juvenile and adult fish condition (K), (8) mean growth rate, and (9) survival between key life stages.

Differences in productivity associated with nutrient supplementation will be tested using mean separation procedures, potentially adjusted by covariates to determine if treatment effects are present. We will also used regression techniques to evaluate what independent variables are best associated with the variability in production metrics.

Objectives 2 and 3 only

Initial responses to nutrient addition – Analysis of Variance (ANOVA) will be used to assess aggregated algal/periphyton, invertebrate and fish abundance and biomass, richness, and taxonomic order composition.

Expected results - We anticipate that pre-treatment sampling will indicate nutrient deficiency as reflected in low algal abundances, low macroinvertebrate density and diversity, low juvenile densities, low fish condition factors and growth rates, low smolt-adult ratios, and potentially premature emigration by juvenile salmonids. Nutrient additions may have the greatest influence on primary productivity in terms of increase algal and periphyton biomass, with commensurate increases seen in grazers and tertiary predators. Higher food availability may increase summer parr fish condition and translate to higher juvenile abundances in late summer and possibly as outmigrants the following spring.

WE 132 - Produce (Annual) Progress Report

This work element covers written reports of results that typically are submitted to BPA at the end of a contract period for dissemination to the public. Previously called "Annual" reports, these progress reports may cover less than a year or multiple years. They are not required or appropriate for all contracts in all years, but are particularly important when useful results are not captured by standard Pisces metrics or status reports, or prior to project-based publications in the peer-reviewed literature.

WE 183 - Produce Journal Article

This work element applies to manuscripts being submitted for publication. Preliminary analyses towards the publication of a journal article can be covered by WE# 132 (above): Produce (Annual) Progress Report.

WE 44 - Enhance Nutrients Instream

This work element addresses possible actions for Objectives 2 and 3 if satisfaction of Objective 1 confirms significant nutrient limitation. This section will be further developed if and when baseline monitoring data for water quality, nutrients and the algae, periphyton, and invertebrate and fish communities indicate nutrient limitation during pre-treatment years.

For project planning purposes most nutrient enrichment programs will be adequately described by characterizing the following seven variables as recommended by Ashley and Stockner (2003), after quantifying baseline conditions in all project trophic levels.

1. Desired nutrient concentrations;
2. Formulation of nutrient source;
3. Seasonal timing of application;
4. Frequency or duration of nutrient addition;
5. Location of application;
6. DIN:TDP ratio of nutrients to be added; and
7. Application techniques.

10.F.4 Metrics

A large series of metrics are involved in the multiple trophic level bio-assessment program proposed for implementation in this project. A comprehensive list of all metrics is presented in table 1 of Section 10.A.2 "Executive Summary:, and are listed below as requested.

Water quality and nutrient metrics will include: standard metals and water chemistry parameters, soluble reactive phosphorous (SRP), total phosphorous (TP), total dissolved phosphorous (TDP), NO3+NO2, N:P ratios, NH4, total organic carbon (TOC) and Chlorophyll a.

Metrics for the algae/periphyton community may include: abundance, biomass, species richness, diversity indices (e.g. Shannon Weaver), nitrogen uptake, oxygen tolerance, trophic state, richness by trophic state, and morphological type.

Metrics for the benthic macroinvertebrate community may include up to 19 variables provided in the following table.

Metrics	Units
Abundance	Numbers/m^2
Biomass	g/m^2
Richness	Overall number of species sampled
EPT_Richness	Number of species in the Orders Ephemeroptera, Plecoptera and Trichoptera
E_richness	Number of species in the Order Ephemeroptera
P_richness	Number of species in the Order Plecoptera
T_richness	Number of species in the Order Trichoptera
Filterer_richness	Number of species in "Filterer" functional group
Gatherer_richness	Number of species in "Gatherer" functional group
Predator_ richness	Number of species in "Predator" functional group
Scraper_richness	Number of species in "Scraper" functional group
p_ Ephemeroptera	% of Order Ephemeroptera
p_ Plecoptera	% of Order Plecoptera
p_ Trichoptera	% of Order Trichoptera
p_ Filterers	% of "Filterer" functional group
p_ Gatherers	% of "Gatherer" functional group
p_ Predator	% of "Predator" functional group
p- Scraper	% of "Scraper" functional group
Shannon	Shannon's index of diversity

Because the main objective of this project is to increase the production and condition of juvenile anadromous salmon species in project waters, a series of standard, diagnostic fish metrics will be evaluated before and after nutrient addition. These metrics include:

1. Annual escapement/run size
2. Redd counts
3. Relative abundance and density
4. Individual fish length, weight and biological condition factor
5. Growth rates

6. Gut fullness
7. Diet composition
8. Annual smolt production
9. Smolt outmigration timing
10. Number of juveniles per redd
11. Egg to immigrant survival

10.G Monitoring and evaluation

The objectives of this project are to:

(1) Determine the nutrient status of the Methow and Twisp rivers, and if productivity is nutrient limiting. If found to be limiting then:

(2) Conduct an experimental manipulation to supplement nutrients and evaluate the effectiveness to increase primary, secondary and tertiary productivity in the system, with the ultimate goal of restoring ecological processes to something approaching historical levels. Finally, we propose to:

(3) Use results from the first two objectives to evaluate the feasibility of scaling up these methods to larger geographical areas and/or applying them to additional rivers within the Columbia River Basin.

Data from Objective 1 of this study will be used to determine if the Methow basin is currently nutrient deficient. See proposal section entitled "Approach to identifying nutrient limitation" on Page 15).

Appropriate null hypotheses to test include:

$H_{o1.1}$: Algal abundance is within acceptable limits for salmon systems not considered to be nutrient limited.

$H_{o1.2}$: Macroinvertebrate abundance and diversity indices are within acceptable limits for salmon systems not considered to be nutrient limited.

$H_{o1.3}$: Fish production, growth rates, and adult escapement numbers are within acceptable limits for salmon systems not considered to be nutrient limited.

The second phase (Objective 2) of the proposed study involves evaluating the effectiveness of nutrient supplementation to improve system productivity. This evaluation would involve comparing data from pre- and post-treatment time periods using inferential statistics. Appropriate null hypotheses include:

$H_{o2.1}$: Water nutrient levels are not significantly different between pre- and post-treatment periods.

$H_{o2.2}$: Algal and periphyton abundance and diversity are not significantly different between pre- and post-treatment periods.

$H_{o2.3}$ Primary productivity rates are not significantly different between pre- and post-treatment periods.

$H_{o2.4}$: Benthic macroinvertebrate abundance and diversity are not significantly different between pre- and post-treatment periods.

$H_{o2.5}$: Salmon productivity metrics are not significantly different between pre- and post-treatment periods.

Comparisons between sites or grouped sites upstream and downstream from an experimental nutrient addition site could also be performed within years to further characterize effects of nutrient addition.

Finally;

$H_{o3.1}$: Results from Objectives 1 & 2 cannot be scaled up to large geographical areas or applied to other subbasins of the upper Columbia River.

Sampling, collecting, and storing data will be done using existing tribal field office resources (vehicles, computers, microscope, waders, Hess samplers etc.)

Stored samples needing further lab analysis will be sent to appropriate contractors. Proposed contractors include: Aquatic Research Institute, the Holm Center, University of Idaho and Eco Analysts. Further Statistical analysis and database development will completed by Statistical Consulting Services. These contractors will be responsible for the equipment to complete their tasks.

If after the assessment period has been completed (up to 3 years) and a nutrient prescription is needed, additional qualified subcontractors (See Key Personnel section, 10.J) will be needed to complete those specific tasks. Tribal facilities will grow to meet the need of the project at that time.

10.H Facilities and equipment

Sampling, collecting, and storing data will involve existing tribal field office and program resources (vehicles, computers, microscope, waders, Hess samplers etc.).

Stored samples needing further lab analysis will be sent to appropriate contractors. These contractors are Aquatic Research Institute, the Holm Center, University of Idaho and Eco Analysts, both in Moscow, ID. Further Statistical analysis and database development, operations, and maintenance will be performed by Statistical Consulting Services, in Clarkston WA.. These contractors are responsible for the necessary equipment to complete their tasks.

After the assessment period has been completed (up to 3 years), if a nutrient prescription is needed, additional contractors may be needed to complete those specific tasks (Ward and Associates, other key personnel (see Section 10.J, “Key Personnel”). Associated tasks may include cost-benefit analysis, site selection, and interaction with the regulatory agencies. Some of these activities could occur during the first three years of the project as directed by empirical data analysis. For example, if study waters are found to be nutrient limited or imbalanced, forecasting approximate experimental nutrient addition loads (by weight/volume) and assessing requirements of holding and dosing site facilities could be required. Tribal facilities may need to be expanded as needed to meet all the needs of this project as future data analysis warrants.

10.I References

Ashley, K. I., and J. G. Stockner. 2003. Protocol for applying limiting nutrients to inland waters. American Fisheries Society Symposium 34: 245-258.

Barbour, M.T., et.al. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish, second edition. EPA 841-B-99-002. Office of Water, U.S. EPA, Washington, D.C. http://www.epa.gov/owow/monitoring/rbp/

Ben-David, M., T. A. Hanley, T. R. Klein, and D. M. Schell. 1997. Timing of reproduction in wild mink: the influence of spawning Pacific salmon. Canadian Journal of Zoology 75:376–382.

Benke, A.C.1996. Secondary production of macroinvertebrates. Pages 557 – 578 *in* Methods in stream ecology. Academic Press, San Diego.

Bilby, R.E., E.W. Beach, B.R. Fransen, J.K. Walter, and P.A. Bisson. 2003. Transfer of nutrients from spawning salmon to riparian vegetation in western Washington. Transactions of the American Fisheries Society. 132:733-745.

Borchardt, M.A. 1996. Nutrients. Pages 183-227 *In:* R. Stevenson, M. L. Bothwell, and R.L. Lowe editors. Algal ecology: Freshwater benthic ecosystems. Academic Press Inc. San Diego, California.

Bothwell. M. L. 1989. Phosphorus-limited growth dynamics of lotic periphyton diatom communities: aerial biomass and cellular growth rate responses. Canadian Journal of Fisheries and Aquatic Sciences 49:1890-1901.

Cederholm C.J., M.D. Kunze, T. Murota, and A. Sibatani. 1999. Pacific salmon carcasses: essential contributions of nutrients and energy for aquatic and terrestrial ecosystems. Fisheries, 24, 6–15.

Cederholm, C. J., M. D. Kunze, T. Murota, and A. Sibatani. 2000. Pacific salmon carcasses. Fisheries. 24:6-15

Cederholm, C.J., and thirteen co-authors. 2001. Pacific salmon and wildlife — ecological contexts, relationships, and implications for management. *In* Wildlife–habitat relationships in Oregon and Washington. *Edited by* D.H. Johnson and T.A. O'Neil. Oregon State University Press, Corvallis, Oregon. pp. 628–684.

Gende, S. M., R.T. Edwards, M.F. Willson, and M.S. Wipfli. 2002. Pacific salmon in aquatic and terrestrial ecosystems. BioScience 52:917-928.

Gresh, T., J. Lichatowich, and P. Schoonmaker. 2000. An estimation of historic and current levels of salmon production in the northeast Pacific ecosystem: evidence of a nutrient deficit in the freshwater systems of the Pacific Northwest. Fisheries 25: 15-21.

Halbert, C. L. 1993. How adaptive is adaptive management? Implementing adaptive management in Washington state and British Columbia. *Reviews in Fisheries Science* 1:261-283.

Hauer, F.R., and G.A. Lamberti, eds., 2007. Methods in Stream Ecology. Second Edition. Academic Press. 877 pp.

Hilborn, R. 1992. Hatcheries and the future of salmon in the northwest. Fisheries 17:5-8.

Hildebrand, G.V., C.C. Schwartz, C.T. Robbins, M.E. Jacoby, S.M. Arthur and C. Servheen. 1999b. The importance of meat, particularly salmon, to body size, population productivity, and conservation of North American brown bears. Canadian Journal of Zoology 77:132-138.

Hildebrand, G.V., T.A. Hanley, C.T. Robbins, and C.C. Schwartz.1999a. Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia. 121:546-550.

Hillman, T., M. Miller, C. Peven, M. Tonseth, T. Miller, K. Truscott, and A. Murdoch. 2007. Monitoring and Evaluation of the Chelan County PUD Hatchery Programs: 2006 Annual Report. *Prepared for:* Public Utility District Number 1 of Chelan County, Wenatchee WA.

Holderman, C., P. Anders, B. Shafii and G. Lester. 2009a. Characterization of the Kootenai River aquatic macroinvertebrate community before and after experimental nutrient addition, 2003-2006. Report to Kootenai Tribe of Idaho and Bonneville Power Administration. 94 pp.

Holderman, C., P. Anders and B. Shafii. 2009b. Characterization of the Kootenai River algae and periphyton community before and after experimental nutrient addition, 2003-2006. Report to Kootenai Tribe of Idaho and Bonneville Power Administration. 76 pp.

Holling, C. S. 1978. Adaptive Environmental Assessment and Management. John Wiley and Sons, London. http://www.consecol.org/vol1/iss2/art1

Johnson, N.T., C. J. Perrin, P.A. Slaney, and B.R. Ward. 1990. Increased juvenile growth by whole river fertilization. Canadian Journal of Fisheries and Aquatic Sciences 47:136-144.

Kline, T.C., C.A. Woody, M.A. Bishop, S.P. Powers, and E.E. Knudsen. 2007. Assessment of Marine-Derived Nutrients in the Copper River Delta, Alaska, Using Natural Abundance of the Stable Isotopes of Nitrogen, Sulfur, and Carbon. American Fisheries Society Symposium 54:51-60.

Kohler, A.E., A. Rugenski, and D. Taki. 2008. Stream Food web response to a salmon carcass analogue addition in two central Idaho, U.S.A. streams. Freshwater Biology (53): 446-460.

Martens, K.D. and Connolly, P.J. 2008. Lower Methow tributaries intensive effectiveness monitoring study. Interim Report. U.S.G.S., Cook, WA. 71 p

Merrit, R.W. and K.W. Cummins. 1996. Aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Moore J.W. and D.E. Schindler. 2004. Nutrient export from freshwater ecosystems by anadromous sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences, 61, 1582–1589.

Mullan, J. W., K. R. Williams, G. Rhoduc, T. W. Hillman, J. D. McIntyre. 1992. Production and habitat of salmonids in mid-Columbia River tributary streams. Monograph 1 U.S. Fish and Wildlife Service, Leavenworth, WA.

Mundie, J.H, K. S. Simpson, and C.J. Perrin. 1991. Responses of stream periphyton and benthic insects to increases in dissolved inorganic phosphorus in a mesocosm. Canadian Journal of Fisheries and Aquatic Sciences 48:2061-2072.

Naiman, R.J., R.E. Bilby, D.E. Schindler, and J.M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems 5:399-417.

Northwest Power Planning Council (NPPC) 2004. Columbia Basin Fish and Wildlife Program. Portland, ORhttp://www.nwcouncil.org/fw/Default.htm

NRC (National Research Council). 1996. Upstream: salmon and society in the Pacific Northwest. National Academy Press, Washington D.C.

O'Keefe, T.C., and R.T. Edwards. 2003. Evidence for hyporheic transfer and removal of marine-derived nutrients in a sockeye stream in Southwest Alaska. American Fisheries Society Symposium 34: 99-107.

Peery, C.A, K. Kavanaugh, and J.M. Scott. 2003. Pacific salmon: Setting biological defensible recovery goals. Bioscience 53:622-623.

Peterson, B. J. and 16 coauthors. 1993. Biological responses of a tundra river to fertilization. Ecology 74:653-672

Quamme, D. L. and P.A. Slaney. 2003. The relationship between nutrient concentration and stream insect abundance. American Fisheries Society Symposium 34: 163-175.

Reimchen, T. E., D. D. Mathewson, M. D. Hocking, and J. Moran. 2003. Isotopic evidence for enrichment of salmon-derived nutrients in vegetation, soil, and insects in riparian zones in coastal British Columbia. American Fisheries Society Symposium 34:59-69.

Ruckelshaus, M.H., P. Levin, J.B. Johnson, and P.M. Kareiva. 2002. The Pacific salmon wars: what science brings to the challenge of recovering species. Annu. Rev. Ecol. Syst. 33:665-7-6.

Sanderson, BL, H Coe, CD Tran, K Macneale, D Harstad, A Goodwin. In press. Nutrient limitation of periphyton in Idaho streams: results from nutrient diffusing substrate experiments. Journal of the North American Benthological Society.

Scheuerell M.D., P.S. Levin, R.W. Zabel, J.G. Williams, and B.L. Sanderson. 2005. A new perspective of marine derived nutrients to threatened stocks of Pacific salmon (*Oncorhynchus spp*.). Canadian Journal of Fisheries and Aquatic Sciences, 62, 961–964. Stockner, J. G. (editor). 2003. Nutrients in salmonid ecosystems: sustaining production and biodiversity. American Fisheries Society Symposium 34, Bethesda, MD.

Snow, C., C. Frady, A. Fowler, and A. Murdoch. 2007. Monitoring and evaluation of Wells and Methow hatchery programs in 2006. Prepared for Douglas County Public Utility District and Wells Habitat Conservation Plan Hatchery Committee, by Washington Department of Fish and Wildlife, Methow Field Office, Twisp, WA.

Snow, C., C. Frady, A. Fowler, and A. Murdoch. 2008. Monitoring and evaluation of Wells and Methow hatchery programs in 2007. Prepared for Douglas County Public Utility District and Wells Habitat Conservation Plan Hatchery Committee, by Washington Department of Fish and Wildlife, Methow Field Office, Twisp, WA.

Stephenson, J.M., G. Carr, U. Gruenert and A. Morin. 2007. Evaluation of two timesaving techniques for processing benthic invertebrate samples for estimating secondary production. Journal of the North American Benthological Society 26(4):611-619.

Stockner, J. Editor. 2003. Nutrients in salmonid ecosystems: Sustaining production and biodiversity. American Fisheries Society Symposium 34.

Stockner, J.G. and K.I. Ashley. 2003. Salmon nutrients: Closing the circle. Pages 3-15 *In*: Stockner, J. Editor. 2003 Nutrient s in salmonid ecosystems: Sustaining production and biodiversity. American Fisheries Society Symposium 34.

Tank, J.L., M.J. Bernot, and E.J. Rosi-Marshall. 2007. Nitrogen limitation and uptake. Chapter 10, pgs 213-238, *In*: Hauer, F.R., and G.A. Lamberti, eds., 2007. Methods in Stream Ecology. Second Edition. Academic Press. 877 pp.

Upper Columbia Salmon Recovery Board (UCSRB) 2007. Upper Columbia Spring Chinook Salmon and Steelhead Recovery Plan, August 2007 http://www.ucsrb.com/UCSRP%20Final% 209-13-2007.pdf

Van Winkle, W., Jager, H.I., Railsback, S.F., Holcomb, B.D.,Studley, T.K., Baldrige, J.E., 1997. Individual-based model for sympatric populations of brown and rainbow trout for instream flow assessment: model description and calibration. Ecol. Model. 110, 175–207.

Walters, C. 1997. Challenges in adaptive management of riparian and coastal ecosystems. Conservation Ecology [online]1(2):1. Available from the Internet. URL:

Walters, C. J. 1986. Adaptive management of renewable resources. McMillan, New York, New York, USA

Williams, R.N. 2006. Return to the River: restoring salmon to the Columbia River. Elsevier Academic Press, Burlington, MA.

Wipfli, M. S., J. Hudson, and J. Caouette. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U. S. A. Canadian Journal of Fisheries and Aquatic Sciences 55: 1503-1511.

Wipfli, M. S., J.P. Hudson, D.T. Chaloner, and J P. Caouette. 1999. The influence of salmon spawner densities on stream productivity in Southeast Alaska. Canadian Journal of Fisheries and Aquatic Sciences **56**:1600-1611.

Yanai, S., and K. Kochi. 2004. Effects of salmon carcasses on experimental stream ecosystems in Hokkaido, Japan. Ecol Res 20:471-480.

10.J Key Personnel

The following key personnel are influential for the successful design, implementation, and evaluation of this project. Their resumes are provided below.

Key Personnel	Affiliation	Project Role(s)
John Jorgensen	Yakama Nation Fisheries, Twisp WA	Project Leader
Kraig Mott	Yakama Nation Fisheries, Twisp WA	Fisheries Biologist
Chris Peery, Ph.D.	Cramer Fish Sciences, Moscow ID	Project design, coordination, and evaluation, scientific advisor
Paul Anders, Ph.D.	Cramer Fish Sciences, UI, Moscow ID	Project design, coordination, and evaluation, scientific advisor
Bahman Shafii, Ph.D.	Statistical Consulting Services, UI, Moscow, ID.	Statistical design, oversight, and analysis
Ken Ashley, Ph.D.	BC Ministry of Environment, Ashley and Associates, Vancouver BC	Project design, oversight, scientific advisor
Peter Ward, Ph.D. Hasssen Yassien, Ph.D.	Ward and Associates, Vancouver BC.	Design, installation, and operational oversight of nutrient dosing systems
David Smith, Ph.D.	USACE, ERDC, Vicksburg MS.	Project design, salmonid cognitive ecology, and scientific oversight
Tim Hatten, Ph.D. Russ Bingham Rod Sprague	Invertebrate Ecology Inc., UI, Moscow ID	Invertebrate taxonomic identification and ecological characterization

John Jorgensen

Education:

B.Sc. 1997 Fish and Wildlife Biology, Montana State University

Employment:

2001- Present	Fisheries Biologist III, Yakama Nation, Methow Field Station, Twisp, WA
1999-2000	Fisheries Technician, Alaska Department of Fish and Game, King Salmon, AK
1997-1998	Fisheries Technician, Idaho Department of Fish and Game, Powel, ID
1995-1997	Fisheries Technician, Montana State University, Bozeman, MT

Experience:

Responsible for multiple projects in the Methow Basin, projects include coho reintroduction and habitat restoration. Responsibilities include project development, implementation, monitoring and evaluation, analysis and report writing.

Kraig Mott

Education:

B.Sc. 2005 Biology, Eastern Washington University

Employment:

2008-Present	Fisheries Biologist, Yakama Nation, Methow Field Station, Twisp, WA
2005- 2008	Scientific Technician, Yakama Nation, Methow Field Station, Twisp, WA
2003-2005	Fisheries Technician, Eastern Washington University & WDFW, Spokane, WA

Christopher A. Peery, Ph.D.

A. Professional Preparation

Linfield College, OR	Biology	B.A. w/honors, 1986
College of William and Mary, VA	Biological Oceanography	M.S., 1989
University of Idaho, Moscow	Fisheries Resources	Ph.D., 1995
University of Idaho, Moscow	Fisheries Ecology	Postdoc, 1995-1996

B. Professional Appointments

2008-Present	Fisheries Scientist, Cramer Fish Sciences, Moscow, ID
2004-2008	Assistant Research Professor, Department of Fish and Wildlife, University of Idaho
2001-2003	Research Scientist, Idaho Cooperative Fish and Wildlife Research Unit, Department of Fish and Wildlife, University of Idaho
1996-2001	Research Associate Idaho Cooperative Fish and Wildlife Research Unit, Department of Fish and Wildlife, University of Idaho

C. Publications Most Relevant to the Proposed Project

(*) see http://www.cnr.uidaho.edu/uiferl/Reports.htm for .pdf of abstracts

Peery, C.A, K. Kavanagh, and J.M. Scott. 2003. "Pacific salmon in aquatic and terrestrial ecosystems": Setting biological defensible recovery goals. Bioscience 53:622-623.

Keefer M.L., C.C. Caudill, C.A. Peery, and S.R. Lee. *In press.* Which way home? Unintended consequences of transporting juvenile anadromous salmonids expressed during adult migration. Ecological Applications.

Keefer, M.L., C.A. Peery, and C.C. Caudill. 2008. Migration timing of Columbia River spring Chinook salmon: effects of temperature, river discharge, and ocean environment. Transactions of the American Fisheries Society 137:1120-1133.

Keefer, M.L., C.A. Peery, and M.J. Heinrich 2008. Temperature-mediated en route migration mortality travel rates of endangered Snake River sockeye salmon. Ecology of Freshwater Fishes 17:136-145.

Peery, C. A., and T. C. Bjornn. 2000. Summer dispersal and growth of hatchery chinook salmon parr following release into four Idaho streams. North American Journal of Fisheries Management. 20:19-27

Peery and Bjornn. 2004. Interactions between hatchery and natural chinook salmon juveniles in an artificial stream channel. Fisheries Research 66:311-324.

Keefer, M.L., C.A. Peery, and C.C. Caudill. 2006. Long-distance downstream movements by homing adult Chinook salmon. Journal of Fish Biology 68:1-7.

Keefer, M.L., C.A. Peery, W.R. Daigle, M.A. Jepson, S.R. Lee, C.T. Boggs, K.R. Tolotti, and B.J. Burke. 2005. Escapement, harvest, and unknown loss of radio-tagged adult salmonids in the Columbia-Snake River hydrosystem. Canadian Journal of Fisheries and Aquatic Sciences 62: 930-946.

Keefer, M.L, C.A. Peery, T. C. Bjornn, M.A. Jepson, and L. Stuehrenberg. 2004. Hydrosystem, dam and reservoir passage rates of adult Chinook salmon and steelhead in the Columbia and Snake rivers. Transactions of the American Fisheries Society 133:1413-1439.

Paul J. Anders, Ph.D.

A. Professional Preparation

Ph.D. Natural Resources (Conservation Biology of White Sturgeon), U. of Idaho, 2002.
M.S. Biology (Fisheries), Eastern Washington University, 1991
B.S. Natural Science (Limnology), Saint Norbert College, 1983.

B. Professional Appointments

1. Associate Consultant, Fishery Scientist, Cramer Fish Sciences (Formerly S. P. Cramer and Associates) Moscow, ID. (10/05-Present)
2. Affiliate Faculty, University of Idaho, College of Natural Resources, Fish and Wildlife Department (9/03-present)
3. Senior Fisheries Consultant, S. P. Cramer and Associates, Moscow, ID. (10/02-10/05)
4. Fisheries Scientist (0.5FTE) Columbia River Inter-Tribal Fish Commission, Steelhead kelt reconditioning project (Fall 01 – Fall 02)
5. Research Support Scientist II, University of Idaho, Center for Salmonid and Freshwater Species at Risk Aquaculture Research Institute, Fish Genetics Lab, Moscow, ID. (1/00-10/02)
6. Research Associate, University of Idaho, Center for Salmonid and Freshwater Species at Risk Aquaculture Research Institute, Fish Genetics Lab, Moscow, ID. (1/99-1/00)
7. Independent Fisheries Consultant (1/99-10/02)
8. Doctoral Research Assistant, University of Idaho, Aquaculture Research Institute, Fish Genetics Lab, Moscow, ID. (7/96-12/98)
9. Fisheries Biologist/Administrator, Kootenai Tribe of Idaho, PO. Box 1269, Bonners Ferry, ID. (5/94-7/96)
10. Fisheries Biologist, Kootenai Tribe of Idaho, PO. Box 1269, Bonners Ferry, ID. (2/93-5/94)
11. Fisheries Biologist (GS-9-482), U.S. Fish and Wildlife Service, Columbia River Field Station, Cook WA. (8/90 - 2/93)

C. Publications Most Relevant to the Proposed Project

Holderman, C., G. Hoyle, R. Hardy, P. Anders, P. Ward and H. Yassien. Libby Dam Hydro-electric Project Mitigation: Efforts for Downstream Ecosystem Restoration. 2009. Pages - *In:* Environmental and Water Resources Institute of American Society of Civil Engineers Conference: "*An International Perspective on Environmental and Water Resources*". Thailand, 2009. http://www.fishsciences.net/reports/2009/holderman_et_al_2009_ EWRI_Conference.pdf

Holderman, C., P. Anders, B. Shafii, and G. Lester. 2008. Characterization of the Kootenai River aquatic macroinvertebrate community before and after experimental nutrient addition, 2003–2006. Report to the Kootenai Tribe of Idaho and Bonneville Power Administration. 53 pp.

Idaho Department of Fish and Game, Kootenai Tribe of Idaho, Cramer Fish Sciences, and Ward and Associates. 2008. Best Management Plan for Kootenai River Nutrient Dosing System. Report prepared for U. S. EPA, Region 10, Seattle WA.

Anders, P., Editor. 2007. Kootenai River Nutrient Restoration Project-Annual Implementation Report prepared by Cramer Fish Sciences for the Kootenai Tribe of Idaho, Idaho Department of Fish and Game, and the International Kootenai/y Ecosystem Restoration Team. 69 pgs. http://www.fishsciences.net/reports/2007/KOO04_2007-implementation-report_2008-0400.pdf

Anders, P.J. and K.I. Ashley. 2007. The Clear-water Paradox of Aquatic Ecosystem Restoration. Fisheries 32 (3):125-128. http://www.fishsciences.net/reports/2007/Fisheries0405-Anders_and_Ashley_2007.pdf

Since 1995, Dr. Anders has authored and co-authored over 100 papers, reports, and articles on fisheries and aquatic ecology topics. For a detailed list see: ttp://www.spcramer.com/content/docs/anders_paul.doc

Bahman Shafii, Ph.D.

A. Professional Preparation

B.S. Agronomy/Agricultural Engineering, Rezaeyeh University, 1977.
M.S. Agricultural Economics, University of Idaho, 1980.
M.S. Statistics, University of Idaho, 1982.
Ph.D. Forest Biometrics, University of Idaho, 1988.

B. Professional Appointments

Lecturer, Department of Management and Systems, Washington State University, Pullman, Washington, 1984-1988.

Professor, Plant Science, Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, Idaho, July 2004-present.

Director, Statistical Programs, College of Agricultural and Life Sciences, University of Idaho, Moscow, Idaho, January 1988-present.

Adjunct Full Professor, Department of Statistics, College of Science, University of Idaho.

Adjunct Full Professor, Department of Business, College of Business and Economics, University of Idaho.

C. Publications Most Relevant to the Proposed Project

Mahler, Robert L., B. Shafii, S. Hollenhorst, and B. J. Andersen. 2008. Public perceptions on the ideal balance between natural resource protection and use in the Western USA. *Journal of Extension, 46(1): 1RIB2*

Smith, David L., E. L. Brannon, B. Shafii, and M. Odeh. 2006. Use of the average and fluctuating velocity components for estimation of volitional Rainbow Trout density. Transactions of the American Fisheries Society 135: 431-441.

Shafii, Bahman and W.J. Price. 2005. Bayesian analysis of dose-response calibration curves. *Applied Statistics in Agriculture*, J. E. Boyer (Ed.). Kansas State University, Manhattan, Kansas, pp. 126-136.

Dr. Shafii has authored and coauthored over 100 peer reviewed papers. For more information see: http://www.uidaho.edu/ag/statprog

Ken I. Ashley, Ph.D.

A. Professional Preparation

University of British Columbia	Zoology	B.Sc., 1972
University of British Columbia	Zoology	M.Sc., 1981
University of British Columbia	Civil Engineering	M.A.Sc., 1989
University of British Columbia	Civil Engineering	Ph.D. 2002

B. Professional Appointments

2009-Present	Manager, Special Projects, Fish and Wildlife Branch, BC Ministry of Environment
2005-2008	Senior Engineer, Environmental Management, Greater Vancouver Regional District
1999-2005	Section Head, Bioengineering, Fisheries Research and Development Section, BC Ministry of Environment
1979-1999	Limnologist, Fisheries Research Section, Ministry of Environment

C. Publications Most Relevant to the Proposed Project

Lawrence, G.A., K.I. Ashley, N. Yonemitsu and J.R. Ellis. 1995. Natural dispersion and the fertilization of small lakes. Limnology and Oceanography 40:1519-1526.

Ashley, K.I., L.C. Thompson, D.C. Lasenby, L. McEachern, K.E. Smokorowski and D. Sebastian. 1997. Restoration of an Interior Lake Ecosystem: The Kootenay Lake Experiment. Water Qual. Res. J. Canada (32):295-323.

Rae, R., F.R. Pick, P.B. Hamilton and K.I. Ashley. 1997. Effects of fertilization on phytoplankton in Kootenay Lake, British Columbia. Lake and Reservoir Management 13(1):57-66.

Johnston, N.T., M.D. Stamford, K.I Ashley and K. Tsumura. 1999. Responses of rainbow trout (Oncorhynchus mykiss) and their prey to inorganic fertilization of an oligotrophic montane lake. Can. J. Fish. Aquat. Sci. 56:1011-1025.

Sterling, M.S., K.I. Ashley and A.B. Bautista. 2000. Slow-release fertilizer for rehabilitating oligotrophic streams: a physical characterization. Water Quality Res. J. Canada 35(1): 73-94.

Stockner, J.G. and K.I. Ashley. 2003. Salmon nutrients: Closing the circle. Pages 3-16 In: J.G. Stockner, editor. Nutrients in salmonid ecosystems: sustaining production and biodiversity. American Fisheries Society, Symposium 34, Bethesda, Maryland.

K.I. Ashley and J.G. Stockner. 2003. Protocol for applying limiting nutrients to inland waters. Pages 245-260. In: J.G. Stockner, editor. Nutrients in salmonid ecosystems: sustaining production and biodiversity. American Fisheries Society, Symposium 34, Bethesda, Maryland

Perrin, C.J., M.L. Rosenau, T.B. Stables and K.I. Ashley. 2006. Restoration of a montane reservoir fishery using biomanipulation and nutrient addition. North Amer. J. Fish. Management 26:391-407.

Anders, P.J. and K.I. Ashley. 2007. The Clear-water Paradox of Aquatic Ecosystem Restoration. Fisheries 32 (3):125-128.

Matzinger, A., R. Pieters, K. I. Ashley, G. A. Lawrence, and A. Wüest. 2007. Effects of impoundment on nutrient availability and productivity in lakes. Limnology and Oceanography 52(6):2629-2640.

Peter Ward, Ph.D.

A. Professional Preparation

- B.Sc (Hons), Physics & Mathematics, University of London, 1961
- M.Sc, Physics, University of London, 1967
- Ph.D., Engineering Science, University of California, Berkeley, 1972.

B. Professional Appointments

- Adjunct Professor (1987-present), Department of Civil Eng., University of British Columbia.
- Member of six engineering and scientific professional organisations, including the Association of Professional Engineers and Geoscientists of British Columbia, Canadian Water Resources Association, American Geophysical Union and American Society of Civil Engineers.
- Dr. Ward has spent thirty eight years working in hydrology and water resources engineering, including full-time engineering teaching at university level, consulting work for government with emphasis on water flows and water quality, developing, installing and monitoring nutrient addition systems, and work for the private sector with a focus on conceptual design, installation and monitoring.

C. Publications Most Relevant to the Proposed Project

P.R.B. Ward, H A Yassien, February 1995. Flow Proportional Liquid Fertiliser Injector. Prepared for Fisheries R & D Division, Ministry of Environment. Vancouver, B.C.

P.R.B. Ward, W.G. Dunford, April 1995. Design of Prototype Flow Proportional Liquid Fertilizer Injector. Prepared for Fisheries R & D Division, Ministry of Environment, Lands and Parks. Vancouver, B.C.

P.R.B. Ward, H A Yassien, November 1995. Construction & Field Testing of Flow Proportional Liquid Fertilizer Injector. Prepared for Fisheries Research and Development Section, Ministry of Environment, Lands and Parks. Vancouver, B.C.

P.R.B. Ward, H A Yassien, June 1996. Gravity Fed Liquid Fertilizer Injection for Fish Habitat Improvement: Error Analysis & Prototype Testing. Prepared for Ministry of Environment, Lands & Parks Fisheries Research. Vancouver, B.C.

P.R.B. Ward, H A Yassien, August 2004 . Sheep Creek Nutrient Dosing Pump Design and Installation. Prepared for BC Hydro Environmental Department. Burnaby, B.C.

P.R.B. Ward, H A Yassien, October 2004. Kootenai River Proposed Nutrient Dosing Equipment Ideas and Layout. Prepared for Kootenai Tribe of Idaho. Bonners Ferry, Idaho.

P.R.B. Ward, H A Yassien, December 2005. Kootenai River Nutrient Dosing: As Built System and Performance for 2005 Summer Season. Prepared for Kootenai Tribe of Idaho. Bonners Ferry, Idaho.

P.R.B. Ward, H A Yassien, December 2005. Transverse Mixing Characteristics of Kootenai River Downstream of Dosing Site: Medium Flow Regime. Prepared for Kootenai Tribe of Idaho. Bonners Ferry, Idaho.

Charlie Holderman, Ryan Hardy, P.R.B. Ward, H A Yassien, March 2006. Equipment for Liquid Nutrient Dosing in Small and Large Rivers in the Northwest. For American Fisheries Society 42nd Annual Meeting – 2006. Sunriver Resort, Oregon.

P.R.B. Ward, H A Yassien, July 2006. Dosing System for Chilliwack River 2006. Prepared for British Columbia Conservation Foundation, Surrey, B.C.

P.R.B. Ward, H A Yassien, December 2006. Kootenai River Nutrient Dosing System and N-P Consumption: Year 2006. Prepared for Kootenai Tribe of Idaho. Bonners Ferry, Idaho.

P.R.B. Ward, H A Yassien, January 2008. Kootenai River Nutrient Dosing System and N-P Consumption: Year 2007. Prepared for Kootenai Tribe of Idaho. Bonners Ferry, Idaho.

Hassen Yassein, P. Eng.

Citizenship: Canadian
Profession: Civil Engineer
Specialisation: Water Resources Engineering and Operation Research
Contact information: 9460 Pinewell Cres, Richmond, BC, V7A 2C6, Canada
Email - hassen@telus.net
Phone (604)218-8887

A. Professional Preparation

B.Sc. in Civil Engineering, Addis Ababa University, Ethiopia.
Diploma in Hydrology, Free University of Brussels, Belgium, 1984.
M. S., in Hydrology, Free University of Brussels, Belgium, 1985
Ph.D. in Water Resources Management, Civil Engineering Department, UBC, Vancouver.

B. Professional Appointments

Twenty three years professional experience in Water Resources and Hydrology, setting up and running of a technology institute and water works construction. Experience includes: Establishing hydrological stations, data collection and hydrological data analysis. Estimating and computing floods and water surfaces levels in rivers and lakes. Writing technical reports for engineering firms, government offices and the public. Served in a research team working for the development of new technology to improve fish habitat in lakes and rivers. Taught at higher education institute, administered workshops and oversaw operation of hydraulic and water treatment laboratories. Designing and constructing of rural water supply systems, pump testing of deep wells and spring developments. Supervised water supply systems, spring developments and drilling water wells. Directed and supervised surveyors, draftsmen and construction technicians.

C. Publications Most Relevant to the Proposed Project

Publications most relevant to the proposed project include reports on design, development, installation and monitoring and maintenance of nutrient addition systems from the following nutrient addition projects.

See relevant publication list above for Peter Ward and Ward Associates

David L. Smith, Ph.D.

A. Professional Preparation

Washington State University, WA	Environmental Science	B.S., 1990
Washington State University, WA	Environmental Science	M.S., 1996
University of Idaho, Moscow	Natural Resources	Ph.D., 2003
University of Idaho, Moscow	Civil Engineering	Postdoc, 2003-2004

B. Professional Appointments

2006-Present	US Army Engineer Research and Development Center, Ecohydraulics and Cognitive Ecology Team, Vicksburg, MS
2006-present	Adjunct Faculty, Department of Biological Systems Engineering, University of Idaho
2006-2004	Senior Scientist, Crammer Fish Sciences, Moscow, ID
1997-1996	Senior Field Engineer, Bechtel Hanford, Inc, Richland, WA
1996-1994	Field Engineer, IT Hanford Inc, Richland WA
1994-1990	Project Engineer, Westinghouse Hanford Inc, Richland, WA

C. Publications Most Relevant to the Proposed Project

Smith, D.L., M. Allen, and E.L. Brannon. 2008. Characterization of velocity gradients inhabited by juvenile chinook salmon by habitat type and season. Pages 53-70 *in* S.V. Amaral, D. Mathur, and E.P. Taft, III, editors. Advances in fisheries bioengineering. American Fisheries Society, Symposium 61, Bethesda, Maryland.

Nestler, J.M., R.A. Goodwin, D.L. Smith, J.J. Anderson, and S. Li. 2008. Flow Field Distortion, Sensory Biology, Hydrogeomorphology, and Cognitive Ecology: Elements Describing Juvenile Salmon Movement Behavior and Passage. River Research and Applications. 24(2): 148-168

Smith, D.L. and E.L. Brannon. 2007. Influence of cover on mean column hydraulic characteristics in small pool riffle morphology streams. Rivers Research and Applications 23: 125–139.

Smith, D.L. E.L. Brannon , B. Shafii, and M. Odeh. 2006. Use of the average and fluctuating velocity components for estimation of volitional rainbow trout density. Transactions of the American Fisheries Society 135: 431-441.

Smith, D.L., E.L. Brannon, and M. Odeh. 2005. Response of juvenile rainbow trout to turbulence produced by prismatoidal shapes. Transactions of the American Fisheries Society 134: 741-753.

Russell C. Biggam

A. Professional Preparation

University of Idaho, ID, Entomology and Biology, B.S., 1973

B. Professional Appointments

1981-Present	University of Idaho, Division of Plant, Soil and Entomological Sciences, aquatic labs under Drs. Brusven and Johnson
1973-1981	University of Idaho, Division of Plant, Soil and Entomological Sciences, aquatic labs with multiple faculty
1968-1972	University of Idaho, Division of Plant, Soil and Entomological Sciences

C. Expertise

- Identifications of larval and adult aquatic and terrestrial invertebrates
- Aquatic sampling techniques
- Biology and ecology in invertebrates
- Data input, formatting and basic analyses

D. Selected Publications

Eigenbrode, S.D., J.D. Andreas, M.G. Cripps, H. Ding, R.D. Biggam, M. Schwarzlaner. 2008. Induced chemical defenses in invasive plants: a case study with *Cynoglossum officinale* L. Biological Invasions 10: 1373-1379.

Bruvsen, M.A. and R. Biggam. 1996. Trend changes in aquatic habitat and benthic macroinvertebrate bioassessment conditions in upper Hangman Creek and tributaries. Project Completion Report.

Bruvsen, M.A. and R. Biggam. 1995. Ecological-economic assessment of a sediment-producing stream behind lower granite dam on the lower Snake River, USA. Regulated Rivers: Research & Management 10:373-387.

Hoiland, W.K., F.W. Rabe and R.C. Biggam. 1994. Recovery of macroinvertebrate communities from metal pollution in the south fork and mainstream of the Coeur d'Alene River, Idaho. Water Environment Research 66: (1)84-88.

Bruvsen, M.A., W.R. Meehan and R.C. Biggam. 1990. The role of aquatic moss on community composition and drift of fish-food organisms. Hydrobiologia: 196:39-50.

Biggam, R.C. and M. A. Brusven. 1989. The Gerridae (water striders) of Idaho (Hemiptera: Gerridae). The Great Basin Naturalist 49:(2) 259-274.

Biggam, R.C. and M.W. Stock. 1988. Pronotal stripes and wing length in *Gerris incurvatus* Drake and *Hottes* (Hemiptera: Gerridae). Pan-Pacific Entomologist. 64(4) 359-363.

Rabe, R.W., R. C. Biggam, R.M. Breckenridge, R.J. Naskali. 1985. A limnological description of selected peatland lakes in Idaho. Journal of the Idaho Academy of Sciences. 22(2) 63-90.

Bruvsen, M.A. and R.C. Biggam, and K.D. Black. 1976. Ecological strategies for assessing impact of water fluctuations on fish food organisms. Project Completion Report, National Marine Fisheries Service Contract No. 03-4-208-243.

Timothy D. Hatten, Ph.D.

President, Invertebrate Ecology Inc., Moscow, ID

A. Professional Preparation

- University of Idaho, Postdoc Entomology, 2007-2009; President Invertebrate Ecology Inc., Moscow, ID.
- University of Idaho, Entomology Ph.D., 2006
- Washington State University M.S., Entomology, 2003
- University of Arizona, B.S. Natural Resources, B.S., 1984

B. Professional Appointments

2006-Present	President, Invertebrate Ecology Inc. Moscow, ID
2007-2009	Postdoctoral Researcher, UI, Moscow, ID
2005	Fellow, NSF Integrated Graduate Education and Research Traineeship Program (IGERT), Moscow, ID
1994-1999	Liaison, Environmental Protection Agency and USDA-NRCS, CA
1988-1993	Conservationist, USDA-NRCS, CA
1986-1988	Peace Corps, Niger, Africa

C. EXPERTIZE

- Landscape and community ecology with emphasis on invertebrate fauna
- All aspects of sampling, collecting, processing and identifying invertebrates, terrestrial and aquatic
- Analysis of parametric, nonparametric, fine- and coarse scale data

D. PUBLICATIONS

Hatten, T.D., S.D. Eigenbrode, N.A. Bosque-Pérez, S. Gebbie, F. Merickel, and C. Looney. 2006. Influence of matrix elements on prairie-inhabiting Curculionidae, Tenebrionidae and Scarabaeidae in the Palouse. Pp. 101-108, *In:* Egan, D. and J. Harrington [eds.], Proceedings of the Nineteenth North American Prairie Conference. August 8-12, 2004, Madison: University Communications, Madison, WI.

Hatten, T.D., N.A. Bosque-Pérez, J.R. LaBonte, S.O. Guy and S. D. Eigenbrode. 2007. Effects of tillage on the activity-density and diversity of carabid beetles in spring and winter crops. Environmental Entomology 36 (2): 356-368.

Hatten, T.D., S. D. Eigenbrode, J. Johnson-Maynard, K. Umiker, J.R. LaBonte and N.A. Bosque-Pérez. 2009. Effect of crops, tillage and soil organic carbon on carabid beetles in commercial agricultural fields of the Inland Pacific Northwest, USA. Agricultural and Forest Entomology (In review).

Umiker, K., J. Johnson-Maynard, T. D. Hatten, S. D. Eigenbrode and N. Bosque-Pérez. 2009. Soil properties and earthworm density as influenced by cropping practices on farms of the Palouse Region, Idaho. Soil and Tillage Research (In press).

Roderick Sprague IV

Taxonomist, Invertebrate Ecology Inc., Moscow, ID

A. Professional Preparation

University of Idaho, B.S. Entomology, 2008

B. Professional Appointments

2008	Taxonomist, Invertebrate Ecology Inc., Moscow, ID
2002-2004	Taxonomist, University of Idaho, Division of Plant, Soil and Entomological Sciences, multiple labs, Moscow, ID
1993-2001	Custodian, University of Idaho, Moscow, ID
1979-1983	Museum Curator, W. F. Barr Insect Museum, University of Idaho, Moscow, ID

C. Expertise

- Identifications of aquatic and terrestrial invertebrates
- Specimen curation and storage, dry or wet

APPENDIX A

Project Task Implementation Schedule and Collaborators

Upper Columbia Nutrient Supplementation Project Implementation Tasks and Schedule

		Field Sampling												Lab Analysis												Report writing											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Trophic level	**Metrics**																																				
WQ nutrients						x	x	x	x	x	x						x	x	x	x	x	x	x	x		x	x	x	x					x	x	x	x
Nutrient diffuser exps.						x	x	x	x	x	x						x	x	x	x	x	x	x	x		x	x	x	x					x	x	x	x
Primary production	*Algae*																																				
	biomass					x	x	x	x	x	x			x	x	x		x	x	x	x	x	x	x		x	x	x	x					x	x	x	x
	taxonomy					x	x	x	x	x	x			x	x	x		x	x	x	x	x	x	x		x	x	x	x					x	x	x	x
	Chlorophyll																																				
	biomass					x	x	x	x	x	x			x	x	x		x	x	x	x	x	x	x		x	x	x	x					x	x	x	x
	accrual rate					x	x	x	x	x	x			x	x	x		x	x	x	x	x	x	x		x	x	x	x					x	x	x	x
Secondary Production	*Aquatic invertebrates*																																				
	biomass					x	x	x	x	x	x			x	x	x									x	x	x	x	x					x	x	x	x
	taxonomy					x	x	x	x	x	x			x	x	x									x	x	x	x	x					x	x	x	x
Fish metrics	*Adults*																																				
	fish counts																									x	x	x	x					x	x	x	x
	Redd counts			x	x	x		x	x	x																x	x	x	x					x	x	x	x
	Juveniles																									x	x	x	x					x	x	x	x
	juv size age				x	x																				x	x	x	x					x	x	x	x
	juv cond				x	x																				x	x	x	x					x	x	x	x
	gut contents					x	x	x	x	x				x	x	x									x	x	x	x	x					x	x	x	x
	juv density and biomass				x	x	x	x	x	x	x			x	x	x									x	x	x	x	x					x	x	x	x
	Smolts																																				
	smolt prod.				x	x								x	x	x										x	x	x	x					x	x	x	x
	migr. Timing				x	x								x	x	x										x	x	x	x					x	x	x	x
	E:E survival				x	x								x	x	x										x	x	x	x					x	x	x	x
Stable isotopes																																					
Bioenergetics																																					

Upper Columbia Nutrient Supplementation Project Implementation Tasks and Schedule

Trophic level	**Metrics**	**Field Sampling**	**Lab Analysis**	**Data Analysis**	**Report writing**
WQ nutrients		YN	YN, ARI	SCS	YN, CFS, SCS
Nutrient diffuser exps.		YN	YN	SCS	YN, CFS, SCS
Primary production	*Algae*				
	biomass	YN	TBD	SCS	YN, CFS, SCS
	taxonomy	YN	TBD	SCS	YN, CFS, SCS
	Chlorophyll				
	biomass	YN	UI-ASL	SCS	YN, CFS, SCS
	accrual rate	YN	UI-ASL	SCS	YN, CFS, SCS
Secondary Production	*Aquatic invertebrate*				
	biomass	YN	YN, IE	SCS, IE	YN, CFS, IE, SCS
	taxonomy	YN	YN, IE	SCS, IE	YN, CFS, IE, SCS
Fish metrics	*Adults*				
	fish counts	WDFW	WDFW	WDFW	WDFW
	Redd counts	WDFW	WDFW	WDFW	WDFW
	Juveniles				
	Juvs/redd	WDFW	WDFW	WDFW	WDFW
	juv size age	WDFW	WDFW		WDFW
	juv cond	WDFW	WDFW	WDFW	WDFW
	gut contents	YN	YN, IE	SCS, IE	YN, CFS, IE, SCS
	juv density and biomass	YN	YN, IE	SCS	YN, CFS, IE, SCS
	Smolts				
	smolt prod.	WDFW	WDFW	WDFW	WDFW
	migr. Timing	WDFW	WDFW	WDFW	WDFW
	E:E survival	WDFW	WDFW	WDFW	WDFW
Stable isotopes		YN	ISIL, UI	UI, SCS	YN, UI, CFS, SCS
Bioenergetics		YN	ISIL, UI	UI, SCS	YN, UI, CFS, SCS

Collaborators:	ARI	Aquaculture Research Inc, Seattle WA.
	CFS	Cramer Fish Sciences, Moscow, ID.
	IE	Invertebrate Ecology Inc, Moscow, ID.
	SCS	Statistical Consulting Services, Clarkston, WA.
	ISIL	Idaho Stable Isotope Lab, University of Idaho,
	UI-ASL	University of Idaho Analytical Services Lab, Moscow,
	WDFW	Washington Department of Fish and Wildlife
	YN	Yakama Nation, Twisp WA.