



Date: October 14, 2009

To: ISRP

**From: John Jorgensen, Yakama Nation, Upper Columbia Nutrient
Supplementation Project Leader (BPA-200847100)**

Re: ISRP Review of project proposal

On behalf of the Yakama Nation and the Upper Columbia Nutrient Supplementation Project (BPA-200847100), we would like to thank the ISRP for your thorough and rigorous review of our recently submitted project proposal. We provide a stand alone document responding point-by point to the ISRP review comments, recommendations, and considerations, and have incorporated this material into the updated project proposal. We feel that our response to the issues raised by your review, and its incorporation into project design and methods, have substantially improved the project.

We appreciate your input and look forward to working together to more efficiently implement this project to improve ecological conditions and increase natural production of anadromous salmonids in the Methow River basin. If necessary, we are available to meet with the ISRP in person regarding clarification of any aspects about the project. We look forward to updating you on project progress and would offer the opportunity for your visit to the project site if feasible following project implementation.

ISRP Comment 1

1.1 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,

A combination of approaches will be used to determine whether nutrient availability is limiting in project streams, including nutrient diffuser experiments and empirical data collection and analyses of various chemical and biological metrics described below that are indicative of ecological condition in study area waters. The use of nutrient diffusing substrates is described in more details on Pages 2 and 3 in response to ISRP Comment 1.2. The current default condition of marine derived nutrient deficits in anadromous salmon streams in the Pacific Northwest (based on weight of evidence) is reflected in biotic community signatures of individual streams throughout the Columbia River basin. Current marine derived nutrient (MDN) loadings from Pacific salmon carcasses have been reported to be as little as 6-7% of historical contributions (Gresh et al. 2000). Subsequently, many forested streams in the region are now classified as ultra-oligotrophic (Stockner 2003; Kohler et al. 2008). A similar condition has been reported for waters in the Methow Basin (Mullan et al. 1992). Data from this project will subsequently be collected and analyzed as support for this hypothesis.

Nutrient limitations for natural production of anadromous salmonids across systems have not been explicitly defined. This study evaluates empirical data from different trophic levels and ecological processes to identify appropriate nutrient limitation levels. Ecological signal to identify nutrient limitation and the possible need for experimental nutrient addition in salmon producing streams includes low metric values of nutrient availability, primary and secondary productivity rates, diversity and richness values, and escapement estimates such values are often reduced by orders of magnitude from known and reconstructed historical conditions, and up to several orders of magnitude less than analogous values from known salmon producing systems (Stockner 2003 and references therein; Stockner and Ashley 2003)

Thus, 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 all be evaluated to assess potential limitation in project streams. Metrics representing these trophic levels and processes will be used to determine whether candidate study streams are nutrient limited, relative to reconstructed historical conditions, compared to current escapement scenarios. Analogous values in streams with production deemed to be healthy and productive will also be assessed

As mentioned above, N:P ratios are typically used to determine whether systems are N-limited, P-limited or co-limited. 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.

References

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

1.2 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.

We agree with this ISRP comment and will include the following information for implementing nutrient diffuser experiments in the updated project proposal. The following information 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):

Sanderson et al. (in press) have recently published an analysis of nutrient limitation in Idaho streams that used similar agar based nutrient diffusing substrates to evaluate whether streams were limited by nitrogen, phosphorus or some combination of both nutrients.

We will modify their protocols developed in our study systems to first characterize the nature of nutrient limitation and subsequently evaluate how limitation shifts over time and with the addition of nutrients. Additional detailed methods for nutrient diffuser apparatus and protocols will be included in the updated proposal.

Nutrient diffusing substrates (NDS) provide a 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) 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 4 replicates is pictured below, referred to as a “periphytometer” (<http://nespal.cpes.peachnet.edu/images/carey%20figure%202.jpg>).

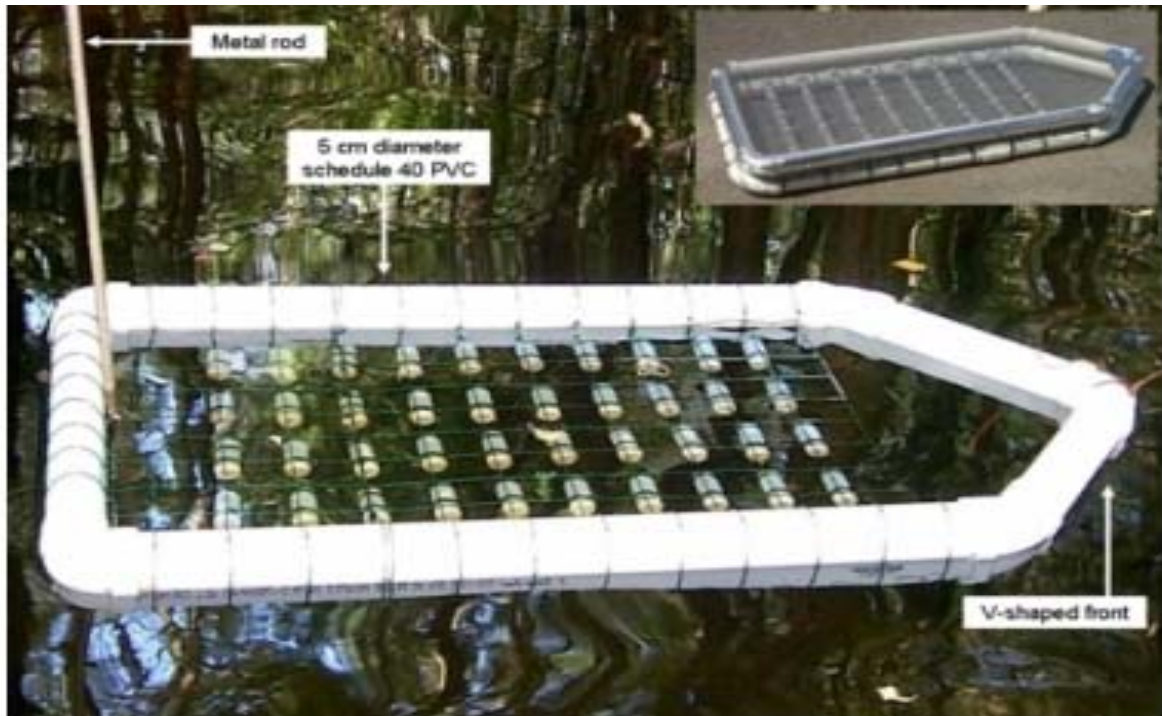


Figure 2. Photographs of periphytometer modified from Matlock et al. (1998) showing primary frame with forty 20 ml scintillation vials and inset showing an inverted picture of primary and secondary frames together; when deployed, the secondary frame lies beneath the primary frame.

Following this in-stream incubation period for periphyton growth, each glass disks 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. Five to eight replicates of each experimental nutrient treatment will be utilized.

A two-factor ANOVA with N, and/or P treatments as the main factors will be used to test 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-colimited
*	*		N- and P-colimited
*	*	*	N- and P-colimited
*		*	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 <i>In</i> ; Hauer and Lamberti 2007)			

References

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

ISRP Comment 2

2.1 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.

We agree with this comment concerning provision of a more rigorous approach for measuring primary production. Accordingly, we will measure primary production using several algal, periphyton, and chlorophyll metrics.

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 ($\text{mg}/\text{m}^2/30\text{d}$); 3) total chlorophyll biomass (chlorophyll *a* and *b* (mg/m^2); and 4) total chlorophyll accrual rate ($\text{mg}/\text{m}^2/30\text{d}$).

In addition, algal taxa will be identified and taxonomically grouped as Cyanophyta (blue-greens), Chlorophyta (greens), Bacillariophyta (diatoms), or Chrysophyta (goldens). Dominant algal species and mean algal densities ($\#/ \text{ml}$) in periphyton samples will also be calculated for each sample site and date.

Finally, as suggested by the ISRP, total periphyton biomass can be calculated as an informative metric of primary productivity. 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.

2.2 A measure of whole-system metabolism would considerably improve this aspect of the study.

Classic light-dark bottle experiments are one example of a low-cost option of assessing whole-system metabolism. Such experiments assess net production and respiration, as a gross measure of metabolism, and can determine if particular river reaches are autotrophic (photosynthesis > respiration) or heterotrophic (photosynthesis < respiration).

Automated, closed-chambered, highly sensitive ecosystem metabolism troughs have been developed and used to quantify and assess compartmentalized river metabolism. However, such apparatus is likely 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.

ISRP Comment 3

3.1 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.

Benthic macroinvertebrates will be sampled at a minimum of six locations within each river in the study area (e.g. Twisp and Methow rivers). Subsequent sample size and power analyses with initial empirical data may result in recommendations to modify this initial sampling regime to ensure adequate sample representation and statistical power to detect treatment effects. At each sampling location transects will be placed perpendicular to the stream along transects on cobble substrate within riffle and run habitats. On each transect the invertebrates will be collected with Hess samplers from the center of the stream (Thalweg), the midpoint between stream center and left bank as well as right bank, for a total of 3 subsamples/transect. However, during the early spring months the Thalweg might be inaccessible due to high flows, in which case two samples will be taken from each site. Sampling is carried out twice per month from March through September for a maximum of 252 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. for processing. Specimens will be identified to the finest level of taxonomic resolution, primarily genus and species level. This will be economically feasible because the number of invertebrates/sample unit is generally < 250 (based on preliminary analysis of recently processed samples). Moreover, 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.

3.2 Why is there no measure of invertebrate density and biomass included?

This was an omission on the author's part in the original proposal. In lotic habitats, Hess samplers do provide a quantitative estimate of benthic macroinvertebrate density (Merrit and Cummins 1996, p. 13). In this case, 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.

Biomass (*B*) of invertebrate taxa and of all taxa combined will be measured directly using standard lab dry weight techniques (Benke 1996). This approach (biomass by taxa groups) will enable us to quantify the relative contribution of individual taxa (e.g. Ephemeroptera, Trichoptera, etc.) to total biomass. Biomass of benthic invertebrates has been shown to be sensitive to nutrient addition, providing a causal linkage for increased abundance of tertiary consumers (e.g. salmonid fry) after such treatments (Johnson et al. 1990). Biomass is also a necessary statistic for calculating secondary production. Secondary production is a measure of biomass, or energy, of the macroinvertebrate community through time (e.g. g/m²/time), whereas biomass is only a snapshot of

production in time (e.g. g/m^2) (Benke 1996). 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 1996).

Sampling multiple times throughout the season will enable calculation of growth rates (gr) for dominant taxa (perhaps EPT), allowing us to calculate secondary production (P), where $P = \text{gr} * B$ (Benke 1996). Empirical growth rate curves are also available to estimate production when biomass is known (see Stephenson et al. 2007 for references). Ryan Bellmore, a collaborating aquatic researcher in the Methow Basin from Idaho State University, has also offered his assistance in calculating secondary production by using growth rate estimates for benthic invertebrates collected on the Methow River not far from the current study site. Estimates of biomass and production will then be used to calculate production to biomass ratios (P/B), with P/B providing a rate of biomass accumulation for any specified unit of time (e.g. week, daily, etc.). Hence, secondary production provides an estimate of biomass flow, whereas P/B provides an estimate of biomass accumulation, or a weighted mean value of biomass, for a desired time period. Biomass measurements and secondary production estimates will give us a standardized, quantitative method to compare 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.

3.3 Fully describe how the Hess samples and kick-net samples will complement each other.

It is unknown at this time whether a kick-net or other sampling devices will be needed to complement the Hess Sampler. Analysis of habitat proportion data from the Twisp River over the study area is underway to determine whether additional sampling methods, such as kick-nets or Surbers, are needed to sample lentic habitats (e.g. pools). Thus far, best professional judgment and data review indicates that the study area is strongly dominated by lotic habitats (e.g. riffles and runs; approximately 76% of study area length). Therefore, the proposed sampling with a Hess sampler along fixed transects remains justified. However, should our analysis reveal that pools occupy a greater proportion of habitat than is currently estimated, we will consider switching to a stratified sampling method, i.e. stratifying by habitat type, that might require the use of a Surber or kick-net. With this method it is common to designate a standardized length (reach) of river at each sample site and then sample it by moving upstream while taking samples from each habitat type (Merritt and Cummins 1996). The intensity of sampling within each habitat type in this scenario is based on the relative proportions of each habitat within the reach (Barbour et al. 1999; Merritt and Cummins (1996). If this method is needed we will select the appropriate sampling apparatus (Merritt and Cummins 1996) for the job, consistent with substrate conditions.

We are also considering sampling a subset of pools within the study area to complement proposed riffle sampling. If this subsampling indicates that benthic fauna differs significantly from that of riffle habitat, then we will consider: 1) switching to a stratified

sampling method, or 2) complementing our transect sampling method with sampling of pool habitats as described by Barbour et al. (1999).

References

- 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/>
- Benke, A.C.1996. Secondary production of macroinvertebrates. Pages 557 – 578 in *Methods in stream ecology*. Academic Press, San Diego.
- Johnson, N.T., C.J. Perrin, P.R. Slaney, and B.R. Ward. 1990. Increased juvenile salmonid growth by whole-river fertilization. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 862-872.
- Merrit, R.W. and K.W. Cummins. 1996. *Aquatic insects of North America*. Kendall/Hunt Publishing Company, Dubuque, Iowa.
- 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.

NOTE: In their review of this project proposal, the ISRP had numerous comments and recommendations concerning the need to increase the rigor of the fish sampling component of the project. Because answers to these concerns were somewhat redundant in response to ISRP Comments 4 and 5, we provide a single response to ISRP comments 4 and 5 below.

ISRP Comment 4

More fully describe the methods to be used in evaluating juvenile fish populations.

One of the greatest needs of this project is the lack of information on smolt production at the Methow River study sites. The proposal indicates that WDFW is conducting adult counts and collecting smolts on the Twisp. But there is no mention of these data being collected for the Methow study site. Nutrient enhancement in the Columbia Basin is being done, primarily, to increase survival of juvenile salmon and steelhead. Without very good data on the number of adult salmon spawning and number of smolts leaving the study sites, this assessment cannot be made. This deficiency constitutes a significant flaw in the current design and needs to be addressed before proceeding with this project.

Will density and biomass be measured? If so, how will these population attributes be measured?

ISRP 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.*

To address the issues presented above in ISRP comments 4 and 5, we provide information about various aspects of fish sampling, including the following suite of fish sampling metrics (in general developmental chronology):

1. Escapement (adult abundance)
2. Juveniles per redd
3. Juvenile size at age, growth
4. Length, weight, and biological condition
5. Stomach content sampling
6. Smolt production
7. Juvenile fish biomass and density
8. Outmigration timing
9. Egg to emigrant survival

The following summaries of methods to evaluate juveniles per redd and smolt production are provided as collaborative efforts by WDFW (2008). Both study rivers (Twisp and Methow rivers) as well as a possible future reference system (Chewuch River) have rotary screw traps collecting juvenile fish. These sites currently provide baseline freshwater production rates (egg to emigrant) as well as future collection and sampling sites for proposed work. Currently there are a number of years of weight to length measurements and biological condition factor data (Fulton's *K*) for hatchery and wild smolts as well as fall wild parr. Scales will be taken on steelhead smolts in order to get age at emigration.

1. *Escapement (Adult abundance)* - We agree with the ISRP that quantifying current contribution to study streams in terms of adult abundance and smolt production is critical to the success of this study. Adult counts from all upstream (adult) passage facilities will be used to estimate adult abundance. Spawning ground surveys performed by WDFW and possibly the USFS, USFWS and USGS may provide data useful for estimating escapement or adult abundance.

2. *Juveniles per redd* – Production estimates for each age class and trapping location (Screw traps currently in the lower Twisp and Methow rivers, with expected data provision from the Chiwuck River) will be summed to produce a total brood year emigration estimate. For spring Chinook salmon, the estimate of fall-migrant spring Chinook salmon parr will be added to the smolt estimate from the following spring to produce a total emigrant estimate for each brood year. Because a single brood of steelhead may require four or more years to completely migrate, the smolt production estimate at each trap location will be multiplied by the proportion of smolts from each brood determined through scale pattern analysis. The number of emigrants per redd for each brood year will be calculated by dividing the total emigrant production estimate by the total number of redds estimated through spawning ground surveys. The number of smolts per redd will be calculated by dividing the total estimated smolt production by the total number of redds for a given brood year.

3. *Size at age and growth* – These variables will be addressed using empirical data from daily fish collections at all outmigrant screw traps in the study area from approximately April through November. Supplemental sampling to further address growth and size at age may be collected by conducting in-stream sampling several times during the same time period, as mentioned by ISRP. Supplemental sampling methods to address growth and size at age could include backpack electroshocking, seining, and minnow traps, with a systematic or stratified sampling design to capture a relatively unbiased representation of the population. Electrofishing is effective in riffle and shallow pool habitat, while seining is more appropriate for collecting fish from pool and run habitats.

4. *Length, weight, and biological condition* (e.g. Fulton's K) values, and other metrics from treated and controlled locations within the project area will be measured as part of a standard fish assessment. Size of fish (including length-weight relationships) and biological condition factor at outmigration will be compared between and among years and within and among pre- and post-treatment years to assess rearing conditions.

5. *Fish stomach content sampling* - A proposed fish stomach content sampling scheme may include a small number of index sampling sites (up to about five per stream, ten total) within each stream that could be sampled at set intervals (i.e. three samplings, one each in late spring, summer, early fall). A consistent level of effort would be used at each sampling site (i.e. three passes with backpack shocker). All fish collected would be measured (TL), weighed, and 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. Although it is currently uncertain whether this sampling would be permitted under ESA take permit limits, it could provide valuable empirical data directly linking available and consumed prey items.

6. *Smolt production* - The number of smolts produced per redd is a metric used to compare the relative productivity of Chinook and steelhead during freshwater rearing. We will use 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.

Utilizing 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.

We will measure actual smolt production in each 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 m² of stream habitat). Smolt production will also be standardized on spawner numbers, as described above. Using estimate smolt production and mean fish weights, we will also be able to 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 designed and adaptively implemented to ensure desired sensitivity of metrics and sampling (based on empirical sample size analysis).

7. *Juvenile fish biomass and density* – These variables will also be addressed using in-stream sampling (e.g. electroshocking or snorkel surveys). Adding this level of sampling would significantly increase costs for the proposed program. Our original intention was to use existing screw trap operations and mark-recapture methods to estimate smolt production and infer annual growth, system productivity and density, based on estimates of available habitat.

Juvenile fish biomass and density can be measured using in-stream, area-based sampling techniques, such as electrofishing, snorkel surveys, or other techniques. 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.

8. *Outmigration timing* – Outmigration timing is affected by in-river conditions such as water temperature and flow. Unsuitable conditions would prompt juveniles to leave the basin in search of more favorable rearing areas downstream. Density dependent relationships would also 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.

9. *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

Spawning ground surveys will identify summer steelhead and spring Chinook salmon redds downstream of the Methow and Twisp River trap sites in some years. We will assume that redds located downstream from each trap site did not contribute to production estimates calculated at upstream smolt traps. To calculate total production and emigration estimates for species, we will apply the egg-to-smolt survival rates calculated for those redds upstream of trap to the estimated number of eggs deposited downstream of the trap. Confidence intervals (95%) will be adjusted in a similar manner. Total brood year smolt production estimates will be calculated by adding the estimated number of smolts produced downstream of the trap to the estimate of smolts produced upstream of the trap location.

Collectively, implementation of the fish sampling activities described above will provide valuable data necessary to assess and compare annual fish production attributes within and among pre- and post-treatment (fertilization) years. Even with this information, as mentioned by the ISRP, adult (spawner) abundance and smolt production numbers are the result of a myriad of factors, many beyond the control and scope of this project. 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 the project are best evaluated within the freshwater rearing area until progeny produced in the study area migrate to the Mainstem Columbia River downstream.

ISRP Comment 6

6.1 Describe how potential density-dependent effects of fish population response to food limitation will be addressed.

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 a limiting factor to smolt production, then little response to nutrient augmentation should be observed relative to fish condition and production rate over time.

6.2 How will the effects of water temperature, flow, and changes in other habitat attributes be accounted for when assessing the responses to nutrient addition?

Flow and temperature directly affect system productivity, habitat suitability, and fish growth and condition. Some of these responses happen 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, 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.

Controlling for potentially confounding factors could also be facilitated by adding a third study stream (such as a tributary of the Methow) not supplemented with nutrients to serve as a control or reference area. Collecting analogous data on adult and smolt abundance (involves purchase and operation of third screw trap at minimum) would enable measurement of system productivity and trends in the absence of nutrient additions. However, care must be taken when considering analogue system comparisons due to inherent differences between systems, including those in close geographic proximity.

ISRP Comment 7

Consider the application of a bioenergetics model to identify appropriate hypotheses and design experiments.

As suggested by the ISRP, bioenergetics models 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.) will 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). A bioenergetics model could then be used to run several scenarios could then be used to population the model. 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, during, and after experimental fertilization

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.

Stable isotope work and bioenergetics modeling components for this project are currently being developed and will be included in subsequent years' project proposals

ISRP Comment 8

Include a more detailed description of the adaptive management process that will be used in moving this study forward.

We agree that adaptive management (AM) 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 that make model predictions suspect”. Typically, the design of management experiments then becomes a key second step in the process of adaptive management, and a new set of management issues arises about 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 8-1). These features differentiate adaptive management from traditional trial-and-error or learn-as-you-go management (Hilborn 1992; Halbert 1993).

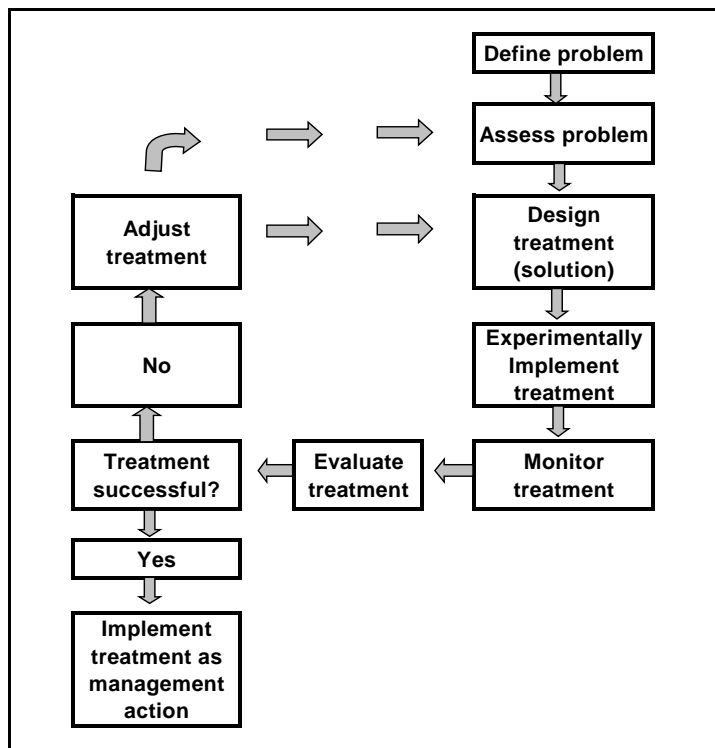


Figure 8-1. A generalized adaptive management model to be used in the Upper Columbia Nutrient Supplementation Project.

In this AM context, project proponents propose the following iterative actions:

- 1) Implement replicated water quality sampling to compare empirical nutrient concentration with defined limiting values, and any reconstructed historical nutrient availability estimates;
- 2) Design and implement a biomonitoring program with appropriate response variables (see proposal) for each trophic level (water quality, including nutrient availability), primary (algae/periphyton), secondary (macro invertebrates), and tertiary (fish) production;
- 3) Perform sample size and power analyses by trophic level to ensure adequate statistical rigor to detect treatment effects, and follow a defined decision tree 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 study river(s).
- 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.
- 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 as needed.

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ISRP 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.

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. Thus, this practice and the behavior of the released fish minimizes 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.

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).

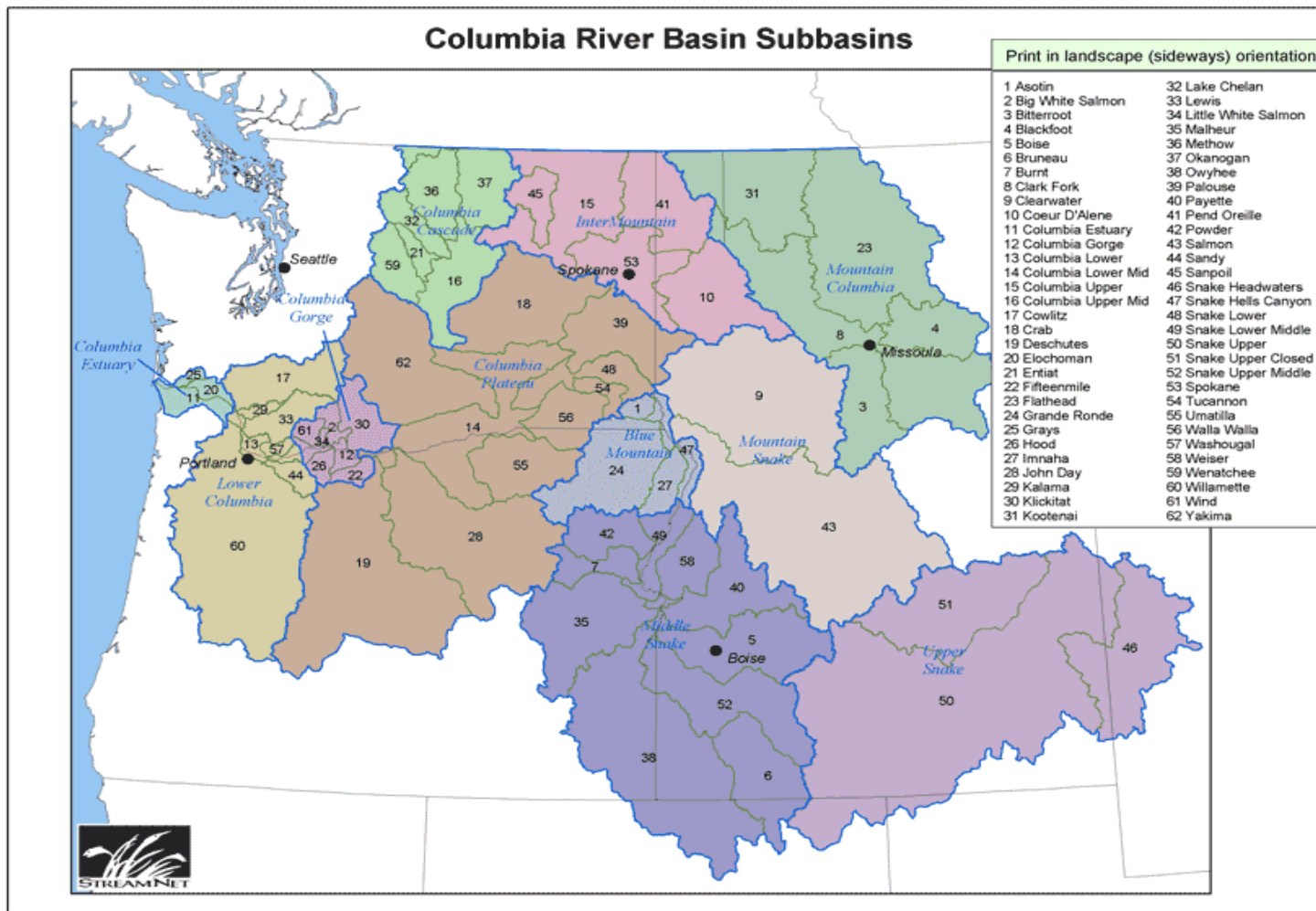


Figure 1. Columbia River Basin map showing all Subbasins, including the Methow River Subbasin (#36) in the upper (Northwest) corner of the Columbia Cascade Ecological Province, bounded on the north by the US-Canada border.

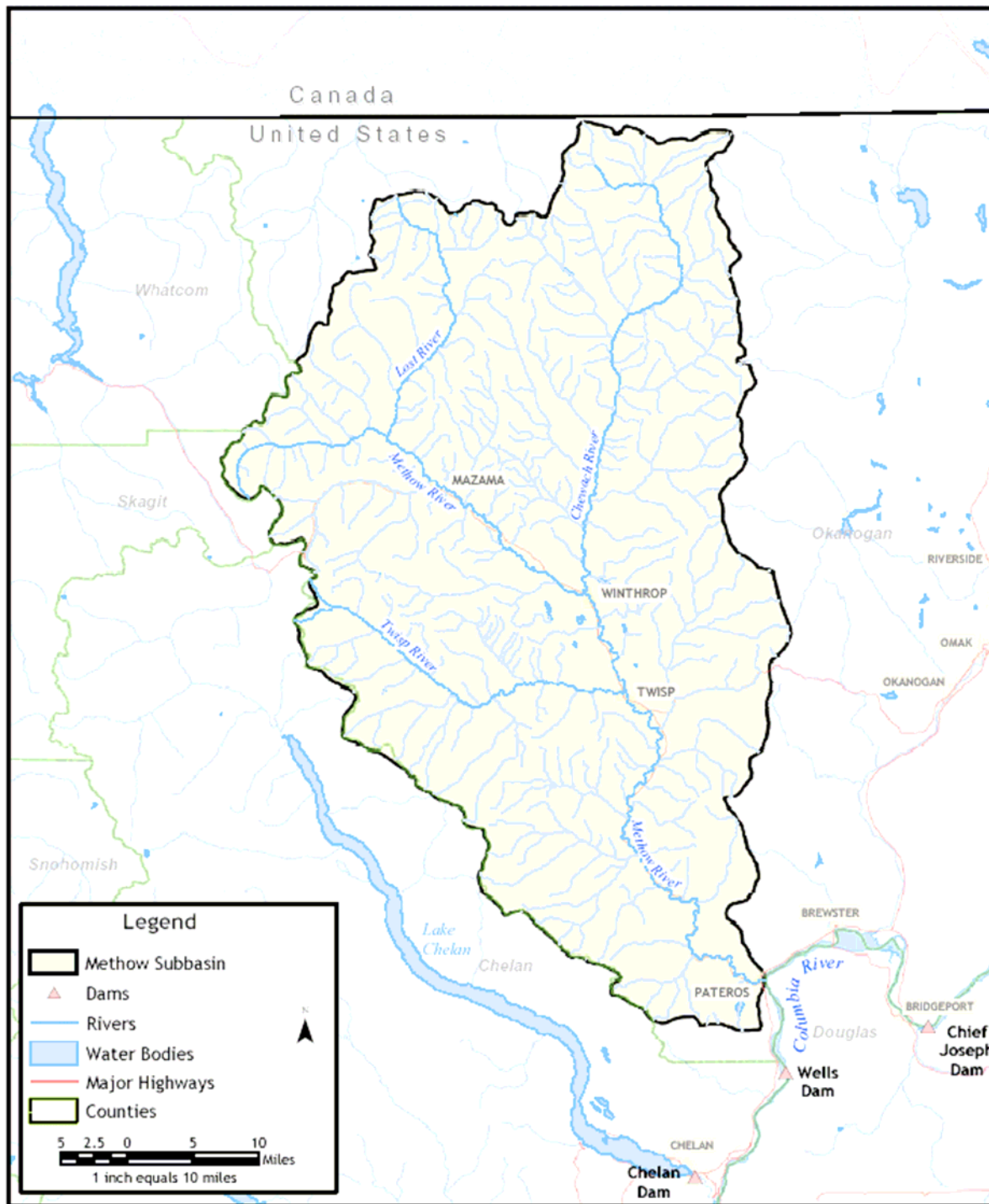


Figure 2. Map of the Methow River Subbasin (shaded) showing the Twisp and Methow rivers, which serve as study areas for this project.

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 (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 four sequential, complementary objectives, to:

- 1) Determine whether nutrient availability and/or imbalance significantly limit natural production of anadromous salmonids in the Methow River Basin (e.g. the Twisp and Methow rivers) (Years 1-3);
- 2) 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);
- 3) Implement and evaluate ongoing nutrient management (Year 8 and beyond as needed); and
- 4) 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
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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).

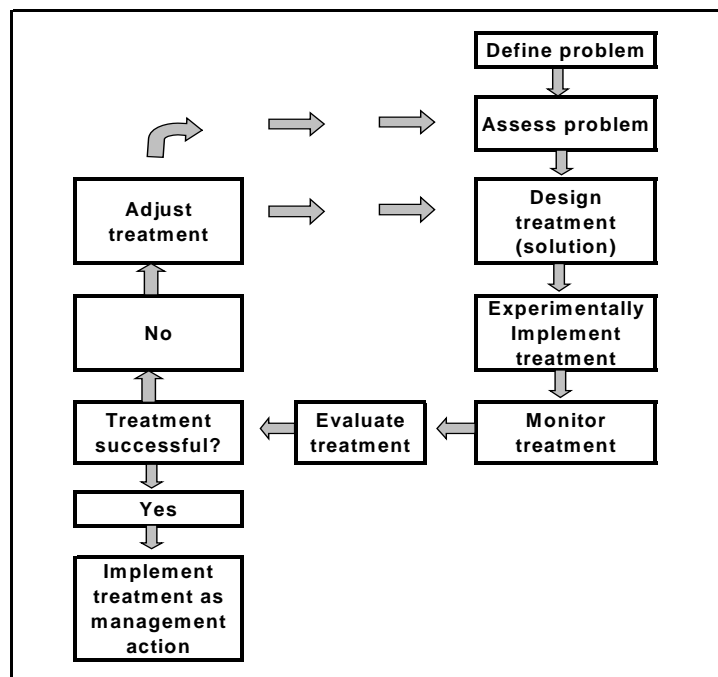


Figure 3. A generalized adaptive management model to be used in this project.

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 water quality 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 study rivers.
- 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 followed by delayed implementation in the Methow River, 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.

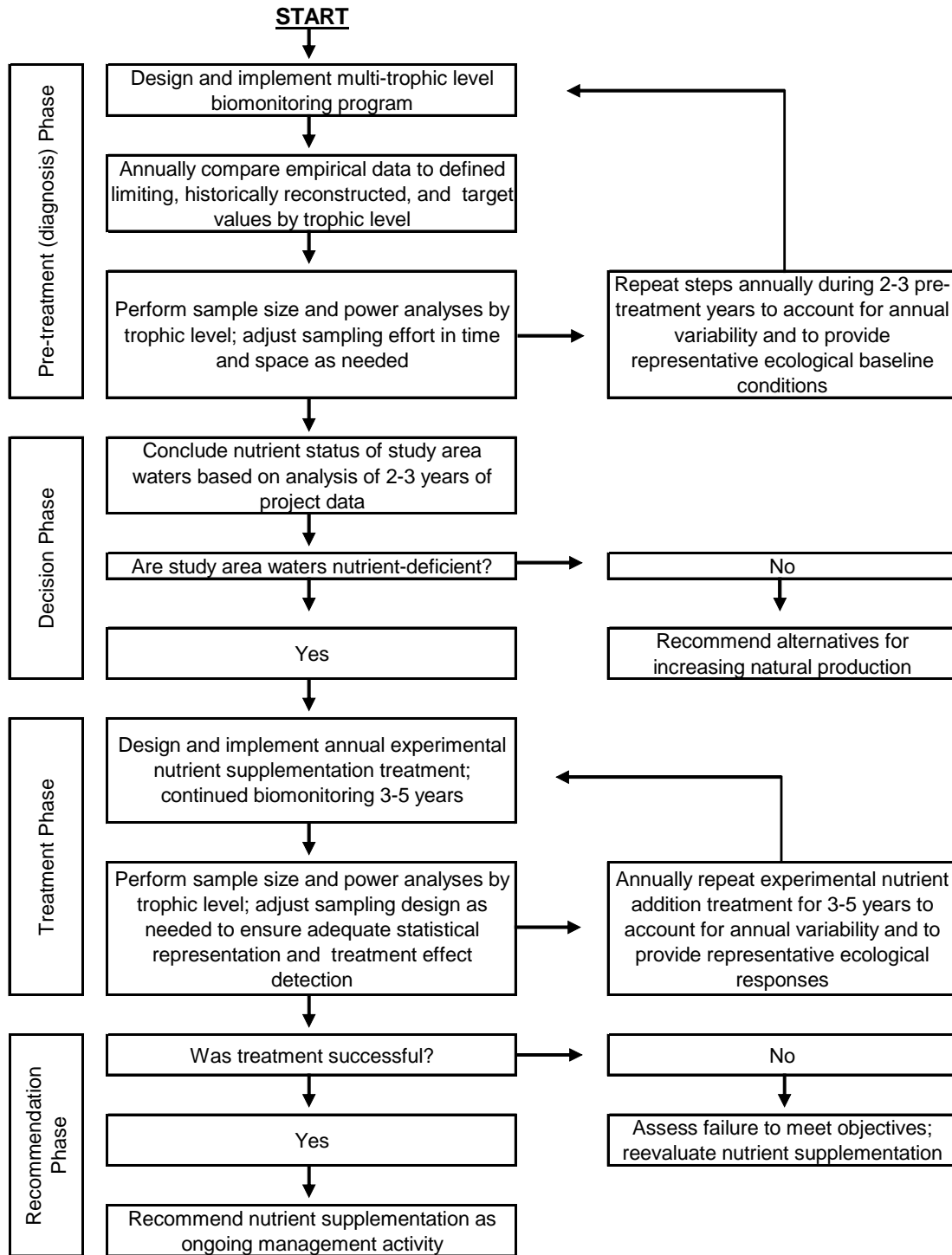


Figure 4. Adaptive project design and implementation flowchart.

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 “perihyrometer” (<http://nespal.cpes.peachnet.edu/images/carey%20figure%202.jpg>).

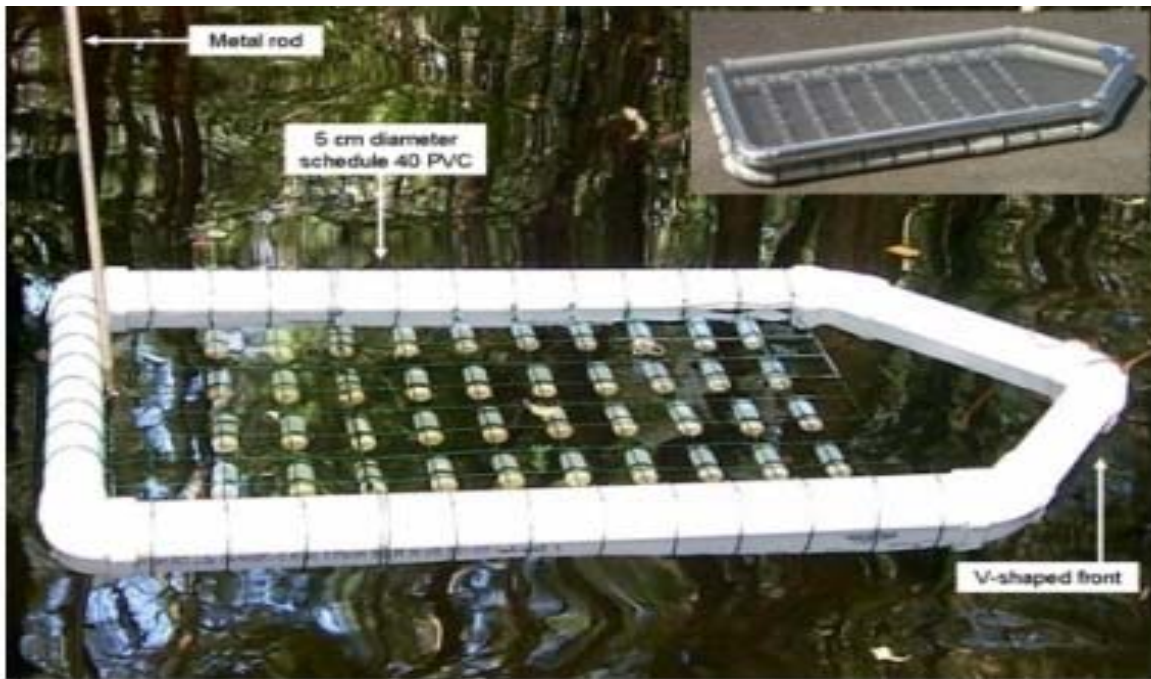


Figure 2. Photographs of periphytometer modified from Matlock et al. (1998) showing primary frame with forty 20 ml scintillation vials and inset showing an inverted picture of primary and secondary frames together; when deployed, the secondary frame lies beneath the primary frame.

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-colimited
*	*		N- and P-colimited
*	*	*	N- and P-colimited
*		*	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 <i>In</i> ; 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

WE 157 - Collect/Generate/Validate Field and Lab Data

An appropriate sampling design will be used to select sites in each of the upper, middle, and lower reaches of the Twisp and Methow rivers. 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. This project is organized by these trophic level categories.

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 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, aquatic benthic macroinvertebrate metrics, and fish community sampling, analysis, and evaluation. 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. Specific fish analysis will include at least standard growth, length, weight, condition factor and age class structure analyses. Furthermore, any community data from other collaborative, sympatric projects will be incorporated into the ecological assessment. We will coordinate with other projects that collect fish stomach content data (e.g. the ISU and USGS study in the Methow Mainstem) to assess effects of nutrient addition. 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 (*Proposium williamsonii*), 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.

More specific methodological information by trophic level is provided below:

Water Quality - Water samples will be collected bi-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 October (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 (NH₄ 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/m²); 2) chlorophyll *a* accrual rate (mg/m²/30d); 3) total chlorophyll biomass (chlorophyll *a+b* (mg/m²); and 4) total chlorophyll accrual rate (mg/m²/30d). 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 October; 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 October 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 the top of the accrual tiles and preserved with Lugols solution and 10% formalin. Algal taxa will be identified and taxonomically grouped as Cyanophyta (blue-greens), Chlorophyta (greens), Bacillariophyta (diatoms), or Chrysophyta (goldens), with further taxonomic identification carried out at least 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 at a minimum of six sites within each river in the study area (e.g. Twisp and Methow rivers). At each site, 4 to 6 replicate invertebrate samples will be collected monthly with standard Hess samplers (or other gear as substrate conditions dictate) from April through October as flow conditions permit; Appendix Table 1).

Transects will be placed perpendicular to the stream at each sampling location on cobble substrate within riffle and run habitats. On each transect the invertebrates will be collected with Hess samplers from the center of the channel (Thalweg), the midpoint between stream center and left bank as well as right bank, for a total of three subsamples/transect. However, during the spring months the Thalweg might be inaccessible due to high flows, in which case two samples will be taken from each site. Sampling is carried out twice per month from April through October for a maximum of 252 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. Specimens will be identified to the finest level of taxonomic resolution, primarily genus and species level. This will be economically feasible because the number of invertebrates/sample unit is generally < 250 (based on preliminary analysis of recently processed samples). Moreover, 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.

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.

Biomass (B) of invertebrate taxa groups and of all taxa combined will be measured directly using standard lab dry weight techniques (Benke 1996). This approach (biomass by taxa groups) will enable us to quantify the relative contribution of individual taxa (e.g. Ephemeroptera, Plecoptera, and Trichoptera) to total biomass. Biomass of benthic invertebrates has been shown to be sensitive to nutrient addition, providing a causal linkage for increased abundance of tertiary consumers (e.g. salmonid fry) after such treatments (Johnson et al. 1990).

Biomass is also a necessary statistic for calculating secondary production. Secondary production is a measure of biomass, or energy, of the macroinvertebrate community through time (e.g. $\text{g/m}^2/\text{time}$), whereas biomass is only a snapshot of production in time (e.g. g/m^2) (Benke 1996). 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 ecologists often calculate secondary production to quantify energy flow or transfer within food webs (Benke 1996). The P/B ratio will be calculated, as a meaningful biological metric.

Biomass measurements, secondary production estimates, and P/B ratio values will give us a standardized, quantitative method to compare benthic invertebrate baseline conditions and invertebrate response(s) between river systems, and longitudinally and before and after fertilization to nutrient addition within study area rivers.

It is unknown at this time whether a kick-net or other sampling devices will be needed to complement the Hess 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). Therefore, the proposed sampling with a Hess sampler along fixed transects remains justified. However, should our analysis reveal that pools occupy a greater proportion of habitat than is currently estimated, we will consider switching to a stratified sampling method, i.e. stratifying by habitat type, that might require the use of a Surber or kick-net. With this method it is common to designate a standardized length (reach) of river at each sample site and then sample it by moving upstream while taking samples from each habitat type (Merrit and Cummins 1996). The intensity of sampling within each habitat type in this scenario

is based on the relative proportions of each habitat within the reach (Barbour et al. 1999; Merrit and Cummins (1996). If this method is needed we will select the appropriate sampling apparatus (Merrit and Cummins 1996) for the job, consistent with substrate conditions.

We are also considering sampling a subset of pools within the study area to complement proposed riffle sampling. If this subsampling indicates that benthic fauna differs significantly from that of riffle habitat, then we will consider: 1) switching to a stratified sampling method, or 2) complementing our transect sampling method with sampling of pool habitats as described by Barbour et al. (1999).

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

Fish community – Due to the central importance of ESA-listed anadromous salmonids in the project area, and the project's focus on them, the following descriptions of proposed fish sampling, metrics, monitoring and evaluation, and analysis is provided below in general developmental chronological order, beginning with spawning adults:

1. Escapement (adult abundance)
2. Juvenile size at age, growth
3. Length, weight, and biological condition
4. Stomach content sampling
5. Smolt production
6. Juvenile fish biomass and density
7. Outmigration timing
8. 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

Upstream passage counts –This project will integrate fish data currently being collected by WDFW to evaluate Chinook salmon and steelhead production within the Methow and Twisp rivers. 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).

Spawning ground surveys - Spawning ground surveys performed by WDFW and possibly the USFS, USFWS and USGS may also provide useful data for estimating escapement or adult abundance. Spawning ground surveys will identify summer steelhead and spring Chinook salmon redds downstream of the Methow and Twisp River trap sites in some years. To calculate total production and emigration estimates for species, we will apply the egg-to-smolt 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.

2. Size at age and growth – These variables will be addressed using empirical data from daily fish collections at all outmigrant screw traps in the study area from approximately April through November. All fish collected at the screw trap will be PIT tagged to assist downstream survival estimates. A portion of these fish will be released upstream so they may be recaptured in the screw trap to estimate capture efficiency and total smolt production.

Depending on the rigor of available data from the screw traps, supplemental sampling may be needed to further address growth and size at age. If needed, sampling would involve in-stream sampling several times during the same time period (April through November). Supplemental sampling methods to address growth and size at age could include backpack electroshocking, seining, and minnow traps, with a systematic or stratified sampling design to capture a relatively unbiased representation of the population. Electrofishing is effective in riffle and shallow pool habitat, whereas seining is more appropriate for collecting fish from pool and run habitats.

3. Length, weight, and biological condition (e.g. Fulton's K) values, and other metrics from treated and controlled locations within the project area will be measured as part of a standard fish assessment. 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.

4. Fish stomach content sampling - A proposed fish stomach content sampling scheme includes a small number of index sampling sites (up to five per stream, ten total) within each stream that could be sampled at set intervals (i.e. three samplings, one each in late spring, summer, early fall). A consistent level of effort would be used at each sampling site (i.e. three passes with backpack shocker) determined by analyzing initial empirical data. All fish collected would be measured (TL), weighed, and 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. Although it is currently uncertain whether this sampling would be permitted under ESA take permit limits, it could provide valuable empirical data to directly link available and consumed prey items.

5. Smolt production - 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 each 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 m² 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).

6. Juvenile fish biomass and density – These variables may also be addressed using in-stream sampling (e.g. electroshocking or snorkel surveys). However, adding this level of sampling would significantly increase costs for the proposed program. Our original intention was to use existing screw trap operations and mark-recapture methods to estimate smolt production and infer annual growth, system productivity and density, based on estimates of available habitat.

Juvenile fish biomass and density can be measured using in-stream, area-based sampling techniques, such as electrofishing, snorkel surveys, or other techniques. Snorkel surveys during summer may be a good approach to estimate fish numbers/densities. However, fish size and condition cannot be accurately estimated from snorkel surveys, whereas seining and trapping will provide size data of adequate resolution to calculate growth and condition values.

In addition to enumeration by routine snorkeling survey techniques, more specific information on numbers, distribution, and size measurements of summer parr (sub-yearlings) could also be collected, if needed, using baited minnow traps and/or beach seining. Minnow traps baited with salmon eggs have been used to successfully collect YOY salmon and trout in streams throughout the Columbia and Snake basin (C. Peery, USFWS, Cramer Fish Sciences, pers. comm. 2009). Mark-recapture techniques will be used to estimate summer rearing densities.

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.

7. Outmigration timing – Outmigration timing is affected by in-river conditions such as water temperature and flow. Unsuitable conditions would prompt juveniles to leave the basin in search of more favorable rearing areas downstream. Density-dependent relationships would also 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.

8. 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.

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 - We will also assess potential confounding factors such as: 1) density-dependent growth effects in response to experimental nutrient addition if implemented by this project, 2) density-independent (environmental) confounding factors such as flow and temperature, and the presence of hatchery fish and hatchery fish carcasses in the study area.

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 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.

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. 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.

Controlling for some of these potentially confounding factors could also be facilitated by adding a third study stream (such as a tributary of the Methow) not supplemented with nutrients to serve as a control or reference area. Collecting analogous data on adult and smolt abundance (involves purchase and operation of third screw trap at minimum) would enable measurement of system productivity and trends in the absence of nutrient additions. However, care must be taken when considering analogue system comparisons due to inherent differences between systems, including those in close geographic proximity.

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.

However, 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 (NH₄ 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/m²) and chlorophyll a accrual rate (mg/m²/ 30 days), and total chlorophyll (chlorophyll a + b; mg/m²) and total chlorophyll accrual (mg/m²/ 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: 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: summer parr abundances, density, size, growth rates, smolt abundances, estimated population size (from mark-recapture calculations), fish size, condition factor (K), growth, estimated survival.

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 Methow and Twisp systems, 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 use 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.

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

The following fish metrics will be used to assess fish condition and system productivity for this project: biomass, abundance, density, and condition factor (*K*) for parr, smolts and returning adults, and survival estimates through various early life stages found in the freshwater spawning, incubation and early rearing environments. Data for these analyses will come from local cooperative fish studies from the study area waters in the Methow River basin and from original sampling efforts where needed. Ongoing studies include: 1) smolt monitoring by WDFW using rotary screw trap and adult escapement estimates from the weir on the Twisp River and spawner surveys, and the local USGS research, (Martens and Connolly 2008) that involves PIT tagging and tracking juvenile chinook salmon in the Methow River. We will coordinate field activities and share data collection efforts with these and other groups.

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_{01.1}: Algal abundance is within acceptable limits for salmon systems not considered to be nutrient limited.
- H_{01.2}: Macroinvertebrate abundance and diversity indices are within acceptable limits for salmon systems not considered to be nutrient limited.
- H_{01.3}: 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_{02.1}: Water nutrient levels are not significantly different between pre- and post-treatment periods.
- H_{02.2}: Algal and periphyton abundance and diversity are not significantly different between pre- and post-treatment periods.
- H_{02.3}: Primary productivity rates are not significantly different between pre- and post-treatment periods.
- H_{02.4}: Benthic macroinvertebrate abundance and diversity are not significantly different between pre- and post-treatment periods.
- H_{02.5}: 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_{03.1}: 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 be 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.

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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. Hassen 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, Povel, 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: http://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 *Hottel* (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																																				
	<i>Algae</i>																																				
	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
	<i>Chlorophyll</i>																																				
	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																																				
	<i>Aquatic invertebrates</i>																																				
	biomass				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
	Fish metrics																																				
	<i>Adults</i>																																				
	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
	<i>Juveniles</i>																								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
	juv density and biomass				X	X	X	X	X	X			X	X	X										X	X	X	X						X	X	X	X
	<i>Smolts</i>																																				
	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

<u>Trophic level</u>	<u>Metrics</u>	<u>Field Sampling</u>	<u>Lab Analysis</u>	<u>Data Analysis</u>	<u>Report writing</u>
WQ nutrients		YN	YN, ARI	SCS	YN, CFS, SCS
Nutrient diffuser exps.		YN	YN	SCS	YN, CFS, SCS
Primary production	<i>Algae</i>				
	biomass	YN	TBD	SCS	YN, CFS, SCS
	taxonomy	YN	TBD	SCS	YN, CFS, SCS
	<i>Chlorophyll</i>				
	biomass	YN	UI-ASL	SCS	YN, CFS, SCS
	accrual rate	YN	UI-ASL	SCS	YN, CFS, SCS
Secondary Production	<i>Aquatic invertebrate</i>				
	biomass	YN	YN, IE	SCS, IE	YN, CFS, IE, SCS
	taxonomy	YN	YN, IE	SCS, IE	YN, CFS, IE, SCS
Fish metrics	<i>Adults</i>				
	fish counts	WDFW	WDFW	WDFW	WDFW
	Redd counts	WDFW	WDFW	WDFW	WDFW
	<i>Juveniles</i>				
	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
	<i>Smolts</i>				
	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.