UPPER COLUMBIA NUTRIENT SUPPLEMENTATION:

CONTRACT YEAR 2010 ANNUAL PROGRESS REPORT

PROJECT NUMBER 2008-471-00

CONTRACT NUMBER 52183

REPORTING PERIOD: APRIL 2009 TO DECEMBER 2011

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23 March 2012

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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 the Twisp watershed of the Methow River Basin, 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.

INTRODUCTION

The problem addressed by this project is the continued low level of natural production of anadromous Pacific salmonids (*Oncorhynchus spp*.) in the Methow River Basin in North Central Washington (Upper Columbia Basin, Figure 1 and Figure 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).

PROJECT GOALS AND OBJECTIVES

Project goal - The goal of the Upper Columbia Nutrient Enhancement Project is to: 1) assess current nutrient concentrations and the trophic status of the Twisp River, relative to nutrient limitation on natural production of native anadromous salmonids; and 2) prescribe, implement, and evaluate a 5-year experimental nutrient addition treatment to increase natural production.

Project objectives - This project has five sequential, complementary objectives, to:

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

PROJECT-LEVEL ADAPTIVE MANAGEMENT

Within the general AM framework previously provided (Figure 2), the following sequence of project actions (Figure 2) will be implemented:

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 standardized, replicated, multi-trophic sampling to compare empirical nutrient concentration with defined limiting values, and available reconstructed historical nutrient availability estimates.
3. Perform sample size and power analyses by metric across trophic levels to ensure adequate statistical rigor to detect treatment effects, Follow a defined logic path (Figure 2), 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 each pre-treatment year to assess baseline trophic status.
6. Conclude nutrient status of the Twisp River.
7. Provide nutrient addition prescription if needed (a 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.

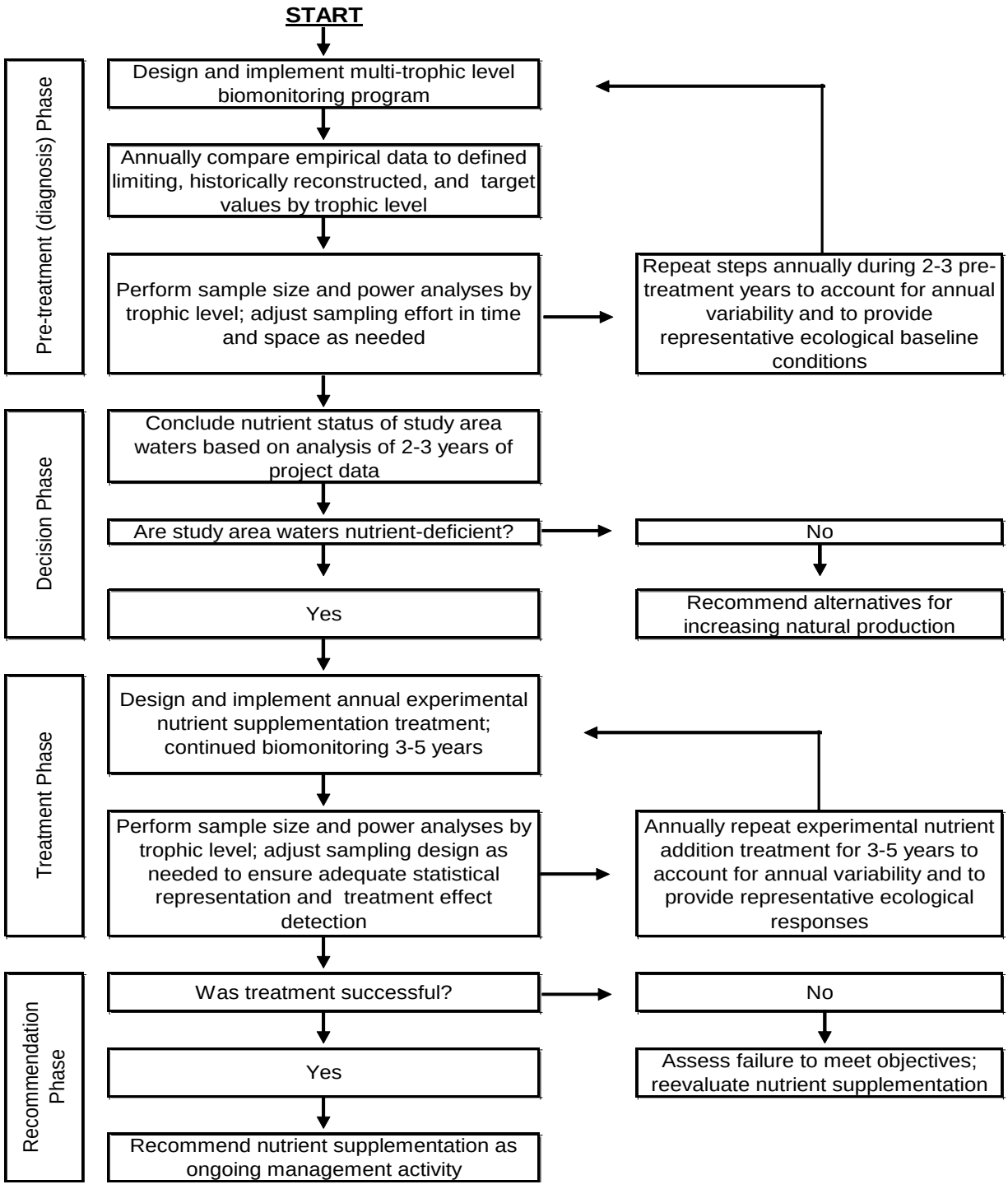

FIGURE 1. ADAPTIVE PROJECT DESIGN AND IMPLEMENTATION FLOWCHART.

STUDY AREA

The Twisp River flows into the Methow River at the town of Twisp in north central Washington (Figure 3). A substantial portion of the upper Twisp River watershed exists in a designated wilderness area and is in nearly pristine condition. Spring Chinook salmon and summer steelhead spawn and rear in the Twisp River for much of its length. Most human activity and resulting habitat changes within the drainage have occurred within the lower 15 miles of the Twisp River, including road placement, bank hardening, and conversion of some riparian areas to agriculture and residential uses have altered habitat conditions in this area.

Sampling sites - This project has six sampling sites over the ~44 km study reach of the Twisp River (TR-1 through TR-6; Figure 3). Each site has two standard transects perpendicular to the river, 100m apart, and three sampling positions: right bank, left bank, and mid-channel (Figure 3). Water quality and the algal, benthic macroinvertebrate, and fish communities will be sampled at all six sites monthly from April through November. An additional (third) transect was established at three of the six sites (TR-2, TR-4, and TR-6), 100m upstream from middle transect at those sites, strictly for stable isotope sampling (Figure 3).

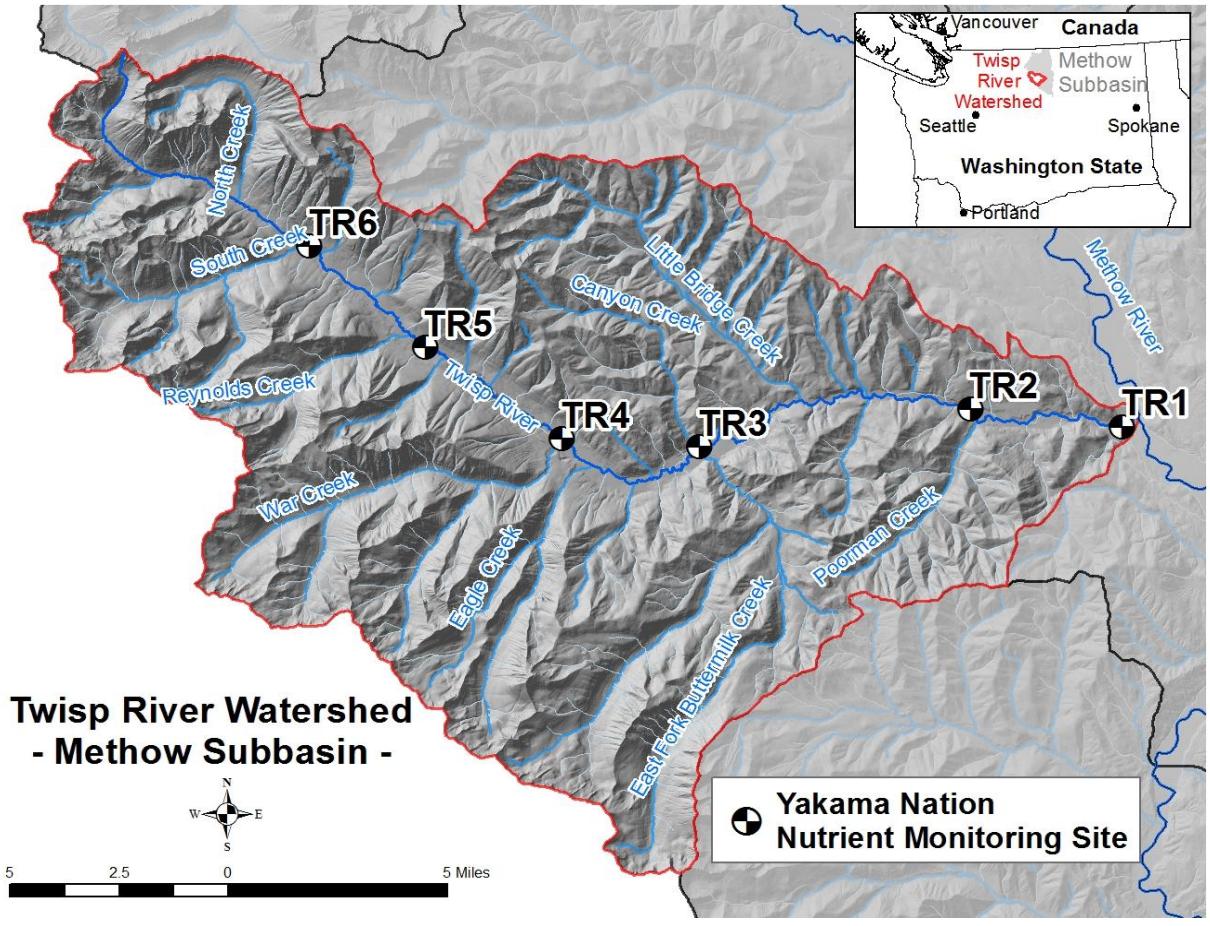

FIGURE 2. TWISP RIVER WATERSHED, STUDY AREA, AND SAMPLING SITES (TR1 THROUGH TR6).

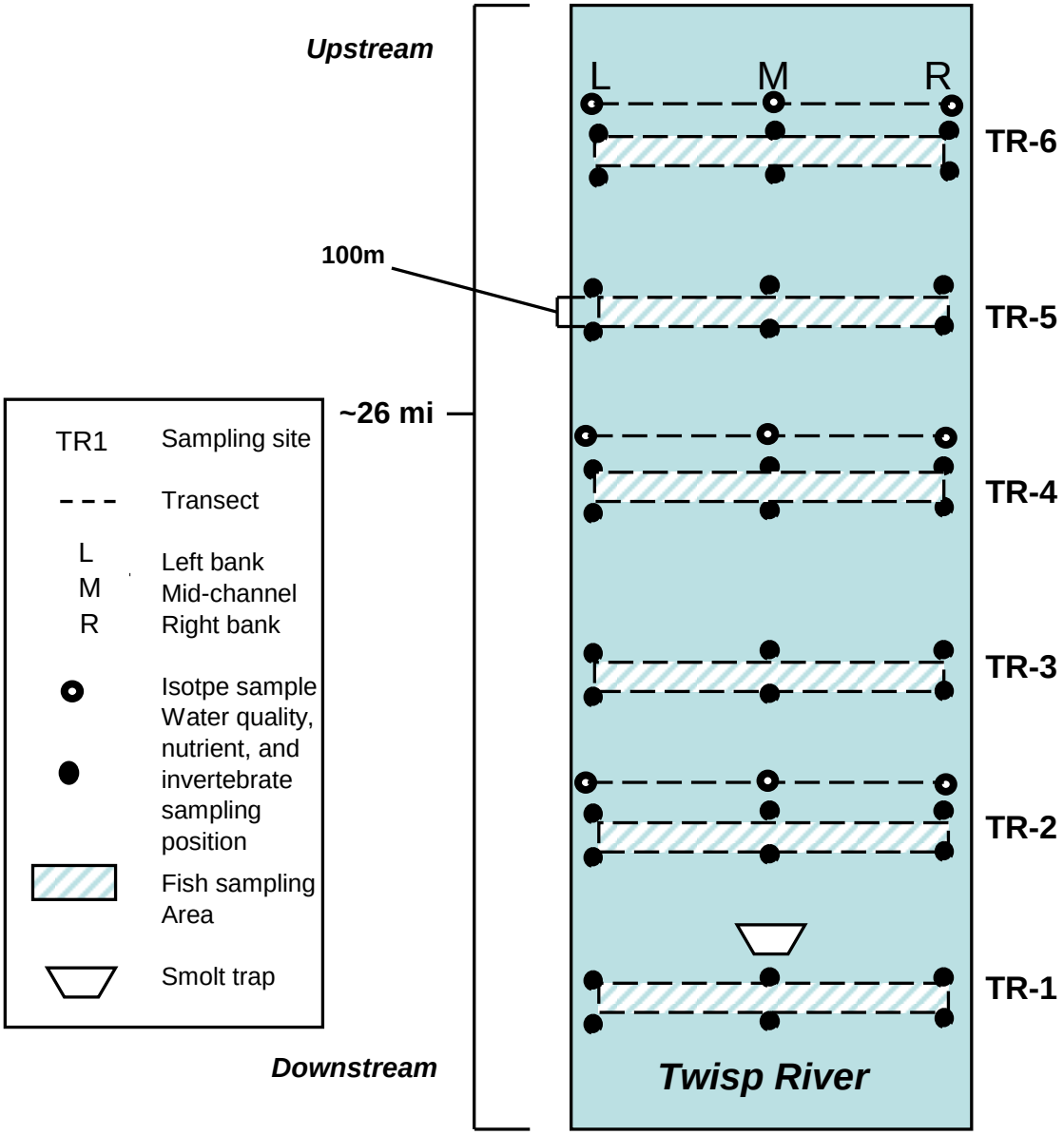

FIGURE 3. SCHEMATIC DIAGRAM OF PROJECT SAMPLING SITES IN THE TWISP RIVER.

METHODS

EXPERIMENTAL TREATMENT STRUCTURE

Temporal - This project has distinct pre- and post-treatment sampling periods, with the treatment being nutrient addition. Although the project has collected some lower trophic level data for more than two years, a minimum of two or three years of concurrent pre-treatment sampling of all variables among all trophic levels is required to evaluate baseline conditions in the Twisp River. Analysis of data collected from all trophic levels during this pre-treatment period will be used to determine whether nutrient addition is warranted, and if so, which type of nutrient sources and treatments is most appropriate. A minimum treatment period of three years would be required to meaningfully assess biological treatment responses with a refined suite of pre-treatment variables (see next section).

Spatial – Nutrient treatments, if warranted, will also have a spatial component, involving one or two biomonitoring sites upstream from nutrient addition and perhaps four or five sites in the treated reach. Monitoring sites and protocols will be held constant between pre- and post-treatment periods. Details regarding nutrient type, source, and locations for addition are currently being developed.

BIOLOGICAL METRICS

A comprehensive suite of biological metrics will be monitored to characterize trophic status, biological production, and community attributes of all trophic levels in the Twisp River and Hancock Springs projects (Table 1). Both projects will collect comparable, spatially and temporally aligned data to characterize current trophic status and biological conditions from each of four general trophic levels:

1) Water quality and nutrient availability;

2) The periphyton community and primary production;

3) The benthic macroinvertebrate community and secondary production; and

4) The fish community and tertiary production.

Estimates of primary (chlorophyll accrual rate), secondary (benthic macroinvertebrate), and tertiary (fish) production will be generated to further characterize trophic status for both projects. Both projects will also develop and quantitatively test hypotheses regarding food web structure and function through a combination of multi-trophic sampling, fish stomach content analyses, and stable isotope analysis.

TABLE 1. BIOLOGICAL METRICS MONITORED IN THE TWISP RIVER FOR THE UPPER COLUMBIA NUTRIENT ENHANCEMENT PROJECT.

Trophic level/ Function	Biological Metrics
Water quality/Nutrients	Dissolved oxygen, temperature, alkalinity, TP, SRP, TDP, TN, NO2+NO3, TN:TP, SIN/TDP
Periphyton	Algal community composition (% composition by taxonomic order)
Primary production	Chlorophyll a, Chlorophyll b, Total chlorophyll (a+b) accrual rate
Benthic macroinvertebrates	19 individual and aggregated taxa and functional group abundance, biomass, and richness metrics (See Appendix 6 for a list of all metrics)
Secondary production	Secondary production estimates (Benke and Huryn 2007)
Fish	Community composition, richness...
Performance and biological condition	Aggregated and single species abundance, biomass, length, weight, and biological condition (*K*), growth, survival, diet composition
Annual production	Redd counts, annual escapement, annual smolt production, egg to emigrant survival, peak outmigration timing, outmigration duration, number of outmigrants

SAMPLING PROTOCOLS

WATER QUALITY AND NUTRIENTS

Metrics

Ten water quality and nutrient metrics will be monitored in the Twisp River (Table 1). In addition to sampling water chemistry, temperature, and dissolved oxygen will be measured throughout the sampling season. Hobo tidbit data loggers will be located at all sampling sites and record temperature every 30 minutes. Two portable Hydrolabs take dissolved oxygen measurements every 60 minutes (June - November). These metrics will be sampled monthly at all three positions on the two standard transects at each of the 6 sites from April through November. All water quality and nutrient samples will be sent to Aquatic Research Inc. (Seattle, WA.) for standard lab analyses. Water samples will be stored in a refrigerator and then shipped overnight to the lab.

Water Quality and Nutrient Hypotheses

The following hypotheses will be tested to assess water quality and nutrient concentrations in the Twisp River before and after experimental nutrient addition in the Twisp River:

H_{o1}: Mean alkalinity...

H_{o2}: Mean TP...

H_{o3}: Mean TN...

H_{o4}: Mean NH4...

(1) ...values were not significantly different among pre-treatment years.

(2) ...values were not significantly different among post-

H_{o5}: Mean NO2+NO3...	treatment years.
H_{o6}: Mean SRP...	
H_{o7}: Mean TDP...	(3) ...values were not significantly different between pre- and post-treatment periods.
H_{o8}: Mean TN:TP...	
H_{o9}: Mean SIN:TDP...	
H_{o10}: Mean water temperature...	

Statistical Analyses for Water Quality and Nutrients

Spatial and temporal contrasts – All water quality and nutrient metric data will be subjected to a standard series of temporal and spatial contrasts. Temporal contrasts will include annual pair-wise comparisons of metric values: 1) among pre-treatment years; 2) among post-treatment years; and 3) between pre- and post-treatment periods. Spatial contrasts will include pair-wise comparisons metric values: 1) among the six sampling sites; and 2) between lower sites (TR1 through TR-3) and upper sites (TR-4 through TR-6). All spatial and temporal contrasts will be performed using ANOVA procedures (SAS 2009).

Sample size determination - Statistical precision of proposed sampling within each trophic level will be assessed using empirical project data and the formula:

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

where n is the desired sample number for desired statistical precision level and s, d, and z represent the variability, desired precision, and confidence levels, respectively. Statistical precision (d) will be set to 10% of each response mean. This designation will allow us to determine the number of samples needed to detect changes in post-treatment metric values down to +/- 10% of the mean for all metric values. The z values for the above equation were chosen to provide a 95% level of confidence, and the variability (s) was determined from the data. Sample size determination as described above will inform us as to whether the chosen sampling scheme meets or exceeds specified precision levels.

PERIPHYTON COMMUNITY AND PRIMARY PRODUCTION

Metrics

Five biological metrics will be monitored to characterize primary production and the algal community in the Twisp River (Table 1). To address algal taxonomic diversity, algal specimens will be identified and grouped by taxonomic Order as Cyanophyta (blue-greens), Chlorophyta (greens), or Bacillariophyta (diatoms).

These metrics were chosen because they quantify algal community composition (including edible vs. inedible forms), and quantify algal biomass and accrual rates, providing standard, comparable

measures of primary production. All algal sampling will occur monthly from April through November at all 3 positions at each of the two standard transects at all 6 sites (6 reps/site). Algal biomass values will be calculated at the University of Idaho's Analytic Services Lab (Moscow, ID.) using a standard PESC-Winterman/DeMots method. This method extracts chlorophyll from algal samples collected from punches of Styrofoam tiles previously placed on the river substrate at all sites and sampling positions along the two standard two transects.

Algae and Primary Production Hypotheses

The following hypotheses will be tested to assess algal composition, chlorophyll biomass, and primary production (algal accrual) in the Twisp River before and after experimental nutrient addition:

H_{o1}: Mean chlorophyll a concentrations...	1) ...values were not significantly among pre-treatment years.
H_{o2}: Mean chlorophyll b concentrations...	(2) ...values were not significantly among post-treatment years.
H_{o3}: Mean total chlorophyll concentrations...	(3) ...values were not significantly different between pre- and post-treatment periods.
$H_{o4;}$ Mean total chlorophyll accrual rate...	
H_{o5}: Algal community composition...	

Statistical Analyses for the Algal Community and Primary Production

Spatial and temporal contrasts – All algae, chlorophyll, and primary production data will be tested using this project's standard series of temporal and spatial contrasts. Temporal contrasts will include annual pair-wise comparisons of metric values: 1) among pre-treatment years; 2) among post-treatment years; and 3) between pre- and post-treatment periods. Spatial contrasts will include pair-wise comparisons metric values: 1) among the six sampling sites; and 2) between lower sites (TR1 through TR-3) and upper sites (TR-4 through TR-6). All spatial and temporal contrasts will be performed using ANOVA procedures (SAS 2009). Chi-square procedures in SAS will be used to compare composition of algal orders between and among pre-treatment years, between and among post-treatment years, and between aggregated pre- and post-treatment year periods (SAS 2009).

Sample size determination – Determination of sampling precision for all algae, chlorophyll, and primary production metrics will occur as described in the "Sample size determination" section of this report (see Page 11).

Invertebrate Community and Secondary Production

Metrics

Nineteen biological metrics will be monitored and calculated where necessary to characterize separate and aggregated species, community, and functional guild attributes of benthic

macroinvertebrates captured in the Twisp River (Table 1). All macroinvertebrates will be measured to the nearest mm in the lab (Invertebrate Ecology Inc., Moscow, ID.). Biomass will be estimated by plugging these length measurements into a length-mass regression model (Benke 1999). Biomass estimates will be used to calculate secondary production of the macroinvertebrates using the size-frequency model (Benke and Huryn 2007). Running the model requires average invertebrate density and biomass data by size class within each sample year. These data will be available from project Hess samples. Length measurements will be taken as a function of estimating biomass with length-mass regression models (see above), the later step facilitated grouping of species by size class.

Secondary production (*P*) will be calculated by the standard formula reported by Benke and Huryn (2007):

$$P = \sum (\hat{W}\Delta N \times No.\ of\ size\ classes)$$

where:

ΔN is the change in density between size classes, and

$\hat{W}$ is the difference in mean biomass between size classes

The formula multiplies ΔN (i.e. changes in density between size classes) by $\hat{W}$ (i.e. mean individual biomass between size classes) and sums the products (i.e. $\Delta N \times \hat{W}$) by size class after first multiplying the products per size class by the number of size classes (the later step is done to fulfill the assumption that the total number of size classes is equal to the number of cohorts per year). Secondary production values for each species will then be corrected based on their cohort production interval (CPI), i.e. the fraction of the year it takes for the species to develop (Benke and Huryn 2007). For example, a species with a CPI of 6 will be adjusted 2 fold (Marchant 1986). With secondary production data, P/B values can then be calculated for any time period, providing information on biomass turnover rates ("growth rates") of macroinvertebrates in the study area, and facilitating comparison of macroinvertebrate turnover rates within and among pre- and post-treatment periods.

Invertebrate sampling will occur monthly from April through November at all sites as flow conditions permit. A total of 6 Hess samples (2 at each position) will be collected monthly at one transect at each of the 6 sites, and subsequently pooled by site per month for a total of 6 samples per month. Additional sampling will occur during December and March to assess invertebrate production, diversity, and life histories (voltinism) at these times of the year.

Benthic Invertebrate and Secondary Production Hypotheses

The following hypotheses will be tested to assess invertebrate taxonomic and community metric values and secondary production in the Twisp River before and after experimental nutrient addition:

H_{o1}: Mean invertebrate density...

H_{o2}: Mean invertebrate richness...

H_{o3}: Mean Ephemeroptera (E) richness...

H_{o4}: Mean Plecoptera (P) richness...

H_{o5}: Mean Trichoptera (T) richness...

H_{o6}: Mean filterer taxa richness...

H_{o7}: Mean predatory taxa richness...

H_{o8}: Mean scraper taxa richness...

H_{o9}: Mean relative abundance (%) of E taxa...

H_{o10}: Mean relative abundance (%) of P taxa...

H_{o11}: Mean relative abundance (%) of T taxa...

H_{o12}: Mean relative abundance (%) of filterer taxa...

H_{o13}: Mean relative abundance (%) of predator taxa...

H_{o14}: Mean relative abundance (%) of scraper taxa...

(1) ...values were not significantly different among pre-treatment years.

(2) ...values were not significantly different among post-treatment years.

(3) ...values were not significantly different between pre- and post-treatment periods.

Statistical Analyses for Benthic Invertebrates and Secondary Production

Spatial and temporal contrasts – All invertebrate and primary production data will be tested using a standard series of temporal and spatial contrasts. Temporal contrasts will include annual pair-wise comparisons of metric values: 1) among pre-treatment years; 2) among post-treatment years; and 3) between pre- and post-treatment periods. Spatial contrasts will include pair-wise comparisons metric values: 1) among the six sampling sites; and 2) between lower sites (TR1 through TR-3) and upper sites (TR-4 through TR-6). All spatial and temporal contrasts will be performed using ANOVA procedures (SAS 2009). Chi-square procedures in SAS will be used to compare composition of dominant benthic macroinvertebrate taxa and functional feeding groups between and among years, and between aggregated pre- and post-treatment year periods (SAS 2009). Ordination techniques and additional community ecology methodologies (e.g. Magurran 2004) may also be employed to detect changes in the composition and structure of the community during the pre- and post-treatment years.

Sample size determination – Determination of sampling precision for all invertebrate and secondary production metrics will occur as described in the "Sample size determination" section of this report (see Page 11).

FISH COMMUNITY AND TERTIARY PRODUCTION

Metrics

Twelve biological metrics will be monitored and calculated where necessary to assess annual production and juvenile performance and condition for dominant anadromous (Chinook, steelhead) and resident species (bull trout, cutthroat trout, sculpin, and suckers) in the Twisp River before and after experimental nutrient addition (**TABLE 1**). Juvenile performance metrics currently include: length (FL) weight (g), and biological condition (*K*) at juvenile emigration, within-season and annual growth rates, juvenile abundance, composition of dominant fish species, and diet composition. Annual production metrics will also be addressed, including: redd counts, smolt production, and egg to emigrant survival, but only for dominant anadromous target species.

Fish sampling - Remote fish sampling will be done 4-5 times (depending on flows) each year at all sites using a combination of backpack electrofishing, snorkeling, and angling. Routine sampling will occur within the 100 m reaches between the lower two transects at each site. Additional sampling outside these areas may occur as needed to increase numbers of fish collected, marked, and recaptured, and to characterize species- and life stage-specific use of additional habitat areas.

All collected fish will be identified to species, measured (FL, mm), and weighed (g). All captured specimens of dominant anadromous and resident species of a suitable size will be tagged with a PIT tag ($\geq$ 65 mm FL recommended by PTAGIS, or $\geq$ 55mm FL with 8 mm tags) to help estimate abundance, growth, and survival. Fish data will be collected by project personnel and by additional field crews from the Washington Department of Fish and Wildlife, the United States Geological Survey, and the U.S. Fish and Wildlife Service. Fish data collected by project personnel will be stored in an electronic database and made available to collaborating agencies.

Electrofishing – Standard upstream single pass backpack electrofishing and multiple pass depletion methods will be employed seasonally in the sampling areas between the lower two transects (100m long x wetted channel width at time of sampling) at each site. Multiple pass depletion electrofishing techniques will follow standard operational guidelines reported by Hankin and Reeves (1988), including conventional abundance estimation techniques consistent with the nature of the collected data as described by Seber (1982). Electrofishing techniques will also be consistent with regionally accepted settings and protocols for sampling small streams (Terraqua 2009).

Snorkeling – Snorkeling will be performed to estimate fish abundance following standard, regionally accepted ISEMP methods. (NOAA; Murdoch et al. 2008; {http://www.nwfsc.noaa.gov/research/divisions/cbd/mathbio/isemp/display_isemp_event.cfm?eventid=32333).

Angling - Angling will be performed within and outside the standard sampling reaches in other representative habitats as needed to collect metric data from adequate numbers of fish.

Fish metrics

Abundance – Abundance of dominant anadromous and resident fishes will be estimated from multiple mark-recapture data by using an open Jolly–Seber model (Seber 1982; Pine et al. 2003) implemented in the POPAN-5 analysis software (Arnason et al. 1998a, 1998b). Abundance estimates from snorkeling observations will be annually calibrated with block-net, multiple pass depletion electrofishing techniques (Hankin and Reeves 1988; Peterson et al. 2004; Terraqua 2009), typically during April and/or July). Analyses will be based on individual fish data obtained by marking fish of appropriate sizes using PIT tags and subsequent recaptures. Initial mark and recapture observations will be paired and reformatted into an encounter history format suitable for the analysis.

Initial sampling results collected in year 1 of this study will provide the basis for power analyses that will be used to refine future sampling efforts. Power analysis will be conducted using the SampleSize 1.1 (Lady et al. 2003) software provided by Columbia Basin Research. This analysis will determine how many tagged individuals are needed to achieve a desired precision in estimates of abundance and survival consistent with sample recapture probabilities that can reasonably be achieved. One outcome from this analysis, for instance, may be to identify the minimum number of fish that need to be tagged in order to estimate of a plausible range of survival probabilities that are useful to the project objectives. *Post-hoc* power analysis for fish sampling will be performed following acquisition of initial empirical data, including numbers of fish marked and recaptured, recapture rates, and initial estimated abundance values. This method was chosen over an *a priori* approach which would be a strictly theoretical construct in the absence of empirical data.

A set of candidate Jolly-Seber models varying in complexity (*e.g.* modeling survival as constant or varying across time) will be fit to the capture-recapture data. Models fit from this set of candidate models will be evaluated using Akaike's information criteria (AIC). This approach to model selection has been validated by simulation studies (Anderson et al. 1994, Burnham et al. 1995) and is strongly recommended for capture-recapture studies (Leberton et al. 1992, Burnham and Anderson 2002).

Biological condition - Fulton's condition factor (*K*) will be used to characterize changes in body form as a proxy for biological condition for dominant fish species. *K* is defined as the ratio between the observed weight and an expected weight dependent on the fish's length (Blackwell et al. 2000). Fulton's *K* will be calculated using the following formula:

$$K = (W/L^3) \times 10^5,$$

where:

W is the weight of the fish in grams,

L is the length in millimeters, and

10^5 is a constant used for scaling purposes.

K will be calculated for all dominant resident fish species and for anadromous species at emigration. A *K* value of 1 will be assumed to represent optimal growth for each species. Because fish exhibit allometric growth (disproportionate increases in weight and length over time), *K* will be calculated for individual year- or size-classes by species, and will be used as relative index rather than an absolute metric of biological condition.

Growth – Growth of anadromous salmonids will be directly estimated from empirical length at age data. Growth may also be estimated for some resident fish species from scale samples using standard back-calculation methods (Summerfelt and Hall 1987). Estimates of growth from the scale sample back-calculation will be corroborated using individual data from recaptures of PIT tagged dominant resident fish. A von Bertalanffy growth curve will be fit the pooled individual data in which time-at-large will act as a surrogate to age as typically used in this growth curve.

Survival – Age and stage specific survival will be estimated with a combination of catch per unit effort, length or age frequency, cohort reconstructions, and mark-recapture data. Different methods will be evaluated for effectiveness based on data collected in the first year of the study. Annual apparent survival rates and recapture probabilities (*p*) will be estimated from mark-recapture data using Cormack–Jolly–Seber (CJS) and related models implemented in the statistical program MARK (White and Burnham 1999; Cooch and White 2001). Apparent survival (hereby referred to as survival) is distinguished from true survival in that apparent survival combines the probability of survival and the probability of not permanently emigrating out of the study area (i.e., 4 = 1 - mortality - emigration), whereas true survival deals only with mortality. Akin to the Jolly-Seber analysis, a set of candidate models will be fit and evaluated with AIC model selection criteria. Because survival through various life stages of anadromous species is critical to project success, and for identifying important age- or life stage-specific mortality periods, incremental survival rates will be calculated during the summer growth season and for the first overwintering period.

Diet composition – Diet composition of dominant fish species will be assessed by analyzing prey items removed from gut content samples collected by standard non-destructive passive and active (with lavage) techniques. Prey items from gut samples will be taxonomically identified, enumerated, and described according to standard methods (Gelwick and Matthews 2007, and references therein).

Composition of dominant fish species – Because this project focuses on dominant fish species and associated metrics as indicators of response to experimental nutrient addition, it will not characterize composition of the entire fish community. However, fish community composition will be assessed using common population metrics of relative abundance (abundance of each species as proportion of abundance of all species), species richness, evenness, and other diversity measures. In addition, all collected fish will be characterized according to status (native vs. non-native), environmental tolerance (tolerant, sensitive, and intermediate), preferred thermal regime (cold, cool, or warm water species), adult feeding guild (omnivore, insectivore, piscivore, or some combination), and adult habitat orientation (benthic, water column, or hider) for fishes of the Pacific Northwest as defined by Zaroban et al. (1999).

Annual production - The annual number of Chinook and steelhead redds will be estimated using standard spawning ground survey methods (Snow et al. 2010). Annual escapement of target species will be calculated by multiplying the total number of redds by the number of fish per redd (i.e., sex ratio) during a given year. Annual smolt production will be calculated using the number of smolts collected at a rotary screw trap in the lower Twisp River and applying numerical expansion techniques derived from in-river mark/recapture efficiency trials (Snow et al. 2010). Trap efficiency is estimated based on period-specific recapture rates of marked groups of fish released upstream from the trap (Roper and Scarnecchia 2000; Steinhorst et al. 2004). For Chinook stocks, total brood year smolt production will be calculated by adding fall parr emigration estimates to spring smolt production estimates. Because summer steelhead may emigrate over multiple years, smolt production estimates for each brood will be calculated as the sum of fish from brood year X that emigrate as age-1 fish in year Y, age-2 fish in year Z. The estimated number of smolts resulting from redds constructed downstream from the smolt trap will be included in emigration estimates using the egg to smolt ratio for redds constructed upstream of the smolt trap (Snow et al. 2010) to the estimate of smolts produced upstream from the trap location (between TR1 and TR2). The annual number of juveniles per redd will be calculated by dividing the total number of estimated outmigrants by the total number of redds in a given year for each anadromous species. Egg to emigrant survival estimates will be calculated by dividing the estimated number of emigrants per species and brood year by the estimated egg deposition for each species and brood calculated from redd surveys (Snow et al. 2010). Egg deposition will be calculated by estimating the age and origin composition of the spawning females and applying stock- and species-specific fecundity values to each redd. For example, given 100 steelhead redds with 70% wild 2-salt females in the spawning population, we apply the fecundity of wild 2-salt fish to 70% of the observed steelhead redds).

Research Questions and Hypothesis Testing for Fish

This study addresses the following questions concerning performance and biological conditions of dominant anadromous and resident fish species in the Twisp River:

1. Did performance and biological condition of dominant anadromous and resident fish species differ among pre-treatment years?
2. Did performance and biological condition of dominant anadromous and resident fish species differ among post-treatment years?
3. Did performance and biological condition dominant anadromous and resident fish species differ between pre- and post-treatment years?

These questions will be addressed by the following null hypotheses concerning performance and biological condition, as indicated by abundance, length, weight, growth, survival, biological condition, and diet composition.

Abundance (of various life stages or size classes)

H_{oA1}: Did juvenile abundance differ significantly among pre-treatment years?

H_{oA2}: Did juvenile abundance differ significantly among post-treatment years?

H_{oA3}: Did juvenile abundance increase significantly following nutrient addition?

Length

H_{oL1}: Did mean length differ significantly among pre-treatment years?

H_{oL2}: Did mean length differ significantly among post-treatment years?

H_{oL3}: Did mean length increase significantly following nutrient addition?

Weight

H_{oW1}: Did mean weight differ significantly among pre-treatment years?

H_{oW2}: Did mean weight differ significantly among post-treatment years?

H_{oW3}: Did mean weight increase significantly following nutrient addition?

Growth (to various life stages)

H_{oG1}: Did juvenile growth differ significantly among pre-treatment years?

H_{oG2}: Did juvenile growth differ significantly among post-treatment years?

H_{oG3}: Did juvenile growth increase significantly following nutrient addition?

Survival (to various life stages, including overwintering)

H_{oS1}: Did juvenile survival differ significantly among pre-treatment years?

H_{oS2}: Did juvenile survival differ significantly among post-treatment years?

H_{oS3}: Did juvenile survival increase significantly following nutrient addition?

Biological condition (*K*, by life stage or size class)

H_{oK1}: Did juvenile *K* differ significantly among pre-treatment years?

H_{oK2}: Did juvenile *K* differ significantly among post-treatment years?

H_{oK3}: Did juvenile *K* increase significantly following nutrient addition?

Diet composition (by life stage or size class)

$H_{oD1:}$ Did juvenile diet composition differ significantly among pre-treatment years?

$H_{oD2:}$ Did juvenile diet composition differ significantly among post-treatment years?

$H_{oD3:}$ Did juvenile diet composition differ significantly following nutrient addition?

This project also addresses several questions regarding natural production of anadromous fish in the Twisp River:

1. Did annual production of juvenile anadromous salmonids differ among pre-treatment years?
2. Did annual production of juvenile anadromous salmonids differ among post-treatment years?
3. Did annual production of juvenile anadromous salmonids differ between pre- and post-treatment years?

These questions will be addressed by the following null hypotheses concerning natural production, as indicated by redd counts, number of juveniles per redd, annual smolt production, and outmigration timing:

These questions will be addressed by the following hypotheses regarding natural production of anadromous species indicated by numbers of juveniles per redd, annual smolt production, and outmigration timing:

Number of juveniles per redd

$H_{oJ2:}$ Did the number of (CHN, STD) juveniles per redd differ significantly among post-treatment years?

$Ho_{J3:}$ Did the number of (CHN, STD) juveniles per redd increase significantly following nutrient addition?

Annual smolt production

$H_{oP1:}$ Did annual (CHN, STD) smolt production differ significantly among pre-treatment years?

$H_{oP2:}$ Did annual (CHN, STD) smolt production differ significantly among post-treatment years?

$H_{oP3:}$ Did annual (CHN, STD) smolt production increase significantly following nutrient addition?

Outmigration timing

$H_{oO1:}$ Did peak (CHN, STD) outmigration timing differ significantly among pre-treatment years?

$H_{oO2:}$ Did peak (CHN, STD) outmigration timing differ significantly among post-treatment years?

$H_{oO3:}$ Did peak (CHN, STD) outmigration timing differ significantly following nutrient addition?

Statistical Analyses for Fish

Spatial and temporal contrasts – All project fish metric data will be tested using a standard series of temporal and spatial contrasts. Temporal contrasts will include pair-wise annual comparisons of all fish metrics: 1) among pre-treatment years; 2) among post-treatment years; and 3) between pre- and post-treatment periods. Spatial contrasts will include pair-wise comparisons invertebrate metrics: 1) among the six sampling sites; and 2) between aggregated lower sites (TR1 through TR-3) and aggregated upper sites (TR-4 through TR-6).

Spatial and temporal contrasts of fish production, performance, and biological condition metrics will be performed using ANOVA procedures (SAS 2009). Chi-square procedures in SAS will also be used to compare composition of dominant fish species between and among pre-treatment years, between and among post-treatment years, and between aggregated pre- and post-treatment year periods (SAS 2009).

Sample size determination – Sampling precision for the dominant anadromous and resident fish species will be determined using empirical data from the Twisp River collected beginning during

2011 using standard methods described in more detail in the "Sample size determination" section of this report (See Page 11).

FOOD WEB CHARACTERIZATION WITH STABLE ISOTOPES

Introduction

The final component of this study will assess functions, processes, and linkages within, between, and among trophic levels by characterizing the Twisp River food web. The food web will be characterized by integrating three complementary techniques: 1) stable isotope analysis of nitrogen (N) and carbon (C) from each trophic level; 2) fish gut content analysis; and 3) annually replicated experimental nutrient addition involving nutrient routing through the food web.

An initial food web diagram will be constructed for the Twisp River by incorporating results of multi-trophic sampling (i.e. taxonomic and function guild assignment) described in previous chapters of this report, and fish gut content analysis. Although individuals of some taxonomic groups may not fit neatly into a single trophic level, the initial food web diagram will include four general trophic levels: organic nutrient sources, primary producers (the algal/periphyton community), secondary producers (the benthic macroinvertebrate community), and tertiary consumers (the fish community). Putative food web structure, function, and linkages will then be assessed using standard labeled isotope techniques.

Isotope Metrics - Isotope metrics will include stable isotope ratio values for C and N (^{13}C:^{12}C and ^{15}N:^{14}N, or $\delta^{13}C$ and $\delta^{15}N$).

Stable isotope analysis – Stable isotope analysis has become a common component of stream ecology studies (summarized in Hershey et al. 2007). Heavy isotopes of carbon (^{13}C) and nitrogen (^{15}N) are particularly useful for delineating biological transfer of C and N from plants, detritus, or primary producers to primary, secondary, and tertiary consumers. Carbon and N each have heavy and light isotopes, and their respective isotopic ratios (^{13}C:^{12}C and ^{15}N:^{14}N) can be measured very accurately, allowing investigation of food web structure and function. Distinct isotopic ratios of C and N are often associated with different organic nutrient sources and specific functional feeding groups, allowing researchers to characterize prey items in animals' diets and to identify significant changes in diet consumption and feeding patterns (MacAvoy et al. 2001; Phillips and Eldridge 2005; Church et al. 2009). Stable isotope techniques have also been used to generate time-integrated information about feeding relationships in aquatic food webs (Kling et al. 1992; Cabana and Rasmussen 1994; Hobson and Welch 1995; Church et al. 2009), to differentiate pelagic and benthic prey items, and to characterize the trophic positions of aquatic organisms (Vander Zanden et al. 1999). Bilby et al. (2001) reported that relationships between stable isotope values and carcass abundance may provide a useful supplement to traditional methods of establishing escapement goals for Pacific salmon.

Given these informative attributes, stable isotope analysis will be used to characterize vertebrate and invertebrate communities, food web structure, function, and linkage to assess nutrient flow and energy routing through the Twisp River food web before and after experimental nutrient addition.

Isotope sampling **–** Isotopes of C and N will be sampled from all trophic levels at the upper transects at the three sites in the Twisp River (TR-2, TR-4 and TR-6) each year during April, July, September and October (Appendix 5). Up to 10 samples will be collected from each of the three sites during each of the four annual sampling episodes. Each isotope sampling episode from the algal community will involve samples scraped from natural substrates (e.g. rocks) at all three transects at each of the three isotope sampling sites. Isotope sampling from the benthic macroinvertebrate community will include up to 10 samples from each site from each of three invertebrate feeding guilds (predators, scrapers, shredders) to account for potentially different food sources, isotopic signals, and energy pathways through the food web. Taxa representing these invertebrate feeding guilds in the Twisp River are currently being identified from initial sample collections. Three dominant fish species (Chinook, steelhead, and sculpin) will also be sampled for isotopic analysis, with small fin clips and mucous samples supplying material for analysis. Isotopic signatures in fish mucous respond much more quickly to isotopic shifts in food sources compared to fin or other tissue samples (Church et al. 2009). Therefore, we will collect and analyze isotope samples from both sources of fish samples (fin tissue and mucus) to enable detection and comparisons between short- and long-term diet shifts and food sources. Additional investigation may include comparisons of the magnitude and stability of isotopic ratio values of C and N from whole fish, mucous, fins clips, and other tissue samples to address effects of sample origin on isotopic signatures.

All isotope samples will be placed in 1.5 ml snap-top plastic micro-centrifuge tubes and immediately frozen in the field. Samples will remain frozen and will be shipped or transported frozen to the University of Idaho's Stable Isotope Lab (Moscow, ID.) for processing and analysis as quickly after collection as possible. Following arrival at the lab, all samples will be thawed, freeze dried, and pulverized into a fine power to facilitate analysis.

Research Questions and Hypothesis Testing Involving Stable Isotopes

Isotope ratio distinction and stability - Because stable isotope ratio values must be distinct and temporally and spatially stable to be informative, this project will initially address stability and distinction of isotopic signatures of C and N in the Twisp River by testing the following hypotheses for each trophic level:

> $H_{oSI}1$: Mean annual isotopic ratio values of (C, N) are not significantly different within sites (between, among) pre-treatment years.
>
> $H_{oSI}2$: Mean annual isotopic ratio values of (C, N) are not significantly different within sites (between, among) post-treatment years.

$H_{oSI}3$: Mean annual isotopic ratio values of (C, N) are not significantly different within sites between pre- and post-treatment years.

Each of the above three hypotheses will be tested for natural and experimentally added organic nutrient sources and the algal/periphyton, benthic invertebrate, and fish communities consistent with multi-trophic sampling techniques and schedules described in previous chapters of this report. Once the above hypotheses are tested and if spatial and temporal stability of isotope ratio values of C and N are adequate for use in the Twisp River, characterization of the food web and fish diet composition will occur.

Food web characterization – Research questions regarding food web structure and function and fish diet composition are currently being developed. However, they may include questions, and when appropriate testable quantitative hypotheses, concerning significant changes in nutrient routing, isotopic ratio values of taxa from all trophic levels, and fish diet composition: 1) between and among pre-treatment years, 2) between and among post-treatment years, and 3) between pre- and post-treatment periods.

Ultimately, the integration of stable isotope and fish gut content analysis, and the experimental addition of labeled nutrients will address questions of whether and how nutrient addition contributed to production, condition, and performance of listed juvenile steelhead and Chinook, and other resident fish species in the Twisp River, and to additional biotic communities and food web functions within and among essential lower supporting trophic levels.

Statistical Analyses of Isotope Data

Spatial and temporal contrasts – Isotope ratio data from all trophic levels will be tested using a standard series of temporal and spatial contrasts. Temporal contrasts will include pair-wise annual comparisons: 1) between and among pre-treatment years; 2) between and among post-treatment years; and 3) between pre- and post-treatment periods. Spatial contrasts will include pair-wise comparisons of isotopic ratio values between and among the three isotope sampling sites.

Spatial and temporal contrasts of isotope ratios of C and N from all trophic levels will be performed using ANOVA procedures (SAS 2009). Chi-square procedures in SAS will be used to compare fish stomach content composition from target anadromous and resident fish species between and among pre-treatment years, between and among post-treatment years, and between aggregated pre- and post-treatment periods (SAS 2009).

Sample size determination – Determination of sampling precision for stable isotope metrics will be determined using annual empirical data initially collected from the Twisp River during 2011 using standard methods described in the "Sample size determination" section of this report (see Page 11).

RESULTS

WATER QUALITY

DISCHARGE- Being an unimpounded system, the Twisp River exhibited similar annual and season runoff patterns during 2009 and 2010 (**FIGURE 4 AND 5**). Discharge during both years increased abruptly during May, reached peak flows between 1,000 and 2,000 kcfs during June and July, and dropped to annual low flows by September or October (**FIGURES 4 AND 5**). Compared to the single peak flow event around June 1, 2009, runoff conditions during 2010 were protracted, with several peak flow events occurring during June 2010 (**FIGURES 4 AND 5**).

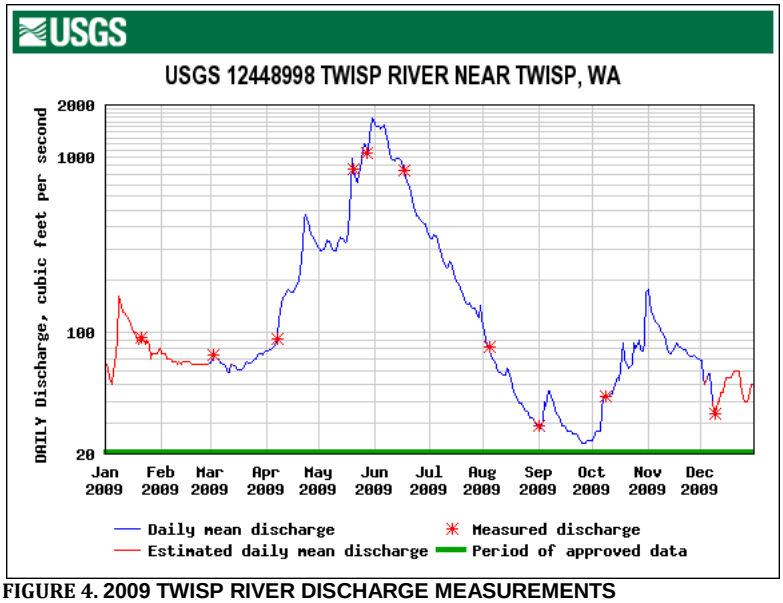

FIGURE 4. 2009 TWISP RIVER DISCHARGE MEASUREMENTS

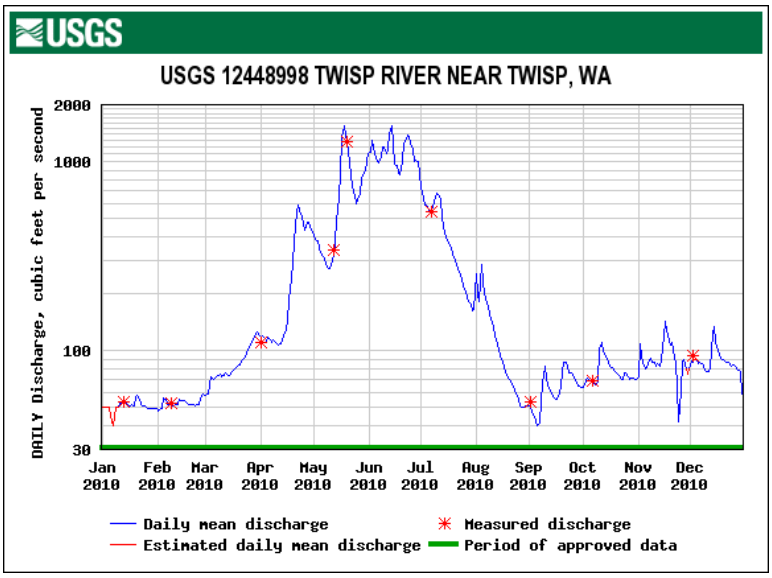

FIGURE 5. 2010 TWISP RIVER DISCHARGE MEASUREMENTS

NUTRIENTS – Total nitrogen (TN) concentrations in the Twisp River exhibited similar patterns during 2009 and 2010. Mean TN values ranged from about 90-100 ug/L at furthest upstream site (TR6), decreased or remained steady downstream until TR2, then abruptly increased to the highest observed values at TR1 (**FIGURE 6**). Mean TN values at each site were greater during 2009 than during 2010, with the exception of TR6, where differences between years were marginal (**FIGURE 6**).

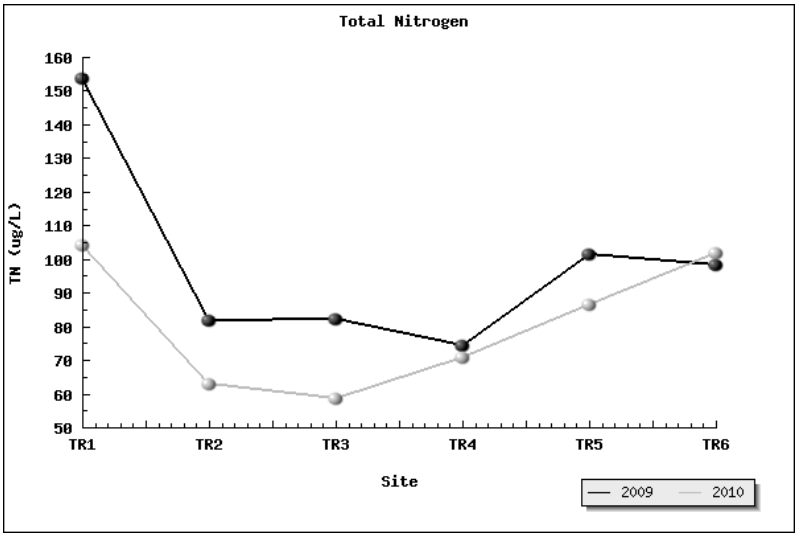

FIGURE 6. ANNUAL MEAN TOTAL NITROGEN PER SITE

Similar total phosphorus (TP) concentrations and longitudinal patterns occurred during both years, with the exception of TR5 during 2009 (FIGURE 7). Total phosphorus exhibited an increasing downstream trend from between 4 and 5.5 ug/L upstream from TR2 to around 7ug/L at TR1 and TR2, with the exception of TR5 during 2009, when TP increased to nearly 7ug/L (FIGURE 7). During 2010, the concentration of soluble reactive phosphorus (SRP), the most biologically available form of phosphorus, averaged at or below the minimum lab detection limit of 1ug/L, while TDP averaged between 2 and 3ug/L (data not shown).

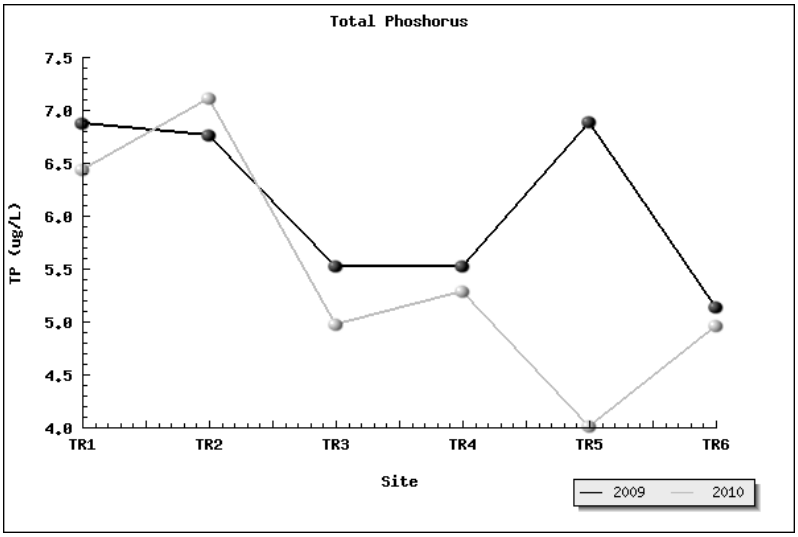

FIGURE 7. 2009 AND 2010 ANNUAL MEAN TOTAL PHOSPHORUS PER SITE

Observed TN:TP ratio values were similar during 2009 and 2010, averaging around 20 at TR6, decreasing to between 12 and 18 downstream at TR2, then abruptly increasing to the highest observed values of 35 and 24 at TR1 in 2009 and 2010 respectively, (FIGURE 8).

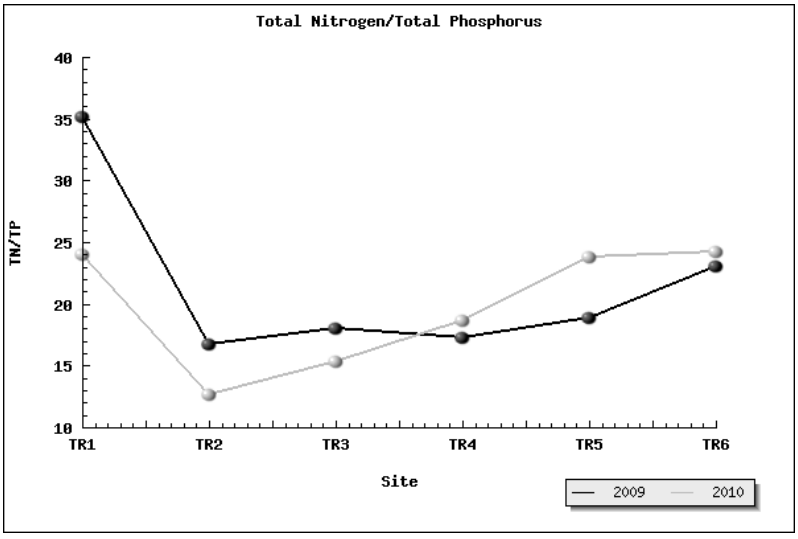

FIGURE 8. 2009 AND 2010 ANNUAL MEAN TOTAL NITROGEN/TOTAL PHOSPHORUS PER SITE

PRIMARY PRODUCTION-Primary production, expressed as chlorophyll accrual rates (ug/m^2/day), exhibited considerable inter-site variation during both years (**FIGURE 9**). Accrual rates generally decreased downstream during both years, however, considerable fluctuations in accrual rates were observed between adjacent sites, especially during 2010 (**FIGURE 9**). Chlorophyll a biomass during 2010 at all sites averaged between 3 and 8 mg/m^2 (data not shown).

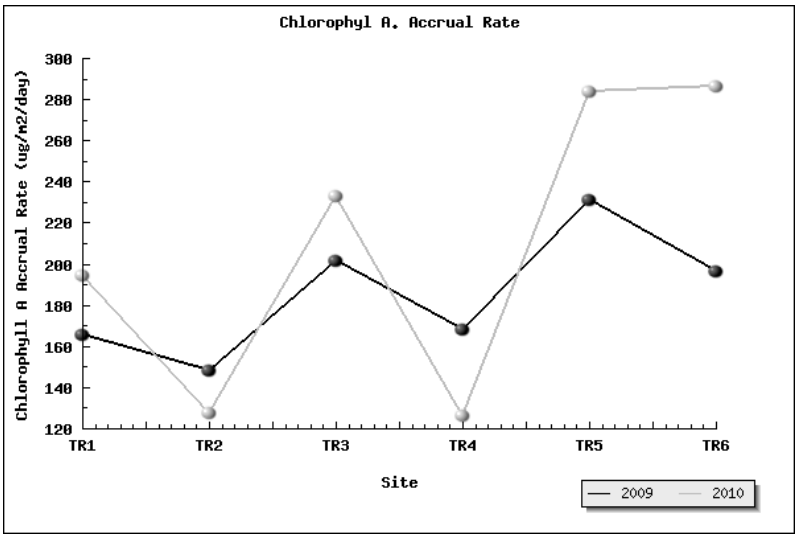

FIGURE 9. 2009 AND 2010 MEAN CHLOROPHYLL A. ACCRUAL RATE PER SITE

BENTHIC MACROINVERTEBRATES – Aggregated benthic macroinvertebrate abundance (all taxa) exhibited a substantial downstream increase during both years. Values were higher at all sites in 2009 than in 2010, with the exception of TR1 (**FIGURE 10**). Aggregated abundance ranged from just over 1,500 organisms/m^2 at TR4 during 2009 to a high of approximately 6,500 and 5,500 organisms/m^2 during 2009 and 2010 respectively at TR1, the furthest downstream site (**FIGURE 10**).

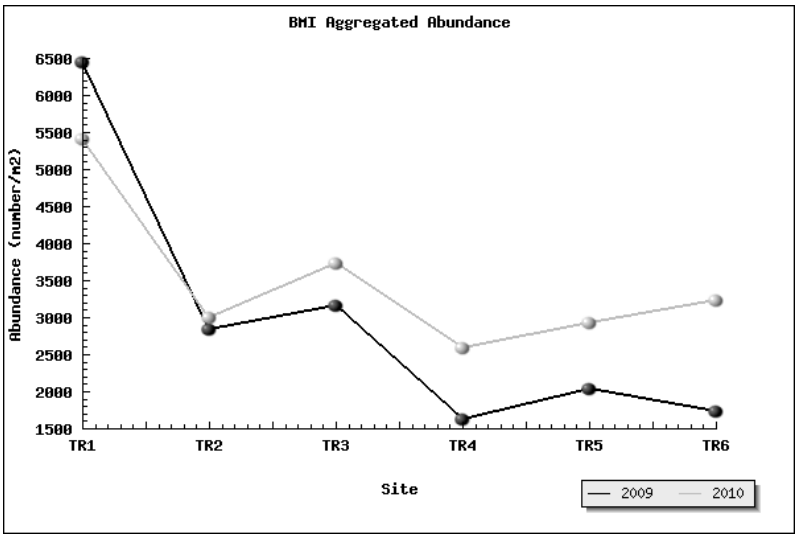

FIGURE 10. 2009 AND 2010 MEAN BMI AGGREGATED ABUNDANCE PER SITE

As seen with aggregated invertebrate abundance, aggregated biomass was greatest at the upstream end of the study area and steadily increased downstream (**FIGURE 11**). Aggregated biomass during 2009 ranged from approximately 1,200-3,300 mg/m^2 during 2009, compared to a range of 1,800-2,300 mg/m^2 during 2010 (**FIGURE 11**). Consistent with aggregated abundance, aggregated biomass of benthic invertebrates was also higher at all sites in 2010 than in 2009, except at TR1 (**FIGURE 11**).

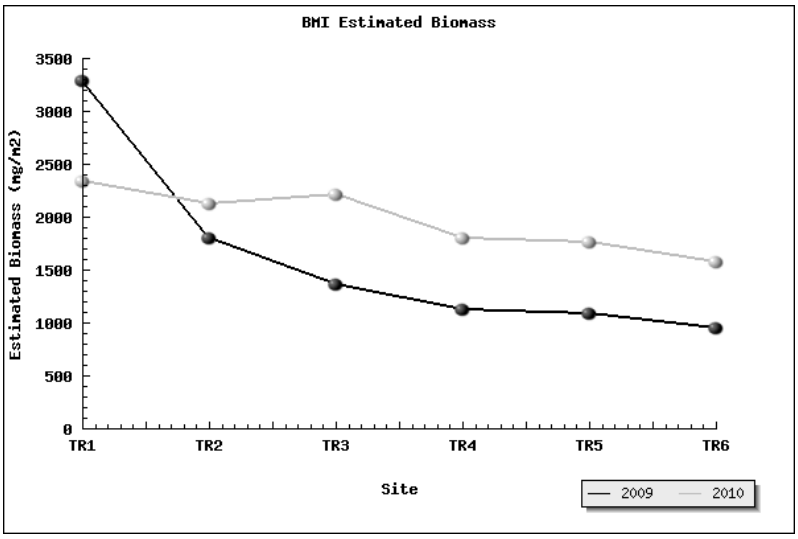

FIGURE 11. 2009 AND 2010 MEAN ESTIMATED BMI BIOMASS PER SITE

Total richness (total number of taxa) exhibited similar increasing downstream trends during both years from TR6 to TR4, considerable variability between adjacent sites, and an increase at TR3 followed by a decrease at TR2 (**FIGURE 12**). Total benthic macroinvertebrate richness per site was higher at all sites during 2010 than during 2009, ranging from approximately 24 to 28 during 2010 and from 21 to 25 during 2009 (**FIGURE 12**).

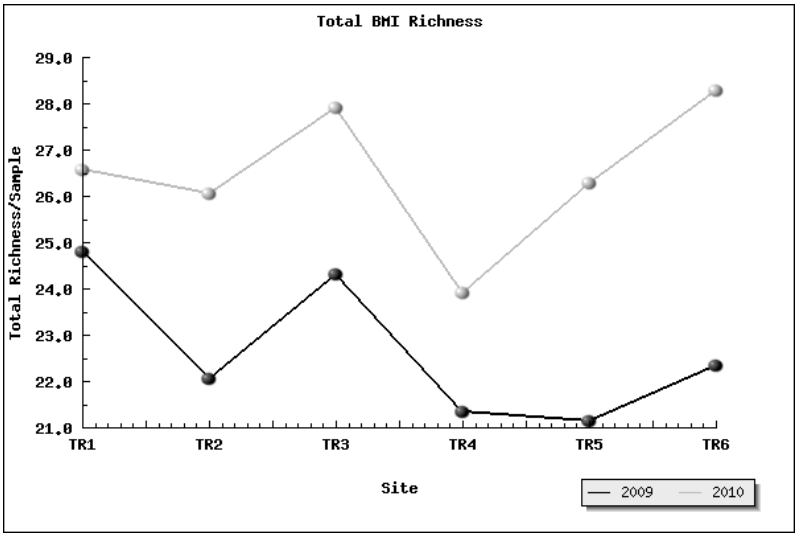

FIGURE 12. 2009 AND 2010 MEAN TOTAL BMI RICHNESS PER SITE

Percent tolerant richness (#tolerant taxa per site/# total taxa per site X 100) exhibited an increasing downstream trend during both years, ranging from approximately 2.5 to 7 taxa during 2009 and 2.5 to 5.5 during 2010 (**FIGURE 13**). Differences between years included higher richness values at TR1, TR2, and TR6 during 2009 than during 2010 (**FIGURE 13**).

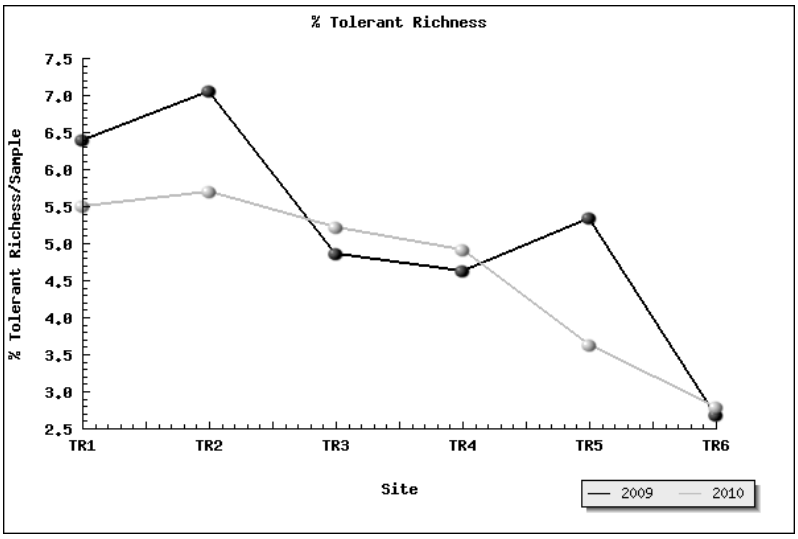

FIGURE 13. 2009 AND 2010 MEAN TOLERANT RICHNESS PER SITE

Mean percent EPT values during 2010 increased upstream from about 57at TR6 to just over 70 at TR4, followed by a steady downstream decrease to 37 at TR1, the farthest downstream site (**FIGURE 14**).

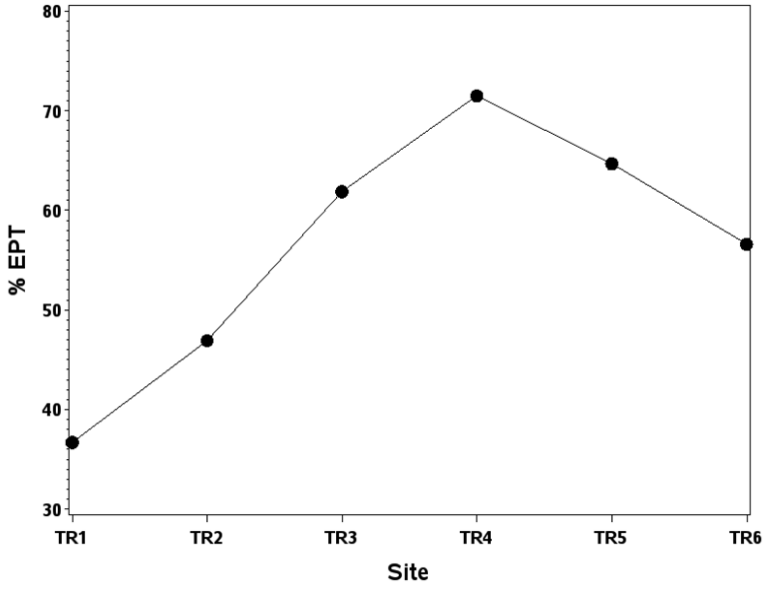

FIGURE 14. 2010 MEAN % EPT PER SITE

Percent Diptera per site during 2010 exhibited the opposite trend shown by percent EPT, with a steady decrease from about 53% to 25% from TR6 to TR4, followed by a steady increase from TR4 to about 53% at TR1 (~42%; **FIGURE 15**).

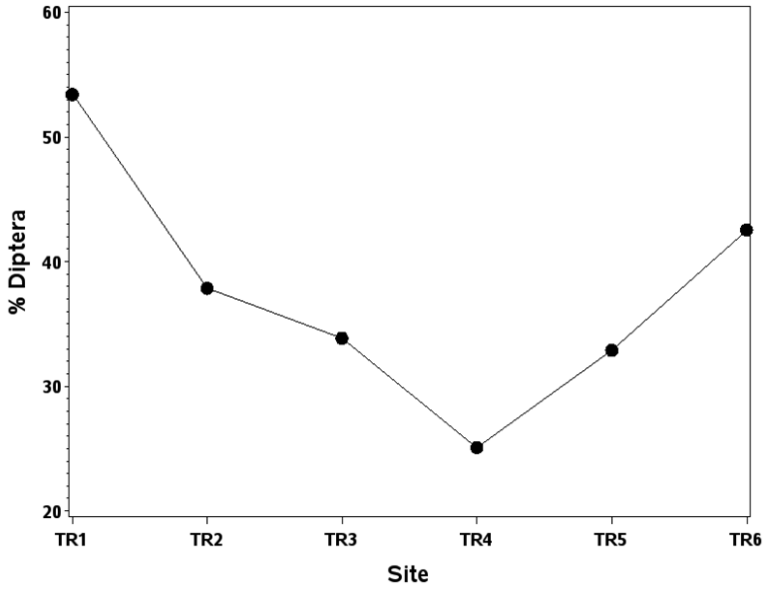

FIGURE 15. 2010 MEAN % DIPTERA PER SITE

DISCUSSION

For this reporting period (2009 and 2010) project tasks addressed the project goal of characterizing baseline nutrient levels in 44km of the Twisp River. Completed tasks included the establishment of sampling sites, development of sampling protocols, and completion of sample size analysis reports to evaluate the effectiveness of the project's sampling regime throughout the study reach.

Sampling sites were chosen systematically by dividing the study reach into 6 sites using public access and major geomorphic reach breaks as criteria for potential locations. When developing sampling protocols, we researched recent relevant literature and spent time with project staff from related programs with similar restoration goals. Using sampling size determination statistics has enabled us to evaluate how well our sampling effectively represents aquatic conditions within the study area. Refinements in sampling protocols (number of sites, replicates etc.) were based on sample size analyses, following collection and verification of annual field data.

Environmental conditions associated with dynamic unregulated rivers are often difficult to predict and generally impossible to control. This being the case in the Twisp River, sampling can be difficult to conduct at pre-planned (monthly) frequencies.

Below we provide an initial (2009 and 2010) discussion of the observed biological metric values and biological trends as presented in the results section of this report. A subset of ecologically diagnostic metrics from several lower trophic levels (water quality, nutrients, primary production, and the benthic macroinvertebrate community) was featured in this report to initially characterize the trophic status and the invertebrate community of the Twisp River. This portrayal is not comprehensive and includes data collected only during 2009 and 2010 with a subset of project metrics reported. Nonetheless, this report provides the first project summary of trophic status in the Twisp River, as the first step in system diagnosis, relative to nutrient effects on natural production of anadromous salmonids and ecosystem functionality.

WATER QUALITY

DISCHARGE – Due to its unregulated nature, annual variation in the Twisp River hydrograph was expected in proportion to the degree of inter-annual variability in snowpack and weather conditions. For example, although outside the period covered by this report, extreme discharge events resulting from a larger than average snowpack and snowmelt conditions during 2011 resulted in delayed project sampling in the Twisp River. However, flexibility has been designed into the project's sampling regimes for all trophic levels to compensate for such variability without losing statistical power or representation of data collected by the project.

NUTRIENTS – Although data from a subset of the projects nutrient metrics from 2009 and 2010 were reported here, the magnitude and longitudinal trends of nutrient concentrations revealed by project sampling helped to characterize the current trophic status of the Twisp River, and supports low amounts of marine derived nutrient or ultra-oligotrophy. For example, average TN values across sites were largely at or below 100 ug/L during 2009 and 2010, despite within-year variation among sites. Total phosphorus concentrations, with the exception of the farthest downstream sites (TR1 and TR2) remained below 6 ug/L, with the exception of TR5 during 2009. The standard Carlson's Trophic Sate index suggests a TP concentration of 12 or less as oligotrophic (Carlson and Simpson 1996). Furthermore, over 95% of the biologically available phosphorus (soluble reactive phosphorus, SRP) samples were at or below a detection limit of 1 ug/L (during most of the sampling season), indicating an ultra-oligotrophy.

In terms of nutrient balance, atomic N:P ratio values < 10:1 were considered N-limited, > 20:1 were P-limited, and at 10 - 20:1 both N and P could be limiting (Redfield 1958; Borchardt 1996). Project TN:TP ratio values calculated for the Twisp River using 2009 and 2010 data were similar between years, showing considerably higher P-limitation at TR1 than at all other upstream sites (TN:TP ratio values of 35 and 24 at TR1 for 2009 and 2010 respectively). Ratio values at TR 2 through TR4 during both years suggested nutrient co-limitation, while upstream conditions at TR5 and TR6 during both years were trending back toward P-limitation as seen at TR1. Nonetheless, at current fertility levels, the lack of extreme nutrient imbalance, which can be associated with problem algal blooms, is encouraging.

PRIMARY PRODUCTION – Observed chlorophyll biomass and accrual rates, as measures of primary production, were consistent with the low nutrient concentrations observed in the Twisp River. Mean chlorophyll biomass values during 2009 and 2010 ranged from approximately 3 to 8 mg/m^2, indicating ultra-oligotrophic status based on literature values (< 20mg/m for ultraoligotrophy, <60 mg/m^2 for oligotrophy, 60-200 mg/m^2 for meostrophy, and > 200 mg/m^2 for eutrophic conditions; (Carlson and Simpson 1996).

BENTHIC MACROINVERTEBRATES

Abundance - Benthic macroinvertebrate abundance is particularly critical during summer months as a biological indicator of system health and secondary production rates and for fish growth, condition, reproduction and juvenile recruitment. Aggregated (all taxa) macroinvertebrate abundance in the Twisp River during 2009 and 2010 ranged from approximately 1,500 to 6,400 organisms/m^2, indicative of an oligotrophic or ultra-oligotrophic system. Compared to other Pacific Northwest rivers, macroinvertebrate densities in the nearby Priest River (an oligotrophic river) were reported to be 3,944/m^2, while macroinvertebrate density in the Coeur d'Alene and Salmon Rivers were 62,938 and 38,233 per m^2, respectively (Royer and Minshall 1997). Wisseman (2003 pers. com.) indicated that 10,000 to 30,000 invertebrates/m^2 was typical for open, larger streams and small rivers in the western United States. These values represent densities up to 15 times higher than those observed in the Twisp River samples collected during 2009 and 2010.

% tolerant richness – Although exhibiting some magnitude variation between years, % tolerant taxa richness per site generally increased downstream during 2009 and 2010, as would be expected from longitudinal river continuum theory (Vannote et al. 1980) and consistent with the increasing degree of habitat alteration and anthropogenic effect.

% EPT per site/%Diptera per site - In 2010 (the only year presented for these metrics), percent EPT abundance increased from TR6 to TR4, then decreased steadily from TR4 through TR1. During the same year, the longitudinal pattern of %Diptera per site was exactly the opposite. These longitudinal distributions of EPT and Diptera abundance suggest the presence of diagnostic gradients in substrate conditions with inverse suitability for both taxa groups in the Twisp River (i.e. the middle sections of the Twisp River (TR3-TR5) are most suitable for EPT and least suitable for Diptera). EPT taxa tend to colonize clean, unembedded gravel and cobble substrates whereas Dipteran taxa overwhelmingly occupy soft-bottomed areas of rivers and streams (Merritt and Cummins 1996), supporting the mirror image plots of longitudinal suitability for the two taxa groups.

CONCLUSIONS

In conclusion, lower trophic level metric values measured during 2009 and 2010 suggest ultra-oligotrophic status for the Twisp River. This conclusion is based on low empirical nutrient concentrations, and generally reduced biological production, abundance, and biomass values within the periphyton and benthic macroinvertebrate communities relative to other Pacific Northwest rivers. These results are not surprising given the low stream order of the Twisp River, the geologic makeup of its watershed, its high elevation headwaters, and the substantial reductions in marine derived nutrient loading rates. Historic MDN loading rates, based on larger historical run sizes and carcass contributions provided the energetic foundation basis for biological productivity in salmon producing streams, especially those in the interior areas of the Columbia River Basin that were naturally oligotrophic. The longitudinal N:P ratio value gradient observed during both years suggests co-limitation at most Twisp River sites and phosphorus limitation at the farthest downstream site (TR1). Based on these initial data analyses, and its relatively intact physical habitat conditions, the Twisp River may be a prime candidate for experimental nutrient addition to increase natural production of anadromous salmonids and the required supporting biological production and ecological functions at lower trophic levels.

ACKNOWLEDGEMENTS

Many people have contributed to the project during this reporting period. I would like to thank our field crew, Dan Russell and John Schaefer, our sub-contractors, Tim Hatten (Invertebrate Ecology), Bahman Shaffi (Statistical Consulting Services) and numerous staff at Cramer Fish Sciences.

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