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1. Executive Summary

2. 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.

3. This project is currently working on quantifying and evaluating nutrient status and availability in the Twisp River and Hancock Spring Creek, within the 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.

5. Introduction

6.

7. The dramatic decline in anadromous salmonid populations throughout the Pacific Northwest during the past century has been accompanied by greatly reduced natural production. The loss and degradation of physical habitat, loss of marine derived nutrient, and the deleterious presence of non-native fishes have been identified as three important factors currently limiting natural production (NRC 1996; Gresh et al. 2000). The goal of this Program is to provide fish managers with options to restore and increase natural production of listed anadromous and resident salmonids. Program objectives are to: 1) define the mechanisms by which these three factors limit natural production, and 2) implement and test restoration measures specifically designed to increase natural production by reducing the effects of these currently limiting factors.
8. This report summarizes the Yakama Nation's Upper Columbia Natural Production Restoration Program (Program) for native anadromous and resident salmonids currently being implemented in the Methow River Subbasin in north central Washington. The report has two parts.
9. Part 1 provides a detailed scientific background and describes how this Program addresses relevant regional management issues and mandates. The scientific background sections discuss anadromous salmon declines in the Pacific Northwest, the roles of anadromy in natural production, factors limiting natural production, the current status of natural production in the Methow Subbasin, and the use of food web studies in restorative ecology. Part 1 then describes relevant ESA issues, the Columbia Basin Accords, Program alignment with ESA recovery and Fish Accord Actions, and the use of food web studies in restoration and management programs for Pacific salmonids. Part 2 describes the Program goals, objectives, and design, along with the project study areas, experimental treatments, and monitoring and evaluation components, including physical habitat and biological sampling, response variables, data collection, hypotheses, and statistical analyses. Current results are then reported and discussed.
10. This Program is being implemented in conjunction with a complementary aquatic trophic production (ARP) food web dynamics model project in the Methow River Subbasin led by the USGS. The modeling project is a collaborative effort involving the USGS, the BOR, the Yakama Nation, the University of Idaho, and Washington State University. Empirical data generated by the Yakama Nation's Program and other regional agency and academic research efforts will be used to populate the food web model. In turn, model output will be used to guide the restoration of natural production. Additional information about the modeling project is provided in Appendix 1 of this report.

a. Tributary Habitat RM&E

Project reporting period covers the baseline data collection, nutrient treatments not yet implemented.

Action Effectiveness Monitoring

Uncertainty Research

Hypothesis

H1 Nutrient enhancement increases fish production

Critical Uncertainties

Do nutrients route dirrectly into fish production or do they move through lower trophic levels?

Project Map:

http://www.cbfish.org/Project.mvc/Map/2008-471-00

Contract Map(s):

http://www.cbfish.org/Contract.mvc/Map/52183

11. Methods: Protocols, Study Designs, and Study Area

Program Design

Because fishery enhancement requires restored natural production at the river scale, and because rigorous evaluation of restoration options may be best achieved through controlled, replicated experimentation at a small scale, this program includes research, monitoring, and evaluation of applied restoration treatments at both the small stream and river scales.

This Program has two projects, the Hancock Springs and Twisp River projects. The Hancock Springs Project occurs in a small spring creek that facilitates research, monitoring, and evaluation at a fine scale, with the goal of detecting nutrient routing pathways. The Twisp River Project evaluates river fertilization at the larger river scale.

Treatment: Nutrient addition

(Hancock Springs 2014 - 2018, Twisp River 2018+)

Objective: Evaluate the effects of nutrient addition on natural production of anadromous and resident salmonids and on supporting trophic ecology in stream reaches with (Reach 1) and without (Reach 2) previous physical habitat restoration treatments.

Justification: Nutrient availability has been extensively reported as an important factor limiting natural production of salmonids in natal habitats during early life stages. Nutrient addition is designed to increase nutrient and food availability in order to increase natural production that may have been previously nutrient-limited.

Design: In Hancock Springs the effects of nutrient addition will be evaluated by statistically comparing mean values from a standard suite of biological response variables before and after treatment, and between Reach 1 (treatment) and Reach 2 (control). Biological responses to nutrient addition will be initially analyzed by reach and subsequently compared between reaches to evaluate the individual and combined effects of physical habitat restoration and nutrient addition treatments. Effects of nutrient addition in the Twisp River will be evaluated by statistically comparing mean values from a standard suite of biological response variables before and after treatment as well as upstream control and downstream impact, scheduled to begin in 2018.

Treatment methods: Nutrient treatments will be designed to simulate the natural seasonality of nutrient contribution from natural spawning and bioturbation events. Nutrient addition treatments will

be implemented using carcass analogs from Aquadine Industries http://www.salmalogs.com/. A loading of 0.15kg/m^{-2} b will be applied to both reaches based on the accepted work of Kohler et al. (2011) and Bibly et al. (2001), consistent with Washing State's protocols and guidelines for distributing salmonid carcasses, carcass analogues, and delayed fertilizers to enhance stream productivity. Additions will take place in early fall (September) to mimic Spring Chinook spawning events. Treatment loading rates in subsequent years will be adjusted based on monitoring data if needed.

Field Sampling

Fish

The fish community is currently being sampled six times per year within the study area in Hancock Springs. Abundance sampling is conducted once per season (March, July, October and December) whereas diet sampling occurs a total six times per year, including the four common seasonal fish sampling events mentioned above. Fish sampling in the Twisp River will begin during 2015, the frequency of sampling events will be dictated by flow, with the goal of capturing seasonal variability within the photo period (March - November). All fish collected are identified to species, measured (FL, mm), and weighed (g). All captured salmonid specimens of the five dominant fish species of a suitable size (> 65 mm FL recommended by PTAGIS, or > 55mm FL with 8 mm tags) will be PIT tagged to estimate abundance and growth. Fish data will be collected by YN project personnel and by additional field crews from the Washington Department of Fish and Wildlife (WDFW), the USGS, and the U.S. Fish and Wildlife Service. Fish data collected by Program personnel will be stored in an electronic database and made available to collaborating agencies.

Electrofishing – Standard upstream multiple pass depletion methods are being employed in both reaches at Hancock Springs. Multiple pass depletion electrofishing techniques 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 (1958) and Zippin (1982). Electrofishing passes are completed until adequate depletion is achieved (following Connolly 1996).Salmonids are depleted to meet meeting regression requirements at a coefficient of variation < 12.5%, where sculpin depletion regressions meet a CV of < 25%. Electrofishing techniques are consistent with regionally accepted settings and protocols for sampling fish in small streams (Terraqua 2009).

Abundance – Abundance estimates for dominant anadromous and resident fish species were generated using standard multiple pass depletion estimate techniques and the K-pass removal package (Ogle 2012) of the R software program.

Biomass – Fish abundance estimates were converted to biomass (g/m2), by multiplying by the average mass (g) of each species within each habitat and then dividing by habitat area (m2). We converted wet biomass to dry mass (DM) by assuming 80% water content for juvenile fish and 75% water content for adult fish and sculpin, as reported by Bellmore et al. (2013).

Growth – Growth rates were used to express growth for an interval of time and are commonly expressed as a percentage. Instantaneous growth rates (Gr) will be calculated using the following formula from Lang et al. (2006):

$$\text{Growth rate } (Gr) = \{[(W_{t+1} - W_t)W_t^{-1}]D\text{-}1\} \times 100$$

where:

Gr =the relative growth rate expressed as the percent weight gained per day over the time period from capture at time (*t*) to recapture at time (*t+1*):

W_t = the weight (g) of an individual at time (*t*);

W_{t+1} = the weight (g) of an individual at time (*t+1*); and

D = the number of days occurring between time (*t*) and time (*t+1*).

Tertiary (fish) production – Production of dominant salmonid fish species will be estimated using the instantaneous growth rate method (Hayes et al. 2007), which estimates production as simply the product of the estimated instantaneous growth rate and estimated mean biomass:

$$P = G * B$$

where:

P = estimated production for a given cohort within a specified interval,

G = estimated instantaneous growth rate for the cohort from time *t* to *t* + 1 (i.e. log*e* *w*–*t* + 1 – log*e* *w*–*t*), and

B **=** estimated arithmetic mean cohort biomass from time *t* to *t* + 1 (i.e. *Bt* + *Bt* + 1)/2).

Size classes for the five dominant fish species are binned into 4 length groups (0-99, 100-149, 150-200 and > 200mm).

Sculpin production will be estimated using published production to biomass ratios from the Methow River (Bellmore et al. 2013).

Fish Diets – Fish gut content sampling is a commonly used method to investigate the diet composition of fishes (Hershey et al. 2006). Stomach content samples from the five dominant stream dwelling salmonids and cottids will be collected using a gastric lavage to flush gut contents from live fishes. This technique has been reported to remove up to 98.9% of the gut contents from the fish with little effect on subsequent survival and condition (Strange et al. 1981). Gut contents will be collected from the five dominant species and distributed from the four size classes. Stomach samples will be collected six times per year - once per season along with other standard fish sampling events and two additional collections during anadromous salmonid spawning periods to assess the effects of egg availability and bioturbation on fish diet compositon. Gut contents are identified to lowest taxa (species in most cases) and biomass values will be calculated using a length-mass regression model (Benke 1999) as described previously. Samples will be stored in 70% ethanol and sent to the lab for taxonomy and biomass analysis. The lengths of fish found in diets will be converted to biomass using length-weight regressions developed using electro-fishing data.

Macroinvertebrates

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

captured in the study areas. Benthic macroinvertebrates will be sampled from riffle and pool habitat in Hancock Springs and riffle habitat in the Twisp River. A Hess sampler (1000um net) modified to include a top net will be used for sampling benthic invertebrates in pool habitats. Samples will be pooled from multiple locations to best represent habitat area sampled. Drifting macroinvertebrates will be sampled during mid-day using drift nets (363 um net) placed across the stream channel (n = 2 per transect) at 2-3 cm above the stream bottom (Smock 2006). Drift density will be expressed as the number of invertebrates drifting per 100 m^3 of water using the following formula:

$$Drift\ density = [(N)(100)]/[(t)(W)(H)(V)(3600\ s/h)]$$

where:

N = the number of invertebrates in a sample;
T = the time of the sampling event (min);
W = net width (m);
H = mean height of the water column in the net mouth (m); and
V = mean water velocity at the net mouth (m/s).

Biomass and secondary production - Benthic and drifting macroinvertebrates will be rinsed, stored in 70% ethanol, sorted, and identified to the lowest feasible taxonomic level (usually species). Macroinvertebrate samples will also be collected for stable isotope analysis, held in freshwater for 24 hr. to allow for gut evacuation, and will be frozen and subsequently analyzed for C and N stable isotopes. 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 a published size-frequency model (Benke and Huryn 2007). Running the model requires average invertebrate density and biomass data by size class from each sample year. Data are available from project Hess samples. Specimen length and weight will be measured to estimate biomass using length-mass regression models (see above), the latter 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 = the change in density between size classes, and
$\hat{W}$ = 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 multiplying the products per size class by the number of size classes (the latter 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 are then calculated for any time period as a simple fraction, 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.

Periphyton

Periphyton standing crop and community diversity will be measured in both study areas. Standing crop will be expressed as chlorophyll a biomass (mg/m^2) and AFDM (ash free dry mass, g/m^2). Biofilm will be scrubbed from the entire surface with a small brush from three representative rocks at each sampling site. Removed biofilm material will be condensed into 300mL of water, with resulting sample slurry vacuum filtered with glass fiber filters (0.45 um) and wrapped in aluminum foil for storage. Filters containing sample material will be placed in dark coolers on ice and frozen as soon as possible. Surface area of the rocks sampled will be determined by tracing the planar area onto paper and weighing the cut-out (Bergey and Getty 2006). Samples will be analyzed for chlorophyll a and AFDM using standard laboratory methods (APHA 1995).

Periphyton slides will be prepared using a standard membrane filtration technique. This technique preserves cell structure and provides good resolution, allowing the samples to be examined at high magnifications. Samples will be thoroughly homogenized as a part of the low pressure filtering process to ensure that the organisms are evenly distributed and undistorted. A Leica DMLB compound microscope (100X, 200X, 400X, 630X, 1000X) will be used to enumerate filtered periphyton samples. The magnification used depended on the size of dominant taxa and presence of particulates. Cell counts will be performed at multiple magnifications to successfully identify and enumerate taxa with cell sizes that vary by several orders of magnitude. If a sample is dominated by cells or natural units below 10-20 µm, or when cells are fragile and difficult to identify, the majority of counting will be completed at 630X.

The abundance of common algal taxa will be estimated by random field counts. A minimum of 400 natural units (colonies, filaments, unicells) will be enumerated to the lowest possible taxonomic level (in most cases, species) from each sample. In addition, an entire strip of the filter will be counted at high magnification (usually 630X) along with half of the filter at a lower magnification (usually 400X) to further ensure complete species reporting. Cell bio-volumes of all identified periphyton taxa will be quantified on a per milliliter basis. Bio-volumes will be estimated using formulae for solid geometric shapes that most closely match the cell shape. Bio-volume calculations will be based on measurements of 10 organisms per taxon for each sample where possible. Mean bio-volumes will then be used to calculate the total biovolume contributed by each taxon to its representative sample.

Ecosystem metabolism

Ongoing efforts to characterize ecosystem metabolism are being coordinated between Program personnel and USGS, University of Idaho, and Washington State University faculty and doctoral researchers performing stream metabolism and hyporheic studies in Hancock Springs. Community metabolism will be determined using single station open-system measurements of dissolved oxygen (O_2) change following the methodologies of McCutchan et al. (2002) and Hall and Tank (2005) that account for groundwater inputs when calculating whole stream metabolism. Two sondes (YSI model Exo2 Yellow Springs, Inc., Yellow Springs, Ohio) will be deployed in the thalweg of the stream, one in the restored reach (Reach 1) and one in the unrestored reach (Reach 2). Dissolved O_2 concentrations and water temperature will also be measured and logged at 10-min intervals from June 2013 to April 2013. Instrument calibration will be conducted every two weeks during the field season to prevent dissolved O_2 concentrations drifting.

Water quality/nutrients

Ten water quality and nutrient response variables will be monitored in the study areas (Tables 5 and 6). In addition to sampling water chemistry, temperature, and dissolved oxygen will be measured throughout the sampling season (March-October). Hobo tidbit data loggers will be located at all sampling sites and record

temperature every 30 minutes. Two portable Hydrolabs located in Hancock Springs will measure dissolved oxygen, PH, conductivity, turbidity as part of a coordinated multi-agency metabolism project. Replicate samples were collected by dipping containers into the thalweg just below the surface at each site. 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 shipped overnight to the lab. Detection limits were 2.0 µg/L for TP and TDP, 1.0 µg/L for SRP, 10.0 µg/L for NO_2+NO_3, 5 µg/L for NH_4 and 50.0 µg/L for TN.

Food Web Characterization and Analyses

Food webs describe the energy pathways through ecosystems and provide insight into the complex, multi-species assemblages within which organisms of interest grow, survive, and reproduce (Elton 1927; Polis and Winemiller 1996). Food webs will be constructed using two distinct, complementary techniques: (1) using fish gut content and invertebrate sample data, and (2) stable isotope analysis. Food flow web diagrams (e.g. Cross et al. 2001; Bellmore et al. 2013) will be constructed to illustrate the scaled contributions of various invertebrate taxa or functional feeding guilds to diets of the five dominant fish species, for which gut content samples will be collected and analyzed. Organic material flows to the fish species will be calculated with the trophic basis of production (TBP) method, which estimates (a) contributions of different prey to fish production, and (b) rates of resource consumption that support measured rates of fish production (Benke and Wallace 1980; Cross et al. 2011) as reported by Bellmore et al. (2013).

Trophic Basis of Production (energy flow webs)

To standardize treatment effects, populate empirical and predictive modeling efforts, and evaluate changes in productivity, biological production within each trophic level will be consistently expressed as values per square meter.

A trophic basis of production (TBP)is currently being constructed to evaluate organic matter flows for fish. The TBP method estimates (1) contributuions of different prey to fish production, and (2) rates of resource consumption that support measured rates of fish production (Benke and Wallace 1980, Cross et al. 2011, Bellemore et al. In press). The relative fraction of annual fish production attributed to each prey type (*Fi*) is calculated as:

$$F_i = G_i \, x \, AE_i \, x \, NPE$$

where:

G_i = proportion of prey type *i* in fish diet,
AE_i = assimilation efficiency of prey type *i*, and
NPE = net production efficiency.

For each fish species j, the proportion of fish production attributed to each prey type (PF_{ij}) is then calculated from the relative fractions (F_i) as:

$$PF_{ij} = \frac{F_i}{\sum_{i=0}^{n} F_i}$$

Lastly, annual flows of each prey type i to fish consumer j (FC_{ij} measured in $gDM \cdot m^{-2} \cdot y^{-1}$) is calculated as:

$$FC_{ij} \; \frac{PF_{ij} x \; P_j}{AE_i x NPE}$$

where:

P_j = annual secondary production (gDM•m^{-2}•y^{-1}) of fish *j*.

The following assimilation efficiencies sere used for all salmonid species: 0.75 for aquatic invertebrates, 0.70 for terrestrial invertebrates, and 0.95 for fish tissue (see Warren 1971, Brocksen and Bugge 1974, Elliot 1976, Warren and Davis 1976). For sculpin we used an assimilation efficiency of 0.82 for aquatic invertebrates (see Davis and Warren 1965, Atmar and Stewart 1972, Eiriksdottir 1974). Net Production efficiency values were set at 0.125 for adult fish and a production efficiency of 0.250 was used for juvenile salmonids (< 150mm) and sculpin (Donner 2011, Cross et al. 2011). Different net production efficiencies for juvenile and adult fish were applied to account for allometric relationship between fish consumption and growth (i.e. larger, older fish spend proportionately more energy and maintenance and growth).

Interaction strength, interspecific competition, and carrying capacity

Interaction strength – The potential strengths of interactions between fish predators and each invertebrate prey *I* were calculated as (Woodward et al. 2005; Benke 2011):

$$I_i = \frac{FC_i}{PP_i}$$

where:

FC_i = total annual consumption of prey type i (g DM•m^{-2}•y^{-1}) by the fish assemblage, and
P = annual production of pre type i.

This metric is a unit-less value, ranging from 0 to 1, which represents the proportion of annual prey-specific production consumed by the fish assemblage. Values greater than 1 (i.e., the fish assemblage is consuming more than is being produced) are energetically impossible, and likely indicate errors in estimates of invertebrate production, fish production, and/or fish dietary proportions.

Competition coefficient – To evaluate potential exploitative competition for prey between each dominant fish species and the rest of the fish assemblage, we will calculate a competition coefficient (CC) as:

$$CC_J = \sum_{i=1}^{n} \frac{FC_{ih}}{PP_i} \; x \; PF_{ij}$$

where FC_{ih} = total annual consumption of prey type *i* (g DM•m^{-2}•y^{-1}) by all members of the fish assemblage exept for the species of interest *J*, and PF_{ij} is the proportion of annual production for species *j* derived from prey item *i*. This index incorporates both the availability of each prey type in the environment, after consumption by the rest of the fish assemblage *h*, and the importance of each prey item to the production of fish species *j*. The output of this index is a unit-less value ranging from 0 to 1 that represents the proportion of prey items important to the species of interest *j* that are consumed by all other members of the fish assemblage (*h*).

Carrying capacity – Carrying capacity with respect to food resources will be calculated by estimating the potential level of production that could be sustained under seperate and additive contributions from treatments. This will be calculated as:

$$Poten = \sum_{i=1}^{n}((PP_1 - FC_{ih}) \; x \; AE_{ij} \; x \; NPE_j \; x \; PF)$$

where AE_{ij} and NPE_j are assimilation and net production effeciencies for prey type *i* by fish *j.*

This metric assumes: (1) that production by all other members of the fish asseblage does not change; (2) that the dietary proportions of all members of fish assemblage remains the same and that fish *j* are able to track the produciton of their prey. These assumptions may not be realistic in all cases, but are imperative for deriving relative per meter estimates of carrying capacity for fish species of interest in terms of food.

Stable isotopes

Isotopes of C and N will be sampled from all trophic levels at multiple sites within the study areas (Twisp River and Hancock Springs). Up to 5 samples will be collected from each trophic level at sites during sampling episodes. Each isotope sampling episode will contain samples from terrestrial vegetation (grasses and deciduous leaves), epilithic organic matter, four aquatic functional feeding guilds of benthic macroinvertebrates (shredders, grazers, collector gathers and predators), terrestrial invertebrates and fins from fish (Chinook, steelhead, and sculpins). Samples will also be obtained from anadromous carcass material, steelhead and Chinook eggs, and any nutrient treatment material (carcasses or carcass analogs) that would be added artificially. Sample collection will follow methods from Bilby et al. (1996, 2001).

Stable isotope samples will be analyzed at the Washington State University or the University of Idaho Stable Isotope labs using an elemental analyzer and a mass spectrometer. Sample values will be calculated using the following formula:

$$\delta \; 15 \; N/ \; 13 \; C = [(R \; sample - R \; standard) / R \; standard] \; x \; 1000$$

where:

R sample = the stable isotope ratio in the sample; and
R standard = the stable isotope ratio in the standard.

Ratio values will be calculated for each trophic level, functional feeding groups of benthic macroinvertebrates, salmon eggs, carcass analogues, dominant fish species, and terrestrial inputs using methods reported by Bilby et al. (1996). Isotope analyses will be conducted following protocols described in Kline et al. (1990), Bilby et al. (1996), and Hershey et al. (2006). Samples will be dried, ground, and prepared in the laboratory. Stable isotope analyses will also help to verify the degree of transfer of marine-derived nutrients to the natal systems being studied, and to inform changes in food web structure and dynamics.

Physical habitat

Columbia Habitat Monitoring Program (CHaMP) protocols will be used classify physical habitat within the study areas (https://www.champmonitoring.org/Program/Details/1#overview). Evaluations included a suite of in-channel and riparian zone metrics, and the construction of a Digital Elevation Model (DEM) at each site. We report results from several CHaMP habitat metrics, including channel unit area (pools vs. fastwater habitats), substrate composition, large wood contribution, fish cover, and pool tail fines.

Statistical Analysis

Statistical analyses performed as parts of both the Hancock Springs and Twisp River projects include: 1) descriptive statistics and exploratory data analysis; 2) analytical and inferential statistics; and 3) sample size analysis.

Descriptive statistics – A series of descriptive statistics involving mean spatial and temporal trend plots of trophic level biological response variables will be constructed and evaluated. This initial qualitative review of all project data represents the most general review of control and treatment conditions of the study areas for both projects, and is intended to provide insight into the temporal and spatial patterns and structure of the data. Results of this initial data evaluation will be numerically summarized using descriptive statistics including the sample mean, minimum, maximum, and range of values for the projects' biological response variables, along with estimates of associated variability such as variance and standard deviation. This initial characterization of the data collected from treatment and control years and from upstream and downstream from treated areas will be followed by a more quantitative investigation in the next tiers of data analyses as described below.

Inferential statistics – Analysis of variance (ANOVA) techniques will be employed for comparison of mean biological response variable values and their associated variability. Water quality, algal, chlorophyll, benthic macroinvertebrate and fish data will be subjected to a series of temporal and spatial contrasts as supported by results of the initial qualitative data review described above. Temporal contrasts will include comparisons of mean values within pre-treatment and post-treatment years, and from upstream and downstream control and treatment reaches. Spatial contrasts will include comparisons of response values between and among sites or a suite of sites as warranted. Chi-square tests will be used to evaluate changes in periphyton, benthic macroinvertebrate, and fish community compositions. Similar analyses will be carried out for fish gut content sample compositions.

Invertebrates

Due to the limited sample size, a nonparametric Wilcoxon-Rank-Sum (Kruskal-Wallis) test was performed to assess the effects of Reach on invertebrate response metrics. This test is analogous to a one-way ANOVA utilizing the data "rank" scores and avoids the statistical issues regarding the distributional assumption of the data (normality, which cannot be accurately assessed with small sample sizes. Both overall abundance and biomass were evaluated. Separate tests were carried out for samples from Pool and Riffle habitats. Chi-square tests were used to assess changes in invertebrate community composition between reaches at the taxonomic Order level.

Fish

Abundance – A one-way ANOVA was used to test the effect of Reach on fish abundance with 2012 fish abundance data and the three seasons (Spring, Summer, and Fall) as replicates. A two-way ANOVA was carried out on 2013 fish abundance data assuming Reach as a main effect and Season as a repeated measures effect. Sites within reaches were used as replicates for testing the effect of Reach with 2013 data (site replicates were not available for 2012). A one-way ANOVA was also used to analyze 2013 sculpin data because this species was only caught during one season. All analyses were performed separately for Chinook, steelhead, brook trout, and sculpin. No abundance analyses by reach were performed for bull trout because none were collected in Reach 2. Power analyses were performed to determine the magnitude of effect needed to achieve reasonable statistical power.

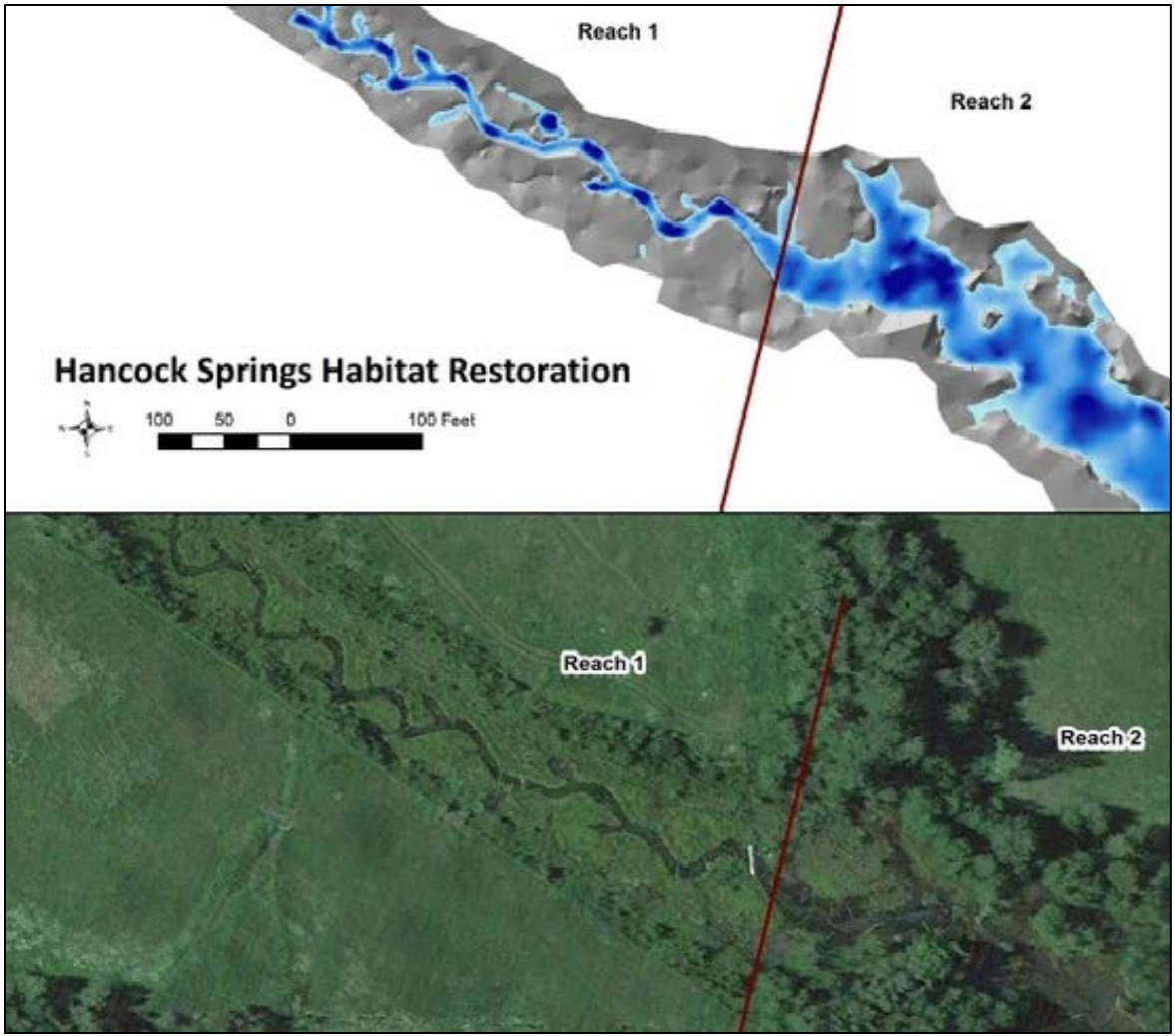
Biomass – A one-way ANOVA was used to test the effect of Reach on biomass using Season (Spring, Summer, and Fall) as replicates. A two-way ANOVA was used to analyze 2013 fish biomass data assuming Reach as a main effect and Season as a repeated measures effect. Sites within reaches were used as replicates for the analysis of 2013 data. All analyses were performed separately for Chinook, steelhead, brook trout, and sculpin. No biomass analyses by reach were performed for bull trout because none were collected in Reach 2. A one-way ANOVA was also used to analyze 2013 sculpin data because sculpins were only collected during one season. Power analysis was used to determine the magnitude of effect (actual difference between Reaches) needed to achieve reasonable statistical power. Power, in this case, represented the probability of detecting a true difference, if one exists.

Growth – A one-way ANOVA was used to analyze 2012 and 2013 fish growth data, expressed as growth per day (g/day) and growth per year (g/yr), using sites within each reach as replicates to test the effect of Reach on growth. All analyses were performed separately for Chinook, steelhead, and brook trout. No growth analyses by reach were performed for bull trout because none were collected in Reach 2. Power analysis was used to determine the magnitude of effect (actual difference between Reaches) needed to achieve reasonable statistical power. Power, in this case, represented the probability of detecting a true difference, if one exists.

12. Results

13. Hancock Springs

14. Aerial imagery simulation illustrates the magnitude of changes in general habitat characteristics of Reach 1 compared to Reach 2 (Figure 4). Overall, the degree of sinuosity, percent composition of major channel units (e.g. pool vs. fastwater habitats), substrate composition, percent cover, large wood presence, and percent of pool tail fines differed to varying degrees between reaches following channel reconstruction



completed during 2011. Details of these physical habitat attributes, as the basis for evaluating biological responses to the habitat restoration treatment, are described in more detail in the following report sections. General channel and floodplain features of Reach 1 (treatment) and Reach 2 (Control) in Hancock Springs in 2012 after Reach 1 channel reconfiguration, completed in 2011.

15. **Figure 1. General channel and riparian habitat features of Reach 1 (treatment) and Reach 2 (Control) in Hancock Springs in 2012 after Reach 1 channel reconfiguration, completed in 2011.**

16. Physical habitat summary

17. The in-stream and riparian habitat restoration treatment in Reach 1 of Hancock Springs during 2011 resulted in considerable differences in physical habitat features between the treatment and control reach. Over 77% of Reach 1 was constituted by pools, with a 3.5:1 pool/riffle ratio, compared to nearly 60% pool coverage and a pool/riffle ratio of 0.2:1 in Reach 2 (Table 9). Substrate composition in Reach 1 was dominated by cobbles and gravels (68%) while Reach 2 substrates were dominated by sand and fine sediments (82%). The substrate composition difference between reaches was larger when expressed as percent pool tail fines, with 9.5% fines in Reach 1 vs. 44.6% in Reach 2 (Table 9). Physical habitat restoration had no effect on the thermal regime, as mean annual water temperature between reaches differed by just 0.2 ^{o}C (Table 9). More detailed comparisons of post-treatment physical habitat comparisons by reach are provided in the following specific habitat results sections.

18. **Table 1. General habitat metric values in Hancock Springs by reach following 2011 habitat restoration in Reach 1.**

Physical Habitat Metrics	Reach 1 (Treatment)	Reach 2 (Control)
% Pools	77.6	58.7
Pool/Riffle Ratio (% area of reach)	3.5:1	0.2:1
% Cobbles and gravels	68	32
% Sand and fines	18	82
% Fish cover (area)	79.4	54.6
Large wood density (pieces/m^2)	0.2	0.02
% Pool tail fines	9.5	44.6
Mean annual water temperature (^{o}C)	7.2	7.4

19.

20. ***Channel units*** – In-channel habitat in Hancock Springs was classified as either pool or fastwater habitat, expressed as percent area by Reach. Reach 1 had more pool area (77.6%) than Reach 2 (58.7%), and both reaches had more pools than fastwater habitat. Reach 1 had over four times more pool area than fastwater habitat, compared to 59% pool vs. 41% fastwater habitat in Reach 2 (Figure 5).

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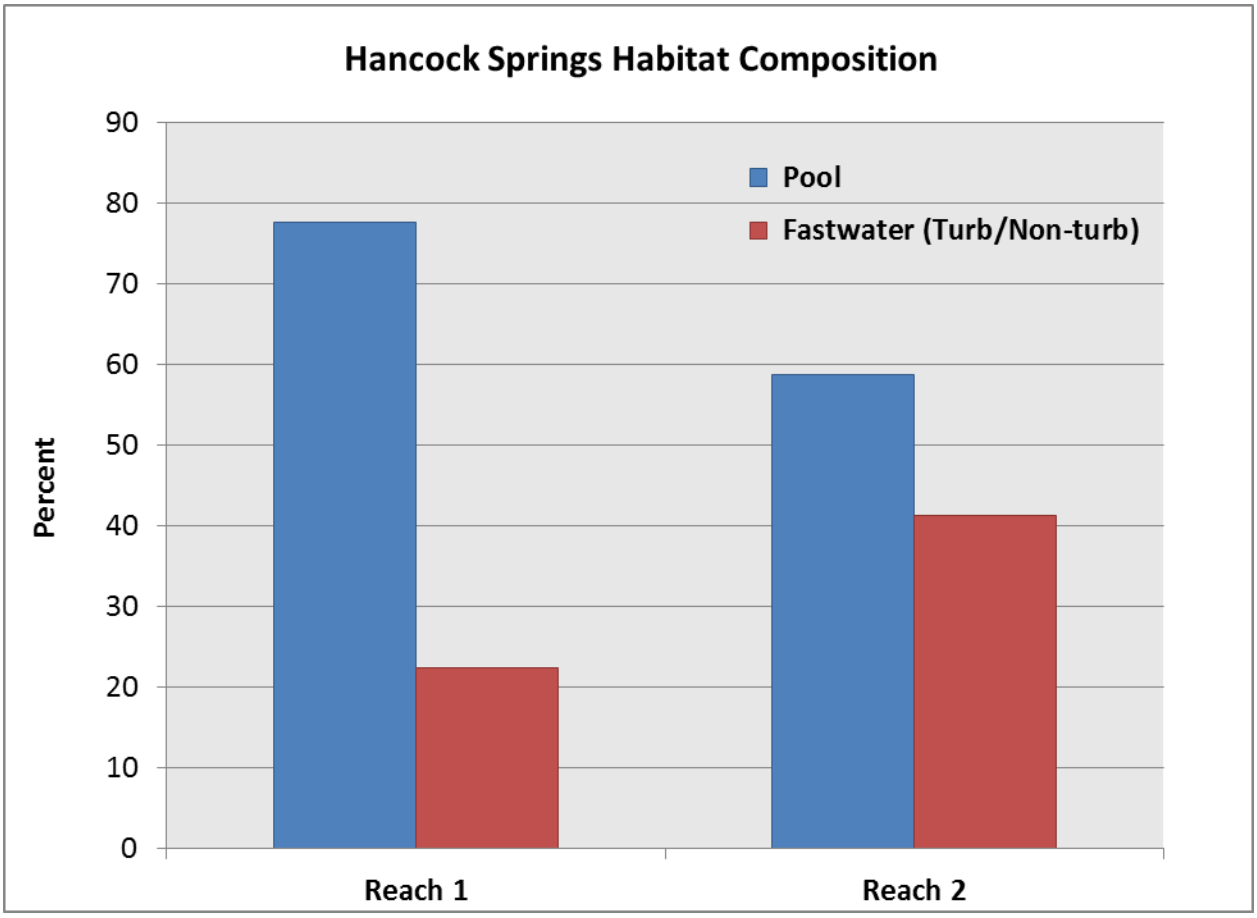
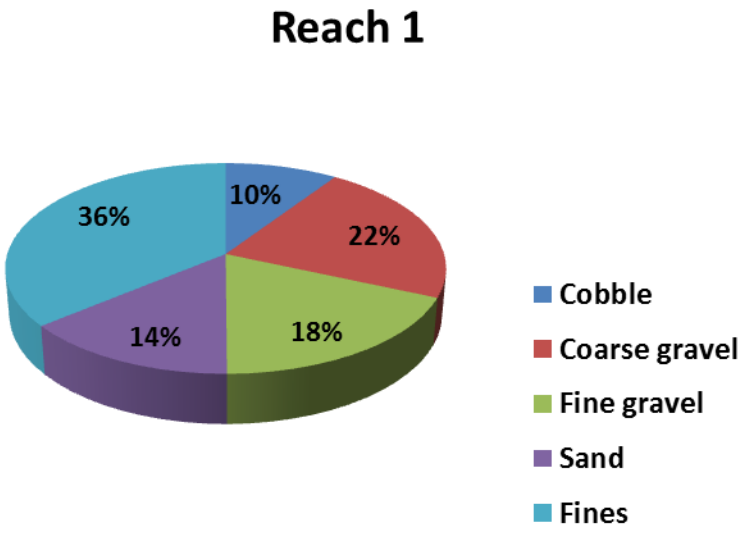


23. **Figure 2. Percent habitat composition (pool and fastwater habitats) in Reach 1 and Reach 2 of Hancock Springs.**

24. ***Substrate composition*** – Cobbles and coarse and fine gravels were more abundant in Reach 1 than in Reach 2, while Reach 2 had slightly more sand and nearly twice as much fine sediment as Reach 1 (Figure 6). In Reach 1 cobble and gravels accounted for 68% of substrate compositon, along with 32% composed of sand and fines. In contrast, substrates in Reach 2 were composed of 82% sand and fines and just 18% cobble and gravels (Figure 6). Reach 1 had nearly twice as much cobble as Reach 2, more than four times as much coarse gravel as Reach 2, and approximately three times as much fine gravel as Reach 2 (Figure 6).



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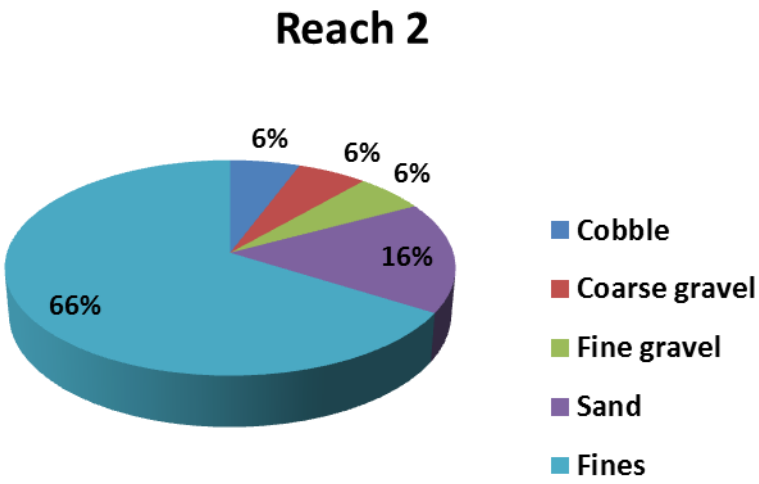

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27. **Figure 3. Percent substrate composition by reach in Hancock Springs, 2013-2013.**

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29. ***Large wood*** – The number of pieces of wood/m^2 in Reach 1 was about 0.1, nearly 10 times greater than that seen in Reach 2 in fastwater habitat and more than twice as high in pool habitats in Reach 1 as in Reach 2 (Figure 7).

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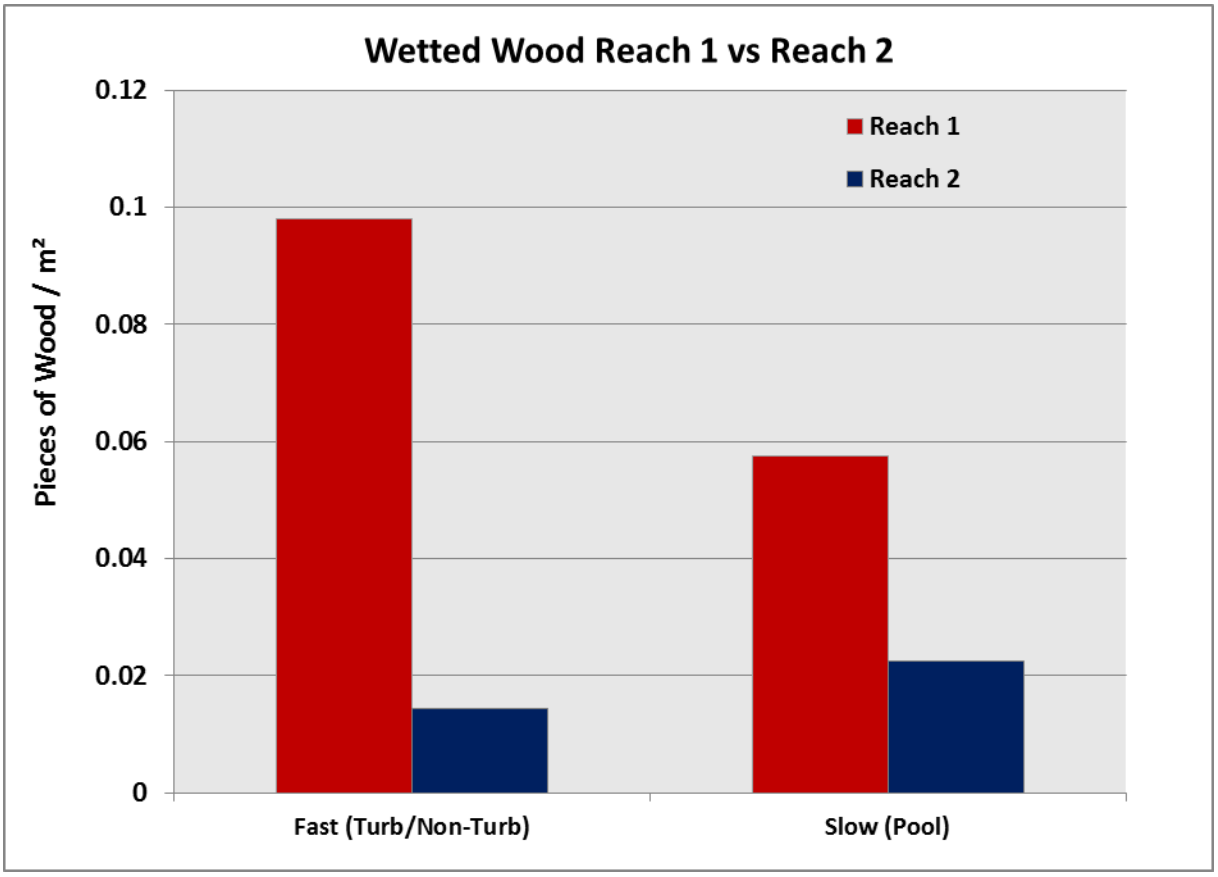
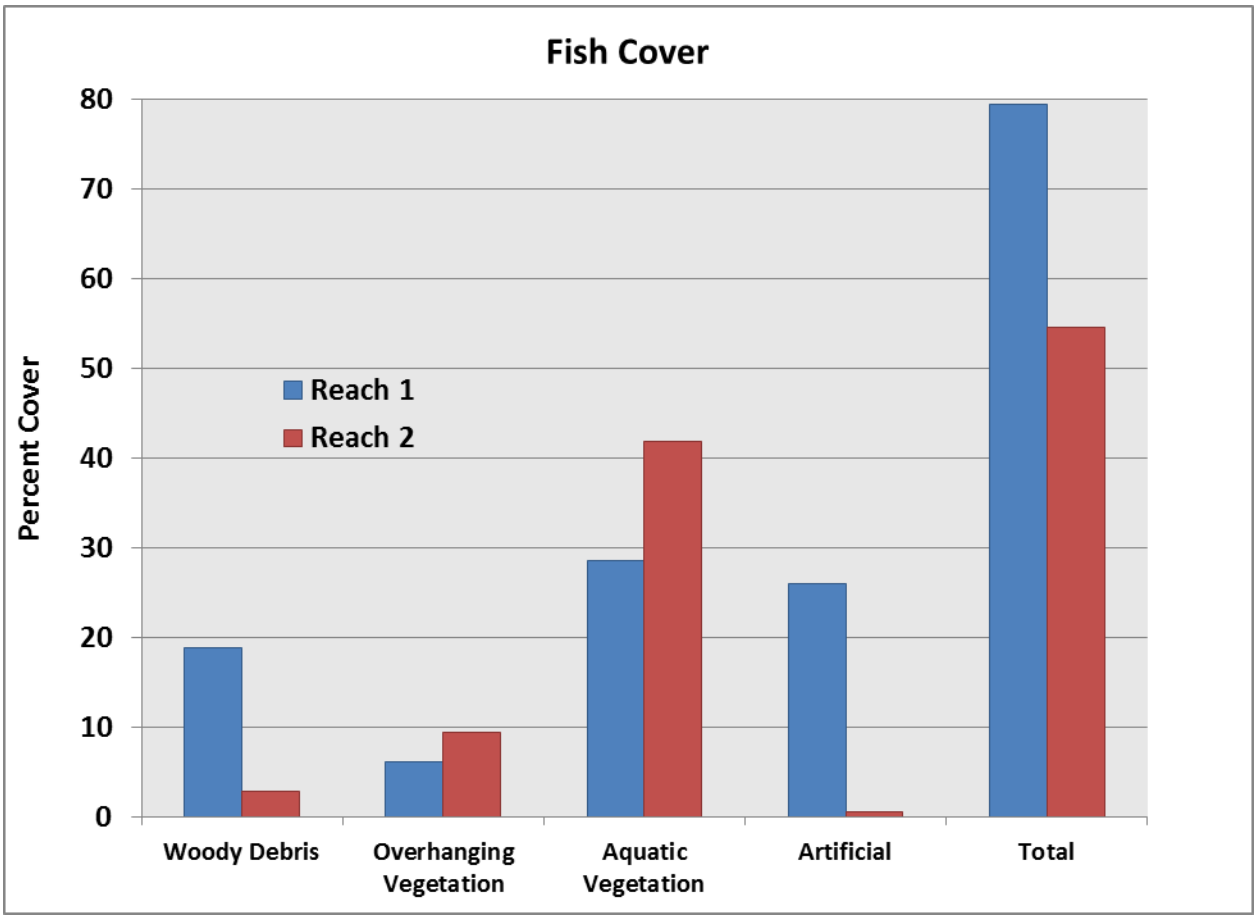


31. **Figure 4. Density (pieces of wood/m^2) in pool and fastwater habitats by reach in Hancock Springs after 2011 Reach 1 channel reconfiguration.**

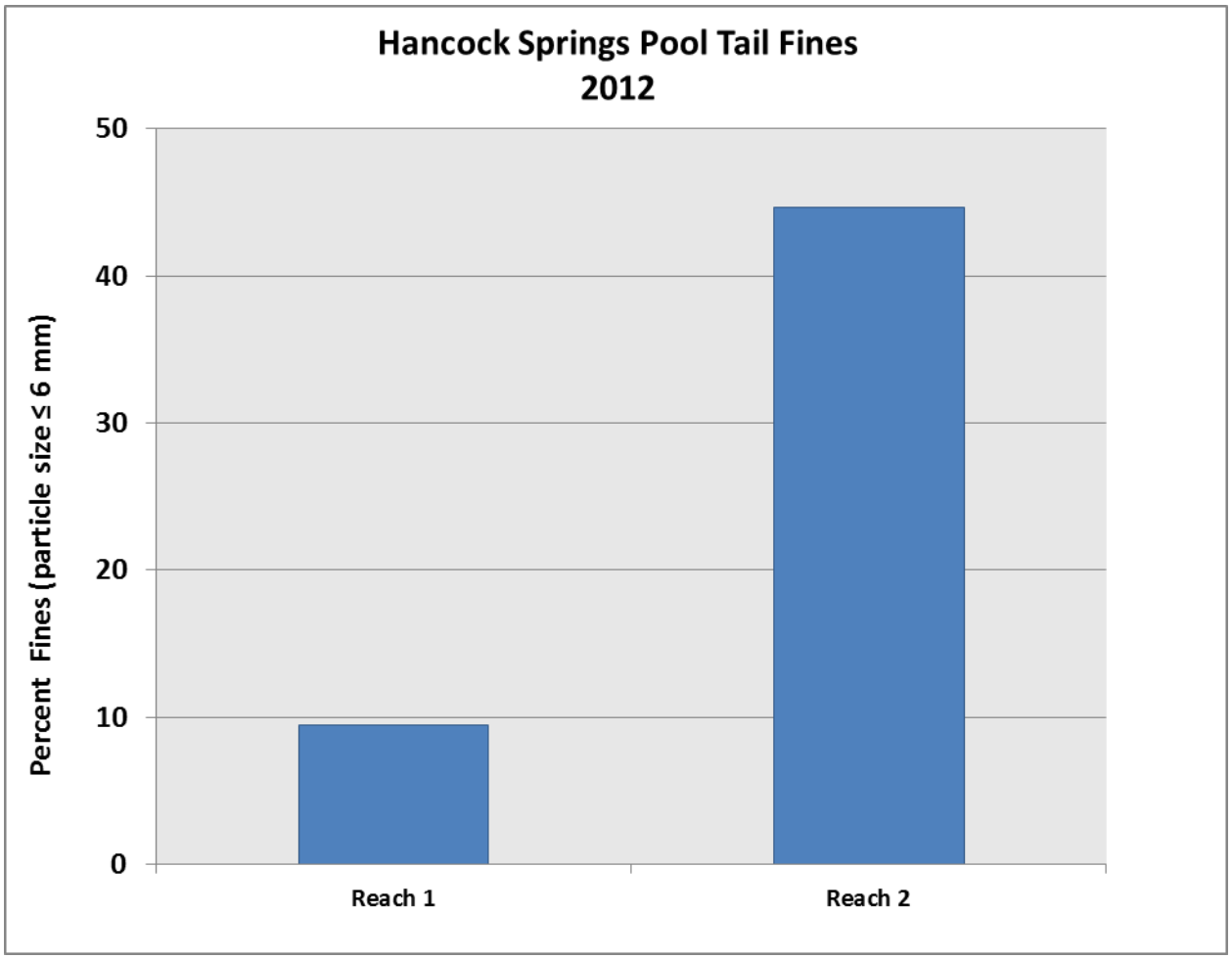
33. ***Fish cover*** – According to analysis of CHaMP data, Reach 1 had a total fish cover value of nearly 80% compared to 54.6% for Reach 2 (Figure 8). When evaluated by cover type, Reach 1 had approximately nine times woody debris coverage than Reach 2 and nearly 25 time more artificial cover than Reach 2 (Figure 8). Alternatively, Reach 2 had about 42% aquatic vegetation coverage compared to 28.5% for Reach 1, and slightly more (9.5%) overhanging vegetation than Reach 1 (6.1%)(Figure 8).

34.



35. **Figure 5. Percent fish cover type by reach in Hancock Springs, 2012.**

37. ***Pool tail fines*** – The percent pool tail fines (substrate particles ≤ 6 mm in diameter) was more than four times higher in Reach 2 (44.6%) than in Reach 1 (9.5%; Figure 9).



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39. **Figure 6. Percent pool tail fines in Hancock Springs by reach, 2012 and 2013.**

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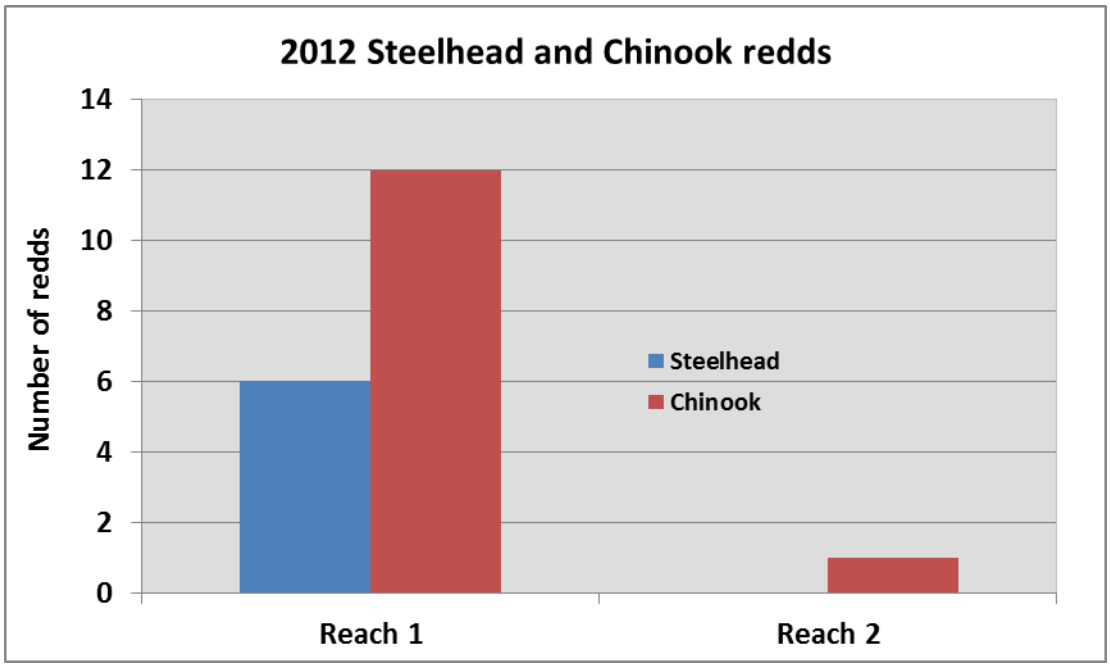
44. Biological response summary

45. ***Fish*** – Post-treatment changes in physical habitat attributes described above by reach also contributed to a wide array of positive biological responses across trophic levels. Eighteen redds (12 Chinook, 6 steelhead) were constructed and used in Reach 1 compared to a single Chinook redd and no steelhead redds in Reach 2. Aggregated fish abundance (all species) was an order of magnitude greater in Reach 1 (0.54/m^2) than in Reach 2 (0.01/m^2), with 91% of aggregated fish biomass and 83% of aggregated fish production in Hancock occurring in Reach 1 (Table10). The aggregated fish community in Reach 1 consumed an estimated 16 gDM/m2/yr of invertebrate production (essentially the entire amount of estimated secondary production), compared to consuming only 2.5 gDM/m2/yr, or about 20% of the estimated 12.8 gDM/m2/yr macroinvertebrate production (Table 10).

46. **Table 2. General biological response metric values for fish and invertebrates in Hancock Springs by reach following 2011 habitat restoration in Reach 1.**

Biological Response Metrics	Reach 1 (Treatment)	Reach 2 (Control)
Fish		
Total Redds (2012)	18	1
Steelhead redds	6	0
Chinook redds	12	1
Total fish abundance (#/m^2)	0.54	0.01
Total fish biomass (gDM/m^2)	1.976 (91%)	0.198 (9%)
Total fish production (gDM/m^2/yr)	1.4 (83%)	0.3 (17%)
Macro Invertebrates and fish (gDM/m^2/yr)		
Aquatic BMI production	13.9	11.1
Aquatic BMI production+ Consumption of terrestrial insects	15.8	12.8
Invertebrate prey consumption by fish	16	2.5
% of total invertebrate production consumed by fish	~100	19.5
% of total invertebrate production **not** consumed by fish	~0%	80.5

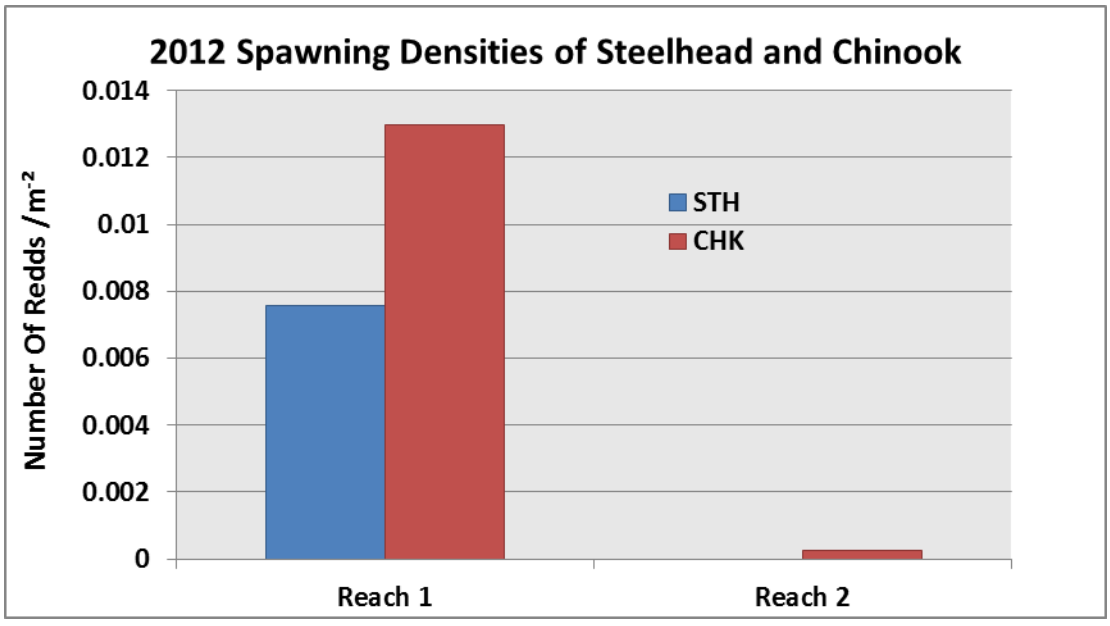
48. ***Redds*** – During 2012, a combined total of 18 Chinook and steelhead redds were found in Reach 1 compared to a single Chinook redd and no Steelhead redds in Reach 2 (Figure 10).



49.

50. **Figure 7. Numbers of Chinook and steelhead redds in Hancock Springs by reach during 2012.**

51. During 2012, the density of Chinooks redds (#/m^2) was two orders of magnitude greater in Reach 1 than in Reach 2 (Figure 11). Within Reach 1, Chinook redds were an order of magnitude more dense than steelhead redds during 2012 (Figure 11).

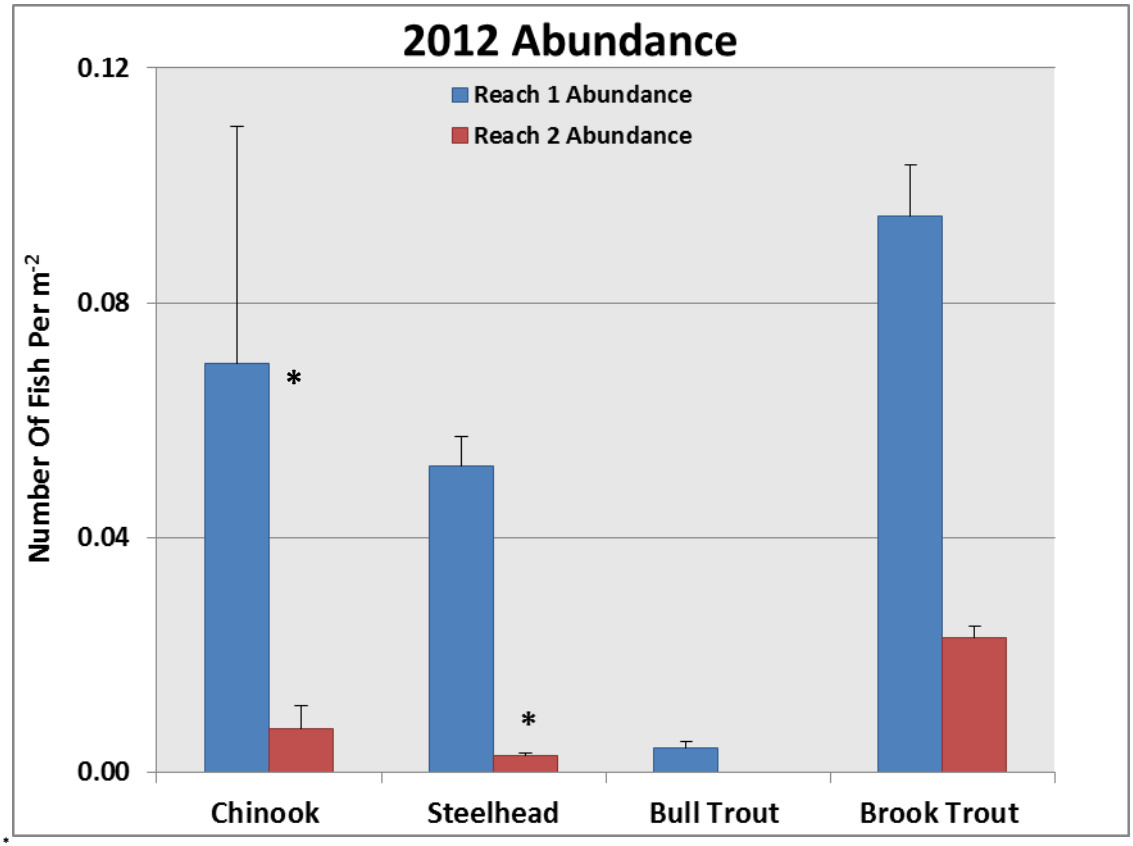


52.

53. **Figure 8. Chinook and Steelhead spawning densities (redds/m^2) in Hancock Springs by reach during 2012.**

54. ***Fish abundance*** – Abundance of the three dominant fish species sampled in Hancock Springs during 2012 (Chinook, Steelhead, and Brook Trout) was generally an order of magnitude higher in Reach 1 than in Reach 2. Abundance for these species ranged from 0.52-0.95 fish/m^2 in Reach 1 and from 0.003 to 0.023 fish/m^2 in Reach 2, with the exception of bull trout, which were only found in Reach 1, in very low abundance (Figure 12). Steelhead were significantly more abundant in Reach 1 than in Reach 2 during 2012 ($p=0.002$), being on average five times more abundant (Figure 12). During 2012, abundance was not significantly different

between reaches for any other fish species tested. However, based on preliminary 2013 data, steelhead (*p=0.009*), brook trout (*p=0.002*) and sculpins (*p=0.003*) were significantly more abundant in Reach 1 than in Reach 2 (data not shown), while brook trout also showed significant season effects during 2013 (*p=0.008*). No bull trout were collected from Reach 2 during 2012 or 2013. No seasonal effects were tested with 2012 data as they lacked seasonal replication; the 2013 Reach*Season interaction was non-significant for abundance in all cases where it could be assessed.



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56. **Figure 9. Abundance of Chinook, Steelhead, Brook Trout, and Bull Trout in Hancock Springs during 2012. Error bars represent one standard error. Asterisks denote statistical significance (*p<0.05*).**

58. ***Fish biomass*** – Steelhead and Chinook biomass were an order of magnitude higher in Reach 1 than in Reach 2, while brook trout biomass was more than three times greater in Reach 1 than in Reach 2 (Figure 13). Biomass ranged from 0.05 to 0.629 gDM/m^2 in Reach 1 compared to 0.01 to 0.18 gDM/m^2 in Reach 2 (Figure 13). Biomass was significantly greater in Reach 1 than in Reach 2 for Steelhead (*p=0.001*) and Brook Trout (*p=0.03*) in 2012, but not for Chinook. Steelhead biomass was also significantly higher in Reach 1 (0.27 g/m^2) than in Reach 2 (0.01 g/m^2) during 2012. During 2013, the Reach and Season effects were non-significant for Chinook but were significant for Steelhead (*p< 0.001*; *p< 0.001*) and Brook Trout (*p=0.002; p=0.03*), while reach was marginally significant for sculpins (*p=0.05*). The Reach*Season interaction was non-significant for biomass in all cases where it could be assessed.

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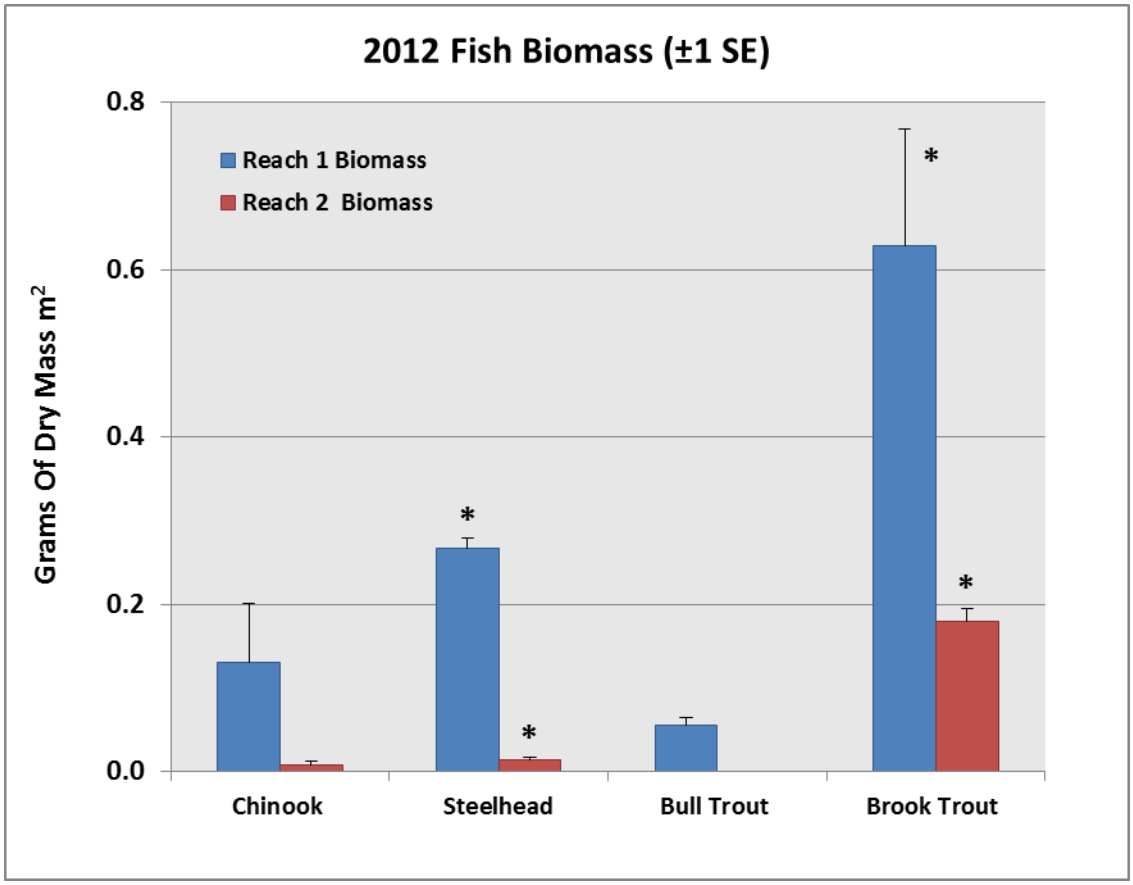

60. **Figure 10. Biomass of Chinook, Steelhead, Brook trout, and Bull Trout in Hancock Springs during 2012. Error bars represent one standard error. Asterisks denote statistical significance (*p<0.05*).**

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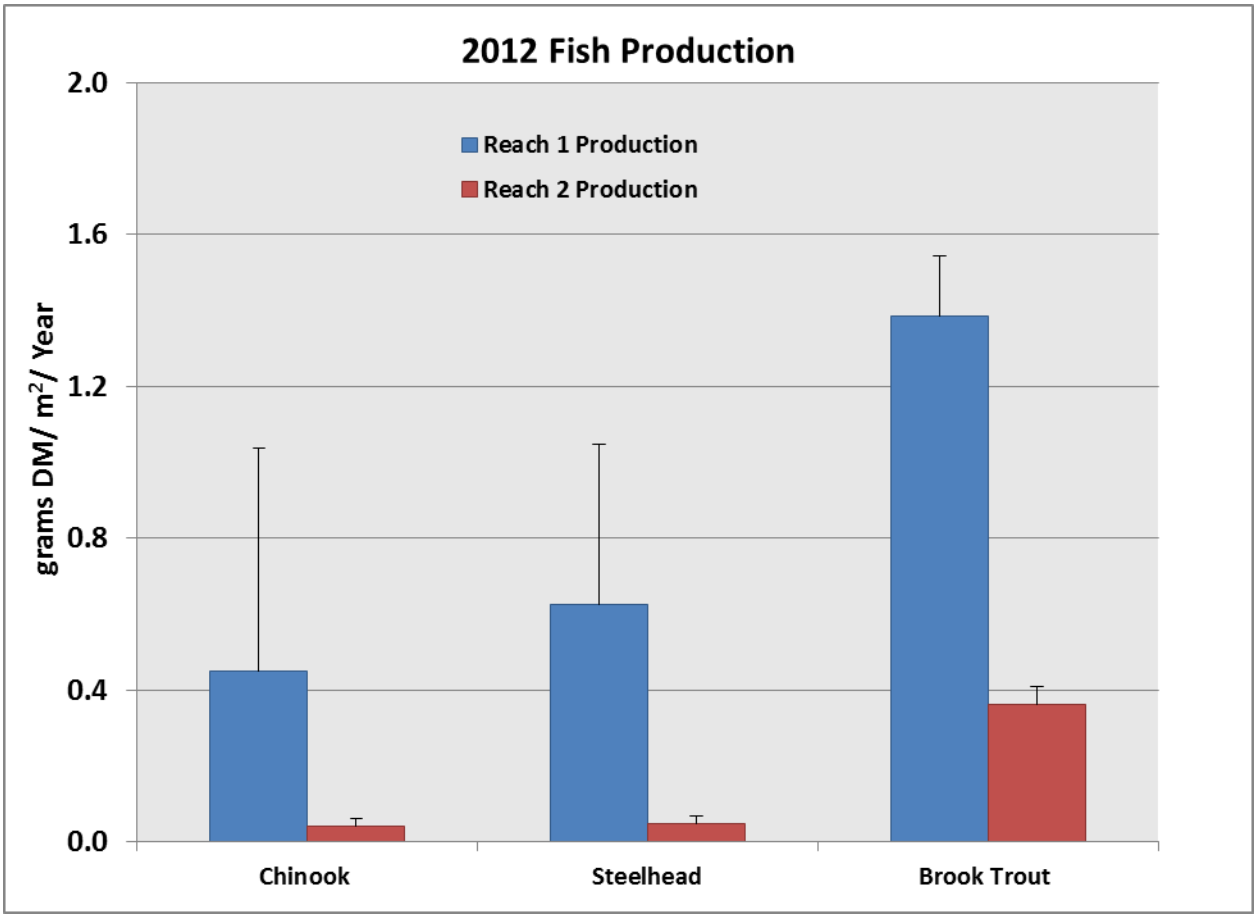
63. ***2012 Fish production*** – Production of the three major fish species collected in both reaches of Hancock Springs during 2012 was an order of magnitude greater in Reach 1 than in Reach 2 (Figure 14). Production ranged from 0.45 to 1.40 g/m^2/yr in Reach 1 compared to 0.04 to 0.36 g/m^2/yr in Reach 2 (Figure 14).



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65. **Figure 11. Production of Chinook, Steelhead, Brook Trout, and Bull Trout in Hancock Springs during 2012.**

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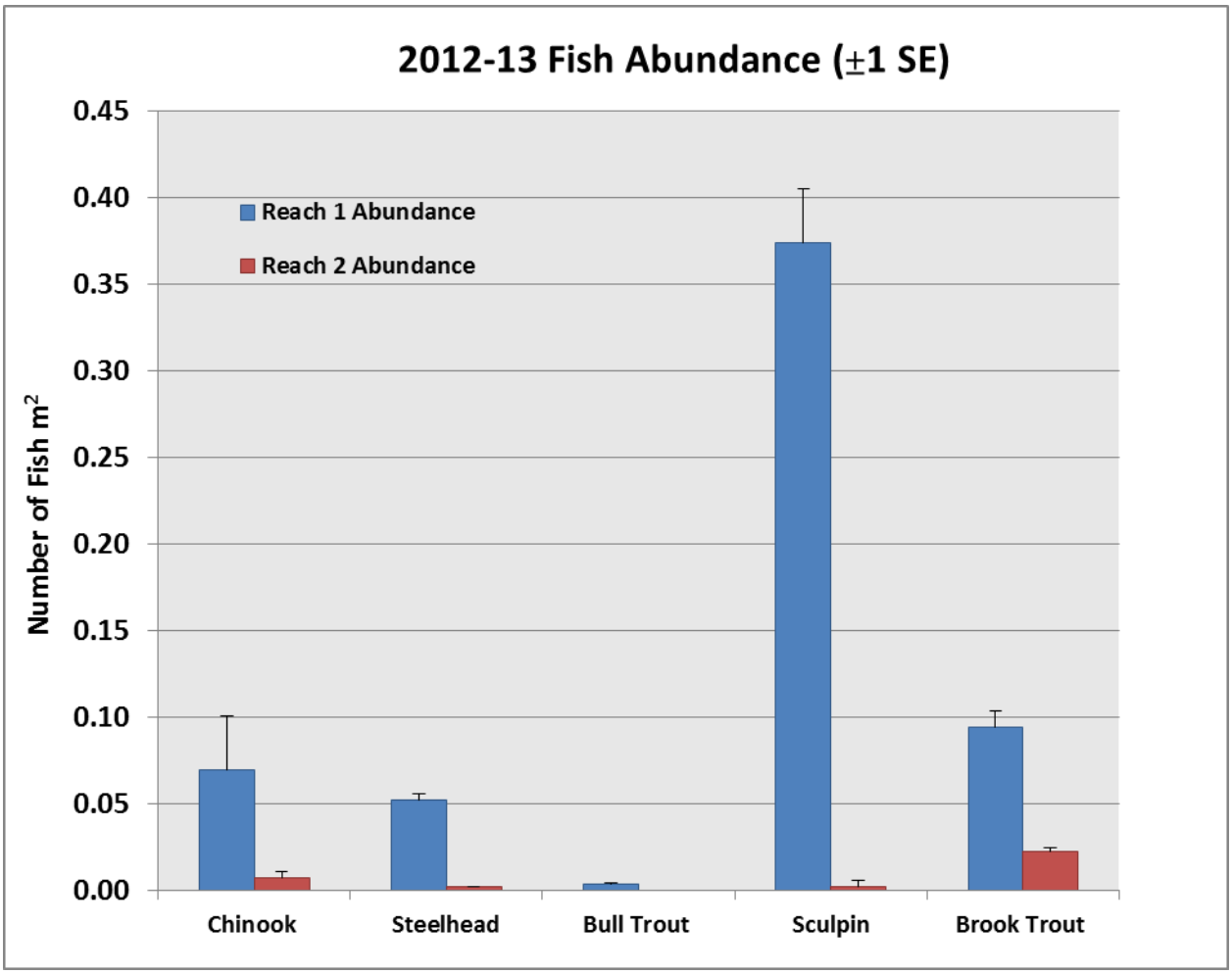
80. ***Fish abundance (2012 and 2013 combined)*** – As seen in 2012, combined data from 2012 and 2013 revealed that abundance was 1 to 2 orders of magnitude higher in Reach 1 than in Reach 2, with the exception of Bull Trout, which were only found in Reach 1 during both years at very low abundance (0.004 fish/m^2)(Figure 15). Abundance among the dominant species in both years ranged from 0.05 to 0.4 fish/m^2 in Reach 1 compared to 0.003 to 0.023 fish /m^2 in Reach 2 (Figure 15). No tests for significance were

performed for combined 2012 and 2013 abundance data because the 2103 data set was incomplete at the time of this reporting.



81.

82. **Figure 12. Biomass of Chinook, Steelhead, Brook Trout, and Bull Trout in Hancock Springs during 2012 and 2013 combined. Error bars represent one standard error. Asterisks denote statistical significance (*p<0.05*).**

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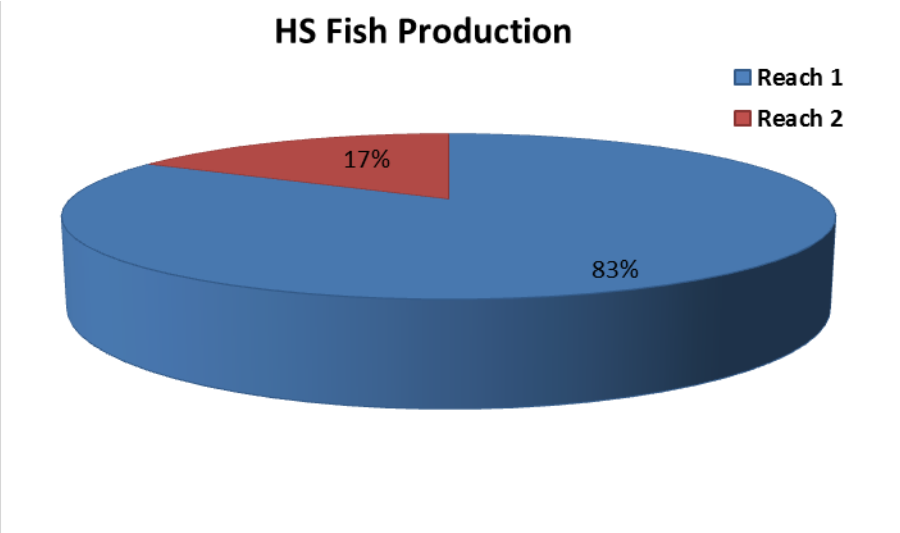
87. ***Fish biomass and production (2012 and 2013 combined)*** – Eighty-three percent of all fish production estimated for Hancock Springs during 2012 and 2013 (1.4 gDM/m^2/yr) occurred in Reach 1, compared to 17% for Reach 2 (0.0048 g/m^3/min Figure 16). Similar to the 2012 data, when combining 2012 and 2013 data, biomass for the dominant fish species was generally an order of magnitude higher in Reach 1 (0.06-0.51 gDM/m^2) than in Reach 2 (0.007-0.14 gDM/m^2)(Figure 17). Combined fish production from 2012 and 2013 was also 1-2 orders of magnitude greater in Reach 1 (0.16-1.39 gDM/m^2/yr) than in Reach 2 (0.03-0.28 gDM/m^2/yr)(Figure 17). No tests for significance were performed for combined 2012 and 2013 biomass because the 2103 data set was incomplete at the time of this reporting.



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89. **Figure 13. Distribution of fish production by reach in Hancock Springs, 2012 and 2013.**

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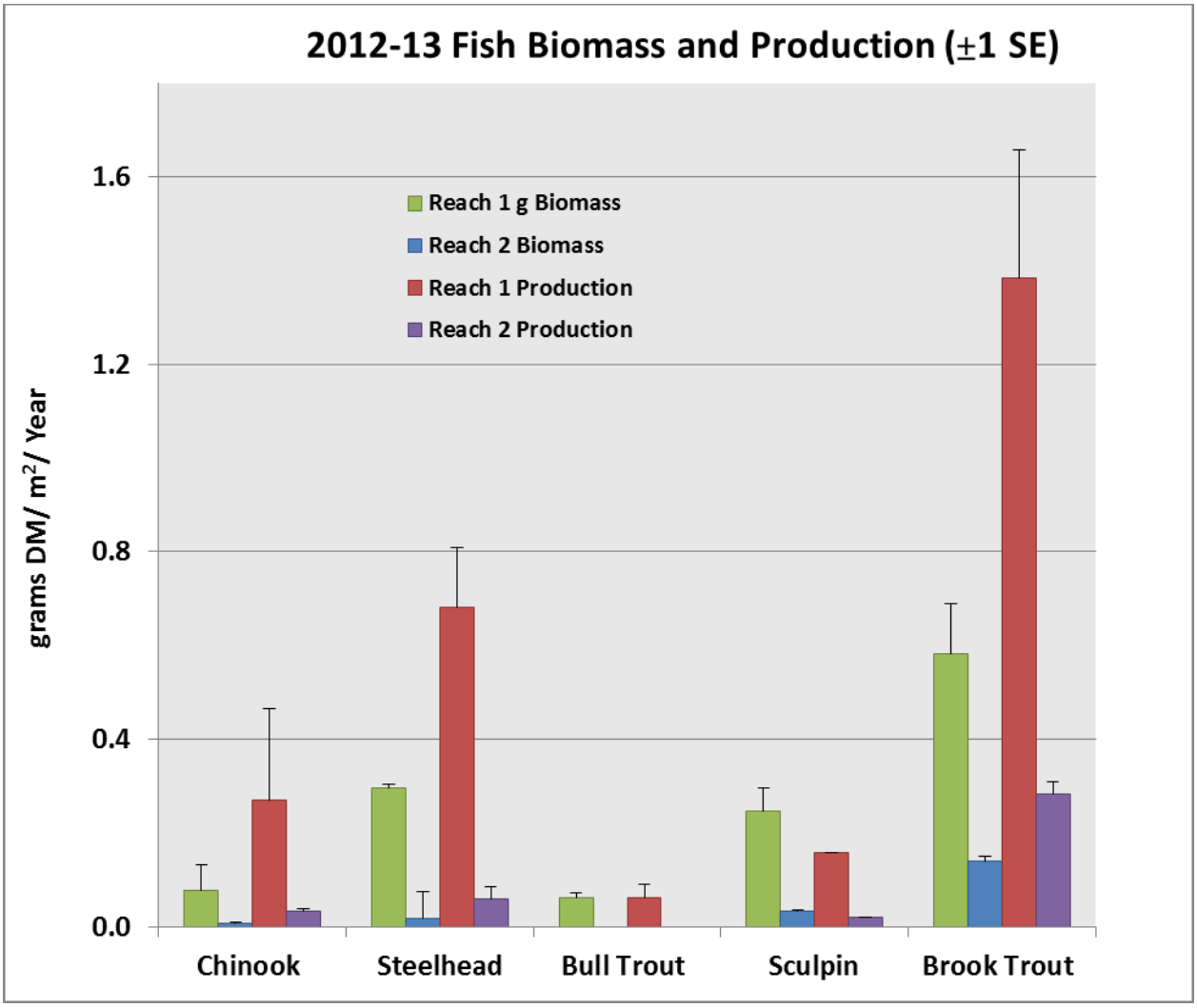

91. **Figure 14. Biomass and production of Chinook, Steelhead, Brook Trout, and Bull Trout in Hancock Springs during 2012 and 2013 combined. Error bars represent one standard error.**

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94. ***Fish growth (2012 and 2013 combined)*** **–** In all cases, no significant differences in daily growth rates occurred between Reaches 1 and 2 for any of the fish species studied. Growth rates were greater in Reach 2 than in Reach 1 for Steelhead and Brook Trout, but not for Chinook (Figure 18). Growth rates (g/week) in Reach 1 ranged from 0.51 to 1.52 g/week, compared to 0.51 to 1.71 for Reach 2 (Figure 18). Alternatively, fish growth measured as length gain (cm/week) was higher in Reach 1 than in Reach 2 for the same three species (Figure 19). Fish growth in length ranged from 1.00 to 2.09 cm/week in Reach 1 compared to 0.74 to 1.83 cm/week in Reach 2 (Figure 19). While no statistical comparisons occurred for daily growth as either g/week or cm/week, annual growth rate (g/yr) was significantly higher in Reach 2 than in Reach 1 for steelhead only (*p=0.02;* data not shown). Linear extrapolation of steelhead growth based on empirical weekly estimates (Figure 18 and Figure 19) produced mean annual steelhead growth rates of 53.0 g/yr in Reach 1 compared to 73.3 g/yr. for Reach 2. Likewise mean annual growth estimates for steelhead expressed as weight gain were lower in Reach 1 (83.72 cm/yr) than in Reach 2 (95.2 cm/yr; data not shown).

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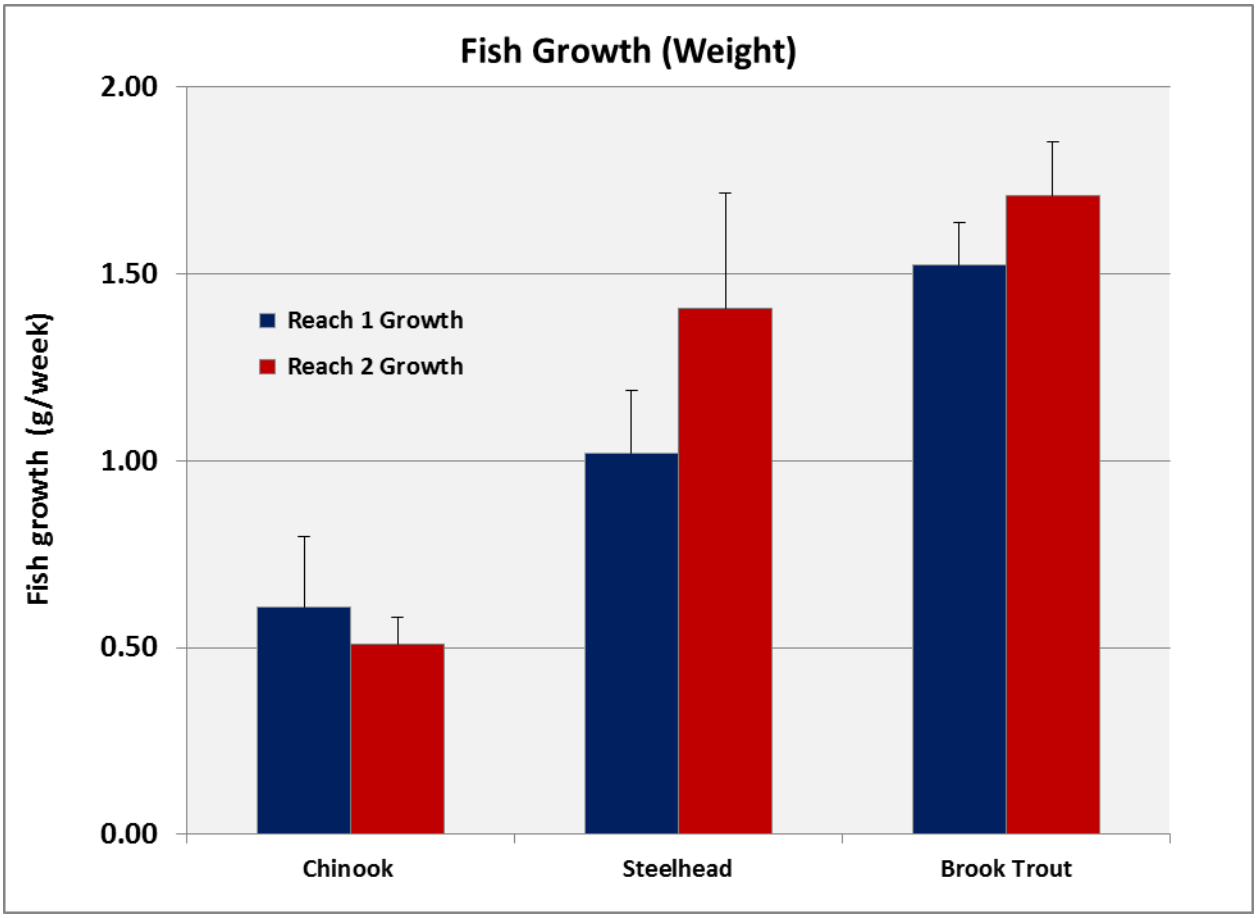

96. **Figure 15. Growth (g/week) of Chinook, Steelhead, and Brook Trout in Hancock Springs during 2012 and 2013 combined. Error bars represent one standard error.**

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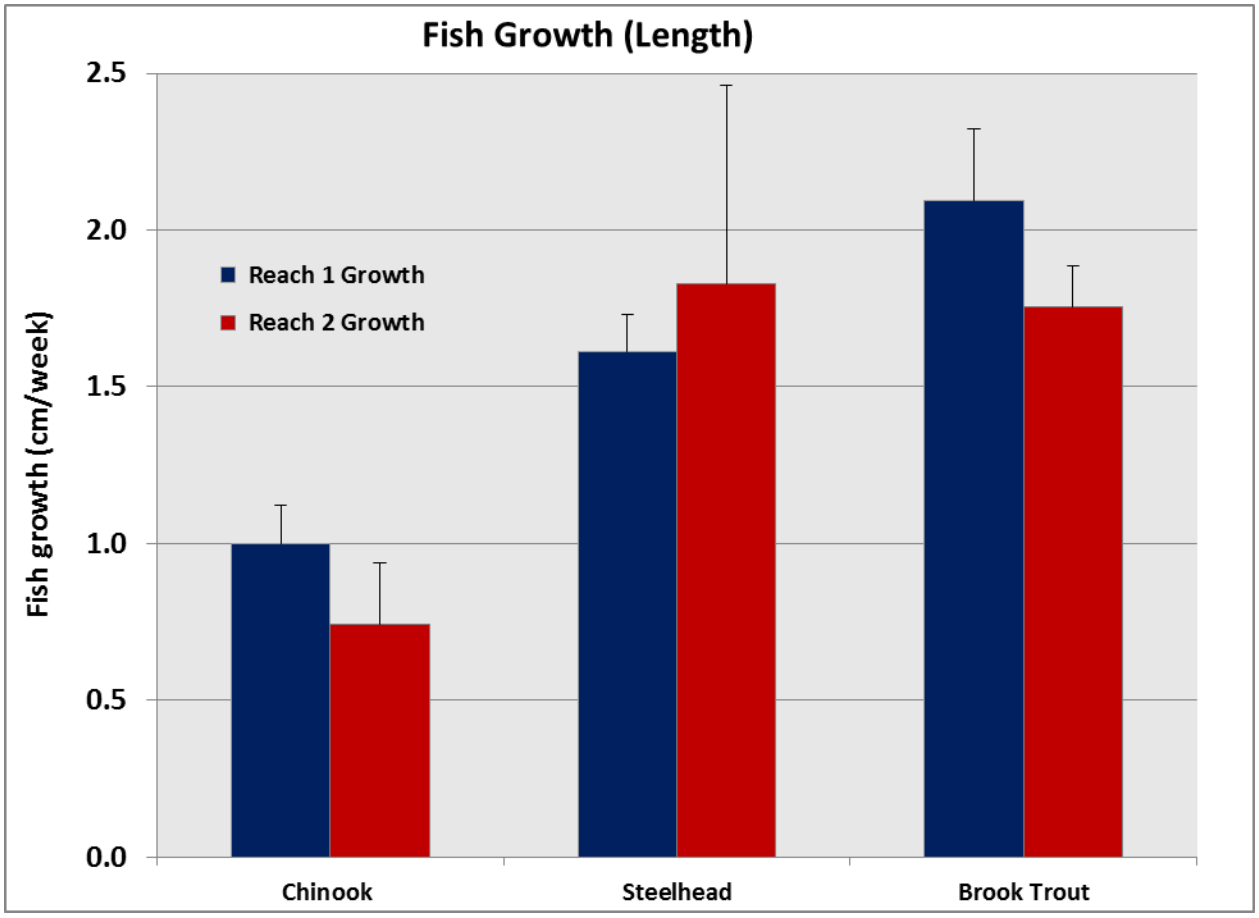

99. **Figure 16. Growth (TL; mm/week) of Chinook, Steelhead, and Brook Trout in Hancock Springs during 2012 and 2013 combined. Error bars represent one standard error.**

100.

101.

103. ***Insect production*** – Aquatic invertebrate taxa accounted for 91.8 % (14.6 of 15.8 gDM/m^2/yr) of estimated secondary production in Reach 1 and > 99% (11.5 of 11.6 gDM/m^2/yr) in Reach 2 (Figure 20). Drift samples contained so few terrestrial insect specimens that the presence of terrestrial insects in the fish gut contents had to be used to account for terrestrial produciton, which could not be directly estimated. Therefore, inclusion of consumed terrestrial insects represented an underestimate of actual terestrial insect production.

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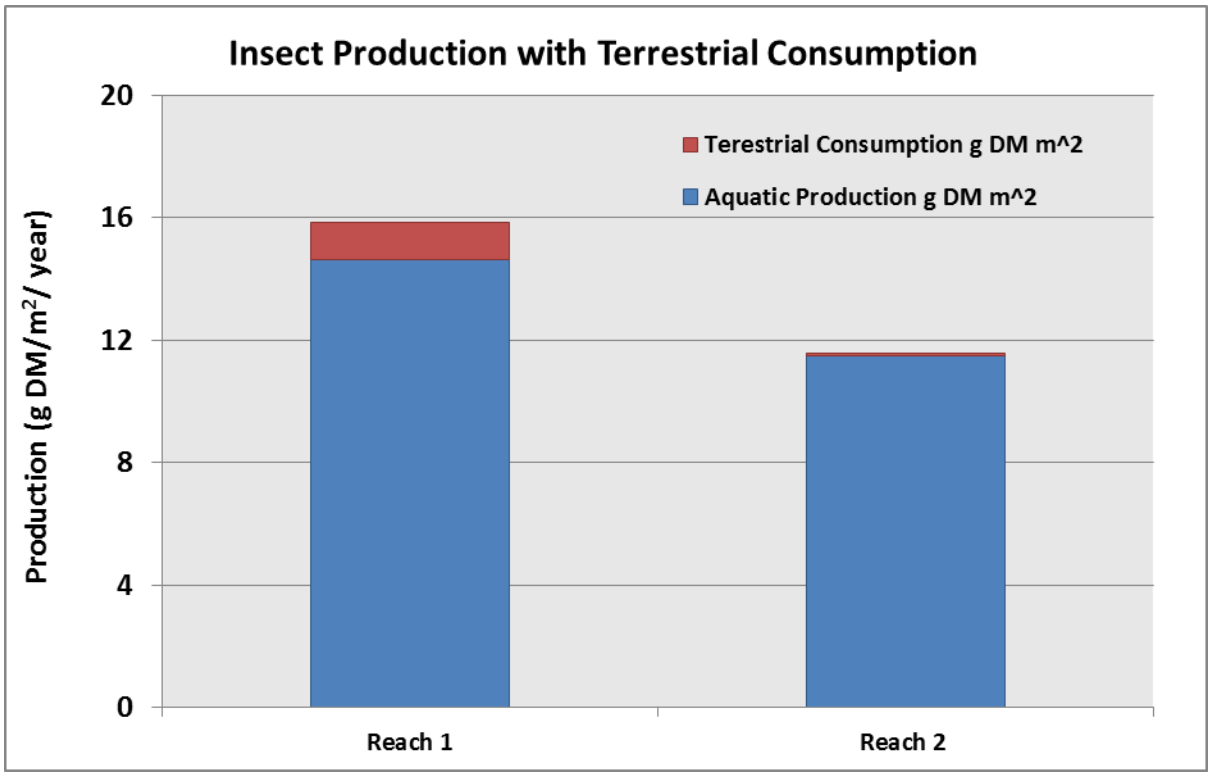

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106. Figure 17. Aquatic and terrestrial insect production in Hancock Springs by reach, 2012 and 2013.

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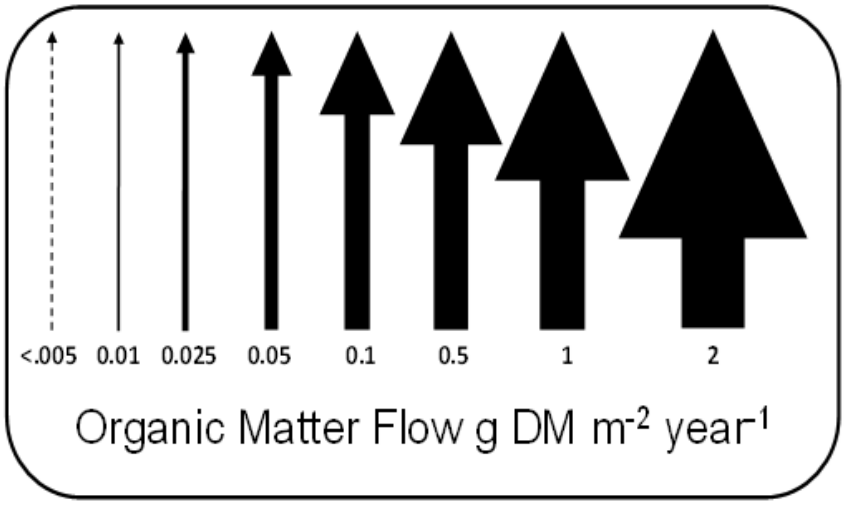
109. Flow webs

110. Food consumption (energy) pathways from invertebrates to fish were very diverse in Reach 1 compared to Reach 2 (Figures 21 and 22). Reach 1, 95% of the documented invertebrate produciton was consumed by four of the five momitored fish species (Chinook, Steelhead, Brook trout, and bull trout), compared to 80% of total invertebrate production being consumed by a single species, Brook Trout (Figure 21). The following legend is provided to quantify food and energy flow from secondary produciton to the fish community illustrated in Figures 21 and 22.



111.

112. ***Reach 1*** – Routing of total invertebrate consumption by fishes (15.2 gDM/m^2/yr) in Reach 1 was dominated by non-native brook trout, which consumed the largest proportion of secondary production (60%) via nine major energy pathways ($\geq$ 0.5 gDM/m^2/yr; Figure 21). Steelhead accounted for 23% of consumed secondary production via 6 major feeding pathways, compared to 9% for Chinook (6 major pathways), 5% for sulpins, and 3% for bull trout (3 major pathways; Figure 21). While the total amount of secondary production consumed by sculpins was reported (5% in both reaches), taxonomic composition of sculpin diets was not completed at the time of reporting.

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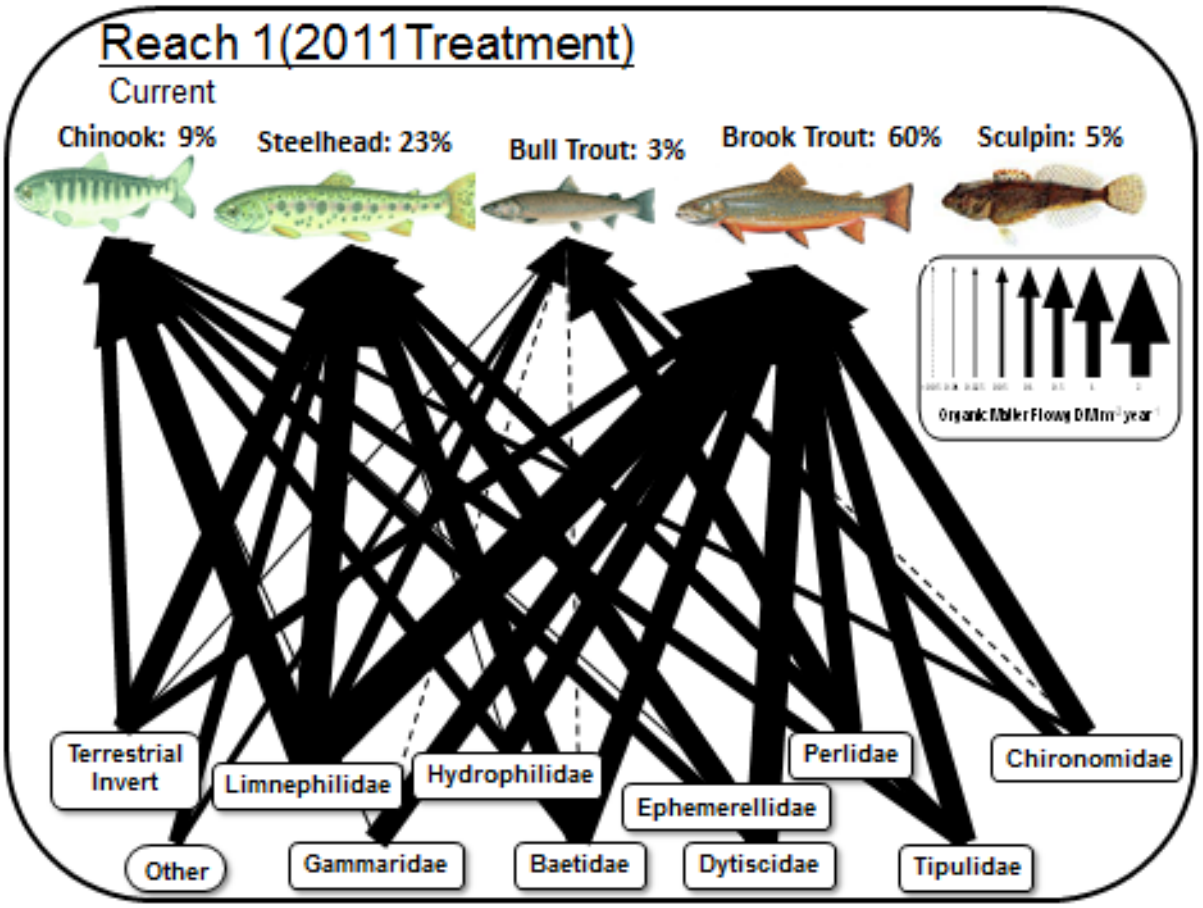

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115. **Figure 18. Flow web illustrating proportions of invertebrate consumption by dominant fish species and invertebrate families in Reach 1 of Hancock Springs, 2012 and 2103.**

116. A total invertebrate biomass of 15.2 gDM/m^2 of invertebrate taxa was consumed in Reach 1 (Table 11). Brook Trout accounted for 9.5 gDM/m2 (62.7%) of total consumed invertebrate biomass, followed by 1.5 gDM/m^2 (9.6%) for Chinook and 3.7 gDM/m^2 (24.2%) for Steelhead, and 0.5 gDM/m^2 (3.5%) for Bull trout (Table 11). Caddisflies of the family Limnophyllidae and Dytisid beetles accounted for approximately 44% and 11% of total consumed invertebrates by family respectively, with terrestrial invertebrate taxa accounting for 7.9% of all taxa consumed in Reach 1 (Table 11).

118. **Table 3. Biomass (gDM/m^2) and percent of invertebrates by family consumed by Brook Trout (BRT), Bull Trout (BULL), Chinook (CHN) and Steelhead (STH) in Reach 1 of Hancock Spring, 2012 and 2013.**

Family	BRT	BULL	CHK	STH	Total	%
LIMNEPHILIDAE	4.708081	0.132606	0.778416	1.064699	6.683802	43.85
DYTISCIDAE	1.173887	0	0.122674	0.429806	1.726367	11.33
TERRESTRIAL	0.244521	0.019824	0.19407	0.749116	1.207531	7.92
PERLIDAE	0.527148	0.309512		0.203504	1.040163	6.82
BAETIDAE	0.565161	0.000544	0.103952	0.324051	0.993709	6.52
CHIRONOMIDAE	0.439494	0.001086	0.073041	0.236235	0.749856	4.92
GAMMARIDAE	0.612978	4.42E-05	0.022655		0.635677	4.17
EPHEMERELLIDAE	0.229492		0.010735	0.299914	0.540142	3.54
TIPULIDAE	0.336348		0.08674	0.080294	0.503381	3.30
HYDROPHILIDAE	0.321119		0.021838	0.010874	0.353831	2.32
OTHER	0.402689	0.066809	0.044046	0.294209	0.807753	5.3
TOTAL CONSUMPTION	**9.56092**	**0.53043**	**1.45817**	**3.6927**	**15.2422**	**100**
Percent	**62.7**	**3.5**	**9.6**	**24.2**		

119.

121. ***Reach 2*** – Routing of total invertebrate production consumed by fishes (2.44 gDM/m^2/yr) was very simplified in Reach 2 compared to Reach 1, and was dominated by non-native Brook Trout at 80%, compared to 8% and 7 % by Steelhead and Chinook respectively, and 5% by sculpins (Figure 21 and Figure 22). No bull trout were sampled in Reach 2 during the two year reporting period (2012-2013). Brook trout consumed invertebrate production via 7 major linkages, compared to $\leq$ 9 minor pathways (<0.01 gDM/m^2/yr; Figure 22). Comparing the food webs (Figure 21 and 22) and associated data from the two reaches (Tables 11 and 12) revealed substantial post-treatment increases in food web diversity (number of pathways) and family level and collective food/energy conveyance in Reach 1 compared to Reach 2.

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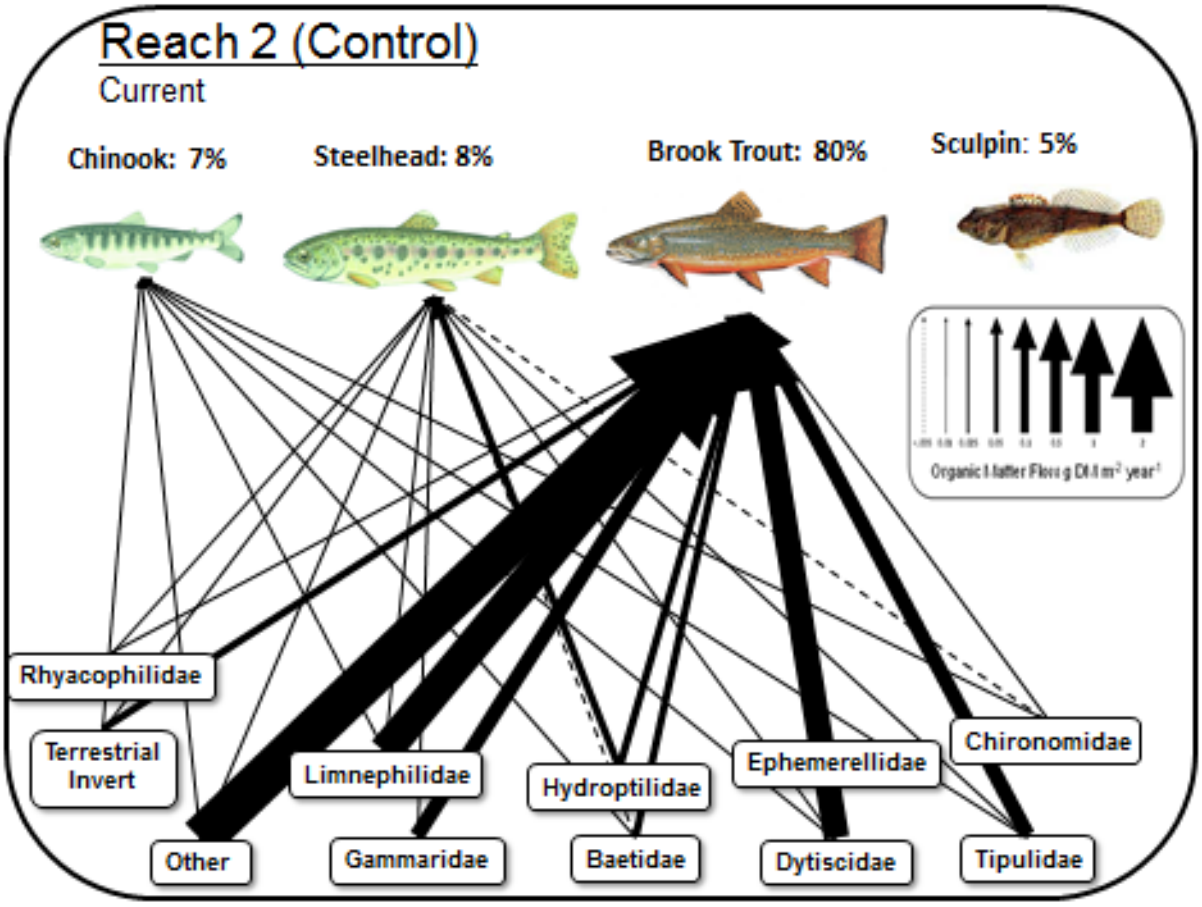

123.

124. **Figure 19. Flow web illustrating proportions of invertebrate consumption by dominant fish species and invertebrate families in Reach 2 of Hancock Springs, 21012 and 2103.**

126. A total invertebrate biomass of 2.4 gDM/m^2 was consumed in Reach 2 (Table 12). Brook Trout accounted for 2.0 gDM/m2 (82.3%) of total consumed invertebrate biomass, followed by 0.2 gDM/m^2 (9.1%) for Chinook and 0.2 gDM/m^2 (7.2% Steelhead (Table 12). Caddisflies of the family Limnophyllidae and Dytisid beetles accounted for approximately 40% and 20% of total consumed invertebrates by family respectively, with terrestrial invertebrate taxa accounting for 4.7% of all taxa consumed in Reach 2 (Table 12). Gammarus, associated with fine sediment substrates, were more common in Reach 2 (8.6%) (Table 12) than in Reach 1 (4.2%) (Table 11).

127. **Table 4. Biomass (gDM/m^2) and percent of invertebrates by family consumed by Brook Trout (BRT), Chinook (CHN) and Steelhead (STH) in Reach 2 of Hancock Spring, 2012 and 2013.**

Family	BRT	CHK	STH	Total	Percent
LIMNEPHILIDAE	0.91	0.04	0.03	0.98	39.96
DYTISCIDAE	0.42	0.01	0.03	0.49	20.12
GAMMARIDAE	0.19		0.02	0.21	8.57
TIPULIDAE	0.10	0.03	0.03	0.16	6.61
TERRESTRIAL	0.07	0.02	0.02	0.12	4.73
HYDROPTILIDAE	0.05		0.04	0.09	3.88
BAETIDAE	0.07	0.01	0.00	0.09	3.73
CHIRONOMIDAE	0.03	0.03	0.00	0.07	2.81
RHYACOPHILIDAE	0.03		0.01	0.04	1.57
OTHER	0.13	0.03	0.03	0.20	8.04
TOTAL CONSUMPTION	**2.01**	**0.18**	**0.22**	**2.44**	**100**
Percent	**82.3**	**7.2**	**9.1**		

128.

129. ***Invertebrate production, consumption, and fish production*** – Aquatic invertebrate production was similar between reaches at 13.8 and 11.4 gDM/m^2/yr for Reach 1 and Reach 2 respectively (Figure 23). Fish consumption of aquatic and terrestrial invertebrates was also about 20% higher in Reach 1 than in Reach 2, at 15.9 and 12.9 gDM/m^2/yr respectively when adding the contribution from consumption of terrestrial origin invertebrate taxa (Figure 23). However, consumption of aquatic and terrestrial origin invertebrate prey was nearly 8 times higher in Reach 1 than in Reach 2, at 16.0 and 2.5 gDM/m^2/yr for these reaches respectively (Figure 23). Likewise, fish production was more than 6 times greater in Reach 1 than in Reach 2, at 2.6 and 0.4 gDM/m^2/yr for these reaches respectively (Figure 23). These findings suggested consumption of virtually all estimated invertebrate production by fish in Reach 1 but consumption of only about 16% of available invertebrate production by fish in Reach 2 (Figure 23).

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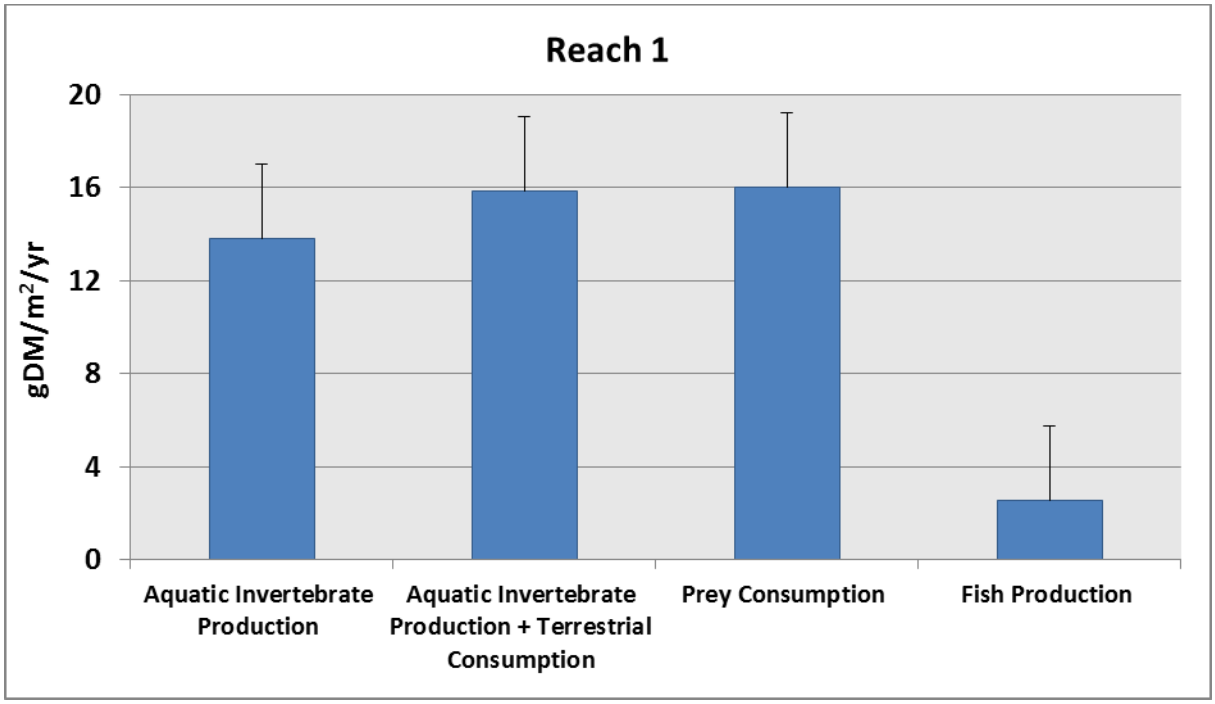
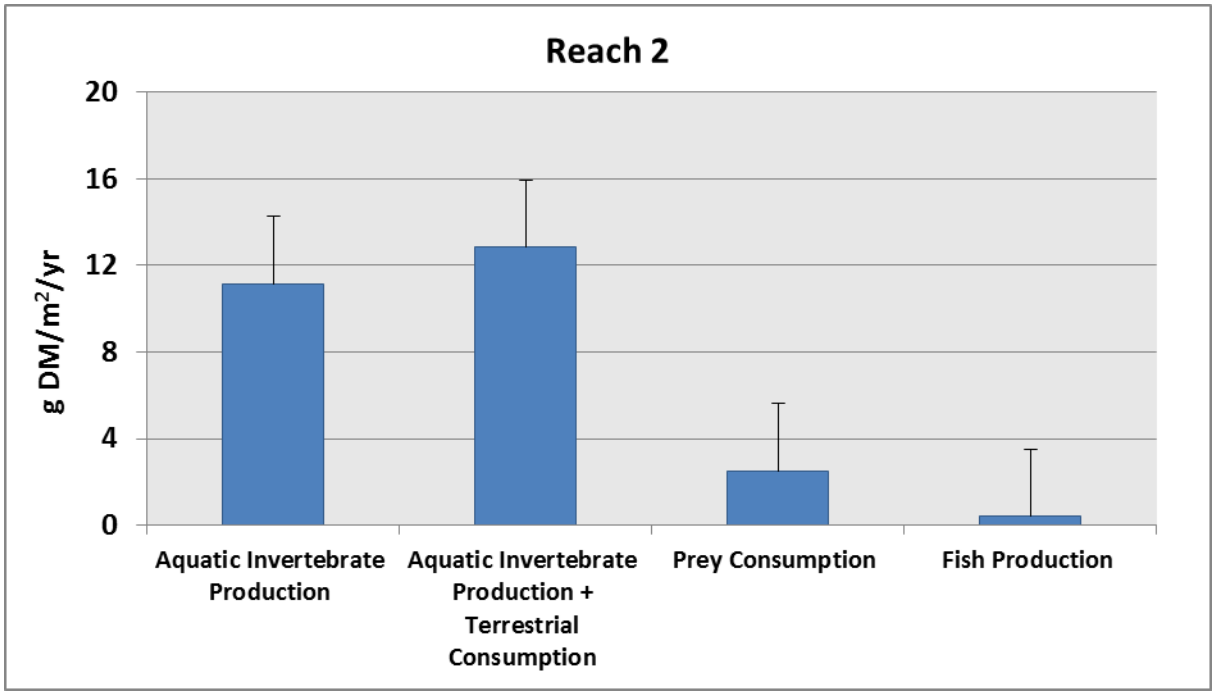




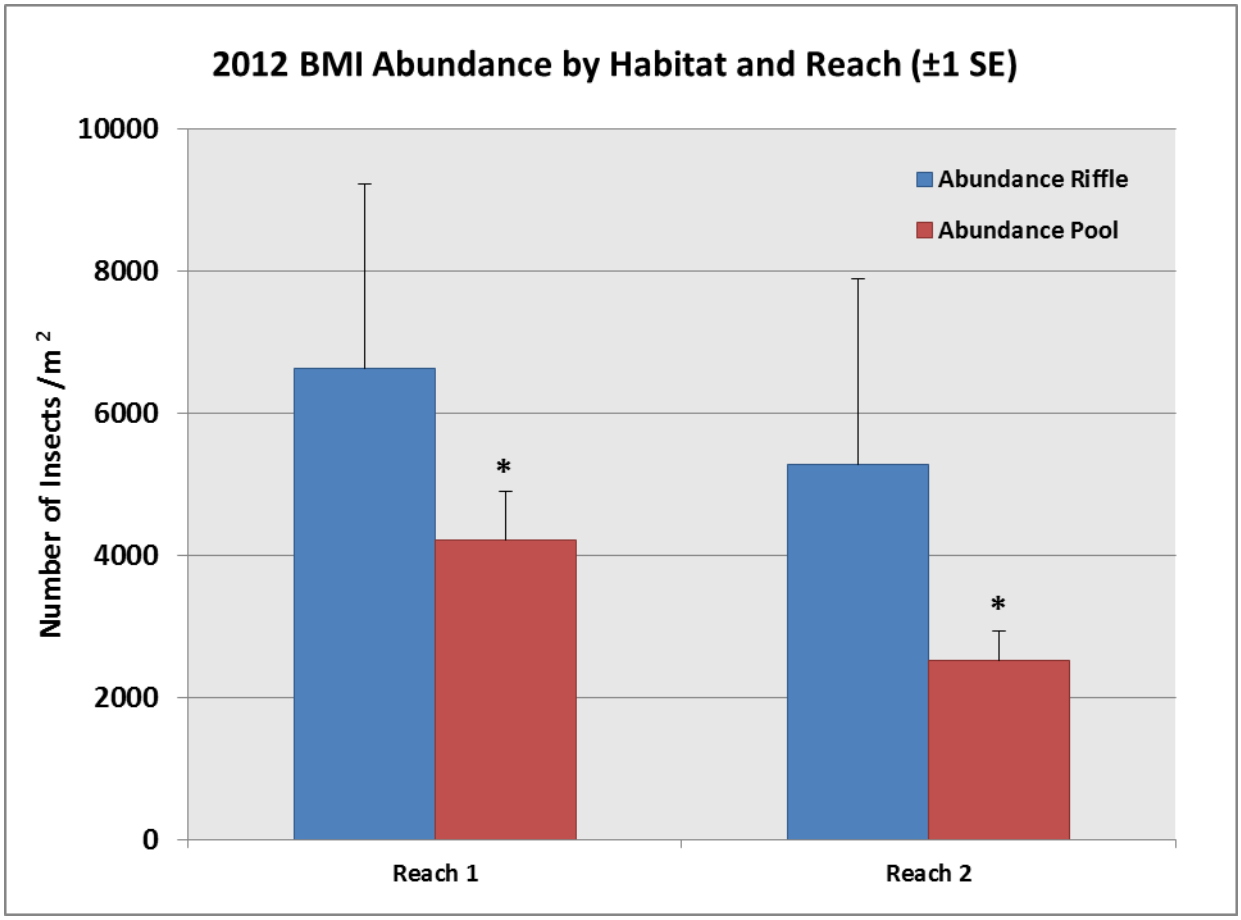
138. **Figure 20. Aquatic invertebrate consumption with and without terrestrial origin taxa, invertebrate consumption by fish, and fish production in Reach 1 and Reach 2 of Hancock Springs during 2012.**

139.

141. Benthic macroinvertebrates (BMI)

142.

143. ***2012 BMI abundance*** **–** Benthic macroinvertebrates were consistently more abundant in riffles (5,290-6,631/m^2) than in pools (2,519-5,290/m^2) in both reaches during 2012 (Figure 24). However, all results were statistically non-significant except that abundance in the pool samples was significantly higher in Reach 1 than in Reach 2 (*p = 0.05*).

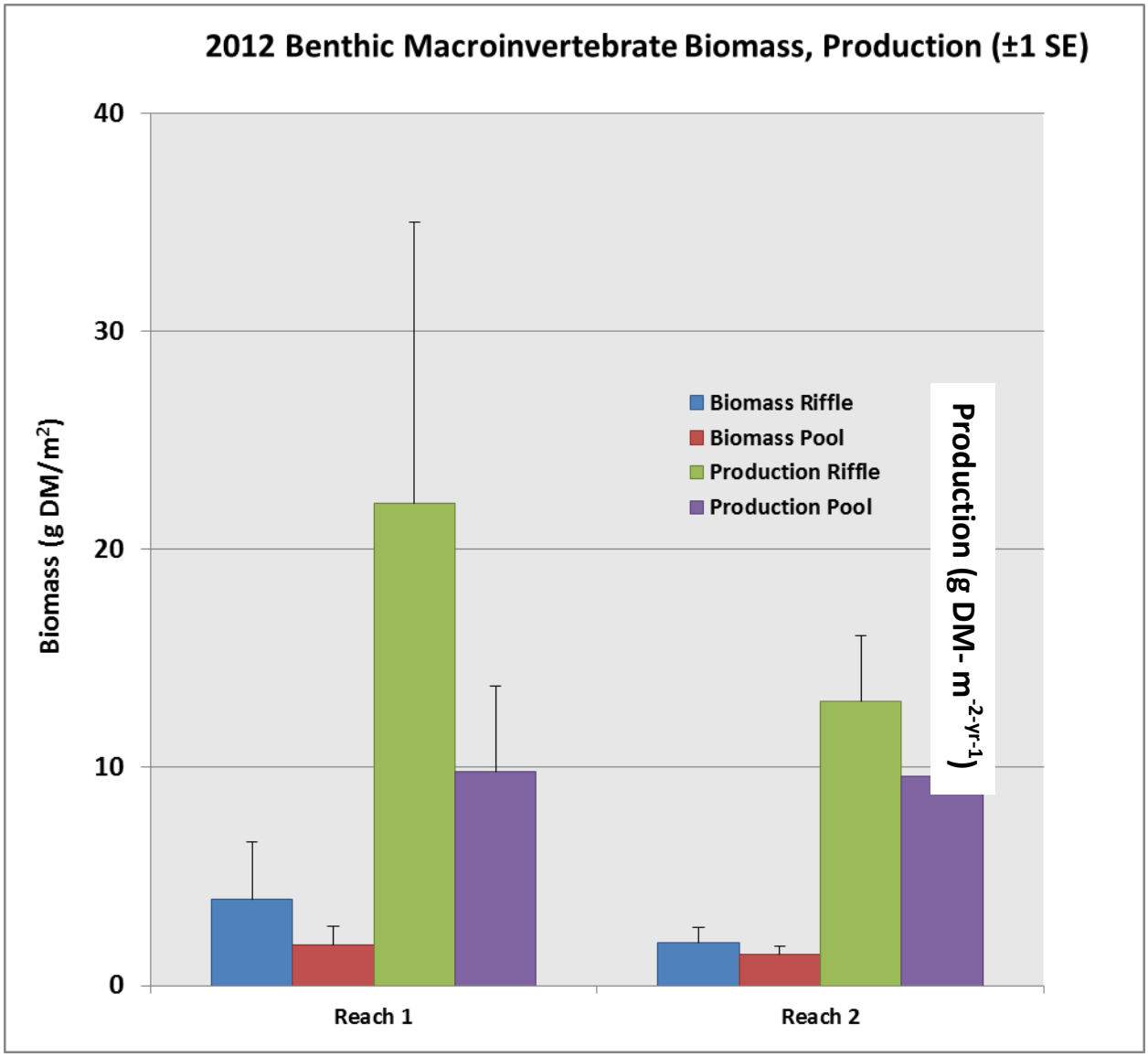


144.

145. **Figure 21. Abundance and biomass of benthic macroinvertebrates in Hancock Springs during 2012 by reach and habitat type (pools, riffles). Error bars represent one standard error. Asterisk denotes statistical significance (*p<0.05*).**

146.

148. ***2012 BMI biomass and production*** – BMI biomass in Hancock Springs during 2012 was consistently but not significantly higher in riffles than in the pools in both reaches (Reach 1 mean 3.7 gDM/m^2, Reach 2 mean 2.0 gDM/m^2)(Figure 25). BMI production, while higher in Reach 1 riffles than in Reach 2 riffles, was essentially equal in pools in both reaches. BMI production was more than twice as high in riffles than in Pools in Reach 1 and about 30% greater in Reach 2 riffles than pools (Figure 25).



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150. **Figure 22. Benthic macroinvertebrate biomass and production in both reaches of Hancock Springs, 2012. Error bars represent one standard error.**

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153. ***Invertebrate drift*** – Biomass of sampled invertebrate drift in Hancock Springs was approximately greater in Reach 1 (0.0048 g/m^3/min) than in Reach 2 (0.0014 g/m^3/min). All but 80 invertebrate specimens identified in the drift samples from both reaches were classified as aquatic vs. terrestrial origin taxa. The contribution of invertebrate biomass in the drift at the Order level was significantly different between Reach 1 and Reach 2 (*p< 0.001*)(Figure 26). Diptera accounted for 60% of the invertebrate biomass in Reach 1 drift samples compared to 75% in Reach 2, Tricladida accounted for 27% of the drift biomass in Reach 1 compared to 0% in Reach 2, and Trichoptera accounted for just over half as much biomass in Reach 1 as in Reach 2 (Figure 26).

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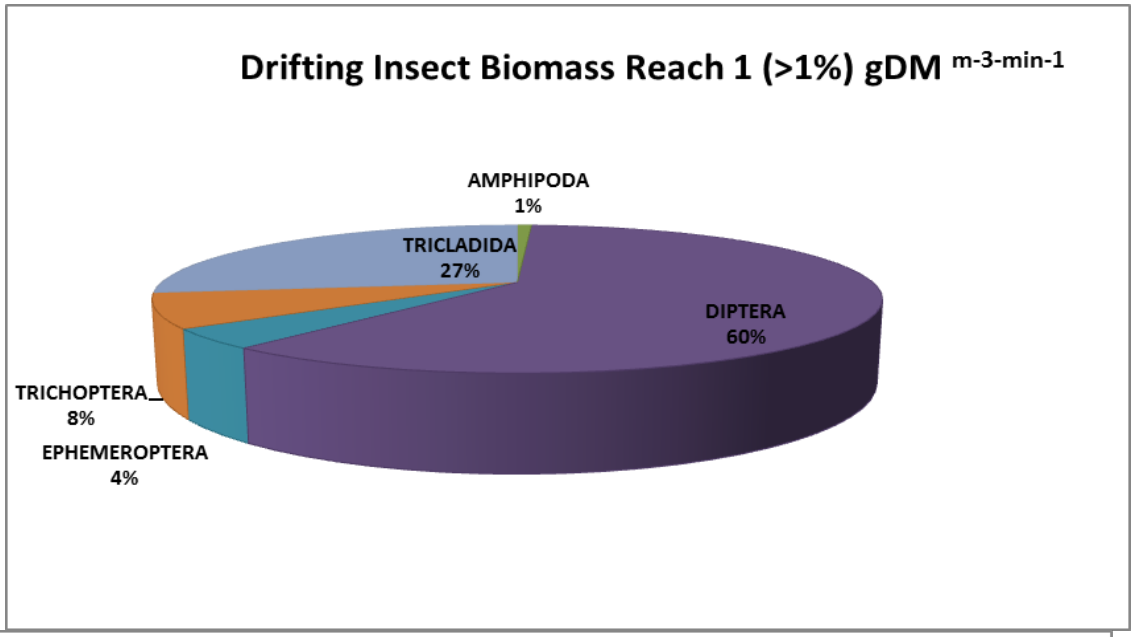

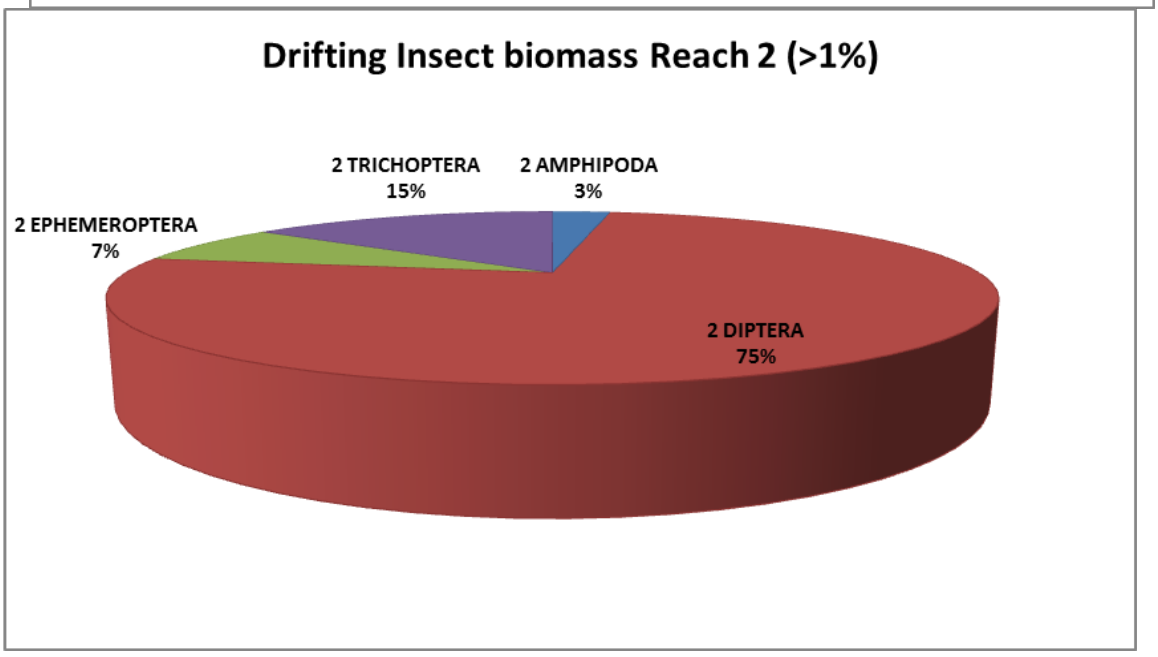

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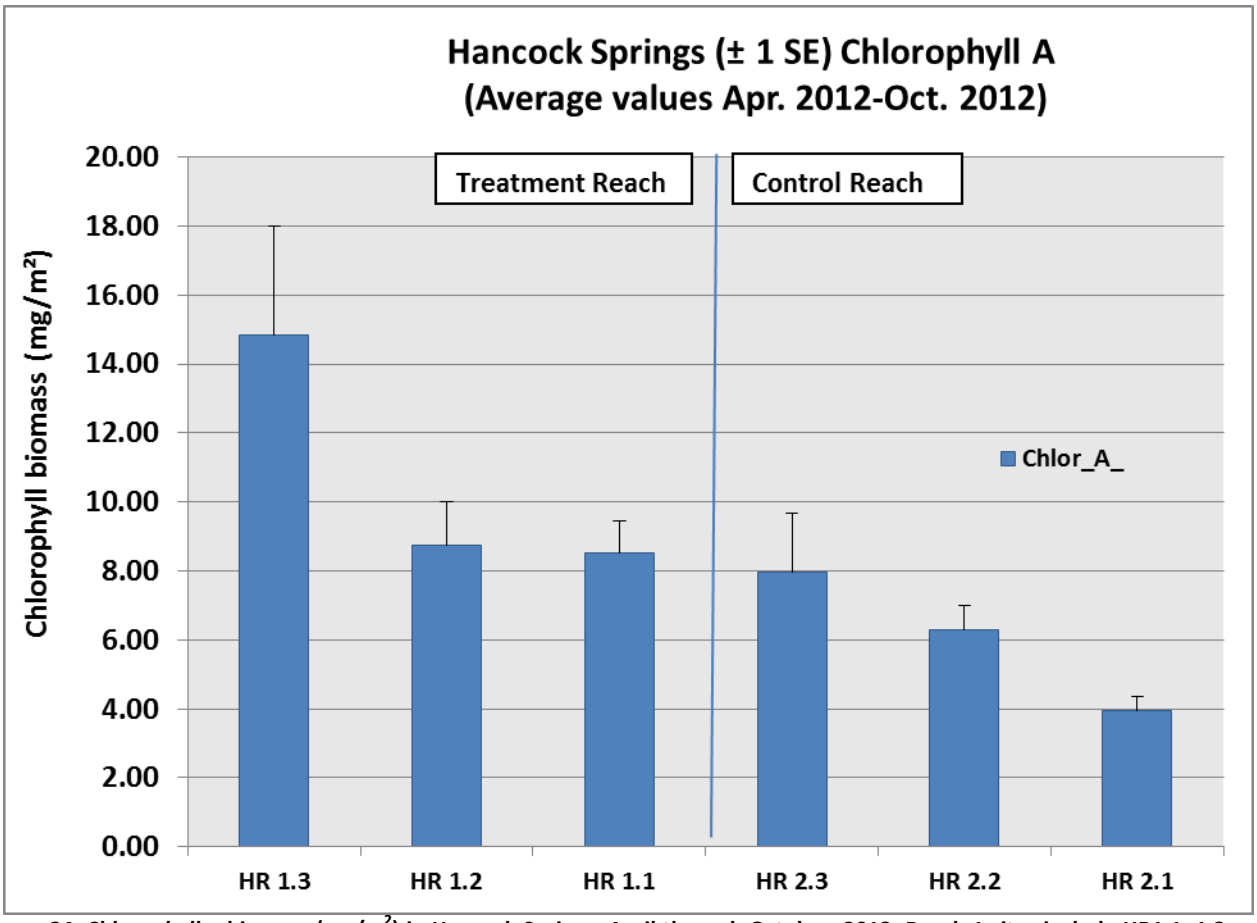
156. **Figure 23. Percent biomass contribution of invertebrate Orders in invertebrate drift samples from Hancock Springs by reach, 2012 and 2103 combined data.**

157. ***Chlorophyll a*** **–** Chlorophyll a biomass in Hancock Springs during 2012 exhibited a marked downstream decline through both reaches, from 14.9 to 3.4 mg/m^2 (Figure 27). Mean chlorophyll a biomass in Reach 1 was 10.7 mg/m^2 (range 8.5-14.9 mg/m^2) compared to 6.1 mg/m^2 (range 3.9-8.0 mg/m^2) in Reach 2 (Figure 27).



158.

159. **Figure 24. Chlorophyll a biomass (mg/m^2) in Hancock Springs, April through October, 2012. Reach 1 sites include HR1.1, 1.2, and 1.3; Reach 2 sites include HR2.1, 2.2, and 2.3. Error bars represent one standard error.**

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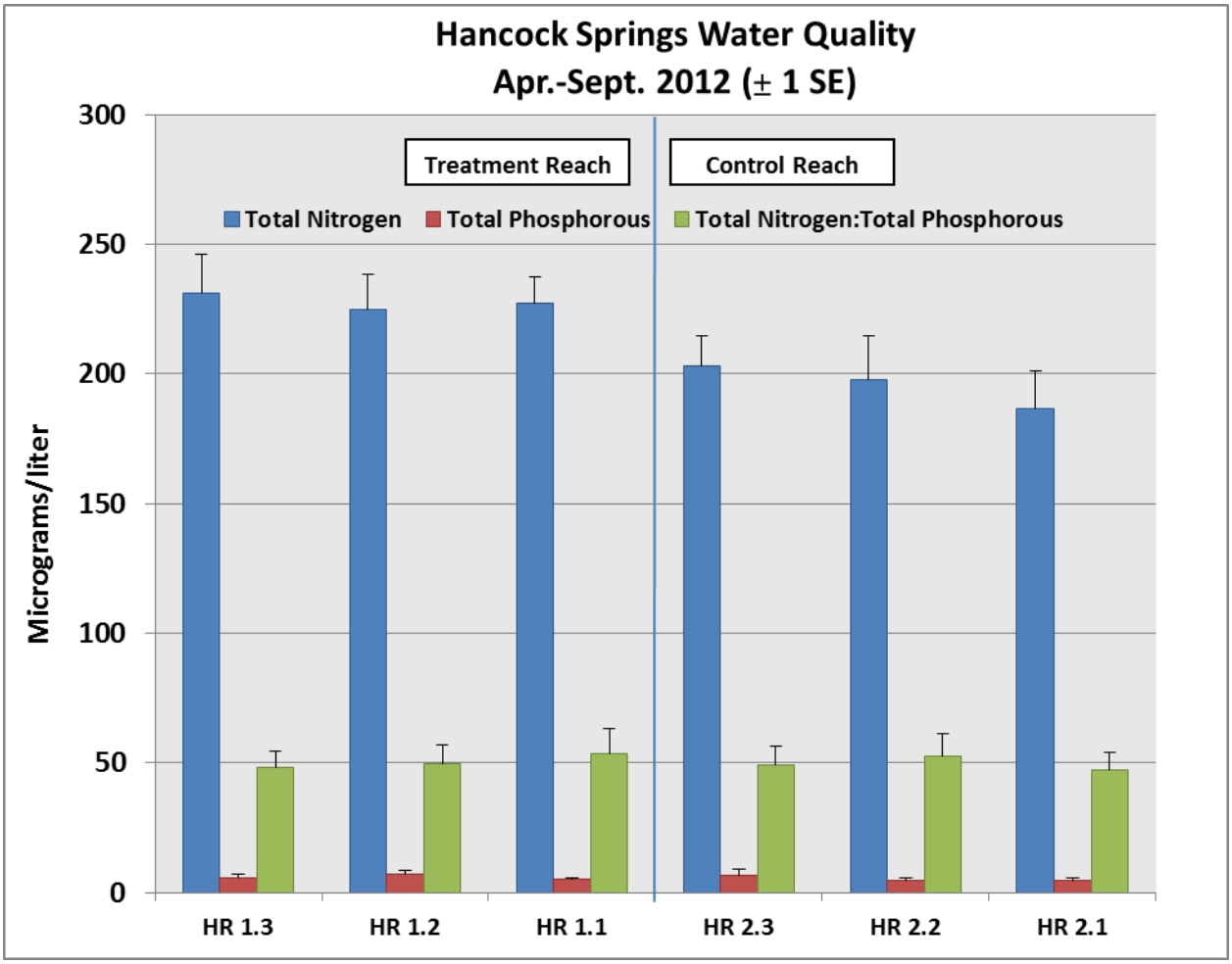
168. ***TN, TP, and TN:TP ratio*** – Total nitrogen ranged from 186.6 to 231.3 ug/L across both reaches and exhibited a general declining downstream trend (Figure 28). Total nitrogen values ranged from 227.4 to 231.3 ug/L (mean 227.8ug/L) in Reach 1 and 186.6 to 202.9 ug/L (mean 195.7ug/L) in Reach 2. TP was low and very stable across both reaches, with a mean value of 6.0ug/L in Reach 1 and 5.5 in Reach 2 (Figure). The TN:TP ratio was also very stable, ranging from 47.4 to 53.4, with a mean of 50.5 in Reach 1 and 49.8 in Reach 2 (Figure 28).



169.

170. **Figure 25. Total nitrogen (TN), total phosphorus (TP) and their ratio values (TN:TP) for Hancock Springs, April through September 2012. Error bars represent one standard error.**

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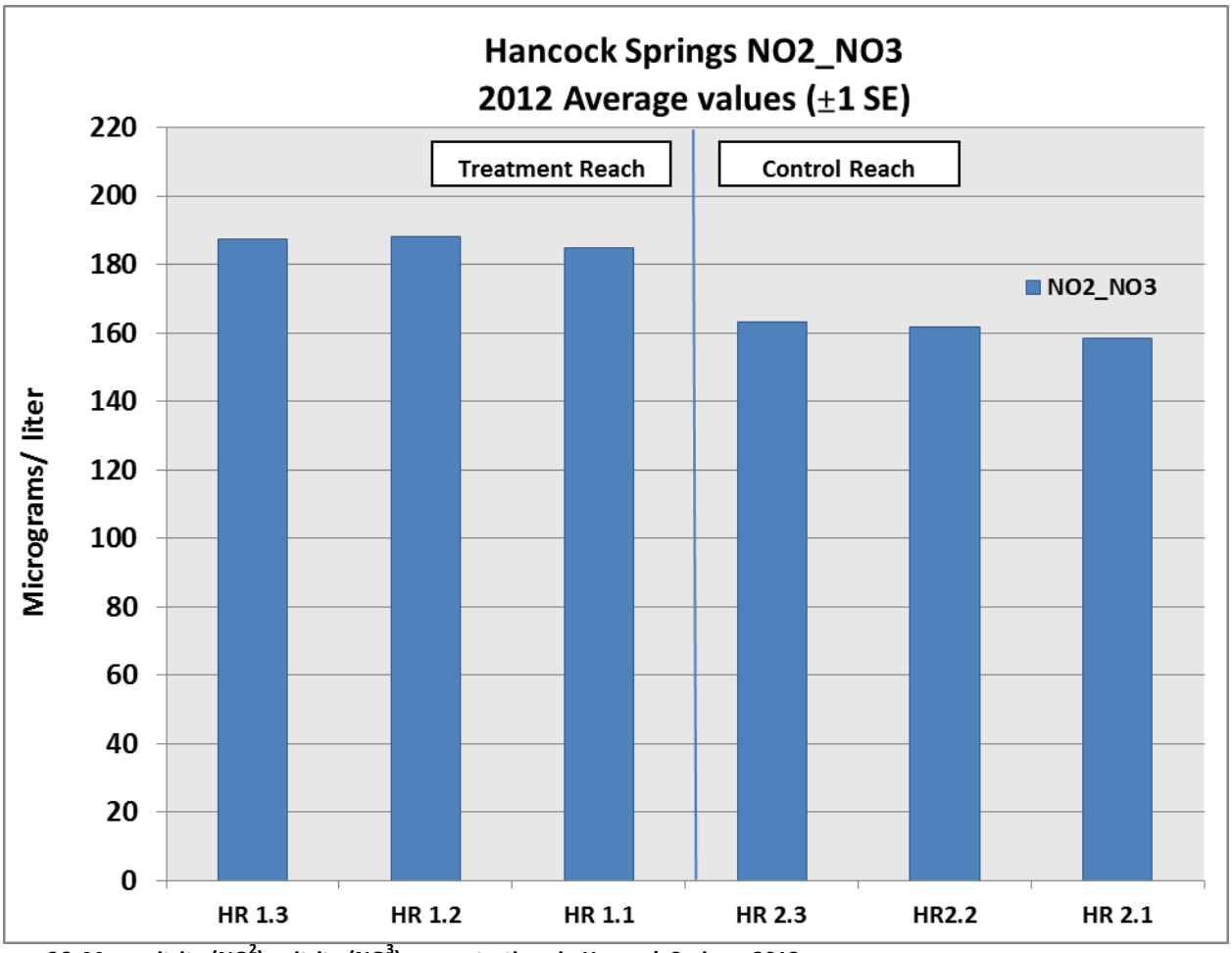
178. ***NO2 +NO3*** – During 2012, NO^2+ NO^3 values were consistently higher in Reach 1 (mean 186.6ug/L, range 184.9-188.0 ug/L) than in Reach 2 (mean 161.1 ug/L, range 158.4-163.2 ug/L)(Figure 29).

179.



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181. **Figure 26. Mean nitrite (NO^2)+ nitrite (NO^3) concentrations in Hancock Springs, 2012.**

182. ***Soluble reactive phosphorus (SRP)*** – SRP values ranged from 1.09 to 2.05 ug/L in Reach 1 and from 1.13 to 2.38 ug/l in reach 2 during 2012. However, a majority (71%) of SRP samples were below the lab detection limit of 1 ug/L (Table 13).

183. **Table 5. Numbers of Hancock Springs SRP samples at and below detection level during 2012.**

	Reach 1				Reach 2				
Sites	HR 1.3	HR 1.2	HR 1.1	Total	HR 2.3	HR 2.2	HR 2.1	Total	Grand Total
No. of samples	12	12	12	36	12	12	12	36	72
No. below detection	10	10	8	28	6	8	9	23	51 (71%)
No. above detection	2	2	4	8	6	4	3	13	21 (29%)

184.

185. ***Ammonia (NH4)*** – No ammonia data were reported because all 36 ammonia samples collected from each reach (total =72) during 2012 were below the lab detection limit of 5.0 ug/L.

186. ***Water temperature*** – Mean monthly water temperature in Hancock Springs was very similar between reaches, with a mean difference between reaches of 0.1°C from January 2012 to January 2013 and a maximum difference between reaches during any given month of 0.8°C. Water temperature ranged from 6.2 to 7.9°C in Reach 1 and 5.8 to 8.6°C in Reach 2 (Figure 30).

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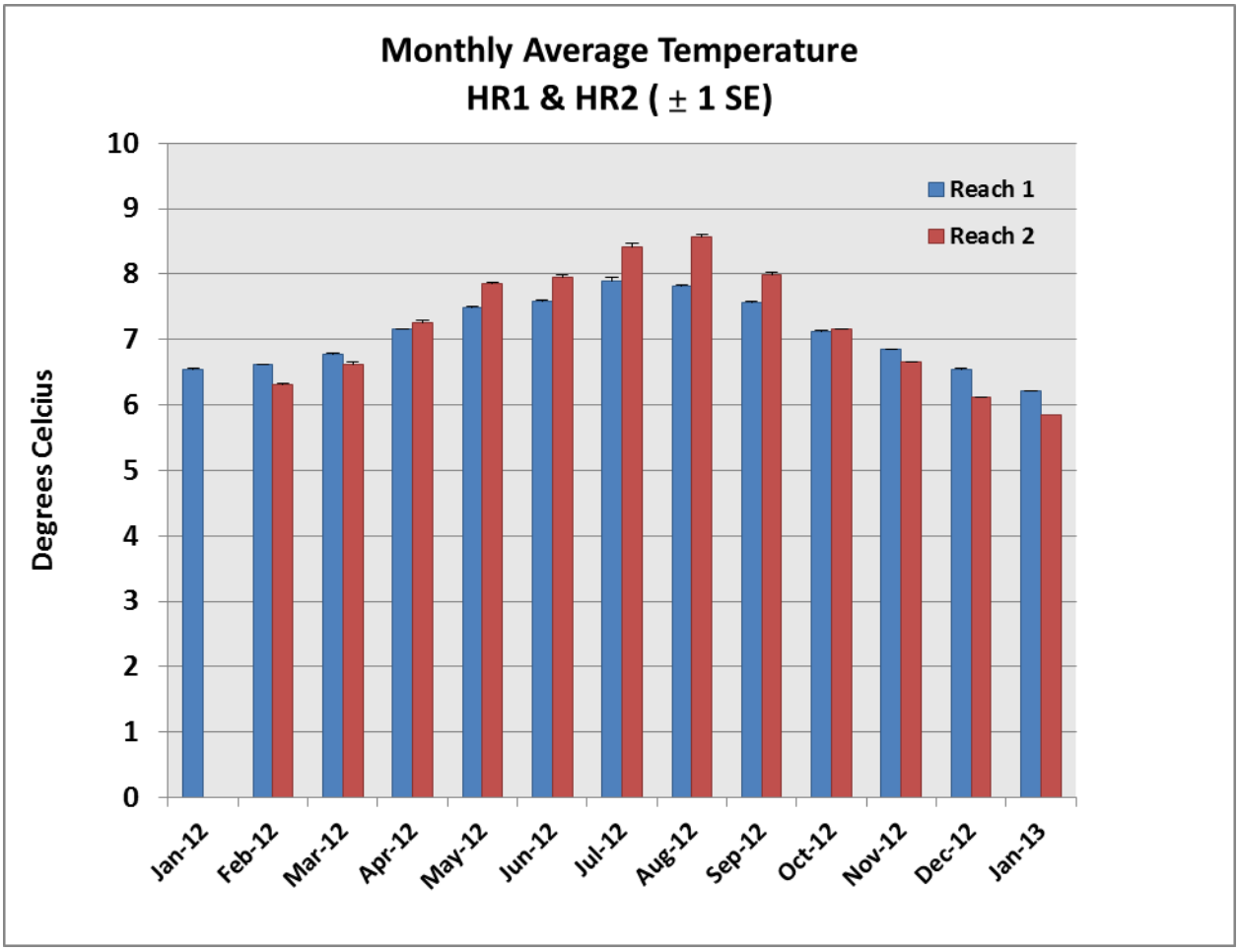

188. **Figure 27. Mean monthly water temperature in Hancock Springs by reach from January 2012 through January 2013. Error bars represent one standard error.**

190. Twisp River

191. Twisp River – Phase 1 (2008-2012)

192. ***BMI abundance –*** Aggregated benthic macroinvertebrate abundance in the Twisp River showed a generally decreasing upstream pattern with considerable variation among sites and years (Figure 31). Abundance was most variable at TR1, the farthest downstream site, ranging from 1,341 to 6,360 individuals/m^2. Most values at TR2 through TR6 ranged from about 1,300 to near 3,000 invertebrates/m^2)(Figure 31).

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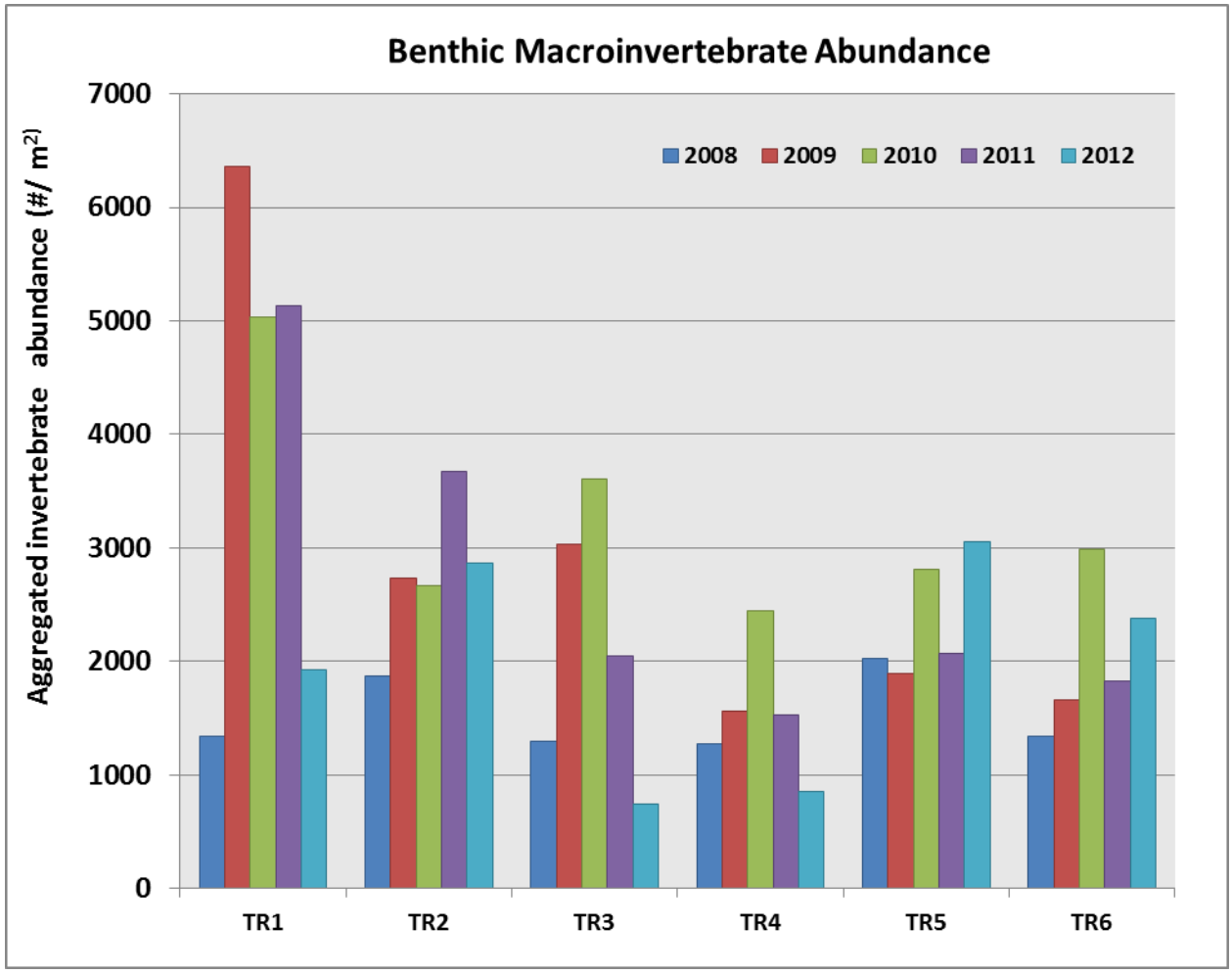

194. **Figure 28. Abundance of aggregated benthic macroinvertebrate taxa (#/m^2) for the Twisp River, sites TR1 through TR6, 2008 through 2012.**

196. ***BMI biomass*** **–** Benthic macroinvertebrate biomass showed a generally decreasing upstream trend along with considerable annual and within-site variability (Figure 32). With the exception of TR1 data during 2009, invertebrate biomass ranged from 0.6 to 2.5 gDM/m^2. Invertebrate biomass at TR1 exhibited the greatest within-site variation, ranging from 0.6 to 3.4 gDM/m^2, while TR2 showed the least within-site variation, with biomass ranging from 1.7 to 2.3 gDM/m^2 (Figure 32).

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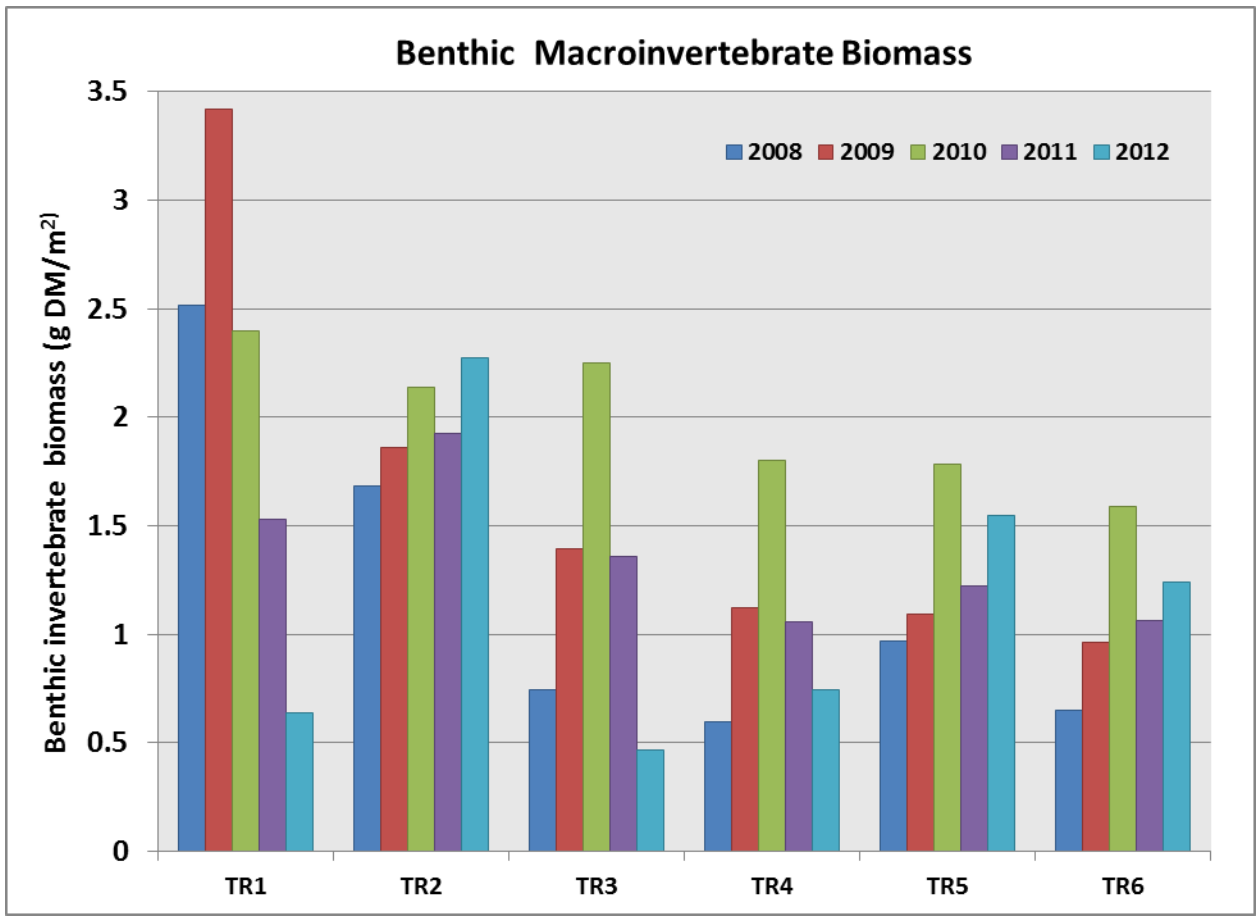
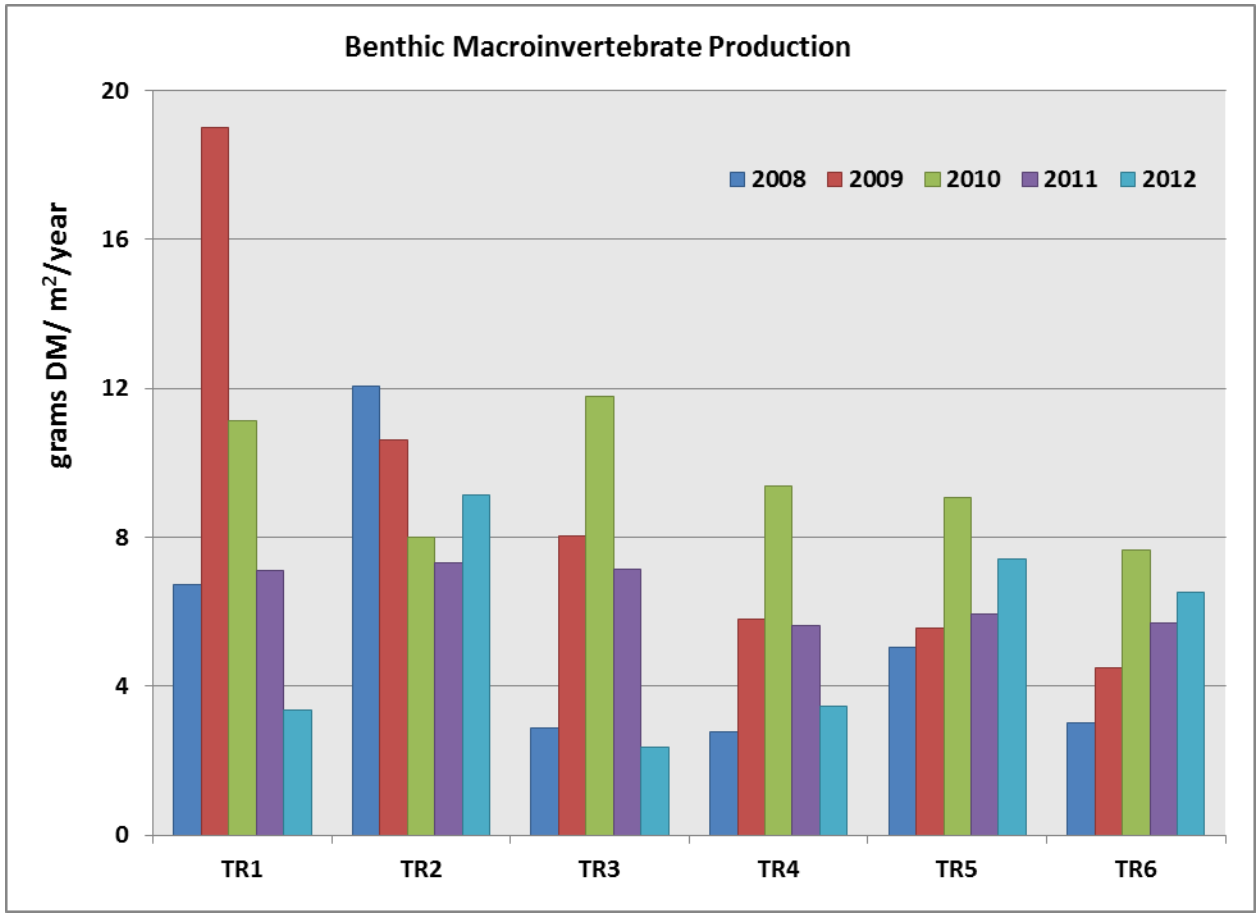


198. **Figure 29. Benthic macroinvertebrate biomass (dry mass, g/m^2) for the Twisp River, sites TR1 through TR6, 2008 through 2012.**

199.

200. ***BMI production*** **–** As with abundance and biomass, invertebrate production was also greatest at TR1 during 2009, but showed considerable within-site and temporal variability (Figure 33). Overall secondary production ranged from 2.4 to 19.0 gDM/m^2/yr. Production during 2009 showed the strongest decrease upstream trend of any of the years studied, followed by 2012 data. Production was the most steady among sites during 2011, ranging from 5.6 to 7.3 gDM/m^2/yr (Figure 33).

201.



202. **Figure 30. Benthic macroinvertebrate production (dry mass, g/m^2/yr) for the Twisp River, sites TR1 through TR6, 2008 through 2012.**

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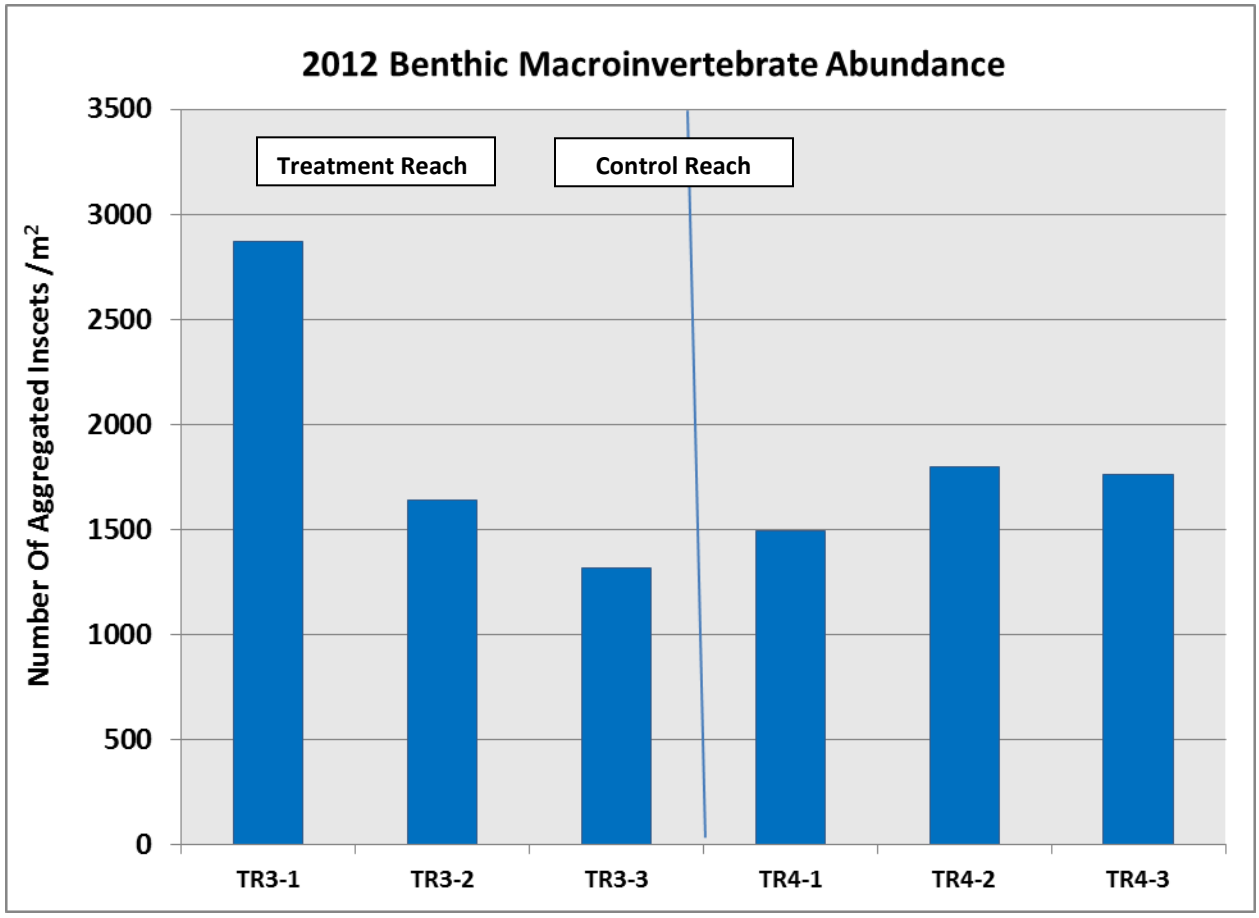
209. Twisp River – Phase 2 (2012-2015)

210. ***BMI abundance*** **-** Aggregated abundance of benthic macroinvertebrates (all taxa) in the Twisp River during 2012 ranged from approximately 1,300 to 1,800 organisms/m^2 from TR3.1 through TR4.3 to 2870/m^2 at the TR1, the farthest downstream site (Figure 34). Mean invertebrate biomass was higher in the Treatment Reach (1,834/m^2; range 1,316-2,870) than in the Control reach (mean 1,684/m^2, range 1,492-1,797) (Figure 34).

211.



212. **Figure 31. Benthic macroinvertebrate abundance (#/m^2) in the Twisp River for the control (TR4.1 through TR4.3) and treatment (TR4.1 through TR4.3) reaches during 2012.**

213.

215. ***BMI biomass –*** Mean benthic macroinvertebrate biomass at the six sites in the Twisp River during 2012 ranged from just under 1 to 1.7 gDM/m2 at all sites except TR3.2, which averaged 2.7 gDM/m2 (Figure 35). Benthic macroinvertebrate biomass averaged 1.8 gDM/m2 (range 1.0-2.7) in the treatment reach and 1.5 gDM/m2 in the control reach (range 1.45-1.56)(Figure 35).

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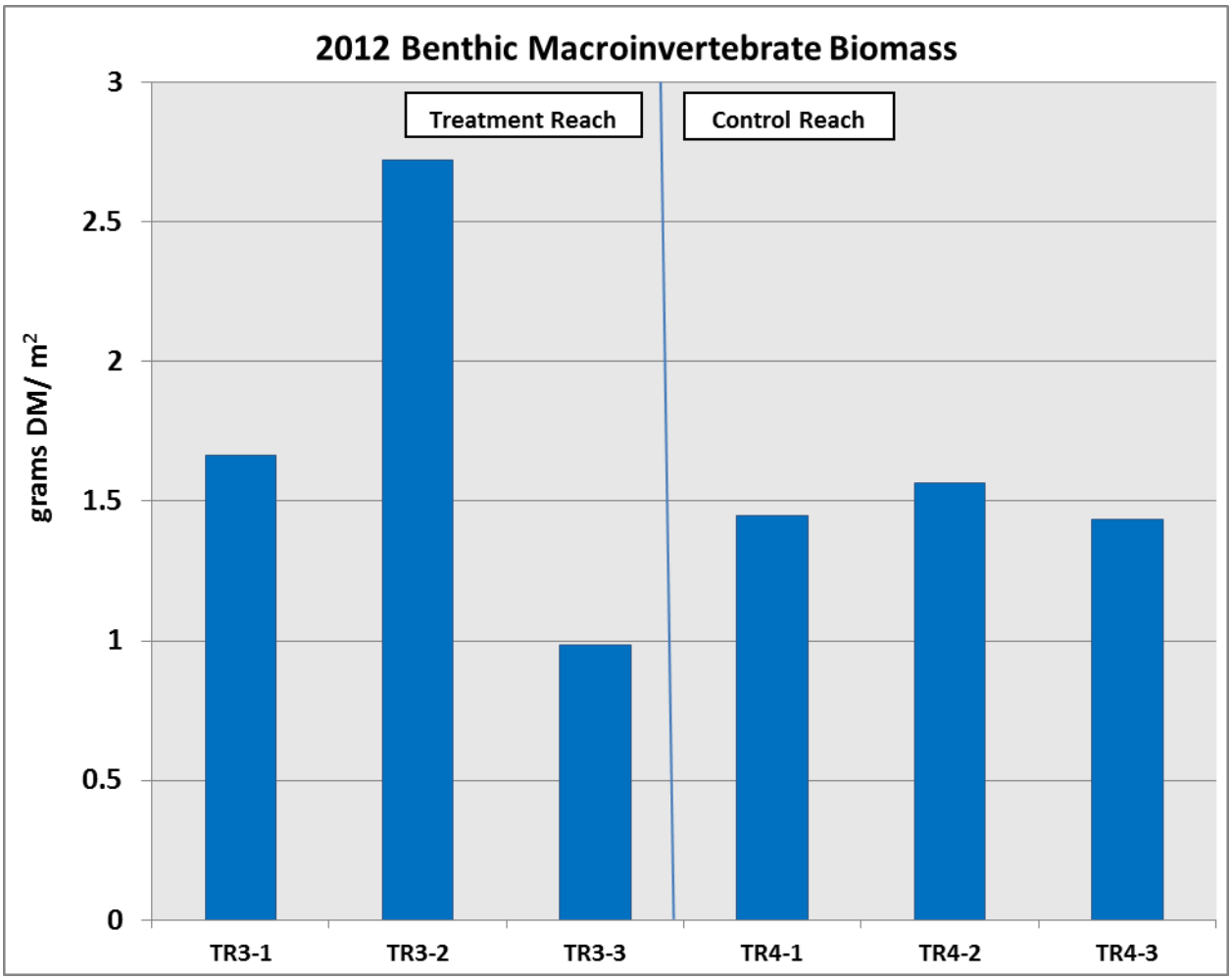

217. Figure 32. Benthic macroinvertebrate biomass (dry weight, g/m^2) in the Twisp River for the control (TR4.1 through TR4.3) and treatment (TR4.1 through TR4.3) reaches during 2012.

218. ***BMI production*** **–** Benthic macroinvertebrate production in the Twisp River during 2012 showed no clear longitudinal pattern across study sites. Secondary production values ranged from 4.5 to 10.7 gDM/m^2/yr at all sites with a mean of 7.9 in the Treatment Reach (range 4.5-10.9) and a mean of 6.8 in the Control Reach (range 6.1-7.3)(Figure 36).

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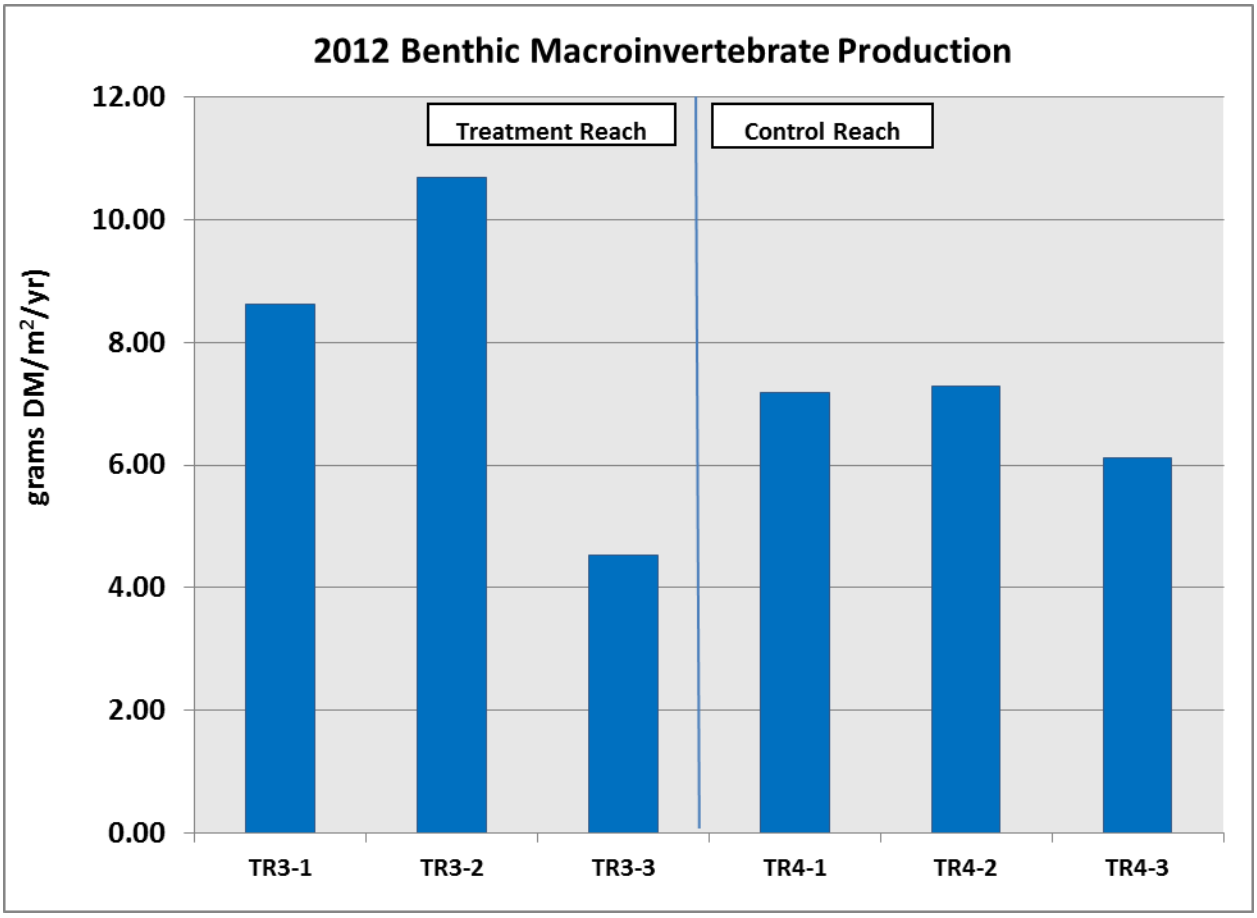

220. **Figure 33. Benthic macroinvertebrate production (dry weight, g/m^2/yr) in the Twisp River for the control (TR4.1 through TR4.3) and treatment (TR4.1 through TR4.3) reaches during 2012.**

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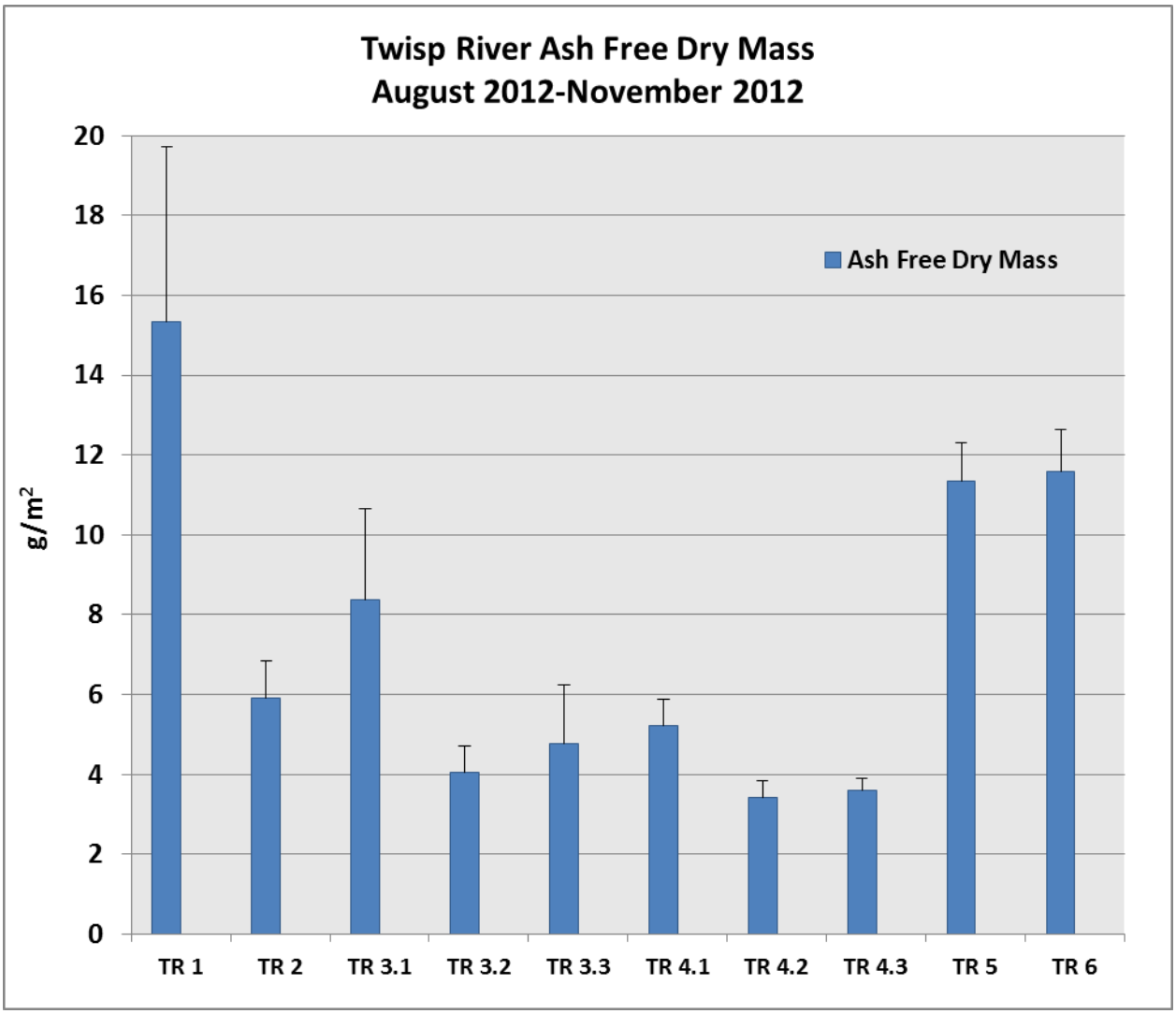
224. ***Periphyton standing crop*** – Periphyton standing crop in the Twisp River during 2012 ranged from 3.6 to 8.4 g/m^2 between TR2 and TR 4.3, but was up to three times higher in far upstream (TR5 and TR6) and downstream (TR1) areas (Figure 37). Periphyton standing crop averaged approximately 11.5 g/m^2 at TR5 and TR6) compared to 15.3 g/m^2 at TR1 (Figure 37).

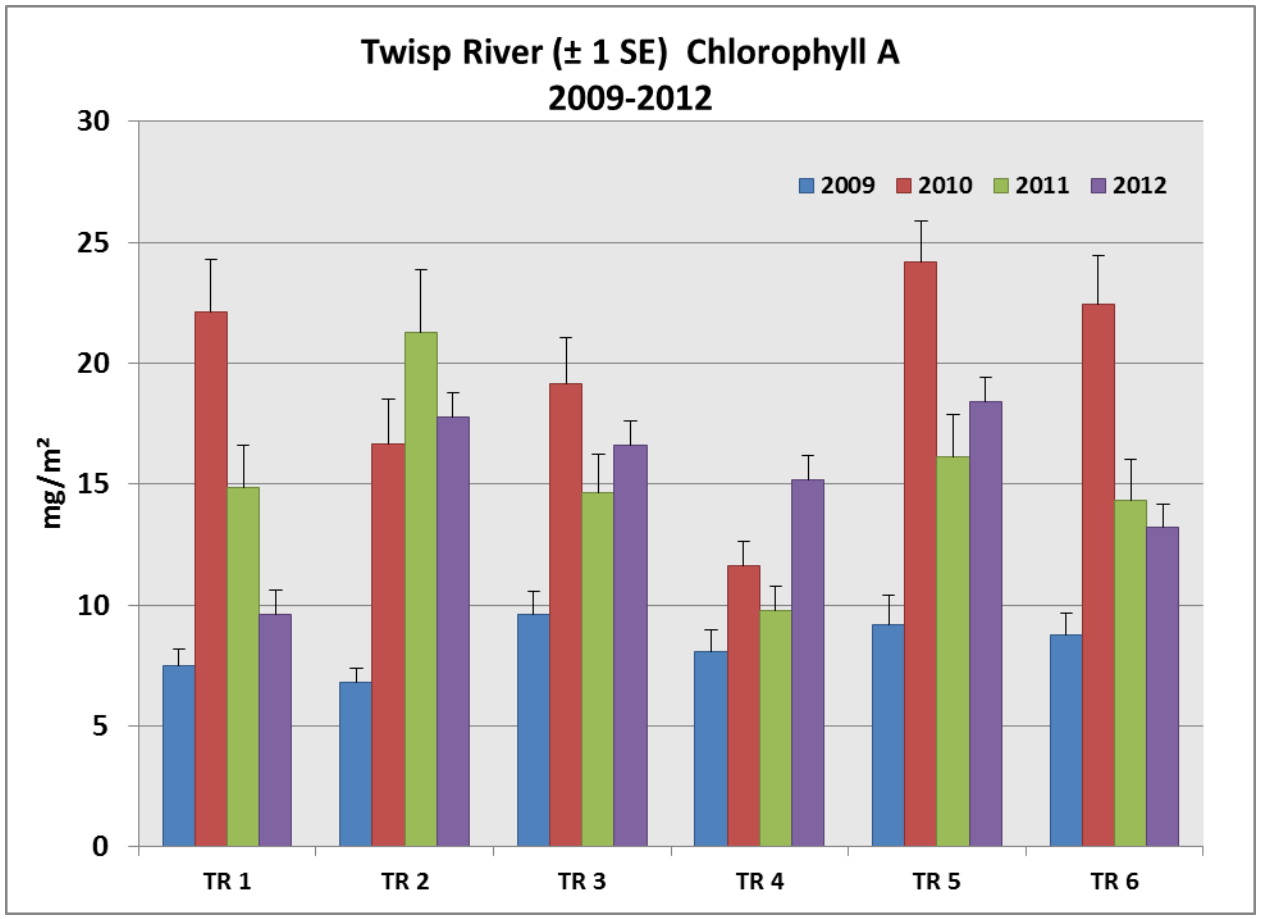
225.



226. Figure 34. Ash free periphyton biomass (g/m^2) from the Twisp River, August through November, 2012. Error bars represent one standard error.

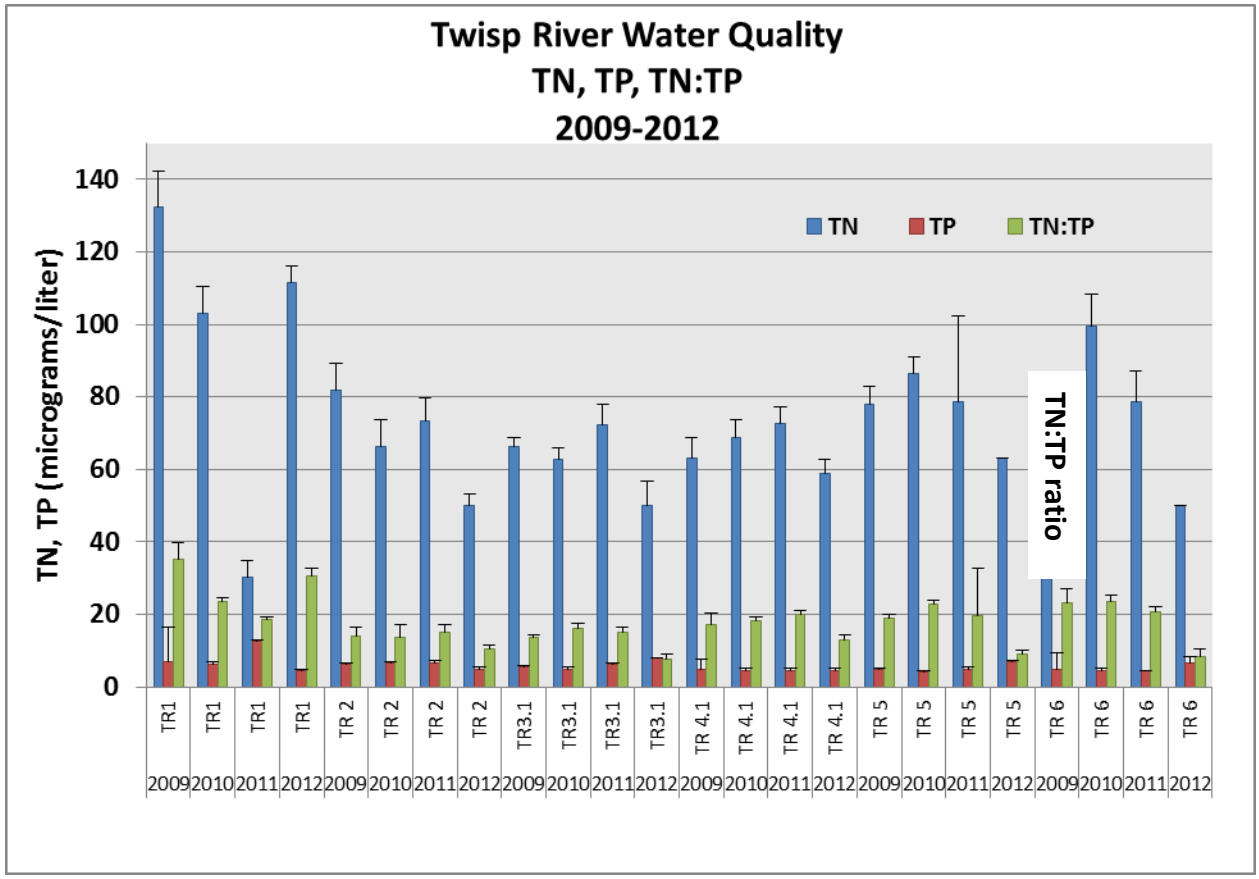
227. ***Chlorophyll a biomass –*** Chlorophyll a biomass was variable across time and space, ranging from 6.8 to 24.2 mg/m2 (Figure 38). Although no particular longitudinal chlorophyll a patterns were evident, within-year variability appeared to be slightly less than among-year variability. For example, mean annual chlorophyll a biomass for all sites was 8.3mg/m2 (SE=0.9) for 2009 compared to 19.3 mg/m2 (SE=1.8) for 2010 (Figure 38).

228.



229. **Figure 35. Chlorophyll a biomass (mg/m^2) in the Twisp River at sites TR1 through TR6 from 2009 through 2012. Error bars represent one standard error.**

231. ***TN, TP, and TN:TP ratio*** – Total nitrogen (TN) in the Twisp River was variable over sites, displayed no particular longitudinal pattern, and ranged from 30.1 to 132.4 ug/L across sites from 2009 through 2012 (Figure 39). Most TN values ranged from about 60 to 100 ug/L, with three (TR2, TR3.1, and TR6) at 50 ug/L (Figure 39). Total phosphorus (TP) concentrations were much lower and much less variable than TN, displayed no particular longitudinal trend, and ranged from 4.8 to 12.4 ug/l across the same sites from 2009 through 2012 (Figure 38). TN:TP ratio values were intermediate to TN and TP values, ranging from 7.8 to 35.1 ug/L at TR1 in 2009, and like their component TN and TP values displayed no particular longitudinal trend (Figure 39). Nine of the 24 TN:TP ratio values (38%) were above 20, indicating slight P-limitation, 3 were < 10, indicating N-limitation, and the remaining 12 were between 10 and 20, indicating co-limitation (Figure 39).



232.

233. **Figure 36. Total nitrogen (TN), total phosphorus (TP) and TN:TP ratio values for the Twisp River, 2009 through 2012. Error bars represent one standard error.**

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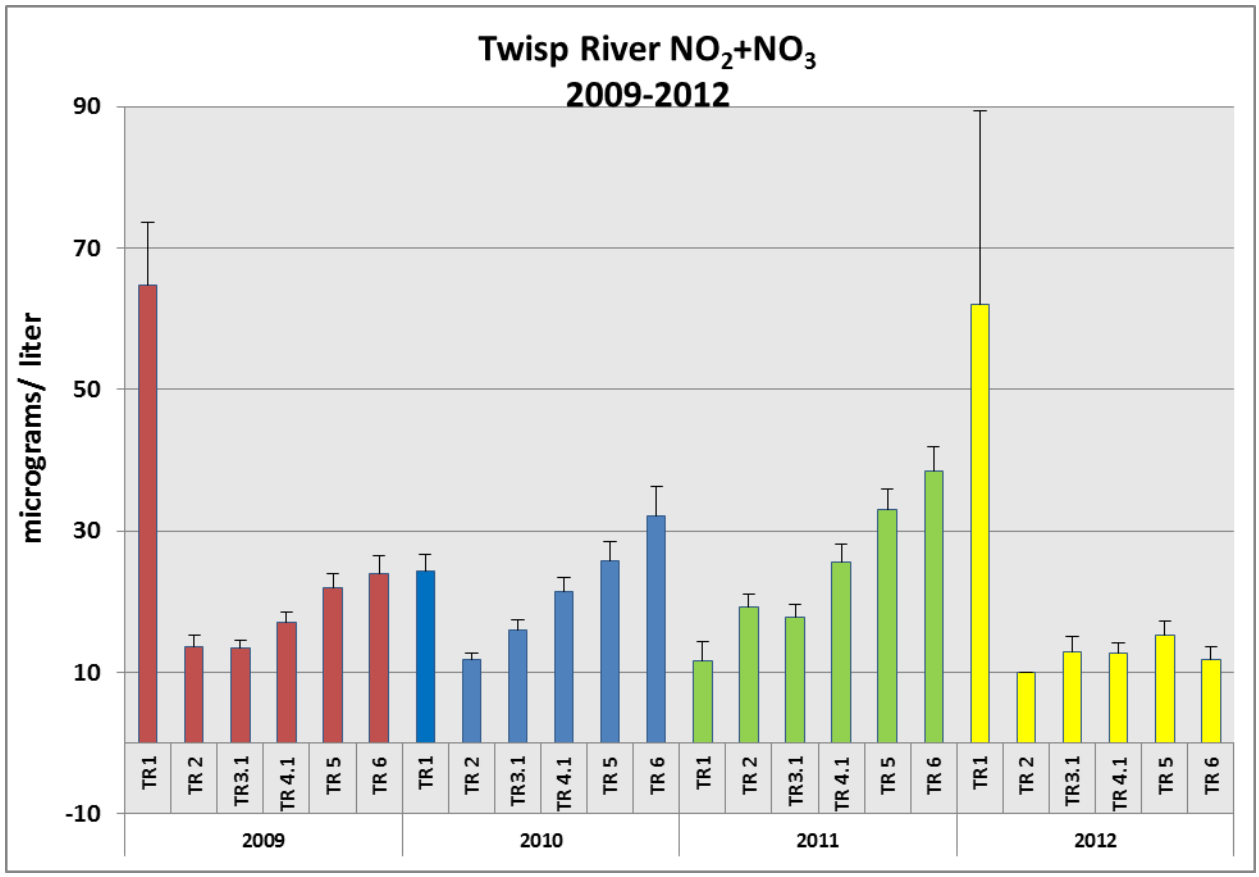
236. ***NO2 +NO3*** – Nitrite+nitrate (NO_2+NO_3) concentrations (ug/L) in the Twisp River during most year from 2009 through 2012 showed a decreasing downstream trend between TR6 and TR2, with values generally ranging between 10 and 38, with the exception of two high values at TR1 of 64.7 ug/L in 2009 and 62.1 ug/L during 2012 (Figure 40).



237.

238. Figure 37. Nitrite (NO_2) + nitrate (NO_3) concentrations (microgram/L) in the Twisp River, 2009 through 2012. Error bars represent one standard error.

239. ***Soluble reactive phosphorus (SRP)*** – SRP values ranged from 1.05 to 2.35 ug/L in the Twisp River for years 2009-2012. However, a majority (94%) of SRP samples were below the lab detection limit of 1 ug/L (Table 14).

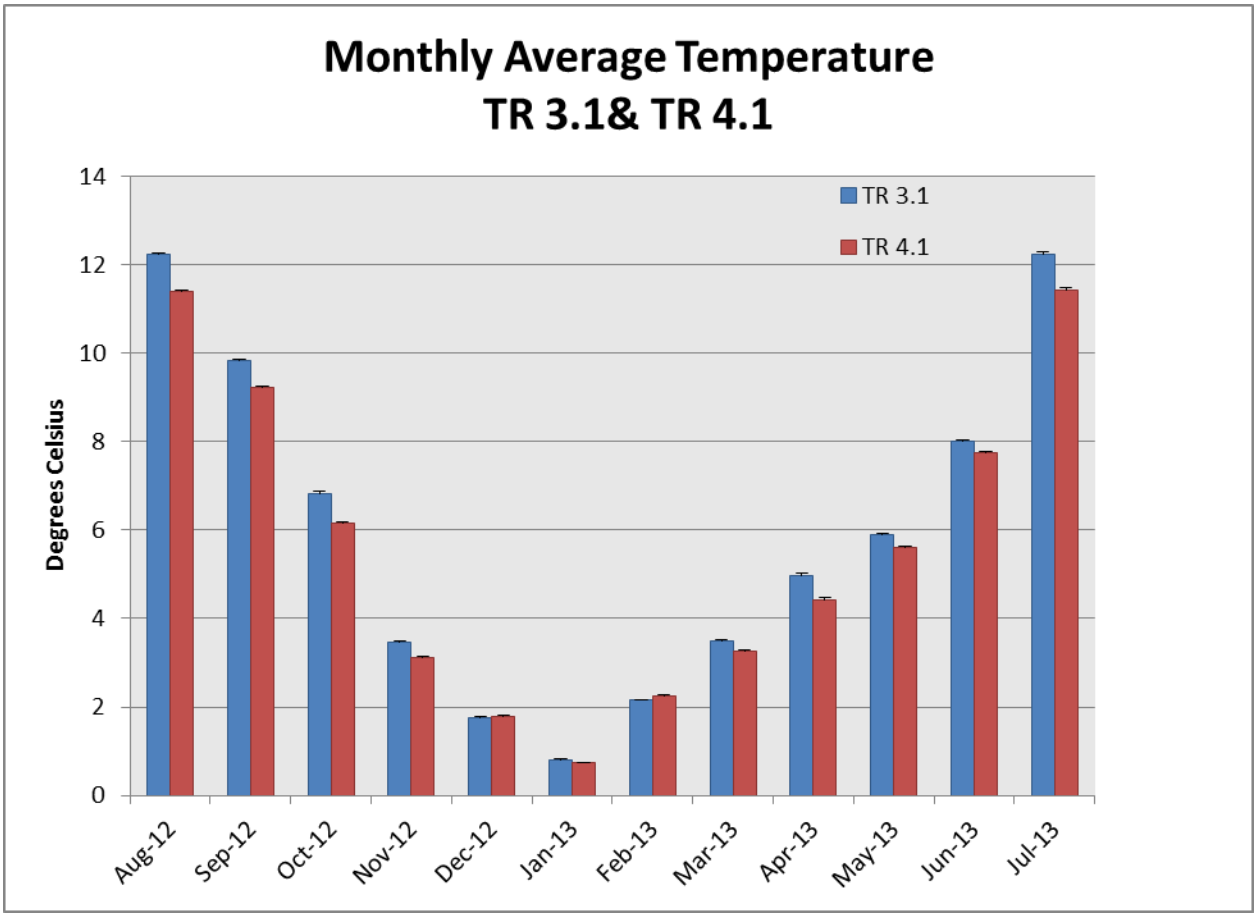
240. Table 14. Numbers of Twisp River SRP samples at and below detection level during 2009-2012.

Sites	TR 1	TR 2	TR 3.1	TR 3.3	TR 4.1	TR 4.3	TR 5	TR 6	Total	Percent
No. of Samples	137	136	137	3	137	3	137	136	826	
No. below detection	132	126	120	2	133	2	130	132	777	94.1
No. above detection	5	10	17	1	4	1	7	4	49	5.9

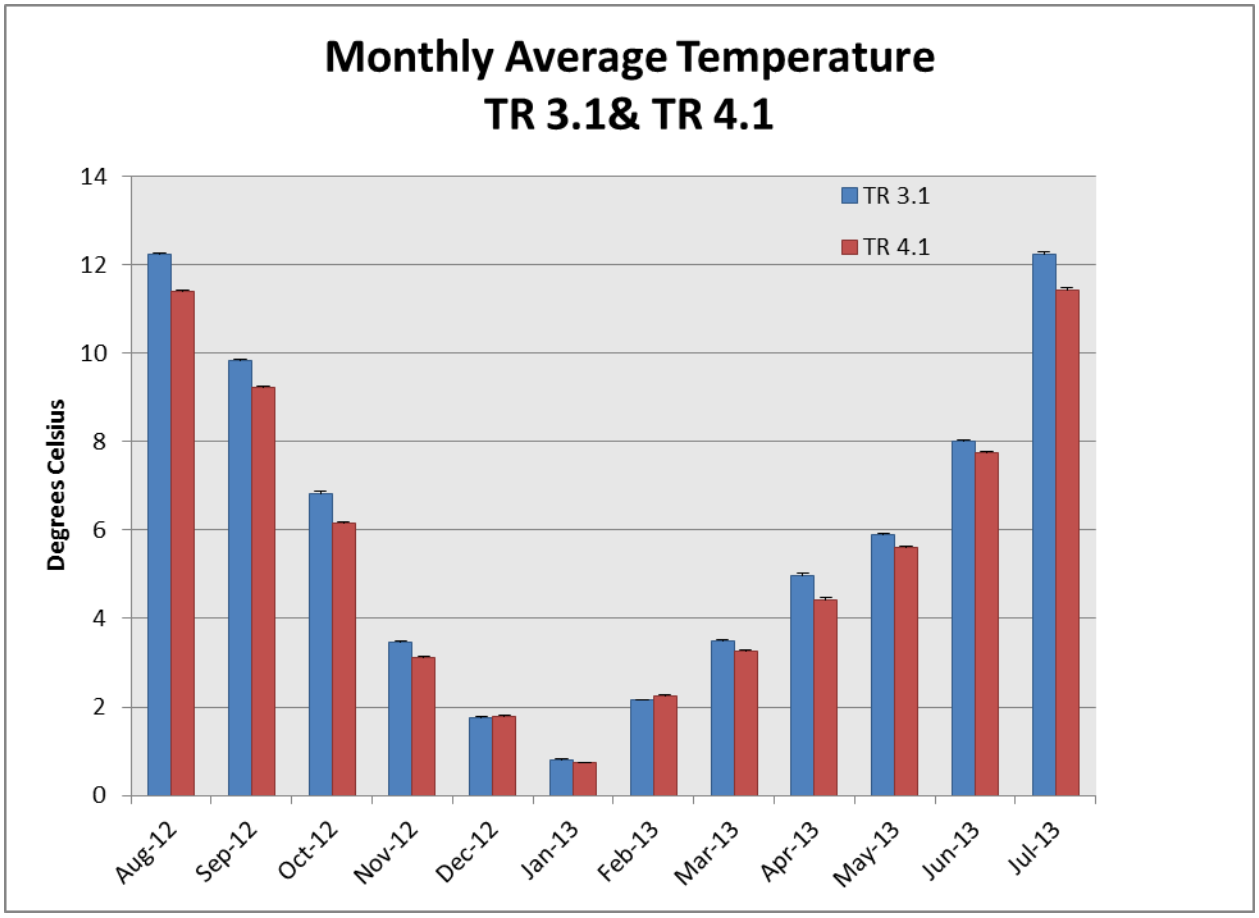
241. ***Ammonia (NH4)*** – No ammonia data were reported because 815 of the 830 samples from years 2009-2012 were below the lab detection limit of 5.0 ug/L.

242. *Water temperature* – Mean monthly water temperature in the Twisp River was very similar between reaches, as reflected by data from TR3.1 and TR4.1, with a mean difference of 0.4oC between sites from August 2012 to July 2013, and a maximum difference of 0.8oC between sites during any given month. Water temperature ranged from 0.8 to 12.2oC at TR3.1 and 0.7 to 11.4oC at TR4.1 (Figure 41). Monthly mean water temperature in the Twisp River was slightly higher in Reach 1 than in Reach 2 from April through September 2012, and but cooler from October to April (Figure 41).

243.



244. Figure 38. Mean water temperature in the Twisp River in the treatment (TR3.1) and control (TR4.1) reaches from August 2012 through July 2013. Error bars represent one standard error.


Monthly Average Temperature
TR 3.1& TR 4.1
Degrees Celsius
14
12
10
8
6
4
2
0
TR 3.1
TR 4.1
Aug-12
Sep-12
Oct-12
Nov-12
Dec-12
Jan-13
Feb-13
Mar-13
Apr-13
May-13
Jun-13
Jul-13

245. Synthesis of Findings: Discussion/Conclusions

246. Review of Pacific salmon ecology and the restoration science literature (Part I of this report) confirms that natural production of anadromous salmonids in the Pacific Northwest and in the Upper Columbia basin can be simultaneously limited by various factors. Limited natural production for anadromous and resident salmonids can occur in different portions of the salmonid life cycle, thereby affecting different life stages, and different factors may also limit natural production through different mechanisms (NRC 1996; Gresh et al. 2000; Naiman et al. 2012). Due to the multivariate nature of restoring natural production of anadromous and resident salmonids in the Pacific Northwest, and because univariate solutions rarely resolve multivariate problems, this Program has implemented a multi-scale empirical research and restoration approach that identifies and tests three restoration strategies to directly counteract three major limiting factors of natural production: 1) physical habitat loss and degradation, 2) reduced nutrient and food availability through loss of MDN; and 3) the deleterious presence of non-native fishes.

247. Implementation of this Program's the first treatment (physical habitat restoration), incorporating a multi-trophic monitoring and evaluation program and the trophic basis of production (TBP) approach, has provided: 1) high resolution characterization of the fish and invertebrate communities and ecological process in improved and unimproved stream habitat conditions, and 2) valuable quantitative pre-treatment multi-tropic baseline and food web characterization to evaluate the Program's second restoration treatment, experimental nutrient addition, scheduled to begin in both reaches of Hancock Springs during 2014. The Program will now (2014-2016) focus on biological responses to nutrient addition in improved (Reach 1) unimproved stream channel and riparian habitat conditions (Reach 2) using a controlled BACI design, while characterizing natural production of anadromous and resident native and non-native salmonids and their supporting biological communities and ecological processes. Ongoing and future monitoring will also provide an additional two to three years of pre-treatment baseline data for non-native brook trout (2014-2016) in both reaches after fertilization. Initial food web characterization of the fish community in Hancock Springs confirmed the importance of this non-native species to the ecology of Hancock Springs, where it currently dominates consumption of invertebrate food resources and fish production fish in both reaches. While dominance of the Hancock Springs fish community by non-native brook trout is not ideal from a fisheries management standpoint, it provide an excellent opportunity to evaluate the effects of brook trout removal on competing native fishes and on food web structure and dynamics.

248. Hancock Springs

249. Responses to physical habitat restoration

250. Results from the first two years after channel and riparian habitat reconstruction in Reach 1 of Hancock Springs revealed an array of biological benefits from physical habitat restoration, expressed as abundance, biomass, and production in the periphyton, invertebrate, and fish communities in a small headwater, spring-fed, salmon producing stream. While mechanisms and habitat conditions reported in the salmonid production and habitat restoration literature support the positive results observed in this study, the large magnitude and wide breadth of observed positive biological responses to physical habitat restoration were somewhat unexpected. Biological benefits in the treated reach (Reach 1) were associated with increased pool density, substrates dominated by cobbles and gravels instead of sand and fines, and more abundant large wood and other forms of fish cover.

251. In terms of fish responses, the production of 18 redds in Reach 1 (12 Chinook, 6 steelhead) compared to a single steelhead redd in Reach 2 during 2012 occurred in the presence of improved post-treatment

substrate conditions in Reach 1 (68% cobble and gravels, 18% sand and fines, and 9.5% pool tail fines). This compared to the presence of a single redd in Reach 2, associated with 32% cobble and gravels, 82% sand and fines, and 44.6% pool tail fines. In addition to improved substrate conditions, groundwater discharge (upwelling) may have further contributed to the observed post-treatment redd densities in Reach 1. Empirical research has highlighted the importance of hyporheic upwelling on the placement and hatching success of eggs in anadromous and resident salmonid redds in the Pacific Northwest (Geist and Dauble 1998; Baxter and Hauer 2000; Tonina and Buffington 2007, 2009). Academic researchers are currently coordinating with Program personnel and monitoring hyporheic conditions in both reaches of Hancock Springs. This work is expected to provide additional insight into the effects of hyporheic connectivity on redd building and hatching success in Hancock Springs. While upwelling may be occurring in both reaches, the presence of degraded substrate conditions in Reach 2, along with a limited amount of suitable spawning gravels may help explain the absence of redds there despite potential upwelling.

252. Many physical habitat and biological factors can affect rearing abundance (density), biomass, and production of stream fishes, including a spatially and temporally dynamic suite of water quality, food and habitat availability, and behavioral (e.g. territoriality) factors (Fausch 1984; Schlosser 1985, 1991; Grant and Kramer 1990; Gresh et al. 2000; Smith et al. 2006; Holtgrieve et al. 2011). Treatment in Reach 1 resulted in greater proportions of pool habitat and fish cover in Reach 1 (77% pools, 79% cover) than in Reach 2 (59% pools, 55% cover). These habitat changes likely contributed to the observed post-treatment increases in fish abundance, biomass, and production in Reach 1. Responses included increased abundance of Steelhead, Chinook and brook trout that was generally an order of magnitude greater in Reach 1 than in Reach 2, and significantly higher steelhead and brook trout biomass. After treatment, aggregated fish abundance was an order of magnitude greater in Reach 1 than in Reach 2, and 91% of the fish biomass and 83% of the fish production from both reaches combined occurred in Reach 1.

253. In addition to suitable physical habitat conditions, adequate food availability is required for successful natural production of salmonids. Production in many freshwater systems currently suffers in this regard, given current basin-wide MDN deficits (Gresh et al. 2000; Holtgrieve et al. 2011; Warren and McClure 2012). Initial post-treatment results in Hancock Springs to date were very encouraging in terms of improved post-treatment food availability. Not only was benthic macroinvertebrate (secondary) production increased in Reach 1 following treatment (16.0 gDM/m^2/yr) compared to Reach 2 (12.9 gDM/m^2/yr), but essentially 100% of the estimated terrestrial and aquatic insect production was consumed by fish in Reach 1, compared to roughly % in Reach 2. Post-treatment consumption of drifting terrestrial insects by fish was about 3.5 times greater in Reach 1 (0.0048 g/m^3/min) than in Reach 2 (0.0014 g/m^3/min), suggesting increased insect production and contribution to the aquatic food web following post-treatment improvements in riparian habitat condition. Although we were unable to empirically estimate terrestrial insect production due to prohibitively low numbers of insects collected in drift samples, greater numbers of terrestrial insects were identified in fish gut samples, indicating high fish foraging efficiency for these diet items by fish.

254. Finally, consumption of aquatic and terrestrial invertebrates by fishes was nearly 8 times higher in Reach 1 than in Reach 2 after treatment (16.0 and 2.5 gDM/m^2/yr for these reaches respectively). Likewise, aggregated fish production (all species) was more than 6 times greater in Reach 1 than in Reach 2 following treatment (2.6 vs. 0.4 gDM/m^2/yr for these reaches respectively). Collectively, these findings indicated that more fish were feeding on more insects from a greater number of taxa, consuming nearly all available secondary production in Reach 1, while only about 16% of available production in Reach 2. These differences in food consumption and energy flow between reaches likely contributed to observed increases in fish abundance, biomass, and production in Reach 1 compared to Reach 2. Future implementation of stable isotope work will help address such hypotheses

255. In terms of ESA-listed fish in Hancock Springs, Chinook and steelhead abundance, biomass, and production were all higher in Reach 1 than in Reach 2 following treatment. Bull trout were collected exclusively in Reach 1, indicating improved habitat suitability for this listed resident species in Reach 1.

256. Although substantial benefits were realized by native anadromous and resident salmonids in Reach 1 following treatment, non-native brook trout dominated the fish community in both reaches of Hancock Springs. However, food web diagrams indicated that brook trout consumption was reduced by 20% in Reach 1 following treatment, where 60% of all secondary production was consumed by brook trout, compared to 80% in Reach 2. While not an ideal condition from a fish management perspective, the dominance of invasive brook trout in Hancock Springs provides an excellent opportunity to evaluate the effects of non-native species removal (Treatment 3, proposed for 2016) on the native fish and invertebrate communities in salmon producing stream in the Upper Columbia Basin. (Brook trout removal is Treatment 3 in the Program, scheduled to begin in both reaches in 2016 following nutrient addition, beginning during 2014 in both reaches).

257. Along with well documented negative effects of habitat degradation on habitat diversity and biological production (Bisson et al. 2009), habitat alteration or degradation can also contribute to food web instability and simplification (Cross et al. 2013). Such changes appear to have occurred in Hancock Springs following decades of past agricultural land use. However, the actual magnitude of these changes remains unknown due to a lack of Program support to collect detailed physical habitat and biological data from both reaches prior to channel and riparian habitat reconstruction in Reach 1 during 2011. Nonetheless, post-treatment data showed substantial increases in overall food web conveyance (the amount of energy/food resources moving from secondary production to the fish community) and complexity in Reach 1 following treatment, compared to untreated conditions in Reach 2. In addition to increased and diversified energy conveyance, food web routing shifted away from dominance by non-native brook trout toward the array of native and non-native salmonid community in Reach 1 compared to Reach 2.

258. Regarding the trophic basis of production, the resulting flow web diagrams revealed major reach-specific differences in food web routing and the amount of secondary production and energy transfer to the fish community. Four of 11 energy pathways each conveyed >1 $gDM/m^2/yr$ from secondary producers to fish as diet items, for a total of 15.2 $g/m^2/yr$ in Reach 1. In Reach 2, no individual pathways conveyed > $1gDM/m^2/yr$ to the fish community, while total conveyance of all 10 food/energy pathways collectively accounted for 2.4 $gDM/m^2/yr$, or just 16% of the estimated food consumed by the fish community in Reach 1.

259. Compared to analogous first-order surface water streams in the Methow Subbasin, elevated thermal and hydrologic stability and nutrient availability from hyporheic discharge in Hancock Springs may have contributed to the magnitude of observed biological responses across trophic levels. However assuming similar hyporheic conditions between reaches prior to analysis of empirical data would further support the array of consistent observed biological benefits associated with physical habitat restoration in Reach 1.

260. Although small stream habitat restoration projects are common among fisheries agencies in the Pacific Northwest, few programs provide the comprehensive monitoring design and high degree of rigor across multiple taxa and trophic levels provided by this Program. The ecologically unique aspects of Hancock Springs (e.g. thermal, hydrologic stability, protected riparian zone, hyporheic contribution) provide a valuable opportunity to study the separate and additive effects of a series of prominent salmon restoration activities, thereby contributing to the refinement and success of future salmon restoration efforts in freshwater spawning and rearing. Continued monitoring, evaluation, and analyses of data generated by this Program are expected to contribute substantially to focused and prioritized salmon recovery efforts in the Upper Columbia Basin and beyond.

261. This Program will now focus on the second of three restoration treatments (experimental nutrient addition) to counteract reduced MDN loading to the study areas. The Program's study design will continue to provide valuable insight into the separate and combined effects of habitat restoration and nutrient addition by evaluating the biological effects of adding nutrients to an improved (Reach 1) and unimproved (Reach 1) channel.

262. Preparation for nutrient addition (Treatment 2)

263. The Program is now focusing on design and implementation of experimental nutrient addition, scheduled to begin in both reaches of Hancock Springs during the fall of 2014 to coincide with timing of Chinook spawning. Updated analyses of data collected during late 2013 not incorporated into this report, along with data collected during winter, spring, and summer of 2014 will further provide a rigorous multivariate pre-treatment baseline condition against which to compare biological responses to nutrient addition. Further increases in abundance, biomass, and diversity, along with increased rates of biological processes such as growth, consumption, and production among trophic levels are expected following nutrient addition. Differences in the distribution and magnitude of biological responses following physical habitat treatment and nutrient addition will be evaluated by reach. Biological responses in Reach 1 will include the additive effects of physical habitat restoration and nutrient addition treatments, while results in Reach 2 following fertilization will be affected solely by nutrient addition in an altered (unimproved) channel. In general we expect to see elevated abundance, biomass, diversity, and production among the invertebrate and fish communities in both reaches, along with possible shifts in community dynamics, competition, and predation illustrated by post-fertilization food webs from both reaches. We also expect to see elevated response magnitudes in Reach 1 compared to Reach 2, since processes in Reach 1 can benefit from both the physical habitat restoration and nutrient addition treatments.

264. Upon quantifying the dominance by brook trout in Hancock Springs, Program personnel considered changing the current order of restoration treatments (i.e. implementing brook trout removal as the second treatment instead of the third, following physical habitat restoration). However, considerable discussion resulted in retaining the current treatment sequence. Fertilizing before removing non-natives will allow us to evaluate the effects of nutrient addition on non-native fishes, with and without improved habitat condition, constituting an important investigation that would be difficult to perform in larger river settings. Retaining the original treatment order (fertilization before removal) will also provide insight into non-native responses to nutrient addition in a very measurable context of a competing fish community, which the scale of Hancock Springs facilitates. Finally, fisheries agencies are increasingly undertaking nutrient addition programs, and a better understanding of responses by non-native fishes to nutrient addition is critical to evaluating the efficacy of nutrient addition as a potential restoration strategy for increasing natural production.

265. Twisp River

266. Benthic macroinvertebrate abundance, biomass, and production in the unimpounded Twisp River typically increased in a downstream direction as would be expected under the River Continuum Concept (Vannote et al. 1980). However, dissolved nutrient concentrations, TN:TP values, Chlorophyll a, and primary production values did not consistently display distinct longitudinal patterns among years. Variation in the nutrient and benthic macroinvertebrate metric values was typically greater among years than within years, such that the lowest and the highest values were year-specific. TN:TP ratio values were intermediate to TN and TP values, and ranged from 7.8 to 35.1 ug/L and like their component TN and TP values displayed no particular longitudinal trend. Nine of the 24 TN:TP ratio values (38%) were above 20, indicating slight P-limitation, 3

were < 10, indicating N-limitation, and the remaining 12 were between 10 and 20, indicating co-limitation. The highest values for some of the nutrient and biological metrics values were recorded at TR1, located near the town of Twisp, where anthropogenic input from human habitation and development may have contributed to increased metric values.

267. Biological and water quality metrics in The Twisp River typically displayed a wider range of values during Phase 1 of the project (2008-2011) than during Phase 2 (2012+). This may have been due to the fact that 6 sites were sampled over a 44 km reach of the Twisp River, compared to 6 sites within an approximate 10 km reach during Phase 2, designed to characterize a shorter upstream control and downstream treatment reach, Phase 1 data were also collected over a series of 4-5 years, compared to 1-2 years for Phase 2 sampling, which could have contributed to a smaller range of values. In some cases the mean and range of metric values during Phase 2 were intermediate to analogous values during Phase 1. These findings were somewhat intuitive given the nested intermediate longitudinal position of Phase 2 sites compared to Phase 1 sites. Such trends were mainly evident with metrics that displayed consistent longitudinal patterns over the 44 km reach sampled during Phase 1.

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Appendix A: Use of Data & Products

Appendix B: Detailed Results

Appendix C: List of Metrics and Indicators

Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2	Specific Metric Title