Upper Columbia Nutrient Supplementation Project
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Table of Contents

Table of Tables

Table of Figures

Authors Note

This progress report provides an updated and detailed summary of methods, activities, and results for the Yakama Nation's Upper Columbia Natural Production Restoration Project (formerly named Upper Columbia Nutrient Supplementation Project). The Program features a multidisciplinary approach that includes the design, implementation, and evaluation of restoration techniques to reestablish and enhance natural production of listed anadromous and resident Pacific salmonids. The Program is designed to separately and additively address three major limiting factors of natural production currently operating in the Upper Columba basin: 1) habitat loss and alteration, 2) nutrient and food deficiency from lost marine derived nutrients (MDN), and 3) the deleterious presence of non-native fishes.

This Project has grown considerably in scope and support from its original form, which was funded solely by the Bonneville Power Administration as the Upper Columbia Nutrient Supplementation Project (BPA Project No. 200847100). Since its inception, this Project has been acquiring significant direct and in-kind support from an array of collaborating agencies, entities, and academic programs to support habitat restoration and evaluation, stream metabolism, hyporheic effects and trophic productivity modeling. Collaborating entities providing direct and/or in-kind monetary, field personnel and analytical support include the USGS, BOR, University of Idaho, Washington State University and PCSRF.

The BPA project support components for the Project's funded component (Upper Columbia Nutrient Enhancement Project) continue to focus on providing rigorous pre- and post-treatment physical and biological assessments of Hancock Springs and the Twisp River, with future support expected for implementing and evaluating experimental nutrient addition. Consistent with the intent of the BPA-funded Project, nutrient addition is proposed to begin in 2014 in Hancock Springs and in 2020 in the Twisp River. As presented in this report, rigorous characterization of pre-treatment physical habitat and ecological conditions has been provided by past and current support and collaborators. Thus, development of this Yakama Nation Program has resulted in very cost-effective implementation of critical, collaborative research and restoration activities within the Methow River Subbasin that continue to greatly exceed the benefits and scope afforded by the original BPA-funded project.

The Project and BPA-funded portions of this reported work have also been greatly improved by integrating recommendations from the Northwest Power and Conservation Council's Independent Scientific Review Panel (ISRP). Specific recommendations involved multi-trophic approaches to track food and energy-routing pathways (e.g. stable isotope tracers, food web diagramming, and trophic basis of production) and whole system stream metabolism modeling techniques to better characterize pre- and post-treatment conditions in project areas. Ongoing and growing collaboration among local and regional agencies briefly mentioned above is also contributing to more comprehensive, diagnostic outcomes for Program activities, collectively designed to restore and sustain more productive and resilient populations, ecosystem conditions, and native fisheries in the Upper Columbia Basin and beyond.

Executive Summary

Review of Pacific salmon ecology and restoration science presented in the first part 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 many different factors. Limitation of natural production can also occur in different portions of the life cycle, thereby affecting different life stages. Different factors may also limit natural production through different mechanisms. Thus, abiding by the axiom that univariate solutions rarely resolve multivariate problems, we designed and implemented a multivariate research and restoration Program that identifies and tests three restoration strategies that directly address three major limiting factors of natural production of Pacific salmonids: 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 (NRC 1996; Gresh et al. 2000; ISAB 2008, 2011; NPCC 2009; Naiman et al. 2012).

The goal of this Program is to provide fish managers with options to restore and increase natural production of listed anadromous and resident salmonids. We understand that achieving this goal requires suitable lower trophic production, nutrient, food and habitat suitability, and sufficient upward routing of food energy to the fish community. Program objectives are to: 1) implement and test three restoration measures (habitat restoration, nutrient addition, and brook trout removal) to increase natural production, and 2) describe the mechanisms by which these three factors limit natural production.

This Project has two components, the Hancock Springs and Twisp River projects. The Hancock Springs Project occurs in a small spring creek that facilitates research, monitoring, and evaluation to restore natural production. The Twisp River Project will evaluate river fertilization at the larger river scale. The Hancock Springs Project will sequentially implement, monitor, and evaluate three restoration treatments and their potential interactions over a 10 year period: Treatment 1: Physical habitat restoration (Reach 1: 2011, Reach 2: 2018), Treatment 2: Nutrient addition (2014-2018), and Treatment 3: Brook trout removal (2016-2018). Based on previous habitat evaluations, the Twisp River is not significantly jeopardized by altered physical habitat conditions or by the deleterious presence of non-native fish species. However, it continues to experience a significant reduction in marine derived nutrient loading from historic levels (Mullan et al. 1992; Snow et al. 2010). Therefore, the Twisp River Project only involves an experimental nutrient addition treatment, currently scheduled to begin in 2018 to incorporate results from the previous nutrient addition experiment in Hancock Springs. This river-scale nutrient addition experiment will occur within a 10km section of the middle Twisp River, approximately 21-31 km upstream from the mouth, with a 5 km upstream control reach and an adjacent 5 km downstream treatment reach. Results from experimental nutrient addition from both systems are expected to guide future nutrient supplementation programs in other salmon producing streams in the Pacific Northwest. An extensive common suite of physical habitat and biological response variables is being used to evaluate treatment effects in both study areas.

The in-stream and riparian habitat restoration treatment in Reach 1 of Hancock Springs completed during 2011 resulted in stark 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. 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 where redds are typically constructed was larger when expressed as percent pool tail fines (9.5% fines in Reach 1 vs. 44.6% in Reach 2). Physical habitat restoration had no effect on the thermal regime, as mean annual water temperature between reaches differed by just 0.2 ^{o}C (7.2^{o}C in Reach 1 vs. 7.4^{o}C in Reach 1).

Post-treatment changes in physical habitat attributes also contributed to a wide array of positive biological responses across trophic levels. Beginning with fish, 18 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. Aggregated fish production was also an order of magnitude greater in Reach 1 (1.4 grams dry mass per meter square per year; gDM/m^2/yr) than in Reach 2 (0.3 gDM/m^2/yr). The aggregated fish community in Reach 1 consumed an estimated 16 gDM/m^2/yr of invertebrate production (essentially the entire amount of estimated secondary production), compared to consuming only 2.5 gDM/m^2/yr, or about 16% of the estimated 12.8 gDM/m^2/yr macroinvertebrate production. Secondary (invertebrate) production of aquatic taxa was also greater in Reach 1 (13.9 gDM/m^2/yr) than in Reach 2 (11.1 gDM/m^2/yr), with consumed terrestrial taxa adding an additional 1.7 and 1.9 gDM/m^2/yr to secondary production in reaches 1 and 2 respectively.

Thus, following habitat restoration, more fish ate more invertebrates from more taxa that contributed to their increased abundance, biomass, and production, via greater foraging efficiency and energetic conversion of secondary production to tertiary (fish) production. Growth of Chinook and brook trout was greater in Reach 2 than in Reach 1; however this was likely due to their low density and increased available forage per individual.

Development and use of the multi-trophic monitoring and evaluation program and results reported here following implementation of the first Program treatment in Hancock Springs (physical habitat restoration) has provided: 1) a high resolution multi-metric characterization of the fish and invertebrate communities and ecological process (e.g. growth, predation, production) in the treatment and control reaches, and 2) a valuable quantitative pre-treatment multi-tropic baseline and food web characterization for the upcoming nutrient addition treatment for both reaches beginning in 2014. The Program will now focus on simultaneously evaluating biological responses to nutrient addition in an improved (Reach 1) and unimproved channel (Reach 2) using a controlled BACI design, while characterizing natural production of anadromous and resident native and non-native salmonids and supporting trophic level dynamics. Ongoing and future monitoring will provide an additional two to three years of pre-treatment baseline data for non-native brook trout (2014-2016) in fertilized improved and unimproved habitat conditions.

In the unimpounded Twisp River, benthic macroinvertebrate (BMI) abundance, biomass, and production typically increased in a downstream direction over the 44 km sampling reach from Phase 1 (2008-2012) 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 display distinct longitudinal patterns among years. Nutrient and benthic macroinvertebrate metric values typically varied more between years than within years, such that the lowest and the highest values across sites usually happened during respective years. 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. Preliminary Phase 2 nutrient and biological data collected from the smaller scale future treatment and control reaches in the Twisp River showed little variation within and among reaches, likely based on their small scale, close proximity, and similarities in physical habitat and substrate conditions. Physical and biological similarities between the future treatment and control reaches for experimental nutrient addition in the Twisp River will strengthen the interpretation of post-treatment responses to fertilization, scheduled to begin in the Twisp River in 2018.

To date, this Program has successfully: 1) implemented, monitored, and evaluated physical, biological, and ecological responses to physical habitat in Reach 1 of Hancock Springs, 2) reported a wide range of important biological responses, 3) characterized the 44 km reach of the Twisp River, and 4) begun to develop pre-treatment baseline conditions in future treatment and control reaches for experimental fertilization in the Twisp River. Additional analysis of data collected during 2013 in Hancock Springs that were not able to be analyzed prior to this reporting period, and data that will be collected during 2014 prior to nutrient addition in the fall of 2014, will continue to provide valuable empirically-based insight on attributes of needed restoration activities for natural production of anadromous and resident salmonids in the Upper Columbia basin.

The Program is now focusing on the design and implementation of experimental nutrient addition in both reaches of Hancock Springs. While the initial work in these areas was originally of a smaller scope, beginning with a BPA-funded project involving the Twisp River, the Program has expanded to prioritizing the rigorous design, implementation and testing of restoration at a smaller scale in Hancock Springs. Results reported here contribute to a better quantitative, mechanistic understanding of the biological and ecological responses to specific experimentally controlled restoration treatments for application to systems and larger scale restoration projects in the Columbia River basin.

Introduction

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.

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.

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.

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.

Part I: Scientific background

Pacific Northwest Salmon Declines

Dramatic declines in the abundance of anadromous salmonids have occurred over the last century in the Columbia River basin (Neilsen et al. 1993; Lichatowich 1999; NPCC 2000; Gresh et al. 2000; NPPC 2001). Declines followed significant commercial harvest that began as early as the middle 1800s (Lackey et al. 2006), hydrosystem and watershed development, habitat loss and degradation, reduced natural production, and reduced survival in rearing freshwater, estuary, and marine environments, (Nehlsen et al. 1991; Welsh et al. 1998; Lichatowich 1999; NPCC 2000; Gresh et al. 2000). Annual numbers of anadromous salmonids returning to the Columbia Basin to spawn, once estimated to range from 10-16 million, were recently reported at about 1 million fish annually (NPCC 2001). In addition to lost fisheries, these population declines have greatly reduced marine derived nutrient (MDN) loads that were historically brought upstream from the ocean by salmon and released into streams by their decomposing carcasses after spawning (Cederholm et al 1999; Gresh et al. 2000; Stockner 2003; Stockner and Ashley 2003; Warren and McClure 2012;). Salmon carcasses historically provided significant amounts of nutrients that supported biological production within the plant and animal communities in and around salmon streams (Wipfli et al. 1998, 1999; Gene et al. 2002; Bilby et al 2003; Moore et al. 2004; Dodds et al. 2012). The contemporary lack of naturally distributed carcasses and their historical MDN contribution are thought to be widely limiting natural production of salmonids in the Columbia River basin (Gresh et al. 2000). This limitation is critical in upstream areas of the basin that are naturally oligotrophic or low in nutrients, such as the Twisp and Methow rivers, over 800 km upstream from the ocean. Based on recently reported salmon escapement levels from anadromous salmon spawning rivers in the Pacific Northwest, Gresh et al. (2000) estimated that as little as 6-7% of the historical MDN inputs are currently being provided. More recently, Scheuerell et al. (2005) suggested that < 2% of historical marine-derived phosphorus is currently returning to the Snake River basin. Moore and Schindler (2004) reported that some historically productive salmon rivers may even be functioning as net nutrient exporters during years of low adult (spawner) escapement.

Roles of Anadromous Salmon in Natural Production

Dramatic increases in body size, fecundity, and energetic reserves of anadromous fish compared to their resident conspecific competitors have enabled salmonids to achieve ecological prominence in Pacific coastal river systems, and historically contributed to expansion of their geographic ranges (Groot and Margolis 1991; Thibault et al. 2010). Gross (1987) reviewed diadromy in fishes and reported that the evolutionary value of migration, of which anadromy is one example, can be measured as the difference in evolutionary fitness of migratory versus non-migratory individuals within conspecific populations. Evolutionary fitness (W) can be calculated as the lifetime summation of an individual's probability to reproduce at any age (lx) multiplied by its fecundity (or male fertility) and breeding success at that age (bx) such that: $W=\sum lx*bx$ (Gross 1987). Thus, retention of anadromy as a dominant population trait should infer increased evolutionary fitness associated with migration compared to the fitness value of residency. Gross et al. (1988) then related the evolutionary fitness of anadromy to large-scale biological productivity gradients and found anadromous life histories to prevail in temperate latitudes, where oceans are more productive than freshwater systems.

Historically, abundant anadromous Pacific salmonid spawning runs contributed large amounts of MDN as nitrogen, phosphorus, and carbon to natal rivers and their associated riparian and upland habitats (Kline et al. 1990; Larkin and Slaney 1997; Cederholm et al. 1999; Gresh et al. 2000; Bilby et al. 2003; Zhang et al. 2003; Schindler et al. 2003, 2005; Rex and Petticrew 2008; Holtgrieve, Gordon and Schindler 2011; Warren and McClure 2012). These generous MDN subsidies can positively affect ecosystem metabolism from the bottom up, in some cases enhancing biological productivity at all trophic levels (Wipfli et al. 1998 Naiman et al. 2002; Thomas et al. 2003; Zhang et al. 2003; Schindler et al. 2005; Kohler et al. 2008, 2011; Rex and Petticrew 2008; Gordon and Schindler 2011). This can largely be attributed to anadromous salmon, which can accumulate over 95% of their biomass in the ocean, setting the stage for substantial energy and nutrient subsidies when they return to their natal habitats to spawn (reviewed in Schindler et al. 2003).

Several MDN pathways have been reported for Pacific salmonid natal streams, including direct and indirect carcass and egg subsidies and bioturbation (the reworking of soils or sediments by animals or plants, in this case, river substrates by spawning salmonids and redd super-imposition). Cederholm et al. (1999) reported three primary nutrient pathways from salmon carcasses to stream biota: 1) uptake of mineralized inorganic nutrients by primary producers and subsequent food web transfer; 2) uptake of dissolved organic matter by microfauna in the streambed and subsequent food web transfer; and 3) direct consumption of eggs and carcasses by secondary consumers (benthic macroinvertebrates) and fishes. In addition to direct egg consumption at the time of spawning, bioturbation can re-suspend incubating embryos, rearing alevins, and benthic invertebrate fauna, making them available for consumption (Moore et al. 2007; Gottesfeld et al. 2008; Moore and Schindler 2008a, 2008b; Gordon and Schindler 2011).

These nutrient pathways may also enhance productivity and diversity of the invertebrate community and juvenile salmonid forage in natal streams (Johnson et al. 1990; Mundie et al. 1991; Quamme and Slaney 2003; Yani and Kochi 2004). Decomposing carcasses can significantly increase the surface area of the streambed available for microbial and invertebrate productivity and diversity, while providing a directly accessible source of nutrients for primary and secondary producers and consumers (Cederholm et al. 1999; Schindler et al. 2003). In turn, increased secondary production can enhance in-stream growth, condition, and survival of juvenile resident and anadromous fishes can ultimately contribute to increased numbers of out-migrating salmonids (Peterson et al. 1993; O'Keefe and Edwards 2003).

Numerous studies suggest broad cycling of salmon-derived nutrients into multiple trophic levels among aquatic, riparian, and terrestrial habitats (Wipfli et al. 1999; Gende et al. 2002; Reimchen et al. 2003; Gende et al. 2002; Naiman et al. 2002; Zhang et al. 2003; Schindler et al. 2003, 2005; Kohler et al. 2008, 2011, 2012; Rex and Petticrew 2008; Gordon and Schindler 2011). MDN has been identified in the hyporheic zone and in riparian and adjacent terrestrial forest soils, vegetation, invertebrate, and vertebrate communities associated with Pacific salmonid ecosystems (Ben-David et al. 1997; Cederholm et al. 2000; Hildebrand et al. 1999a, 1999b; Bilby et al. 2003). Thus, the preponderance of evidence has made it clear that current discussions on restoration efforts must include the role of MDN in restoring salmon populations and the systems on which they rely (Peery et al. 2003; Schindler et al. 2003; Stockner 2003, and references therein).

Factors limiting natural production of Pacific salmonids

Current low levels of natural production of anadromous Pacific salmonids in the Columbia River Basin and other west coast North American river systems have resulted from cumulative effects of multiple anthropogenic factors in the freshwater and marine environments (Nehlsen et al. 1991; Williams 2006; NOAA 2008b). Reduced natural production can occur at various life stages from physical habitat, biological, and ecological limitations (Table 1).

Table 1. Factors and mechanisms limiting natural production of Pacific salmonids in the Upper Columbia Basin from spawning to outmigration.

		Limiting Factors		
Life stage	***Activity***	Physical habitat loss and degradation	Reduced nutrient and food availability	Deleterious presence of non-native fishes
Adult	Spawning	***Mortality Mechanisms***		
Gametes	Fertilization	Not limiting	Not limiting	Egg predation
Embryo	Incubation	Unsuitable substrate properties; deposition of sediment and fines (suffocation)	Not limiting	Embryo predation, remobilization from redd superimposition
Fry	Emergence to < 55 mm TL	Unsuitable substrate properties; deposition of sediment and fines (suffocation)	Not limiting	Predation
Parr	Rearing	Unsuitable substrate properties; deposition of sediment and fines (suffocation)	Food limitation, starvation	Predation, competition
Smolt/ outmigrant		Unsuitable substrate conditions, predation, competition	Food limitation, starvation	Predation, competition

Examples include the degradation of spawning, incubation, and rearing habitats, effects of invasive species through competition and predation, passage restrictions to and from critical habitats, climate change, and nutrient limitation and associated cascading trophic (food web) effects (NRC 1996; Ruckelshaus et al. 2002; Williams 2006; ISAB 2007, 2008, 2011; Naiman et al. 2012).

This Program addresses three prevalent limiting factors for natural production of anadromous salmonids in the Upper Columbia basin: Habitat loss and degradation, nutrient limitation, and the deleterious presence of non-native fishes. Loss and degradation of physical habitat can negatively affect all early life stages of Pacific salmonids following spawning, whereas nutrient and food limitation only affect life

stages following the onset of exogenous feeding (Table 1). Thus, characterizing the timing and mechanisms associated with natural production failure is an important component of its diagnosis and restoration.

These three general limiting factors are described below, along with detailed discussions of how this Program specifically addresses them later in this report.

Habitat loss and degradation – Bisson et al. (2009) provided the following summary regarding the importance of freshwater habitat for restoring Pacific salmonids through natural production:

"The imperilment of many salmon populations is attributed, in large part, to loss of freshwater habitat. Along the Pacific coast of North America, lost or degraded freshwater habitat is identified as a primary contributor to salmon decline more often than any other potential problem, e.g., dams, hatcheries, or overfishing (Nehlsen et al. 1991, National Research Council 1996). Whether habitat is more important than other factors depends on the species and location in question (e.g., Augerot 2005); however, there is broad consensus within the scientific community that the recovery of at-risk salmon cannot be achieved without protecting currently productive freshwater habitat, maintaining watershed processes, and restoring those aquatic ecosystems that have been damaged by human activity (Knudsen et al. 2000, Lackey et al. 2006, Williams 2006)".

Dams associated with the mainstem hydropower system and tributary impoundments have blocked access to large areas of historic salmon spawning and rearing habitat, while habitat degradation has simultaneously contributed to reduced natural production in areas that remain accessible to migrating fish (NRC 1996; Lichatowich 1999; Bisson et al. 2009). Human activities, including forestry, agriculture, grazing, industrial, commercial, residential, and recreational development, and flood control can produce a variety of adverse effects on salmon habitats (Nehlsen et al. 1991; NRC 1996; Williams 1996). Although many of these activities can affect habitat suitability and life cycle completion at different spatial scales and in habitats associated with various life stages for Pacific salmonids (Bisson et al. 2009), this Program focuses on habitat issues that affect spawning and early life stages through outmigration of smolts from project areas.

Within natal freshwater habitats, numerous stream processes can affect the success of spawning and early rearing of anadromous Pacific salmonids. As summarized by NOAA (2012), successful spawning and rearing require a combination of habitat characteristics, including cool, clean water, appropriate water depth, quantity, and velocity, upland and riparian vegetation to stabilize stream banks and provide shade, clean gravel for spawning, incubation, and early rearing, large woody debris to provide resting and hiding cover, and adequate food and habitat diversity. Given these requirements, human activities or habitat alterations that jeopardize suitable conditions can negatively affect natural production.

In addition to adequate water quality, quantity, and hydraulic characteristics, groundwater connectivity can also affect microhabitat (substrate) suitability for salmonid spawning, incubation, and early rearing. As a subset of groundwater connectivity, hyporheic discharge regimes are important because they can substantially improve (or reduce) the suitability of thermal, physical, and chemical conditions for early life stages of salmonids (Geist and Daulble 1998; Geist 2000; Geist et al. 2002; Malcolm et al. 2004;

Toninia 2005; Toninia and Buffington 2007, 2009). Hatching rates of salmonid eggs can vary due to many physical factors, including variation in channel morphology, groundwater connectivity, and substrate permeability (Arntzen et al. 2006).

Salmonid eggs require high dissolved oxygen concentrations and cool water temperatures for optimal growth and metabolism (Groot and Margolis 1991; Brown and Hallock 2009). Following hatch, the developing alevins mature and emerge from the gravels following yolk absorption. Subsequent exogenously feeding fry occupy pool margins and cover provided by woody debris and over-hanging banks to avoid predation and energy expenditures associated with position maintenance in thalweg areas (Groot and Margolis 1991; Roni and Quinn 2001a, 2001b; Cederholm et al. 1997). Developing fry and parr typically move downstream during their freshwater development period, occupying different habitats over time to maximize access to food, feeding efficiency, and concealment from predators (Railsback et al. 2005). As young salmonids develop they may also increase their distance from cover and occupy greater water depths where they can find shelter from the current (Bjornn and Reiser 1991; Keeley and Slaney 1996). Thus, the needs for adequate habitat complexity, food availability, thermal, and hydraulic conditions for early life survival become evident.

Resilience, or the ability of a population or species to persist over long periods of time despite varying environmental conditions, is crucial for perpetuating important short-term benefits of salmon restoration actions. In naturally functioning watersheds, stream processes generate variable environmental conditions that contribute to variation in life history expressions and resulting population responses. This variability serves as the raw material for natural selection, population resilience, and ecological redundancy. Bisson et al. (2009) suggested that a singular definition of resilience for Pacific salmonids may be less important from a habitat restoration standpoint than understanding how natural processes have been altered by human activities and how those impacts can be reversed to promote salmon recovery.

Bisson et al. (2009) also reported several key criteria for restoring Pacific salmon production in freshwater, which are based on managing for natural variability. These include a system's capacity to recover, including food web functionality, habitat diversity, and ecological and habitat connectivity. Regarding a system's capacity to recover, these authors suggested that:

"The resilience of Pacific salmon is influenced by watershed processes that supply structural components of the aquatic environment such as coarse sediment and large wood, as well as those that support the transfer of energy and nutrients through aquatic food webs"

In addition to food web recovery, habitat diversity provides the physical basis for biological diversity and system resilience (Lowe et al. 2006). Bisson et al. (1999) also reported that:

"In fresh water, connectivity includes migratory pathways along rivers and their tributary systems as well as unimpeded lateral connections between main channels, secondary channels, and floodplains. Ecological connectivity is similarly critical for processes essential to the function of freshwater ecosystems, including a wide variety of complex aquatic and terrestrial interactions that regulate

channel dynamics, food webs, and water quality (e.g., Naiman and Bilby 1998, Power and Dietrich 2002)".

Finally, climate change represents an addition environmental variable with potentially significant future effects on habitat suitability and availability. A recent report addressing potential effects of climate change on Columbia basin salmonids (ISAB 2007) summarized the probable consequences along the Pacific coast of North America, including: 1) warmer air temperatures resulting in more precipitation falling as rain rather than snow, 2) diminished snow pack and altered timing of stream flows, 3) increased peak flows in streams, and 4) increased water temperatures. Although the authors concluded that all potential future climatic trends may not be harmful to all aquatic habitats or target species, and may not currently represent primary concerns, they emphasized that predicted climate change scenarios may have important implications for salmon resilience in the Columbia River Basin.

Nutrients and food availability – Nutrient availability is central to biological productivity in aquatic systems in general, and for Pacific salmonids in particular (Wipfli et al. 1999; Gende et al. 2002; Naiman et al. 2002; Zhang et al. 2003; Schindler et al. 2005; Kohler et al. 2008, 2011, 2012; Rex and Petticrew 2008; Gordon and Schindler 2011). Historically, anadromous Pacific salmonids provided significant inputs of MDN to freshwater streams (Cederholm et al. 1999, 2001; Gresh et al. 2000), which served as the metabolic driver for interior, low order, naturally oligotrophic natal streams, such as the Twisp and Methow rivers. These generous MDN subsidies can positively affect ecosystem metabolism from the bottom up, in some cases enhancing biological productivity at all trophic levels (Wipfli et al. 1998 Naiman et al. 2002; Thomas et al. 2003; Zhang et al. 2003; Schindler et al. 2005; Kohler et al. 2008, 2011; Rex and Petticrew 2008; Gordon and Schindler 2011). Thus, from a recovery perspective, failed natural production of Pacific salmonids across the Columbia Basin should not be viewed simply as a problem of demographic limitation. Rather, natural production failure represents an interrupted nutrient cycle that conveys nutrients from the ocean to natal habitats for freshwater fish production and back following emigration to complete the anadromous life cycle.

Non-native fishes – The transfer and range expansions of non-native species, including fishes, remain extensive on regional and global scales (Crowl et al. 2008). In addition to anthropogenic habitat loss, degradation, and nutrient reduction described above, non-native species invasions threaten the status of native species, biological communities, and the overall health of freshwater ecosystems in North America (Richter et al. 1997; Wilcove et al. 1998; reviewed in ISAB 2008). Non-native species invasions have been cited as a major environmental threat to biological diversity (Vitousek et al. 1996, Simberloff et al. 2005), having reportedly contributed to the endangerment of 48% of the species listed under the ESA (Czech and Krausman 1997; Wilcove et al. 1998; reviewed in Sanderson et al. 2008). These authors suggested that while intentional and unintentional introductions account for the initial establishment of non-native taxa, habitat loss, degradation, fragmentation, and climate change can all contribute to the severity of non-native species invasions. For example, the lack of access to previously available mainstem and tributary habitat for native Pacific salmonids can contribute to the rate and scale of range expansion of non-native fish and to their possible domination of native fish communities. In reviewing the effects of non-native species invasions on native salmonids in the Columbia River Basin, the ISAB

(2008) identified predation, competition for food and habitat, food web alteration, interbreeding, and pathogen transmission as key impact mechanisms of concern.

Brook trout (*Salvelinus fontinalis*) are a prevalent non-native species in the Program study areas. Brook trout are particularly abundant in the Program's Hancock Springs area (Yakama Nation, unpublished data), where they have been documented consuming eggs from naturally spawning Chinook salmon and steelhead, and are thought to constitute a substantial threat to native fishes and local food web dynamics. Sanderson et al. (2008) reviewed the status of brook trout in the Pacific Northwest, and reporting that:

"The proliferation of brook trout has led to the decline of native bull trout and cutthroat trout through hybridization, displacement, competition, and predation (Gunckel et al. 2002, Dunham et al. 2004, Peterson et al. 2004). Although the potential impacts of brook trout on salmonids remain virtually unexplored, Levin and colleagues (2002) found that the presence of brook trout was associated with a 12% reduction in the survival of juvenile salmon in Snake River basin streams. The mechanism driving this difference in survival is unknown".

Food webs in restorative ecology

A wide array of restoration efforts has been implemented to mitigate the continuing declines in Pacific salmonid population abundance, production, and resilience across the Columbia River Basin (Naiman et al. 2012). Restoration efforts in the basin have been recently categorized according to "the four H's" (hydrosystem, habitat, hatcheries, and harvest) as part of the Northwest Power and Conservation Council's Columbia Basin Fish and Wildlife Program (NPCC 2000). However, because limiting factors and responses to restoration actions often transcend these categories, an inclusive means to evaluate and integrate the responses and interactions of mitigation actions is needed to evaluate and guide Pacific salmon restoration and management in the Columbia Basin. Furthermore, (Bellmore et al. 2013) suggested the need for studies that evaluate how responses to restoration treatments influence the flows of energy that sustain target species and supporting ecological processes are needed, in addition to ongoing efforts that simply evaluate changes in physical habitat conditions following physical habitat restoration treatments. We also suggest including food web characterization and analyses in salmon restoration monitoring and evaluation programs.

Food webs describe the pathways by which energy and materials move through ecosystems, and provide insight into complex, multi-species assemblages within which organisms of interest grow, survive and reproduce (Elton 1927; Polis and Winemiller 1996). In their comprehensive food web report, the ISAB (2011) reported that food webs generally reflect the range of environmental factors encountered by biological communities, and as such incorporate effects of the myriad of ecological functions and processes, including habitat conditions, carrying capacity, nutrient delivery and cycling, competition, predation, pathogens, and others. These authors emphasized the importance of characterizing food webs as an integral component of evaluating ecological responses to restoration treatments. They further recommended that:

"…. incorporating food web concepts as well as detailed studies would significantly benefit basinwide restoration efforts, help sustain ecological systems, and provide for more productive fisheries" (ISAB 2011, p. 8).

The importance of incorporating food webs into fisheries and habitat restoration programs is becoming increasingly recognized, with the advent of studies that describe them as imperiled species or species of special concern (e.g. Christensen and Pauly 1993; Vander Zanden et al. 2003). Recent food web studies have also focused more broadly on larger fish and macroinvertebrate taxa groups (e.g. Cross et al. 2011, 2013; Bellmore et al. 2013).

Food web studies have been successfully used to characterize and quantify the suite of responses to restoration actions, and to describe and evaluate the mosaic of interactions among physical, biological, and ecological conditions and processes in natural and altered systems (reviewed in ISAB 2011; Naiman et al. 2012; Bellmore et al. 2013). Application of a complementary integrated food web approach can also reveal insights into processes regulating productivity and resilience that cannot be gained by focusing exclusively on the four Hs (Naiman et al. 2012, and references therein). Further, Naiman et al. (2012) reported that:

"*Food webs are integral to the four Hs, because they provide the fuel and direct the flow of energy and material for both productivity and resilience over the long term".*

We suggest that food webs analyses can also provide valuable information regarding biological responses to restoration actions in the short-term, in some cases within the year of treatment, depending on the type of actions implemented and the biological response metrics used. The food web approach also enables quantitative evaluation of biological and ecological conditions and responses to restoration actions that are directly associated with empirical environmental and ecological conditions (e.g. Bellmore et al. 2013).

Thus, the integration of restoration treatments (Part II) and the collaborative food web/trophic production model (Appendix 1), along with biomonitoring efforts and stable isotope techniques described in the following methods sections of this report is designed to improve restoration effectiveness as described by Naiman et al (2012). Finally, this inclusive, adaptive approach simultaneously offers the benefits of identifying and/or precluding unintended consequences of management actions, such as disruptive changes in food webs, biological processes, and species community composition (Minckley et al. 2003; Fausch et al. 2009; Ellis et al. 2011; Naiman et al. 2012).

Status of natural production

Historic anadromous salmonid abundance estimates for the Methow basin followed similar declining patterns as those described for the Columbia Basin (Lichatowich 1999; Gresh et al. 2000; NPCC 2000, 2001). Approximately 64,000 adult anadromous salmonids were estimated to have inhabited the Methow basin during the 1850s (Mullan et al. 1992). Of these, numerous Chinook (24,000), Coho salmon (36,000), and steelhead (3,600) would have contributed corresponding MDN loads to basin habitats during peak annual spawning events (Mullan et al. 1992). It is likely that these spawning events also mobilized aquatic invertebrates and previously buried salmonid eggs as part of the natural

bioturbation processes that supplemented juvenile fish diets with high calorie diet items (Schindler et al. 2003; Moore and Schindler 2008a; Holtgrieve et al. 2011). Although historical natural production numbers of adults and early life stages are uncertain for these populations, one can reasonably assume long-term average replacement rates of >1 given their long-term historical persistence. Alternatively, current natural replacement rates for Upper Columbia River ESA-listed spring Chinook and summer steelhead have typically fallen well below recommended numerical recovery values (NMFS 2001).

Current estimates of mean spawning abundance for Chinook are around 4,000 adults, however, this estimate combines spring and summer runs (Snow et al. 2010). Mean annual spawner abundance for Federally-listed spring Chinook of 2,645 (data from 2001-through 2010) was estimated at 2,645, with only 530 (21%) of those fish being naturally produced. Coho in the upper Columbia basin were extirpated around the turn of the century (Murdoch et al. 2004). However, coho re-introduction efforts in the Upper Columbia Basin began during 1999, with resulting adult escapements over Wells Dam averaging 832 from 1999 through 2010. Mean total basin annual spawner abundance for steelhead (data from 1999 – 2010) was recently estimated to be 4,898 fish, with only 590 (12%) being naturally produced (Snow et al. 2010; Hillman et al. 2012).

Interim recovery abundance values of 2,000 naturally produced Chinook spawners/year and approximately 2,500 for steelhead were reported by NMFS (2001; Table 2). These estimates were described as "values that fall within the range of the habitat capacity estimates, historical run sizes, and simple PVA recommendations".

Table 2. ESA target demographic conditions for Methow River Chinook and steelhead.

Chinook	**Target under ESA**
Interim recovery abundance	2,000[b]
Cautionary (minimum) abundance[a]	500[b]
Population growth rate	>1.0[c]
12-yr average spawning contribution	Spawning in a minimum of three tributary streams, with each contributing ≥ 5% of spawning abundance
Maximum out-of-ESU[e] hatchery fish gene flow	<1%
Steelhead[d]	**Target under ESA**
Interim recovery abundance	~2,500[b]
Cautionary (minimum) abundance[1]	
Population growth rate	>1.0[c]
12-yr average spawning contribution	
Maximum out-of-ESU[e] hatchery fish gene flow	<1%

a: Level below which demographic, genetic, and other risk factors to the population become of increasing concern.
b: Mean numbers of naturally produced spawners per year over 12 brood cycles.
c: Geometric mean natural return ratio (NRR) value.
d: Does not include resident *O. mykiss* individuals.
e: ESU: Upper Columbia River Chinook and steelhead ESUs.

Isotope analyses inform food web models

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

Management Issues

Endangered species management

Regarding the role of natural production in recovery of T&E species listed under the ESA, the National Marine Fisheries Service, in their review of Upper Columbia Chinook and Steelhead population status (NMFS 2001) reported that:

"The stated purposes of the ESA are to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved, to provide a program for the conservation of such endangered species and threatened species, and to take such steps as may be appropriate to achieve these purposes (ESA sec. 2(b)). The ESA's focus is, therefore, on natural populations and the ecosystems upon which they depend. Artificial propagation of a listed salmonid species is therefore not a substitute for eliminating the factors causing or contributing to a species' decline (Hard et al. 1992)".

Previously (May 5, 2008), NOAA Fisheries Service released the final Endangered Species Act documents on the Remand of the 2004 Biological Opinion on the Federal Columbia River Power System (NOAA 2008a). On May 19, 2010, NOAA issued a "Supplemental FCRPS Biological Opinion" intended to protect Columbia/Snake River Basin salmon and steelhead listed under the Endangered Species Act (NOAA 2010). This supplemental biological opinion documented NOAA Fisheries' determination that the

operation of the Federal Columbia River Power System (FCRPS), through 2018, complies with the standards of § 7(a) (2) of the Endangered Species Act.

The 2008 BiOp (Section 8.1.3) included mitigation options for various salmonid life stages as recommended by the Independent Scientific Advisory Board (ISAB (2007). Recommendations relevant to the Upper Columbia Natural Production Restoration Program include the following habitat issues:

- Protect or restore riparian buffers, particularly in headwater tributaries that function as thermal refugia
- Remove barriers to fish passage into thermal refugia, and
- Protect and restore wetlands, floodplains, or other landscape features that store water to provide some mitigation for declining summer flow, including:
    - Identify cool-water refugia (watersheds with extensive groundwater reservoirs)
    - Protect these groundwater systems and restore them where possible
    - May include tributaries functioning as cool-water refugia along the mainstem Columbia where migrating adults congregate

Of particular relevance to this YN Program, the 2010 BiOp reported that the 24-year extinction risk for Upper Columbia River (UCR) Steelhead (Wenatchee, Entiat, and Okanogan populations) "...was unchanged or declined, but remained much greater than 5%". However, extinction risk estimates for Methow River steelhead doubled, increasing from the 2008 BiOp estimate of 47% to 97% in the 2010 BiOp (NOAA 2010). Regarding UCR spring Chinook, the 2010 BiOp stated that:

"The Wenatchee and Methow populations are important for the viability of this ESU. NOAA Fisheries and the Action Agencies are watching this closely with respect to AMIP [Adaptive Management Implementation Plan] triggers and various monitoring protocols".

Recovery actions being implemented under the 2008 and 2010 BiOps are focused on improving fish survival at federal dams and throughout the salmon lifecycle. In addition to dam operation, fish passage, and survival in the estuary and the ocean, both BiOps focus on improving juvenile and adult salmon and steelhead survival by protecting and enhancing habitat. The 2008 FCRPS BiOp, lists:

"Tributary habitat restoration, including new information on evaluating and prioritizing projects to achieve survival and other benefits" as a formal component (Section 2.2.3; NOAA 2010).

In the 2010 BiOp, NOAA Fisheries reviewed the new scientific information on the best methods for achieving the benefits needed from tributary habitat restoration (RPA Actions 34 and 35 including Table 5, in the 2008 BiOp). These studies support the Action Agencies' approach for selecting goals for habitat improvement projects based on addressing limiting factors. New scientific information reviewed by the NOAA review

"....supports its [previous] conclusions that the RPA, as amended, addresses factors that have limited the functioning and conservation value of spawning and rearing habitat, and will increase the survival of the affected populations".

"In summary, the studies reviewed [in the 2010 BiOp] support NOAA Fisheries' assumptions in the 2008 BiOp that the RPA, as amended, will address factors that limit the functioning and conservation value of habitat that Interior basin salmon and steelhead use for spawning and rearing. The PCEs [primary constituent elements under ESA] expected to be improved are water quality, water quantity, cover/shelter, food, riparian vegetation, space, and safe passage/access, as described in the 2008 analysis".

Columbia Basin Fish Accords

As stated in the 2010 BiOp: "The RPA, as amended in 2010, includes the 2008 Columbia Basin Fish Accords (NOAA 2010, Appendix G). The Accords support and enhance the tributary habitat program by securing a number of Columbia Basin Tribes and the State of Idaho as implementing partners. The Accords' habitat improvement objectives are beyond those required by Table 5 of RPA Action 35, which adds to NOAA Fisheries' confidence that habitat improvements over the term of the BiOp will meet or exceed those expectations for the affected populations.

Habitat project selection and implementation in the Accords prioritizes the treatment of limiting factors that demonstrably improve salmon and steelhead survival and are based on recovery plans. This approach is supported by extensive research, monitoring, and evaluation to confirm estimates of biological benefit and allow mid-course adjustments through the adaptive management process resulting in a disciplined process of informed and accountable implementation".

Program alignment with ESA recovery and Fish Accord actions

Research and restoration measures reported in the YN Program report directly address many of the needs reported in the BiOps and in the Fish Accords. For example, both the Hancock Springs and Twisp River projects address limiting factors to improve natural production and juvenile survival by enhancing and protecting habitat (Table 3). The Hancock Springs Project also addresses increased natural production through the protection and restoration of coldwater refugia associated with groundwater discharge. Furthermore, both projects involve research, monitoring, and evaluation designed to quantify biological benefits to salmon and steelhead in tributary habitat in an adaptive management setting as requested in the Accords (Table 3).

Table 3. Program components that address BiOp and Fish Accord attributes.

BiOp attributes	Natural Production Restoration Program	
	Hancock Springs Project	Twisp River Project
Tributary habitat restoration, including new information on evaluating and prioritizing projects to achieve survival and other benefits	X	X
Improve juvenile and adult survival by protecting and enhancing habitat	X	X
Implement habitat projects that address limiting factors	X	X
Protect or restore riparian buffers, particularly those that function as thermal refugia	X	
Remove barriers to fish passage into thermal refugia	X	
Identify cool-water refugia	X	
Protect groundwater systems and restore them where possible	X	
Columbia Basin Fish Accord attributes		
Enhance tributary habitat	X	X
Address limiting factors that demonstrably improve salmon and steelhead survival	X	X
Involves research, monitoring, and evaluation to confirm estimates of biological benefit and allow mid-course adjustments through the adaptive management process	X	X

Incorporating food webs into restoration and management programs

In addition to the scientific value of food web characterization described earlier in this report, the inclusion of food web studies also directly addresses biological aspects of the Northwest Power and Conservation Council's Columbia Basin Fish and Wildlife Program. A major goal of the Council's program is to establish and maintain an ecosystem that sustains abundant, productive, and diverse fish and wildlife communities (NPCC 2009). Incorporating food web assessments into restoration and management programs also provides valuable information regarding the basic ecological mechanisms that regulate biological productivity, community structure and dynamics, and resilience that cannot be acquired by focusing solely on the NPPC's four H's (hydrosystem, habitat, hatcheries, and harvest; NPCC 2000; Naiman et al. 2012). Prior to recent comprehensive reports and papers on the value of food webs for restoration and management (e.g. Cross et al. 2011, 2013; ISAB 2011; Naiman et al. 2012), the ISAB and ISRP reported on the importance of integrating food web assessments into Columbia Basin restoration and management programs on 22 separate occasions in 22 Council reports between 1999 and 2009 (See Table A.2.1. in ISAB 2011).

By their very nature, food webs or food web networks portray the integrated structure that links crucial biological components and functions of ecosystems, enabling their use as evaluation templates of ecological conditions and responses. Food web networks also provide a de facto adaptive management construct to characterize ecosystem status and evaluate responses to perturbations or restoration activities. Finally, and perhaps most importantly in the fisheries management realm, incorporating food web characterization into restoration and management plans can help to restore and sustain more productive and resilient fisheries (ISAB 2011; Naiman et al. 2012).

Part II: Program Components

Restoration and maintenance of natural production of listed anadromous and resident salmonids in the Pacific Northwest and the required supporting ecological conditions in freshwater natal streams are critical components of regional salmon restoration programs (NRC 1996; NPCC 2009). The loss and degradation of physical habitat, loss of marine derived nutrient loading, and the deleterious presence of non-native fishes are three important factors currently limiting natural production (NRC 1996; Gresh et al. 2000; ISAB 2008, 2011; NPCC 2009; Naiman et al. 2012).

Program Goals and Objectives

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) implement and test three restoration measures (habitat restoration, nutrient addition, and brook trout removal) to increase natural production, and 2) describe the mechanisms by which these three factors limit natural production.

Study Area

This Program includes two study areas: Hancock Springs and the Twisp River (Figure 1).

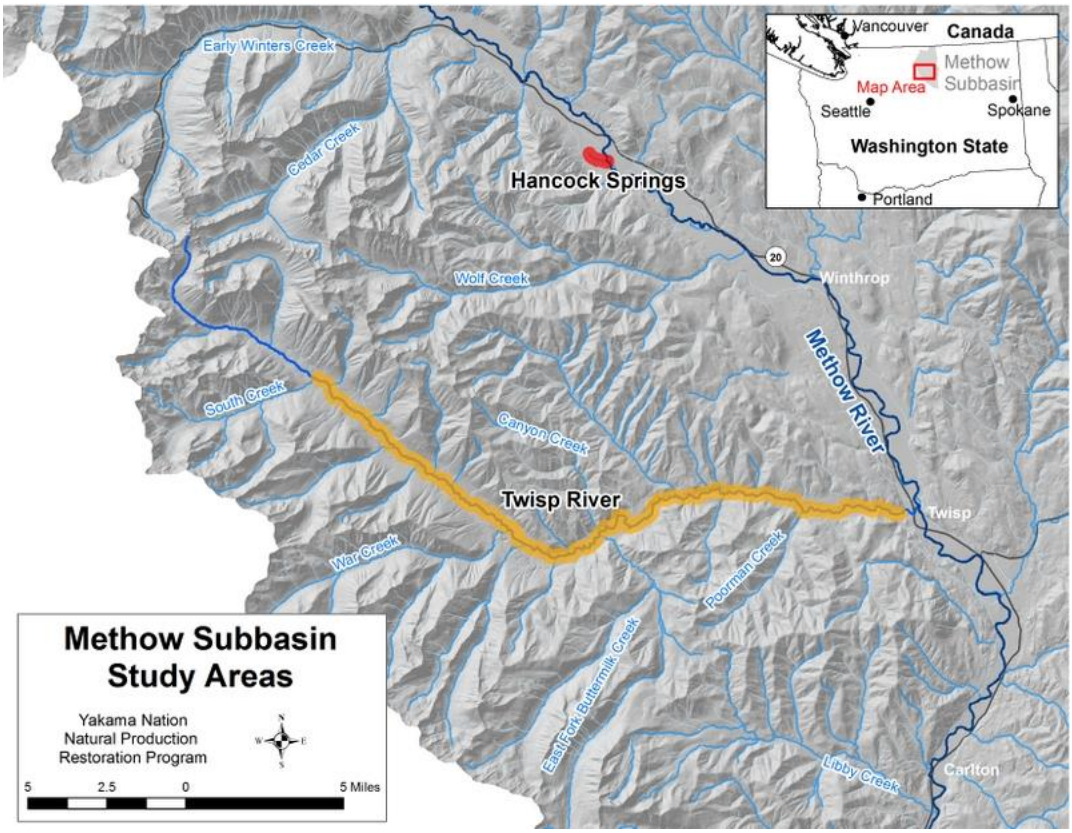


Figure 1. Locations of Hancock Springs, the Twisp River, and the Methow River Subbasin.

Hancock Springs

Hancock Springs is a small (1.6 km-long) spring creek located at RKM 96 on the Methow River (Figure 2). This opportunity enables the Yakama Nation Fisheries Program to design and carry out long-term studies and restoration treatments of interest.

Its small size and unique ecological conditions (annually stable hydrograph and thermograph) provide an ideal opportunity to evaluate individual and additive effects of multiple restoration treatments in a small salmon producing stream. Unlike most larger tributaries used for natural production throughout the Columbia Basin, Hancock Spring's physical and biological attributes are ideal for high resolution fine-scale sampling to assess key limiting factors and the mechanisms and conditions needed for successful natural production. A secured conservation easement along the entire length of this tributary provides long-term system stability and the rare opportunity to apply and evaluate a series of restoration treatments over a 10-year period. This opportunity enables the Yakama Nation Fisheries Program to design and carry out long-term studies and restoration treatments of interest.

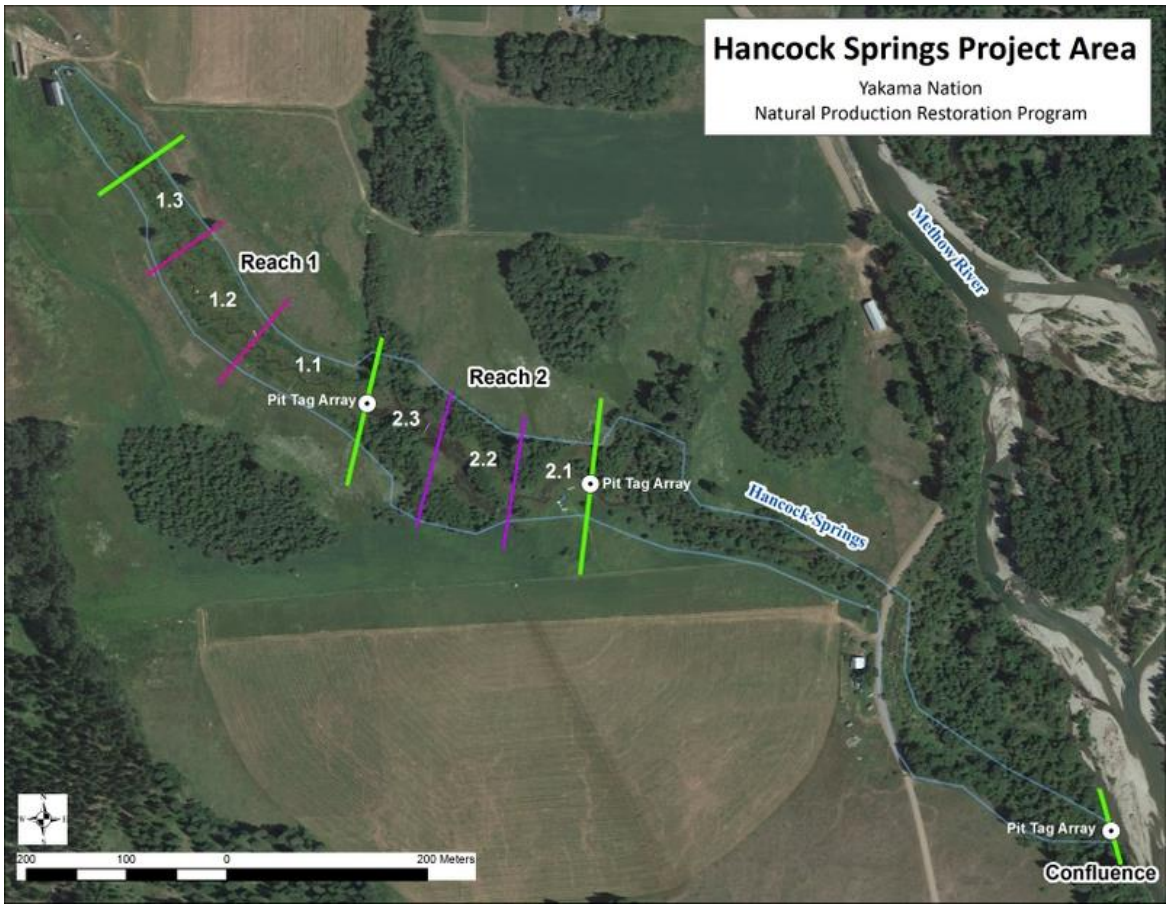


Figure 2. Hancock Springs Project area, illustrating treatment and control reaches and sampling sites.

Twisp River

The Twisp River is an unimpounded 4th order river that flows into the Methow River at the town of Twisp in north central Washington (Figure 3) with a natural hydrograph that typically peaks during May June, or later based largely on snowmelt runoff and annual climatic conditions. Mean annual discharge in the Twisp River was 266 cfs over a 28 year period (1974-2012), with mean monthly discharge ranging from 41 to 1,200 cfs (USGS data). A substantial portion of the upper Twisp River watershed exists in a designated wilderness area and is in nearly pristine condition. Spring Chinook salmon, summer steelhead, and bull trout spawn and rear in the Twisp River over much of its 55 km length. Most of the human activity and resulting habitat changes within the drainage, such as road placement, bank hardening, and conversion of some riparian areas to agriculture and residential uses, have occurred along the lower 15 miles of the Twisp River.

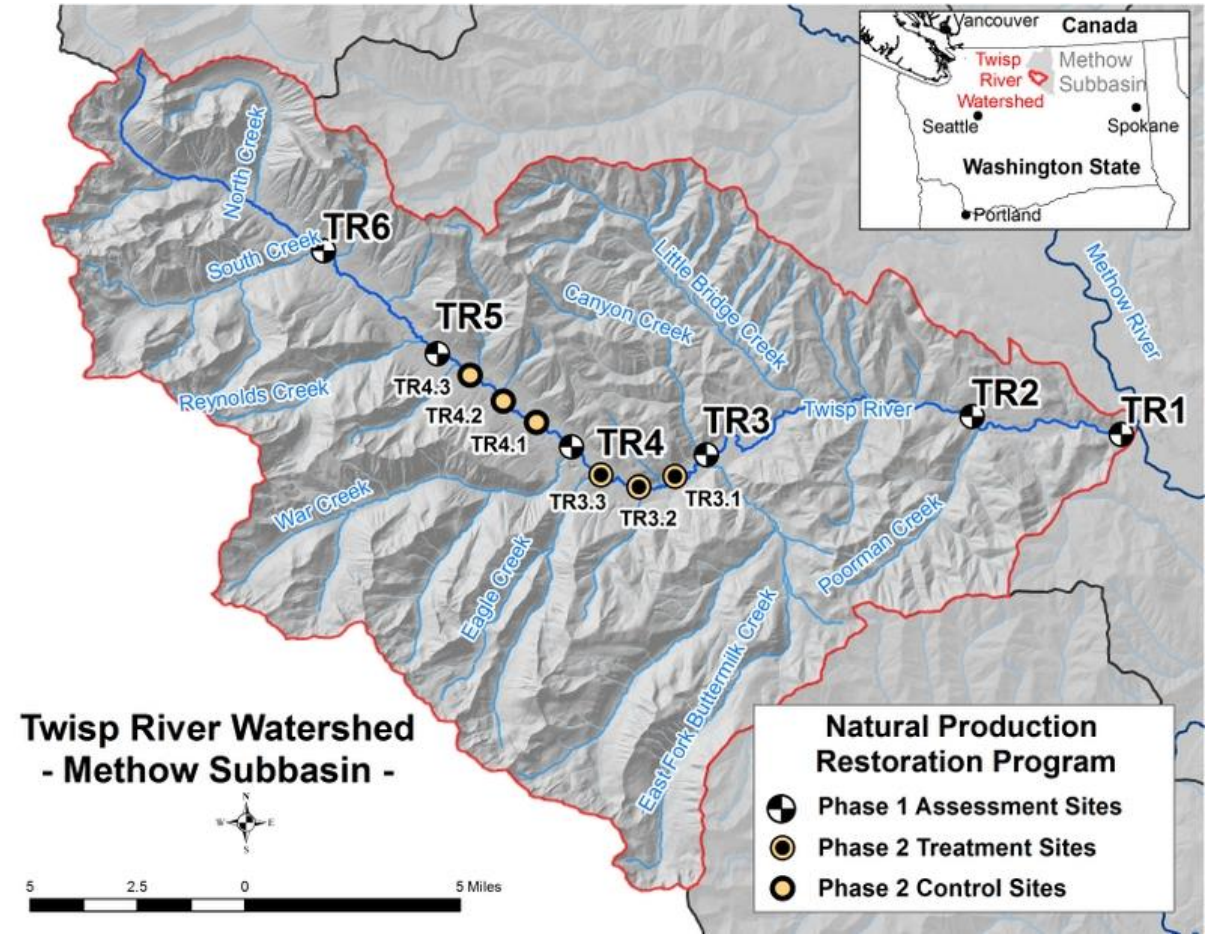


Figure 3. Twisp River watershed, study area, and sampling sites for Phase 1 (TR1 through TR6) and Phase 2 of the project (Sites TR3.1, TR3.2, TR3.3, TR4.1, TR4.2, and TR4.3).

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 is also unique in that it sequentially quantifies the individual and combined effects of physical habitat restoration, nutrient addition, and the removal of non-native brook trout on natural production.

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 of efforts to restore natural production. The Twisp River Project evaluates river fertilization at the larger river scale.

Methods

Hancock Springs Project – The Hancock Springs Project will sequentially implement, monitor, and evaluate three restoration treatments and their potential interactions over a 10 year period:
Treatment 1: Physical habitat restoration (Reach 1: 2011, Reach 2: 2018)

Treatment 2: Nutrient addition (2014-2018)

Treatment 3: Brook trout removal (2016-2018)

Hancock Springs is divided into two adjacent 300m reaches: Reach 1 (upstream) and Reach 2 (downstream, Figure 2). Physical habitat restoration occurred in Reach 1 during 2011, and will occur again in Reach 2 in 2018, followed by annual nutrient addition beginning in 2014, and brook trout removal beginning in 2016 (Table 4). Nutrient addition and invasive species removal will occur in both reaches. A standardized multi-trophic level biomonitoring program is ongoing in Hancock Springs during all years to address pre- and post-treatment conditions as described if the following sections

Table 4. Treatment structure and monitoring schedule for the Hancock Springs Project, 2011-2018.

	Hancock Springs Project					
	Reach 1			Reach 2		
Year	Habitat restoration	Nutrient addition	Brook trout removal	Habitat restoration	Nutrient addition	Brook trout removal
2011	I					
2012	M	PTM	PTM	M	PTM	PTM
2013	M	PTM	PTM	M	PTM	PTM
2014	M	I, M	PTM	M	I, M	PTM
2015	M	I, M	PTM	M	I, M	PTM
2016	M	I, M	I, M	M	I, M	I, M
2017	M	I, M	I, M	M	I, M	I, M
2018	M	I, M	I, M	I, M	I, M	I, M

I: Implement ; M: Monitor; PTM: Pre-treatment monitoring

Analytical designs differ slightly between treatments and study areas. For example, responses to habitat restoration in Hancock Springs are determined by within-year comparisons between Reach 1 and Reach 2, while responses to nutrient addition and brook trout removal are compared between pre- and post-treatment periods (Table 5).

Table 5. General design for testing responses to restoration treatments in Hancock Springs Project.

Project	Treatment	Comparison	Design
Hancock Springs	Habitat restoration	Within-year spatial comparison (2012) Temporal comparison (2018)	Reach 1 (treatment) vs. Reach 2 (control) Before and after treatment
	Nutrient addition	Temporal comparison Effect of nutrient addition in complex, restored habitat vs. simple altered habitat	Pre-treatment vs. Post-treatment years Reach 1 vs. Reach 2
	Nonnative spp. removal	Temporal comparison Effect of brook trout removal in complex, restored habitat vs. simple altered habitat, with and without nutrient addition	Pre-treatment vs. Post-treatment years Reach 1 vs. Reach 2

Twisp River Project – Based on previous habitat evaluations, the Twisp River is not significantly jeopardized by altered physical habitat conditions or by the deleterious presence of non-native fish species. However, it continues to experience a significant reduction in marine derived nutrient loading from historic levels (Mullan et al. 1992; Snow et al. 2010). Therefore, the Twisp River Project only involves an experimental nutrient addition treatment, currently scheduled to begin in 2020 to incorporate results from the previous nutrient addition experiment in Hancock Springs (Table 4). This river-scale nutrient addition experiment will occur within a 10km section of the middle Twisp River, 55 km upstream from the mouth, with a 5 km upstream control reach and an adjacent 5km downstream treatment reach. Experimental nutrient addition in the Twisp River is intended to provide guidance for future nutrient supplementation programs in other salmon producing streams in the Pacific Northwest.

The Twisp River Project has three sequential phases (Table 6):

Phase 1: Whole river trophic characterization, 44 km (2008-2011)

Phase 2: Fine scale trophic characterization 10 km (2012-2013, 2018-+).

Phase 3: Nutrient addition (2020+).

Table 6. Treatment structure and monitoring schedule for the Twisp River Project, 2009-2018.

Twisp River Project (nutrient addition)			
	Phase 1	Phase 2	Phase 3
Year	Whole river assessment	Pre-treatment monitoring	Post-treatment monitoring
2009	PTM		
2010	PTM		
2011	PTM		
2012		PTM	
2013		PTM	
2014			
2015			
2016			
2017			
2018			

Treatment design in the Twisp River will involve temporal and spatial comparisons using a BACI design (Table 7).

Table 7. General design for testing responses to nutrient addition in the Twisp River.

Project	Treatment	Comparison	Design
Twisp River	Nutrient addition	Temporal and spatial comparisons	BACI (Before After Control Impact) (Pre-treatment vs. Post-treatment years *and* within-year upstream control reach vs. downstream treated reach)

During Phase 1, sampling occurred throughout the 44 km anadromous production zone in the Twisp River at six sampling sites (TR 1-6, Figure 3. 3). Biomonitoring during Phase 1 included sampling of water chemistry, periphyton, chlorophyll accrual, and benthic macroinvertebrates. Sampling during Phase 2 will also occur at 6 sites over a 10km river reach, with three sites in a 5km upstream control reach (TR 4.1-4.3) and three in an adjacent a 5km downstream treatment reach (TR 3.1-3.3, Figure 3. 3). Phase 2 will involve a BACI (Before-After-Control-Impact) design, along with an upstream control and downstream treatment reach. All pre-treatment data will be collected during Phase 2. Phase 3 is the nutrient addition phase. A standard suite of biological response variables (Table 8) will be measured during Phase 2 and Phase 3 upstream and downstream as well as before (2012-2017) and after nutrient addition (2020+). Stable isotopes, insect drift, fish, and fish gut content sampling will be added to the standard sampling regime from Phase 1 at each site during Phase 2 and Phase 3 to better characterize food web and trophic production responses to nutrient addition.

Experimental Treatments

Treatment 1: Physical habitat restoration

(Hancock Springs, Reach 1, 2011, Reach 2, 2018)

Objective: Evaluate the effects of habitat restoration (engineered channel reconfiguration) on natural production of anadromous and resident salmonids and on the supporting trophic ecology in Hancock Springs.

Justification: The extent and condition of available physical habitat in the Pacific Northwest have been extensively reported as important factors limiting natural production of salmonids in natal habitats during early life stages. The engineered channel reconfiguration performed in Reach 1 of Hancock Springs during 2011 was designed to increase habitat quality and quantity to increase natural production that may have been previously habitat-limited.

Design: Due to limited monitoring funding, only course fish sampling (redd counts, presence of juveniles) was done prior to Reach 1 channel reconfiguration. With no pre-restoration trophic monitoring available, treatment effects of Reach 1 are compared to current physical and trophic conditions of Reach 2. Thus, we have a spatial control but lack a temporal control when assessing biological responses to channel reconstruction in Reach 1. Effects of the second habitat restoration treatment (in Reach 2 during 2018) will involve analogous compare conditions in Reach 2 between pre- and post-treatment periods (BACI).

Treatment methods: Reach 1 channel reconfiguration treatment was performed during 2011 by regional U.S. Fish and Wildlife Service hydrologists and engineers using an empirical reference reach methodology. Quantitative values for existing, impaired channel morphology parameters were compared to reference conditions in two east Cascades streams of like stream type (Rosgen C4), with spring-fed hydrology, geologic control (glacial trough valleys), and local boundary conditions (sedge-rush community with secondary shrub component). Initial reach-scale design parameters were developed by assigning target values for stream slope and sinuosity. An iterative process was used to refine morphological variables including channel length, depth, and width-depth ratios. Final design parameters were set using bed grain size, predicted velocities, pool-riffle facet slopes, and measured intra- and inter-annual discharge information. The 2018 Reach 2 channel reconstruction design will use the same methodology and similar slope, discharge, and particle size values as used in Reach 1, and will incorporate lessons from construction and observed stability in Reach 1 from 2011 through 2017.

Treatment 2: 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.

Treatment 3: Non-native brook trout removal

(Hancock Springs only, both reaches, 2016-2018)

Objective: Evaluate the effects of brook trout removal on natural production and diet composition of anadromous and resident salmonids and on the supporting trophic ecology in Hancock Springs.

Justification: Along with habitat loss, degradation and nutrient limitation from loss of historic MDN contributions, competition and predation by non-native fishes have also been reported as important factors limiting natural production of salmonids in natal habitats during early life stages. Brook trout removal is designed to increase natural production that may have been previously limited by non-native brook trout predation or competition.

Design: The effects of non-native brook trout removal will be evaluated by statistically comparing mean values from a standard suite of biological response variables before and after removal in two stream reaches: one with (Reach 1) and one without a prior physical habitat restoration treatment (Reach 2).

Treatment methods: Brook trout removal will occur 6 times per year using standard multi-pass electrofishing depletion techniques from 2016 through 2018 with methods described in detail in the following methods sections. All collected brook trout will be measured (FL, mm), and weighed (g), and will be euthanized in MS222 to facilitate gut content sampling. Removing all Brook Trout will be difficult, but we expect to drastically reduce overall biomass, given our success with depletion estimates within the study are thus far.

Biological Response Variables

Hypotheses will be used to test for statistically significant biological responses to experimental restoration treatments using a series of biological response variables that describe water quality and nutrients, as well as primary, secondary, fish production, and conditions of the periphyton, benthic macroinvertebrate, and fish communities (Table 8). Response variable values will be calculated for five dominant fish species: Chinook (*Oncorhynchus tshawytscha*), steelhead (*O. mykiss*), bull trout (*Salvelinus confluentus*), brook trout (*S. fontinalis*), and sculpin (*Cottid spp.).*

Table 8. Biological response variables associated with treatments and monitoring in the Hancock Springs and Twisp River projects.

		Hancock Springs			Twisp River
Response Variable categories	**Response Variables**	**Physical habitat restoration**	**Nutrient addition**	**Brook trout removal**	**Nutrient addition**
Fish	Abundance	X	X	X	X
Tertiary production	Length	X	X	X	X
	Weight	X	X	X	X
	Condition factor	X	X	X	X
	Growth rate	X	X	X	X
	Gut sample composition and biomass	X	X	X	X
	Annual fecundity	X	X	X	X
	Carcass measurement	X	X	X	X
	Tertiary production (P)	X	X	X	X
	Interaction strength (I)	X	X	X	X
	Competition coefficient (CC)	X	X	X	X
	Carrying capacity (PotenP)	X	X	X	X
Macroinvertebrates	Total abundance	X	X	X	X
Secondary production	Total biomass	X	X	X	X
	Total richness	X	X	X	X
	EPT variables	X	X	X	X
	Feeding guild variables	X	X	X	X
	Diversity and composition	X	X	X	X
	Secondary production				
Periphyton	Standing crop (AFDM)	X	X	X	X
Primary production	Chlorophyll a biomass	X	X	X	X
	Algal community composition	X	X	X	X
	Primary production[1]				
WQ/Nutrients	D.O., temp, alkalinity, pH, TP, SRP TDP, TN, NH4, NO2+NO3, TN:TP	X	X	X	X
Stable Isotopes[2]	Isotope ratio (δ 15 N/ 13 C)		X		X

1: Provided by UI/WSU

2: Stable isotope values will be calculated for periphyton, invertebrate, and fish communities, salmon eggs and carcasses, terrestrial plant and invertebrate material, and nutrient addition sources.

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 $gDM \bullet m^{-2} \bullet y^{-1}$) is calculated as:

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

where:

P_j = annual secondary production ($gDM \bullet m^{-2} \bullet y^{-1}$) 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 ($g\ DM \bullet m^{-2} \bullet y^{-1}$) 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:

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

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.

Ordination techniques, such as principal component analysis (PCA) and nonmetric multidimensional scaling (NMDS) will be used for clustering of sampling units, based on multiple community metrics (e.g. benthic macroinvertebrate and fish). NMDS will be particularly useful for visualizing the similarities and dissimilarities in the data. Adequacy of the NMDS analyses will be assessed using diagnostic scree plots and predicted correlations.

In addition, before-after control impact analysis (BACI) will be considered to subsequently test for effects of nutrient addition on the trophic level responses. This repeated measures type of ANOVA will be used to test various responses or response patterns across the periods, i.e. before and after nutrient addition, between treated and untreated sections of the river/streams.

Statistical analyses will be carried out using SAS ver. 9.3.

Sample size analysis – For each trophic level data, including water chemistry, algal, periphyton standing crop, benthic macroinvertebrate, and fish responses, standard sample size and power analyses were performed to assess the adequacy of sampling scheme and intensity over time. Statistical precision of proposed sampling within each trophic level was set to an arbitrary value (customarily 10% of the each response mean) and the significance level for both sample size determination and power analysis were set at 5%, corresponding to a 95% confidence coefficient.

Results

Hancock Springs

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.

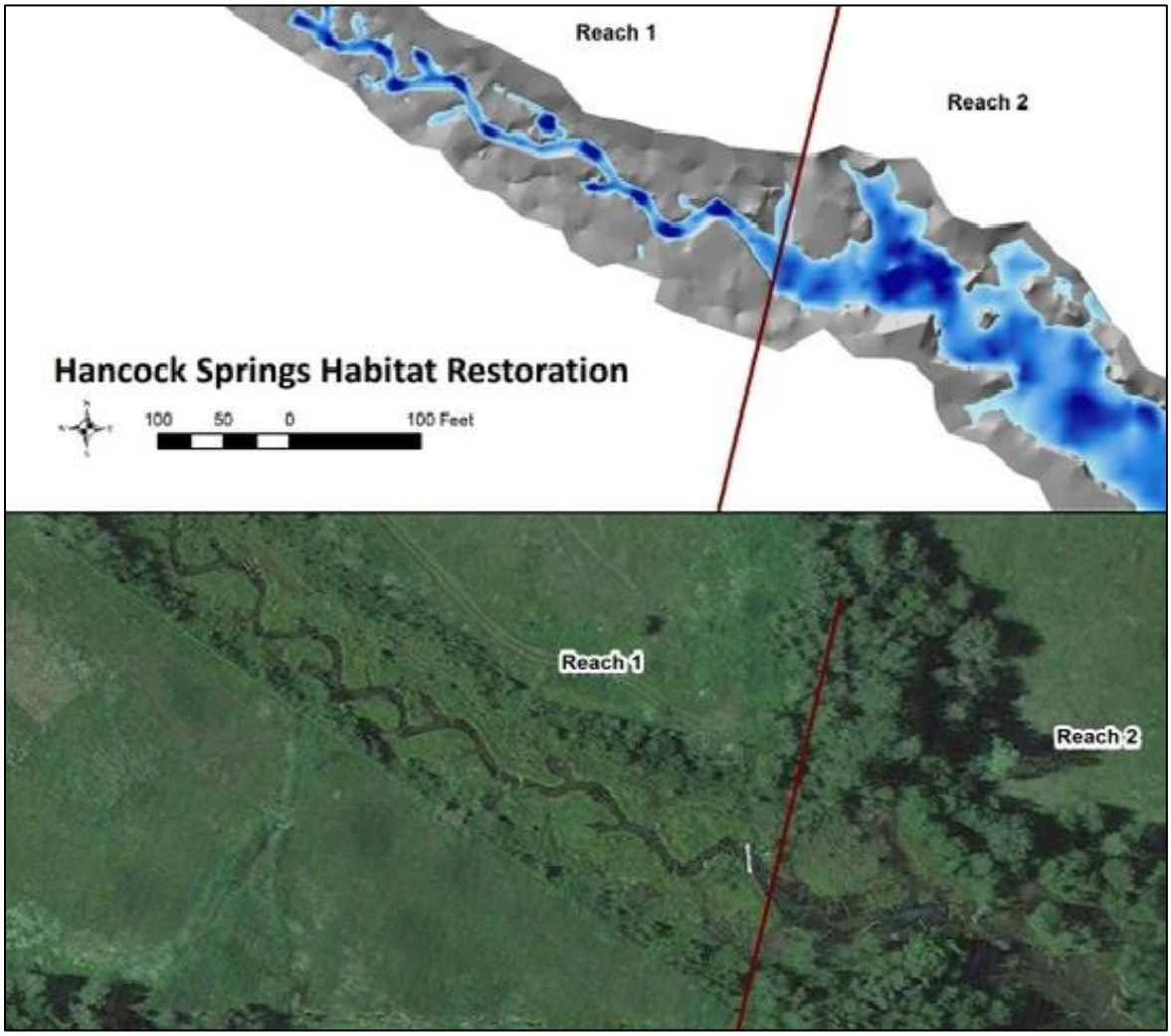


Figure 4. General channel and riparian habitat features of Reach 1 (treatment) and Reach 2 (Control) in Hancock Springs in 2012 after Reach 1 channel reconfiguration, completed in 2011.

Physical habitat summary

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.

Table 9. 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

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).

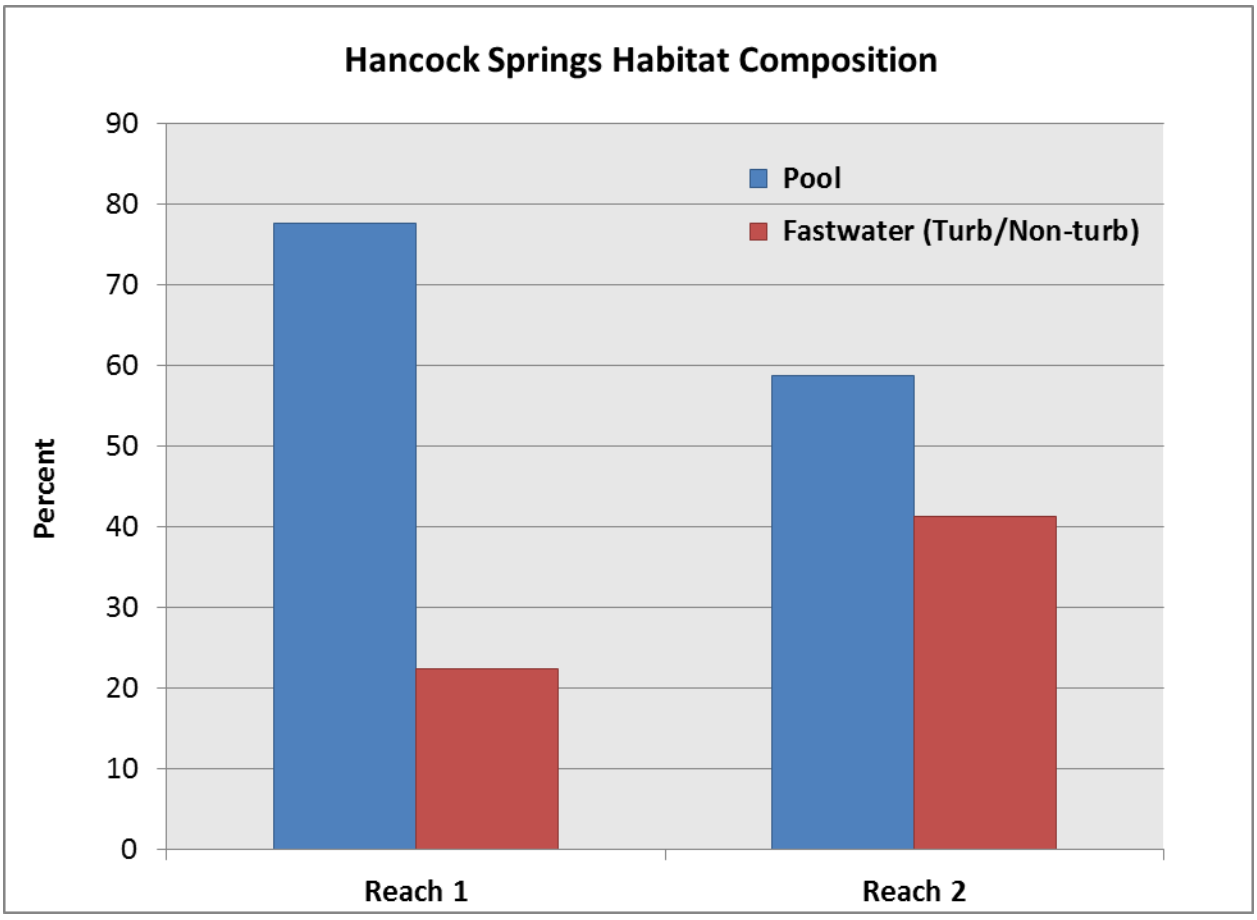


Figure 5. Percent habitat composition (pool and fastwater habitats) in Reach 1 and Reach 2 of Hancock Springs.

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).

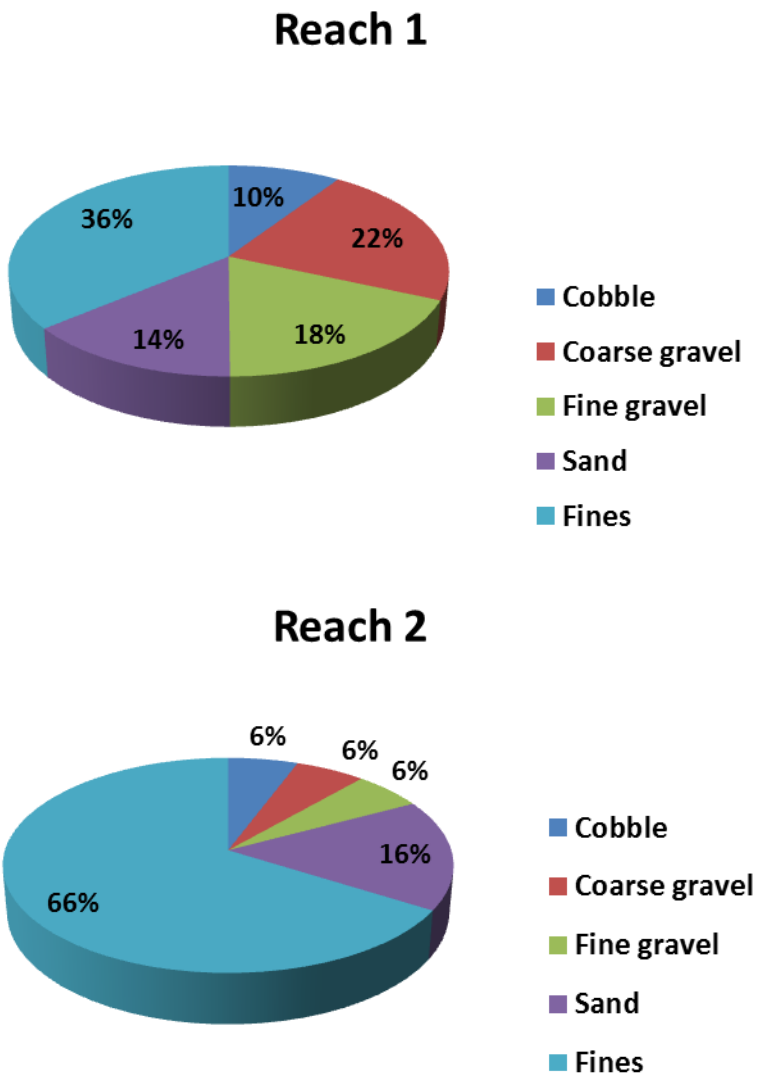


Figure 6. Percent substrate composition by reach in Hancock Springs, 2013-2013.

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).

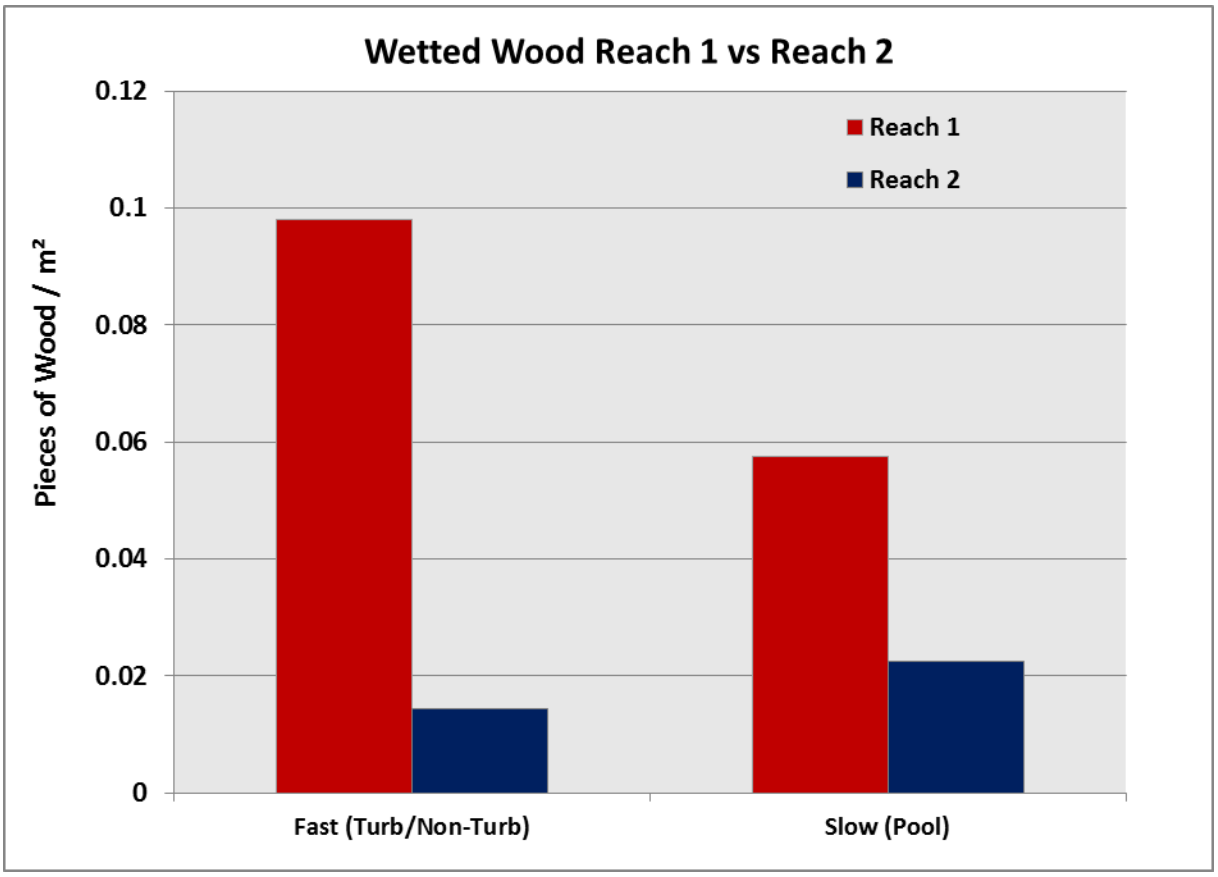


Figure 7. Density (pieces of wood/m^2) in pool and fastwater habitats by reach in Hancock Springs after 2011 Reach 1 channel reconfiguration.

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).

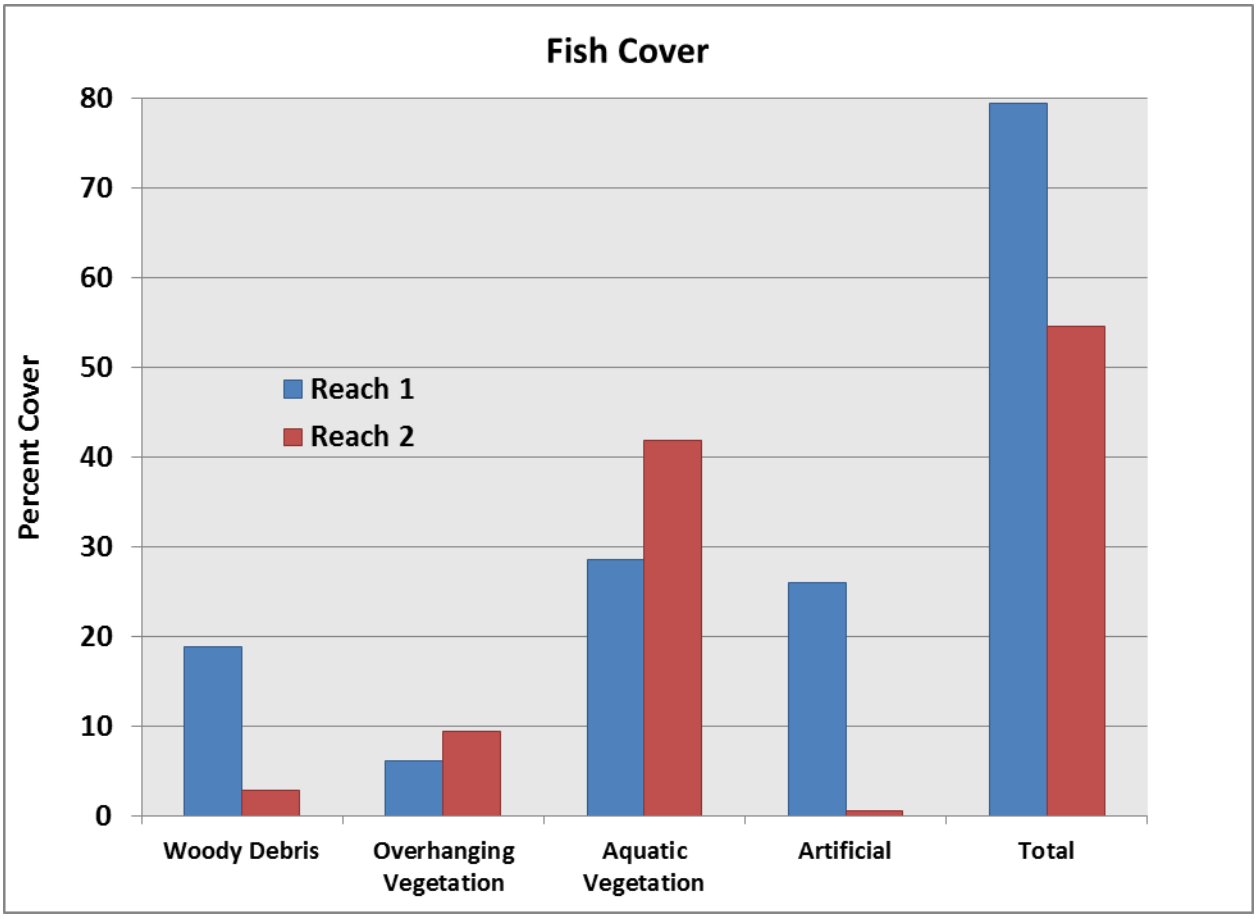


Figure 8. Percent fish cover type by reach in Hancock Springs, 2012.

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).

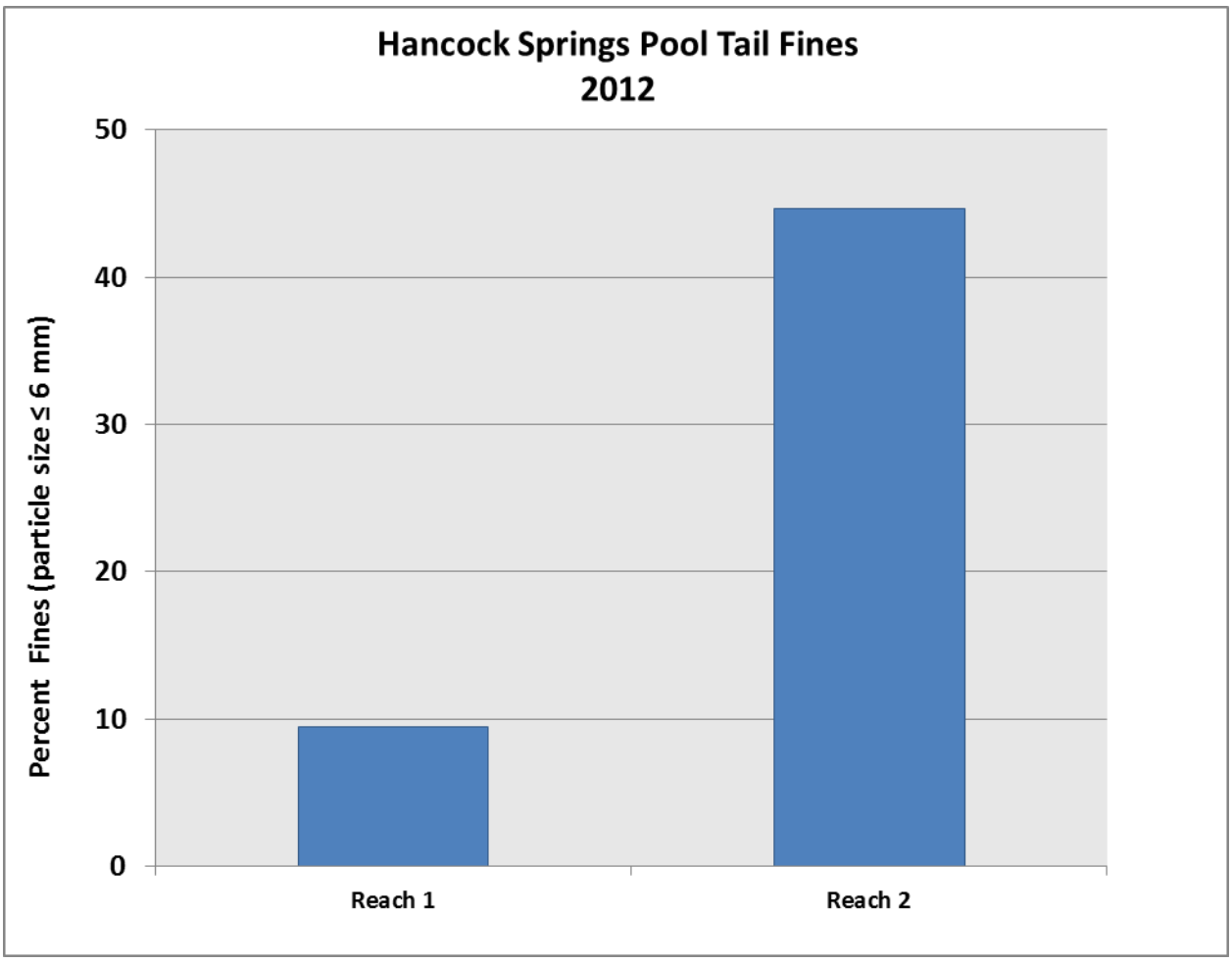


Figure 9. Percent pool tail fines in Hancock Springs by reach, 2012 and 2013.

Biological response summary

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).

Table 10. 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

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).

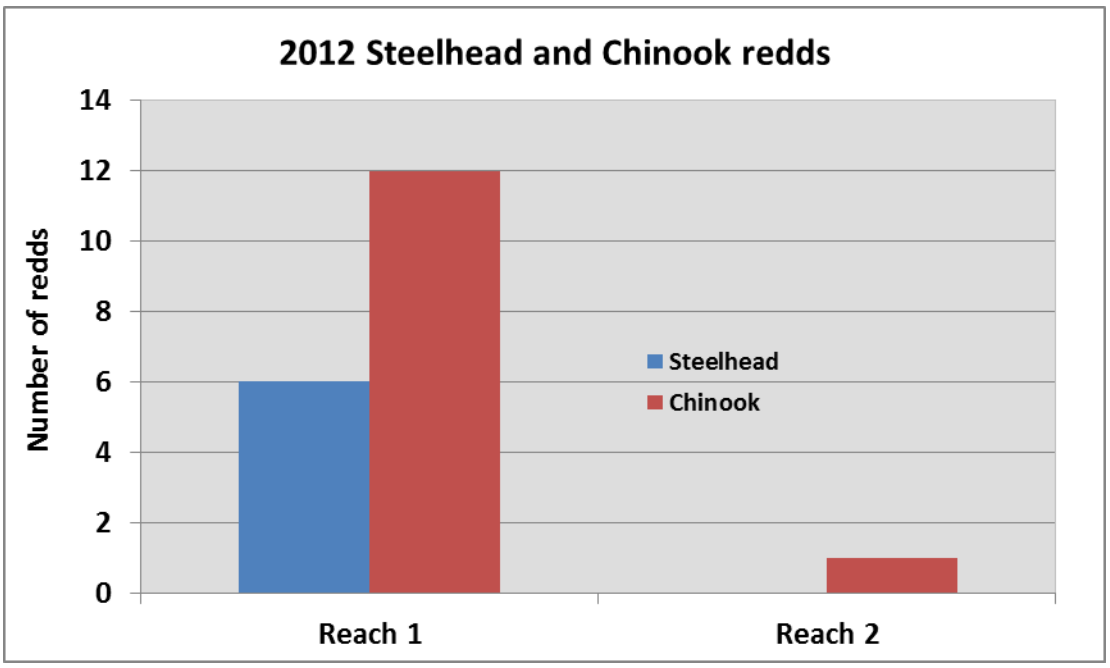


Figure 10. Numbers of Chinook and steelhead redds in Hancock Springs by reach during 2012.

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).

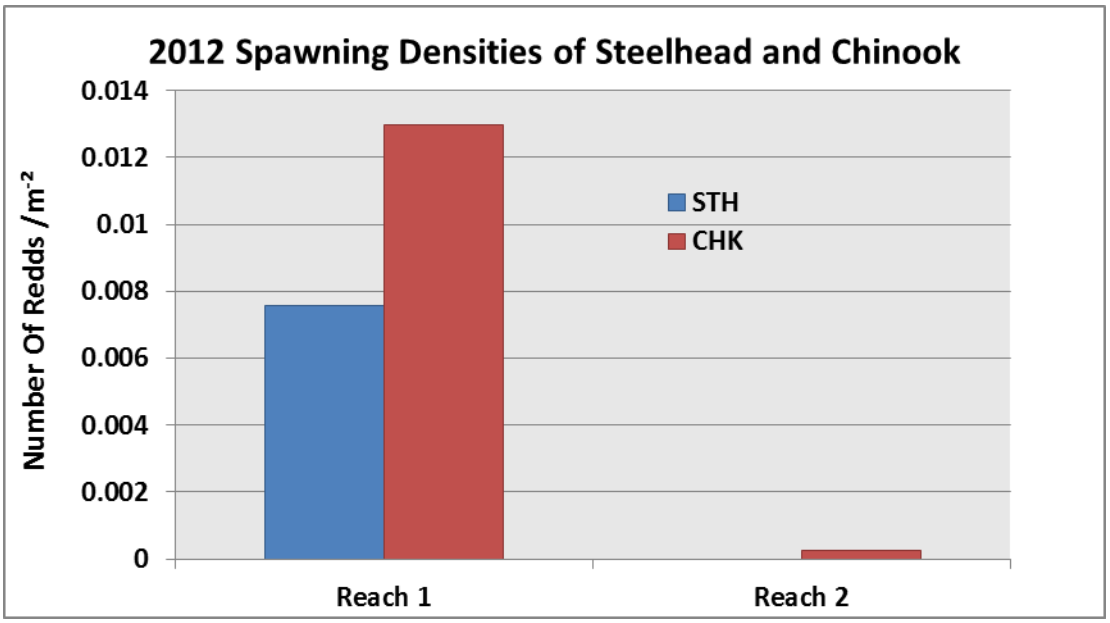


Figure 11. Chinook and Steelhead spawning densities (redds/m^2) in Hancock Springs by reach during 2012.

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.

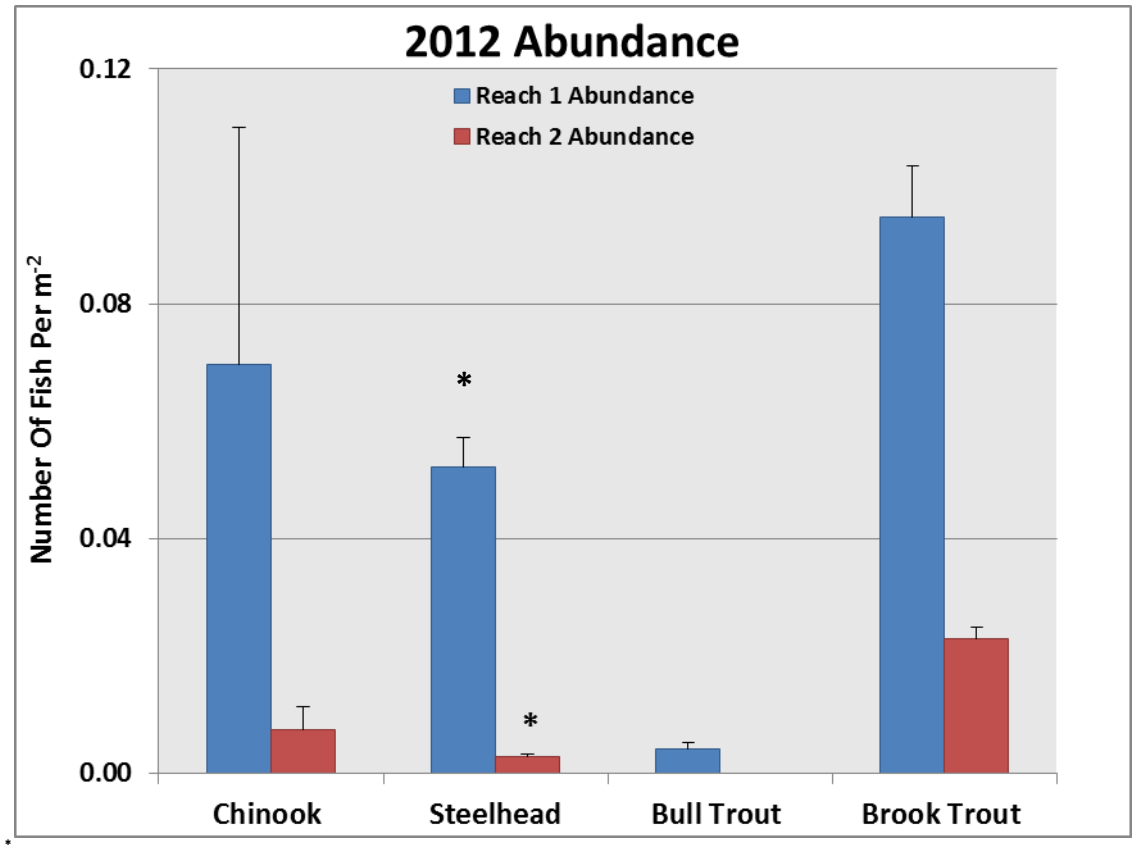


Figure 12. Abundance of Chinook, Steelhead, Brook Trout, and Bull Trout in Hancock Springs during 2012. Error bars represent one standard error. Asterisks denote statistical significance (*p<0.05*).

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.

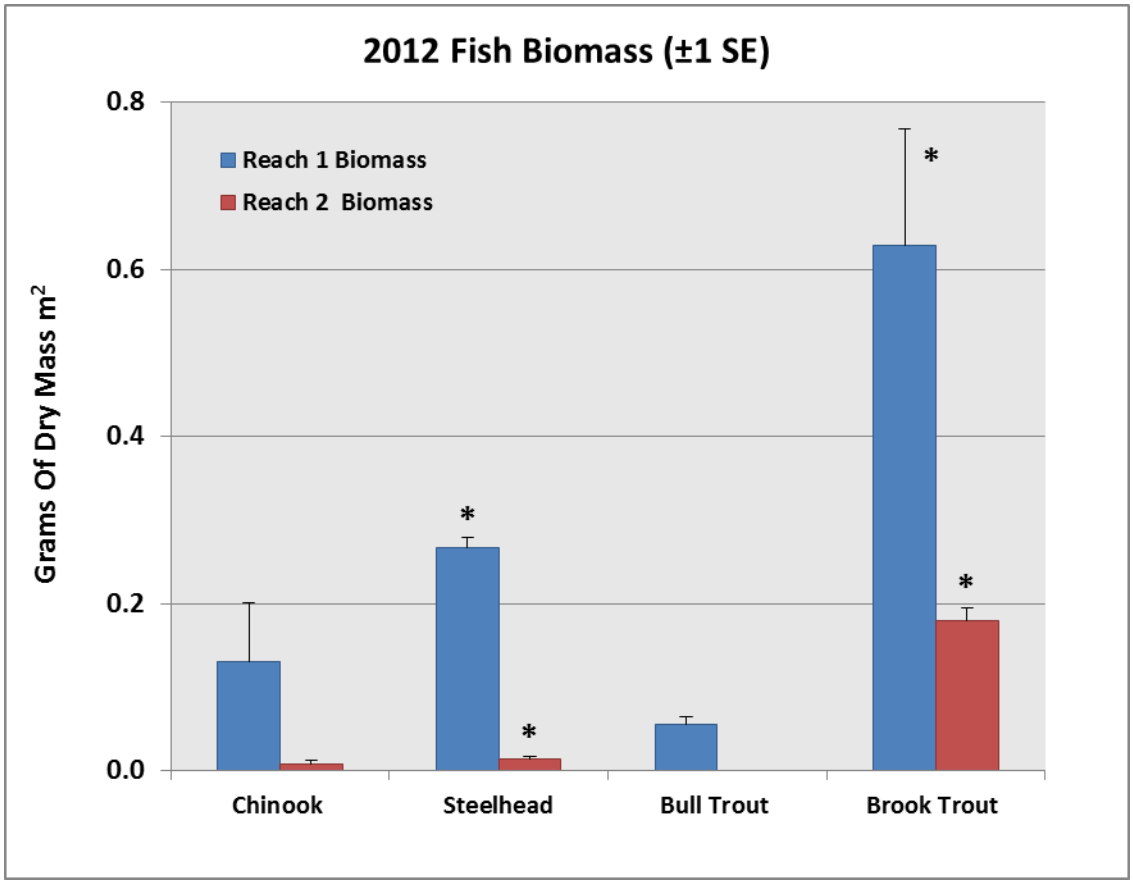


Figure 13. Biomass of Chinook, Steelhead, Brook trout, and Bull Trout in Hancock Springs during 2012. Error bars represent one standard error. Asterisks denote statistical significance (*p<0.05*).

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).

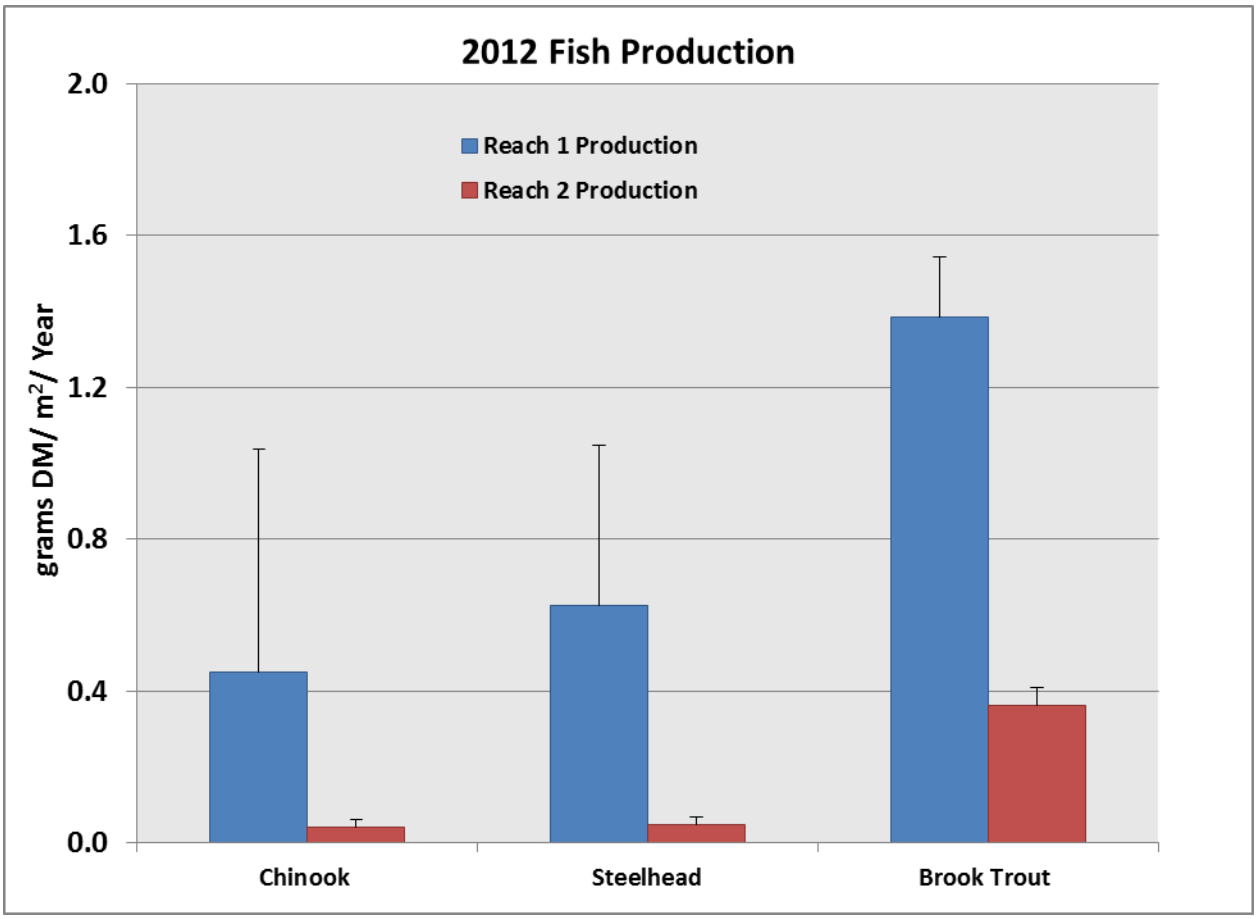


Figure 14. Production of Chinook, Steelhead, Brook Trout, and Bull Trout in Hancock Springs during 2012.

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.

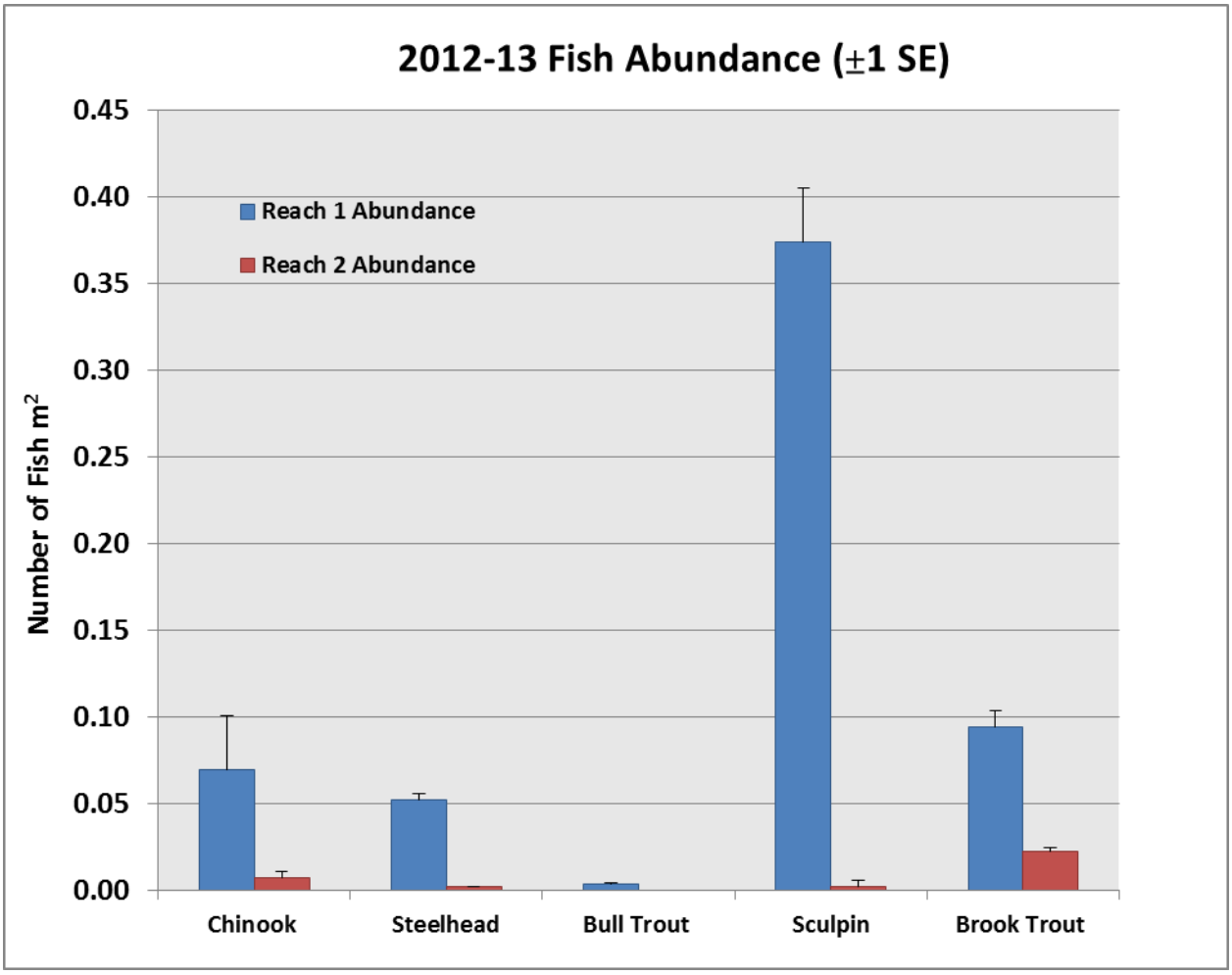


Figure 15. Biomass of Chinook, Steelhead, Brook Trout, and Bull Trout in Hancock Springs during 2012 and 2013 combined. Error bars represent one standard error. Asterisks denote statistical significance (*p<0.05*).

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.

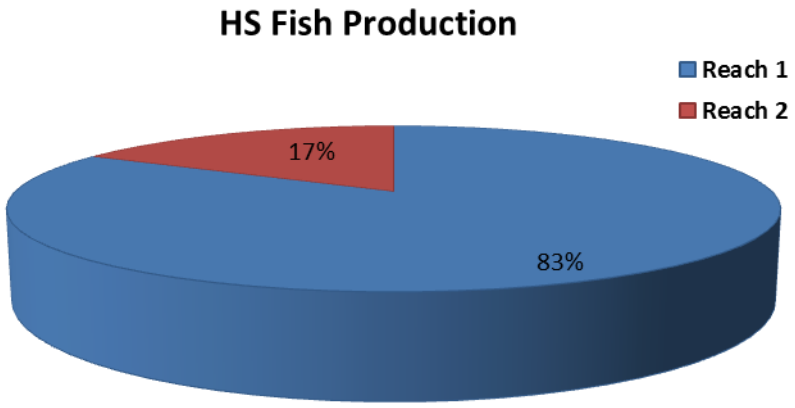


Figure 16. Distribution of fish production by reach in Hancock Springs, 2012 and 2013.

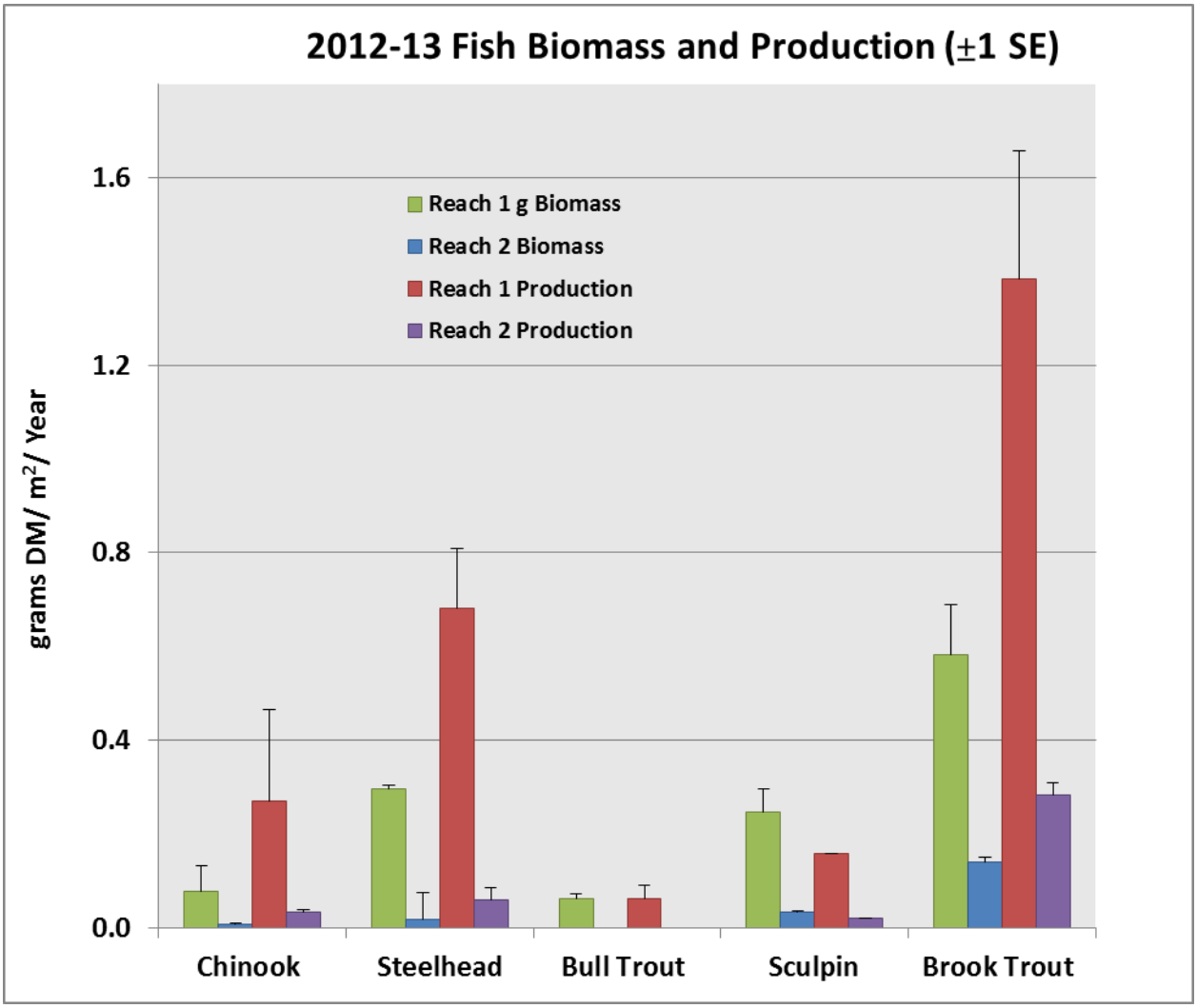


Figure 17. Biomass and production of Chinook, Steelhead, Brook Trout, and Bull Trout in Hancock Springs during 2012 and 2013 combined. Error bars represent one standard error.

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).

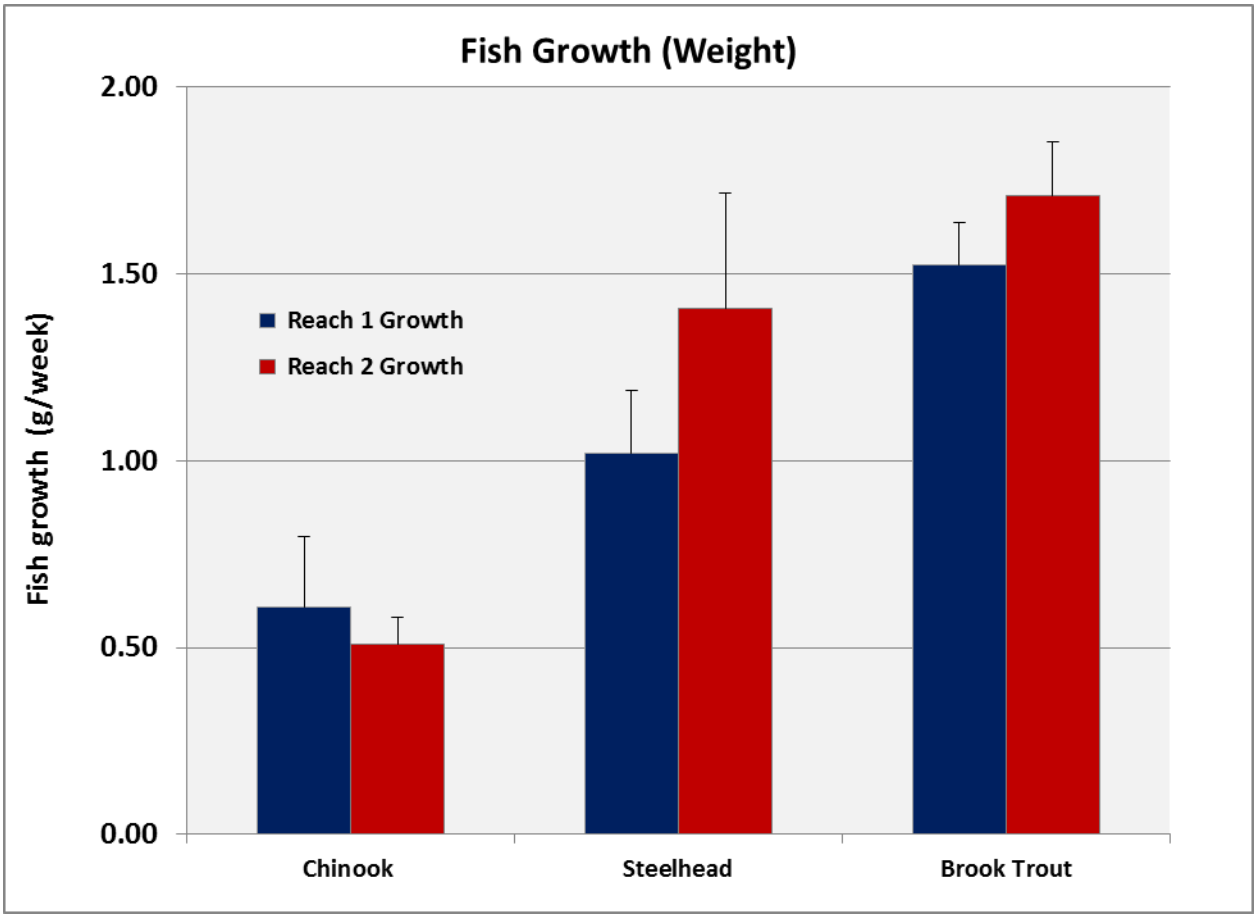


Figure 18. Growth (g/week) of Chinook, Steelhead, and Brook Trout in Hancock Springs during 2012 and 2013 combined. Error bars represent one standard error.

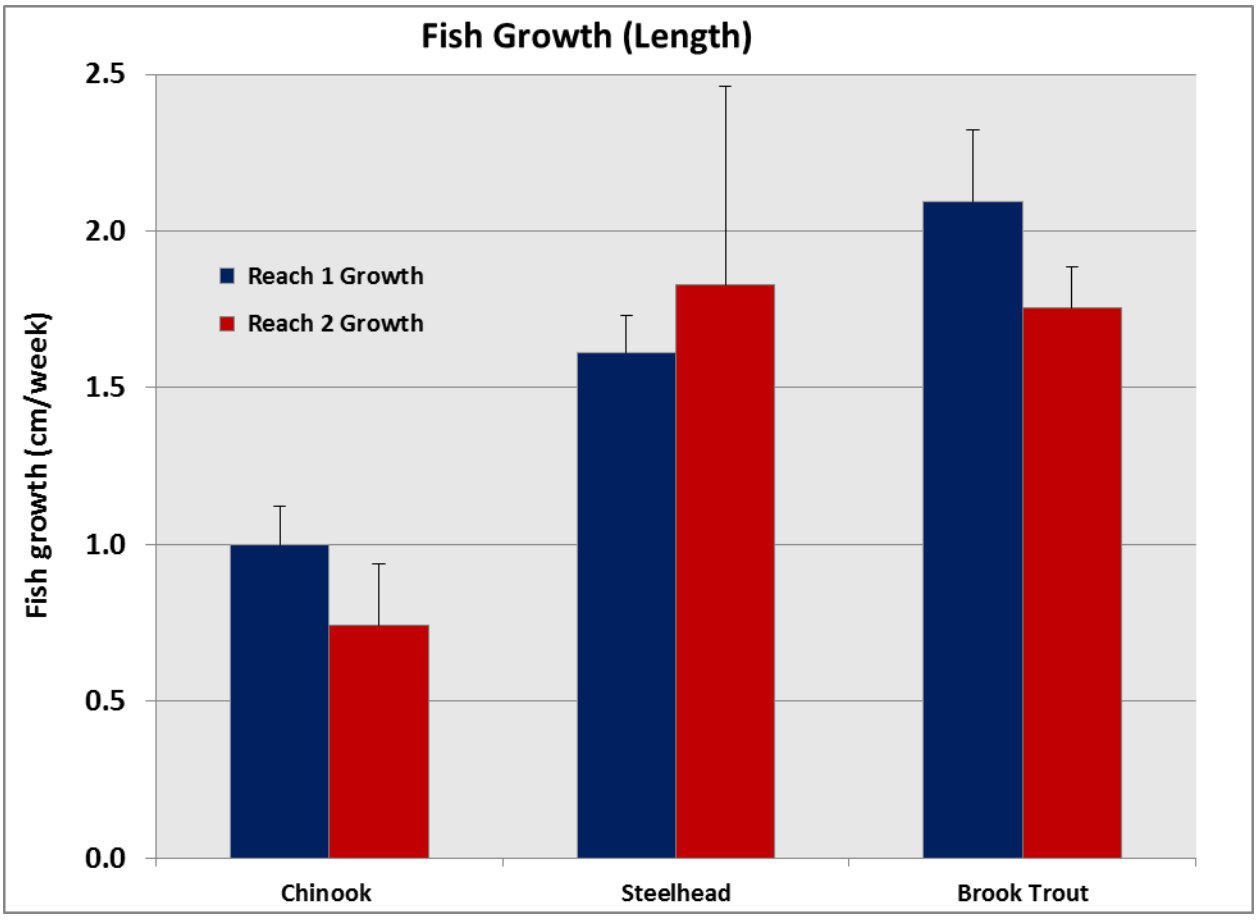


Figure 19. Growth (TL; mm/week) of Chinook, Steelhead, and Brook Trout in Hancock Springs during 2012 and 2013 combined. Error bars represent one standard error.

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.

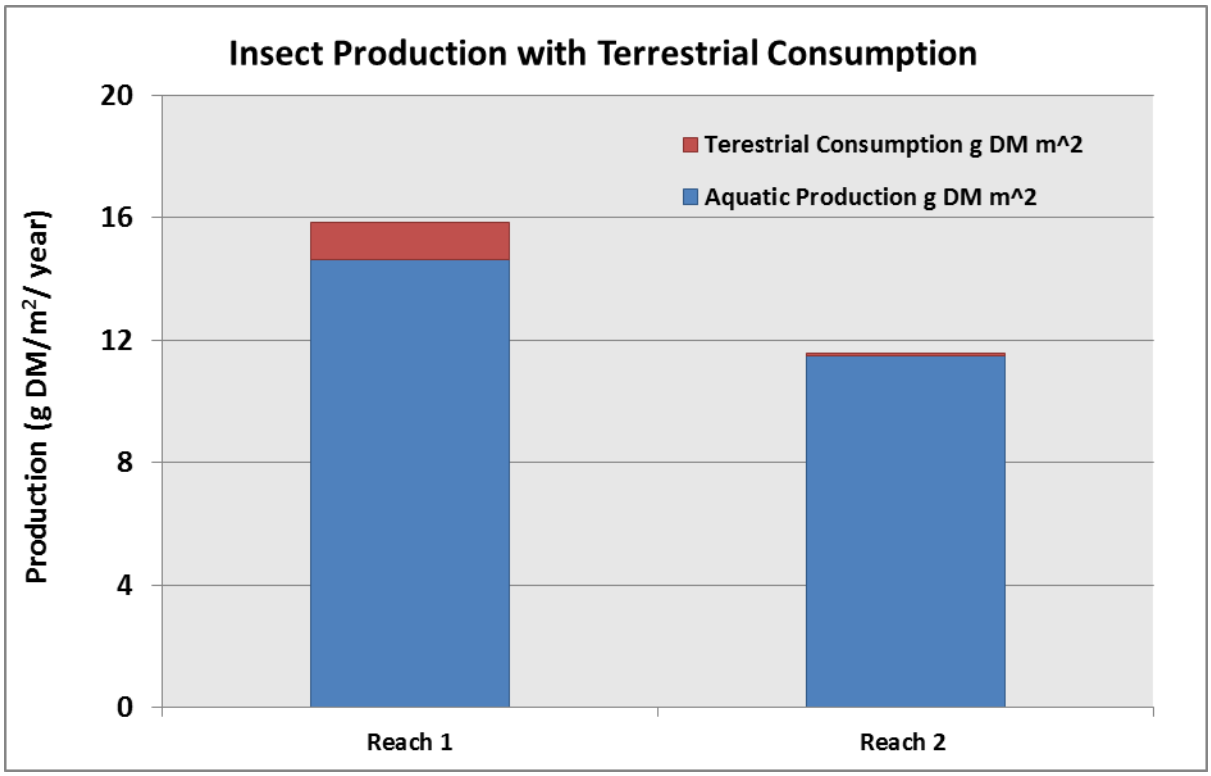
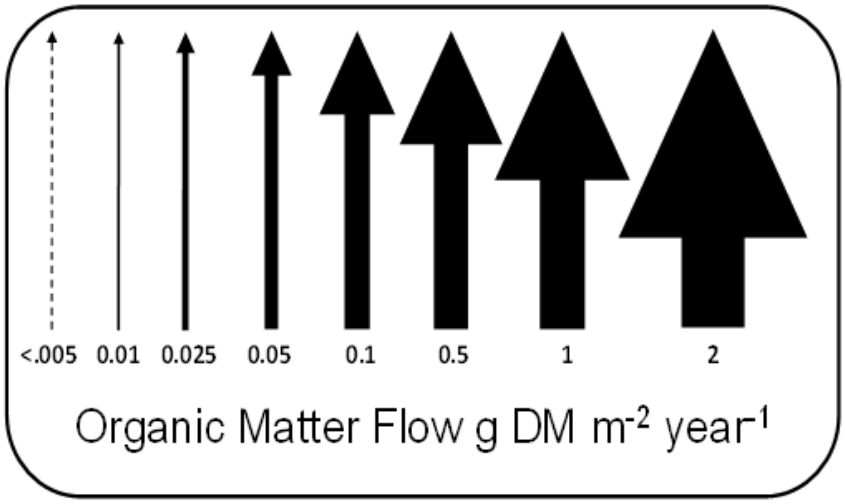


Figure 20. Aquatic and terrestrial insect production in Hancock Springs by reach, 2012 and 2013.

Flow webs

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.



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.

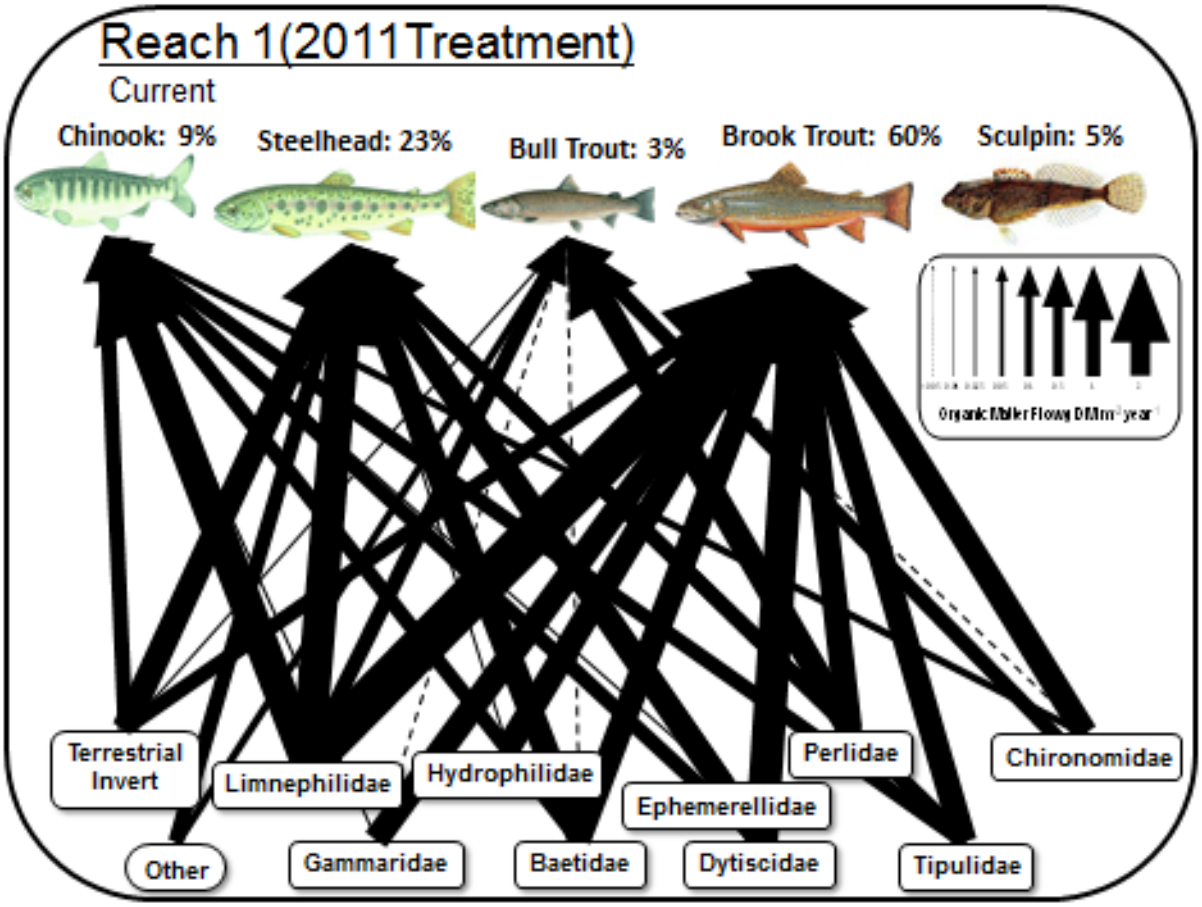


Figure 21. Flow web illustrating proportions of invertebrate consumption by dominant fish species and invertebrate families in Reach 1 of Hancock Springs, 2012 and 2103.

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).

Table 11. 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**		

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.

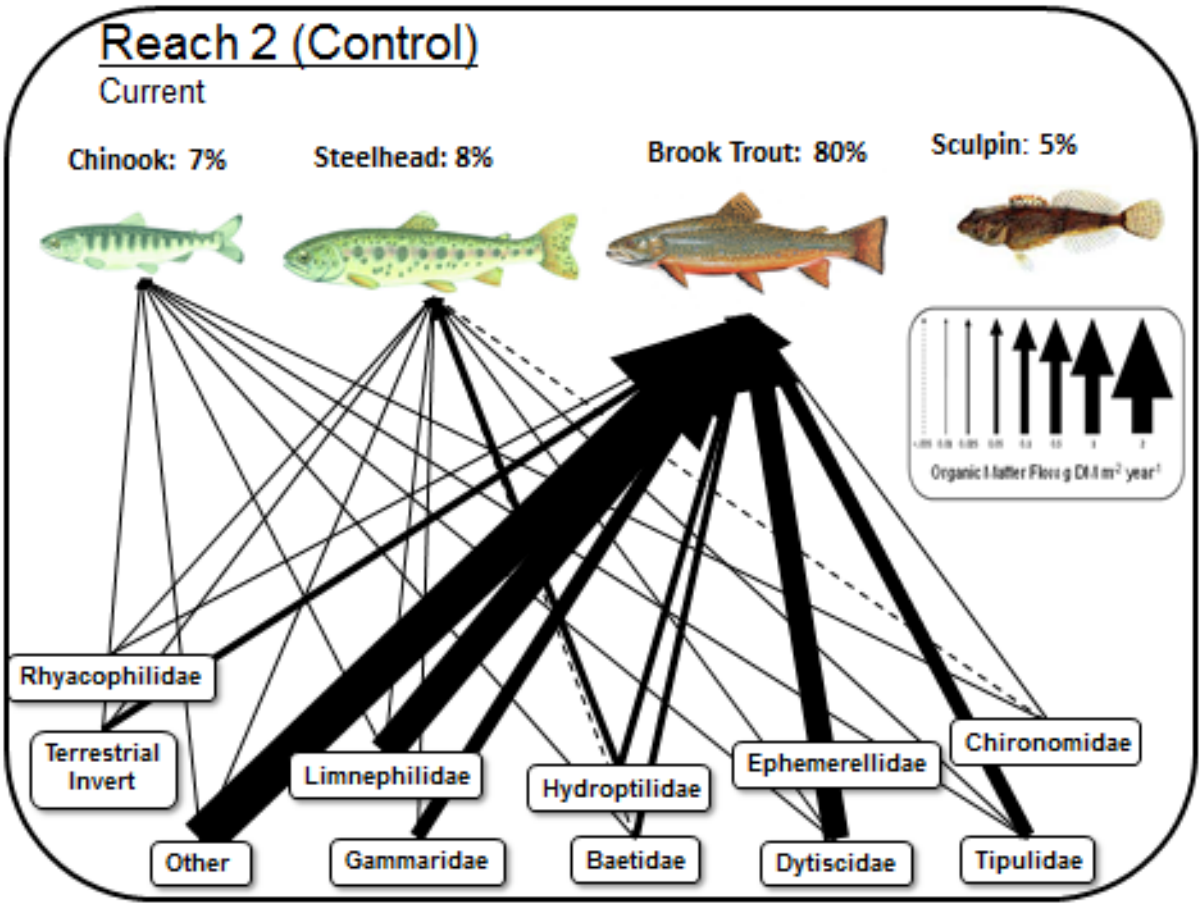


Figure 22. Flow web illustrating proportions of invertebrate consumption by dominant fish species and invertebrate families in Reach 2 of Hancock Springs, 21012 and 2103.

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).

Table 12. 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**		

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).

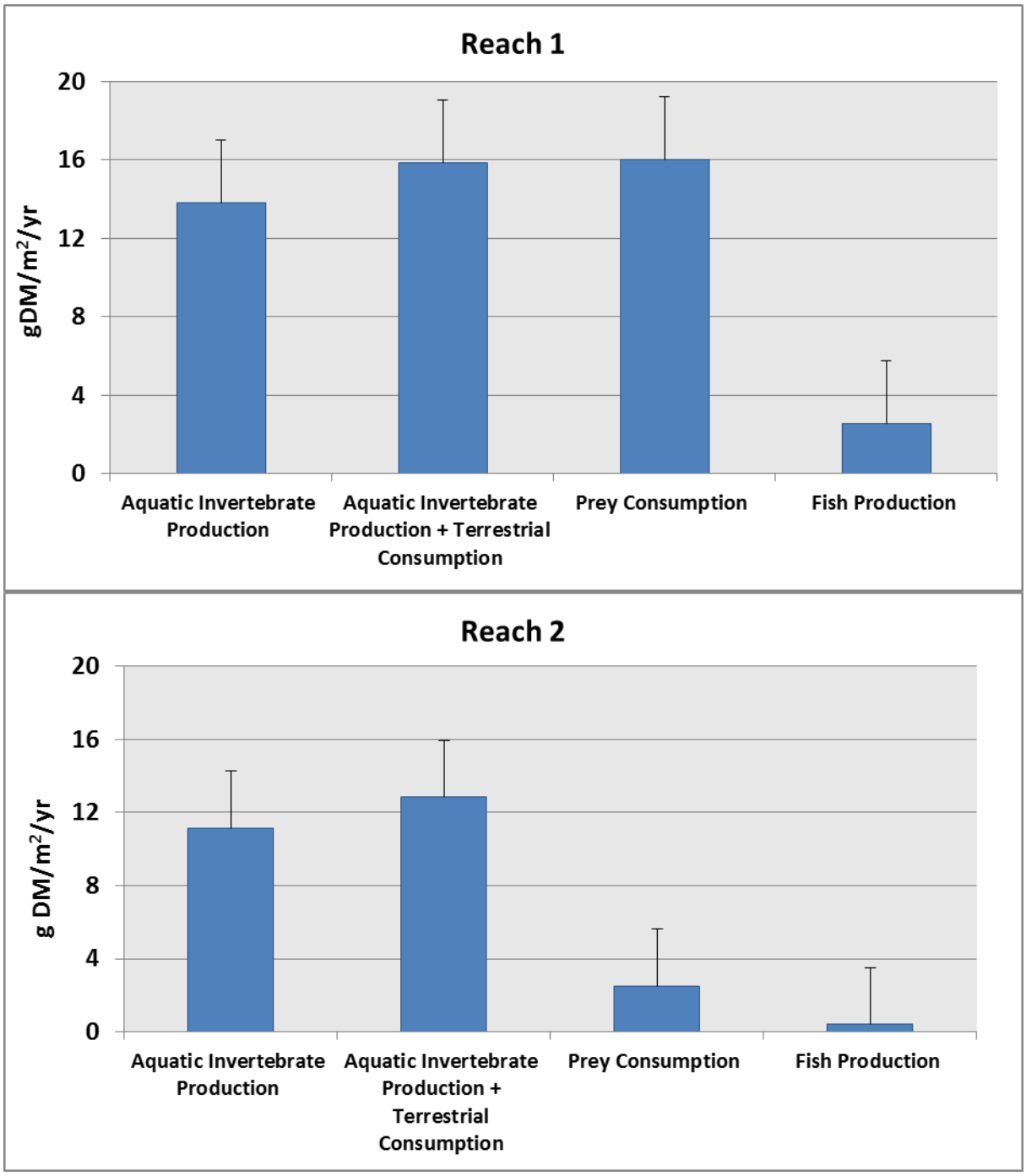


Figure 23. Aquatic invertebrate consumption with and without terrestrial origin taxa, invertebrate consumption by fish, and fish production in Reach 1 and Reach 2 of Hancock Springs during 2012.

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*).

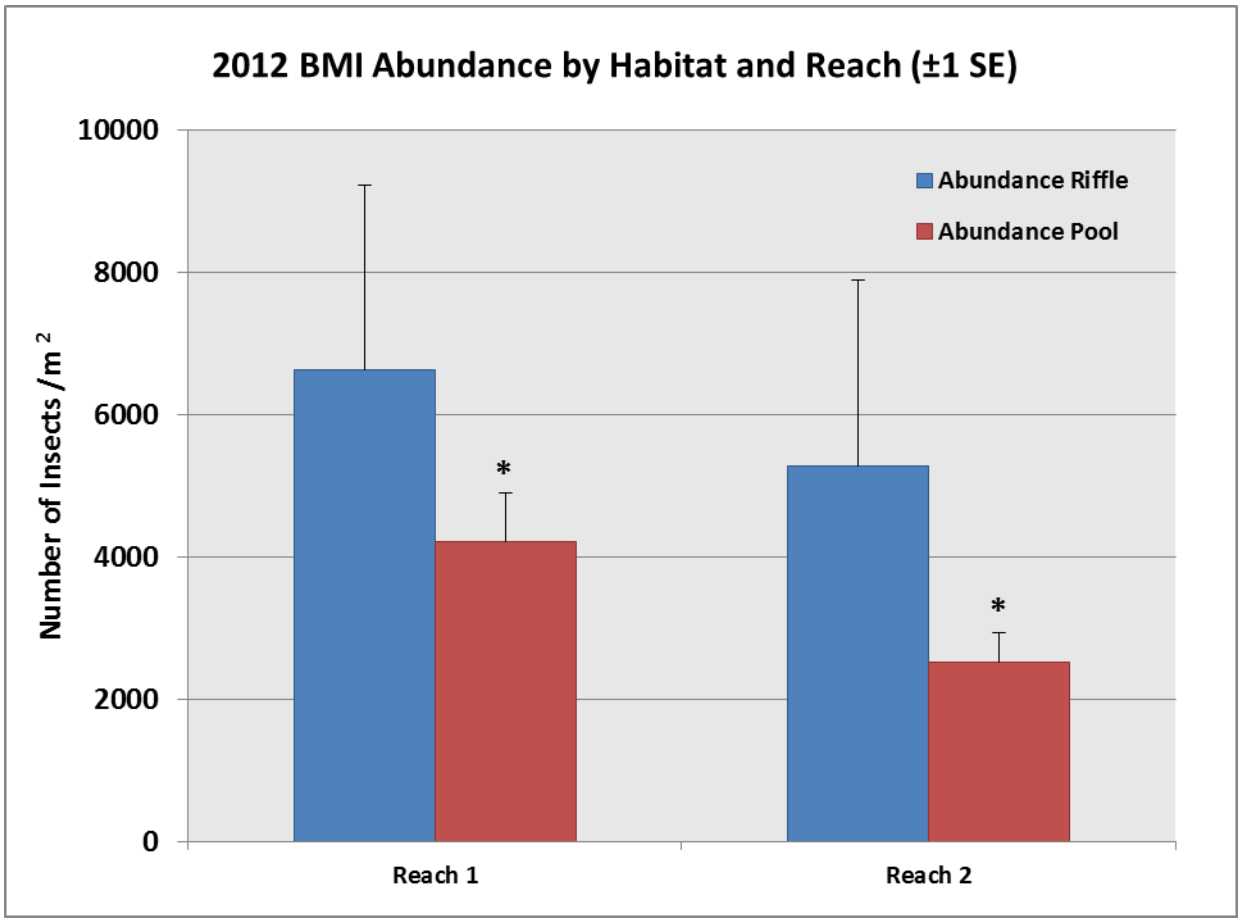


Figure 24. Abundance and biomass of benthic macroinvertebrates in Hancock Springs during 2012 by reach and habitat type (pools, riffles). Error bars represent one standard error. Asterisk denotes statistical significance (*p<0.05*).

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).

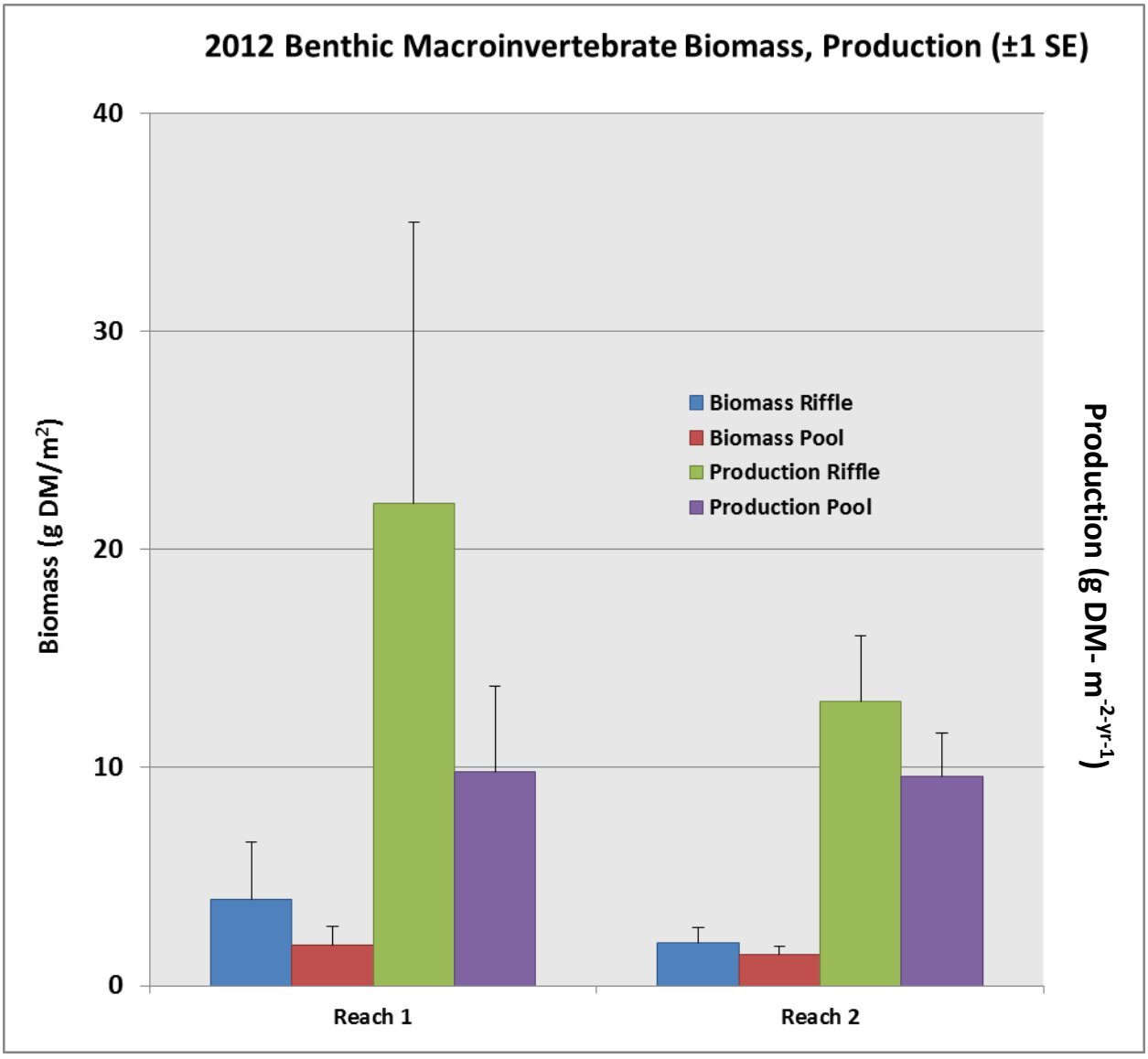


Figure 25. Benthic macroinvertebrate biomass and production in both reaches of Hancock Springs, 2012. Error bars represent one standard error.

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).

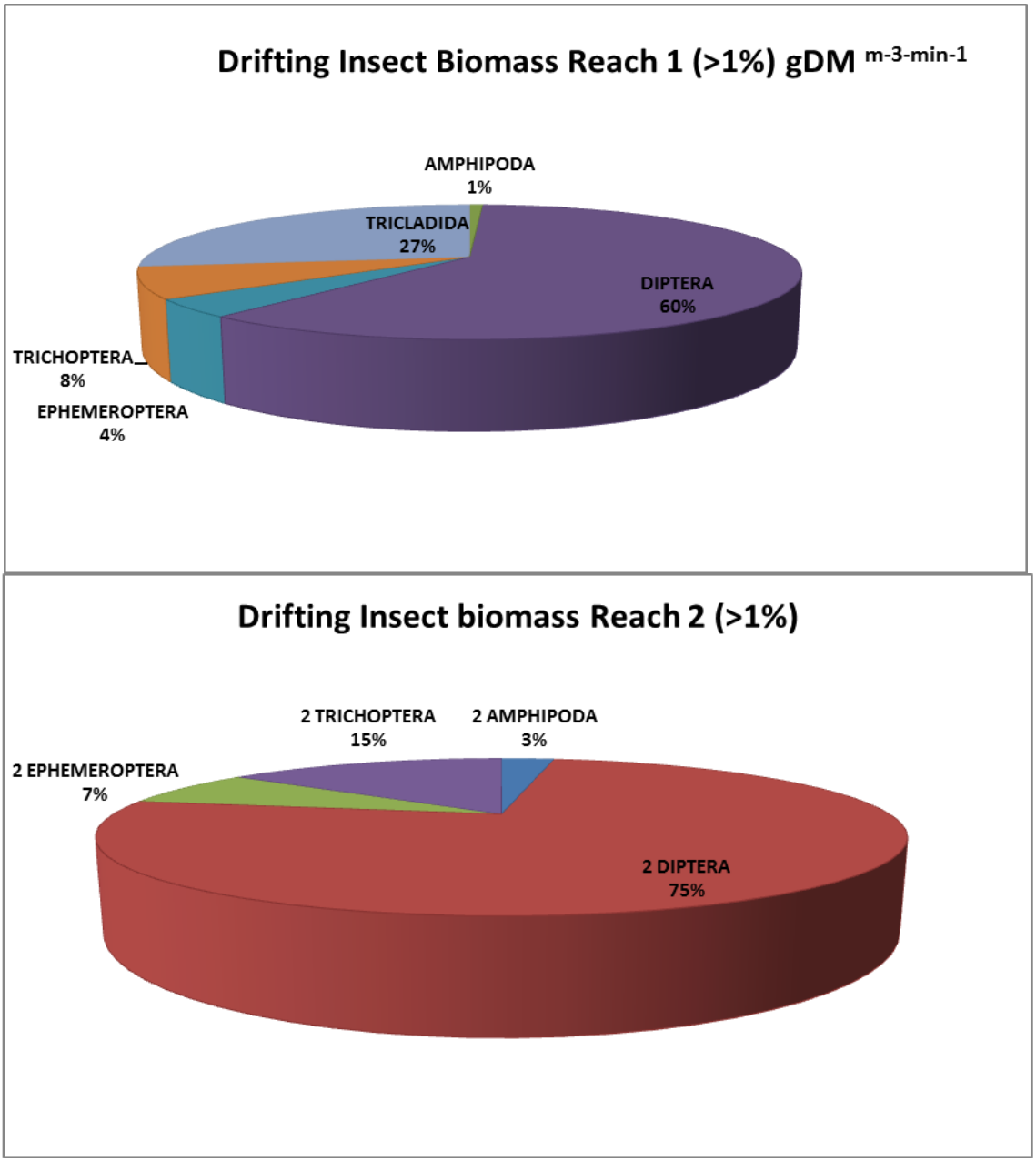


Figure 26. Percent biomass contribution of invertebrate Orders in invertebrate drift samples from Hancock Springs by reach, 2012 and 2103 combined data.

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).

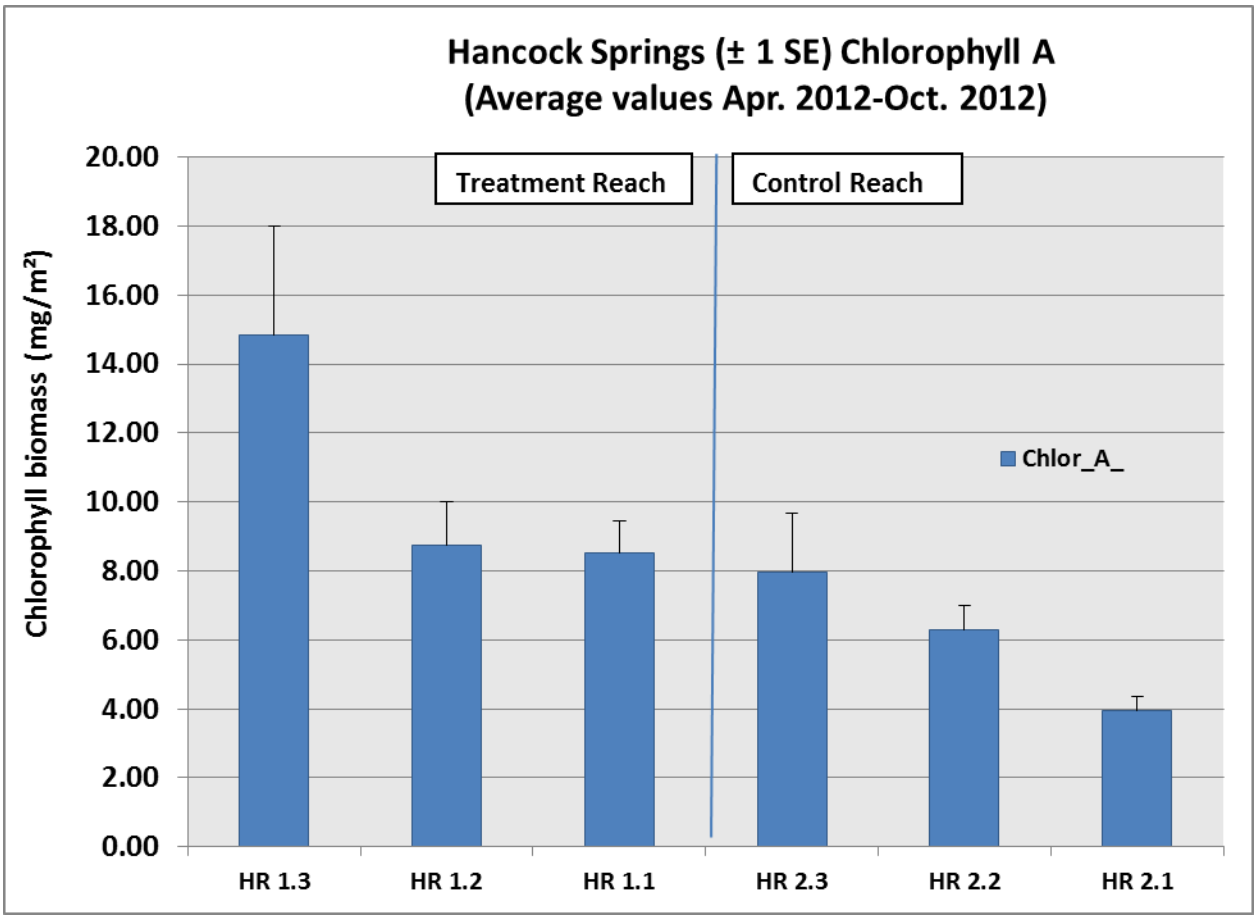


Figure 27. Chlorophyll a biomass (mg/m^2) in Hancock Springs, April through October, 2012. Reach 1 sites include HR1.1, 1.2, and 1.3; Reach 2 sites include HR2.1, 2.2, and 2.3. Error bars represent one standard error.

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).

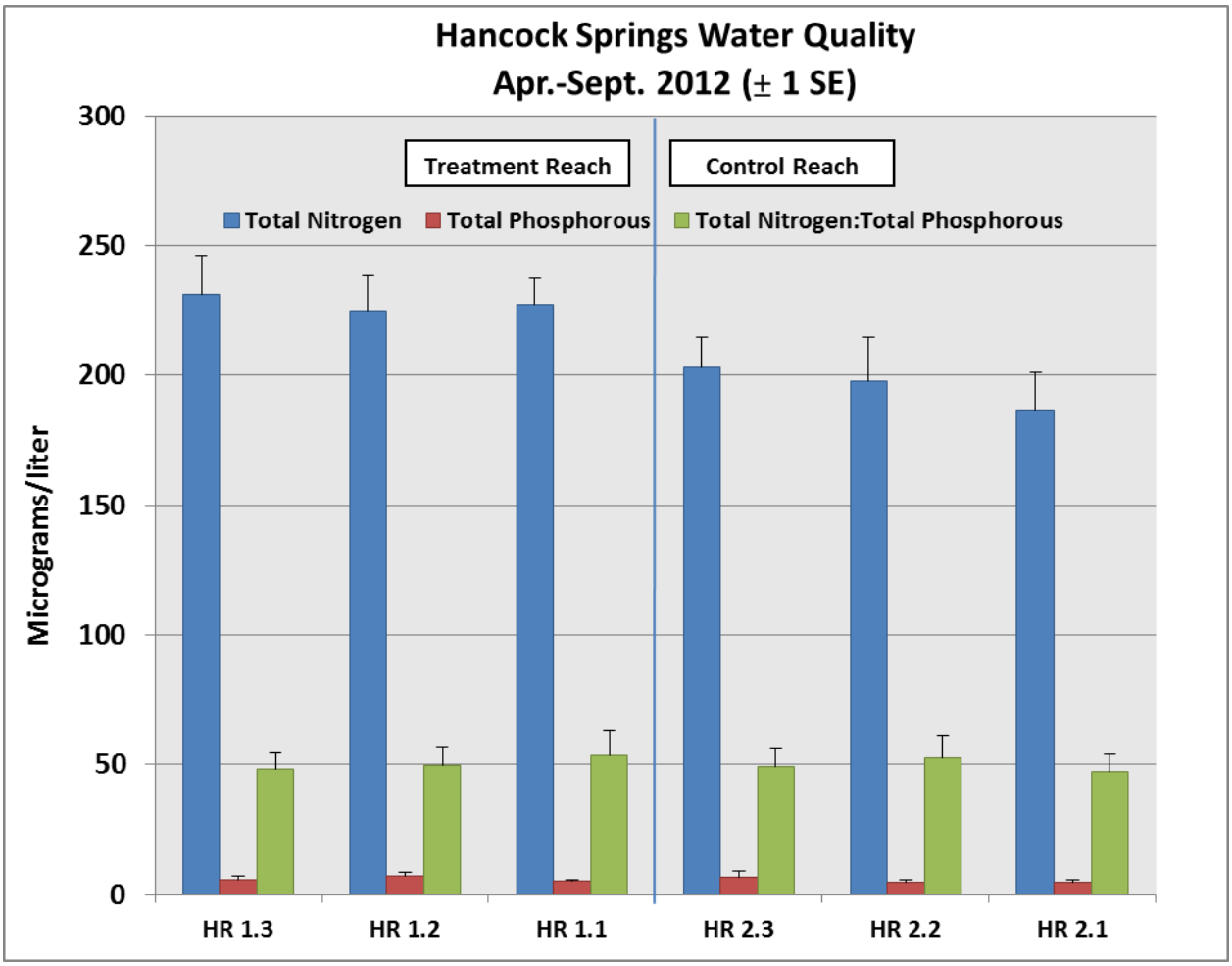


Figure 28. Total nitrogen (TN), total phosphorus (TP) and their ratio values (TN:TP) for Hancock Springs, April through September 2012. Error bars represent one standard error.

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).

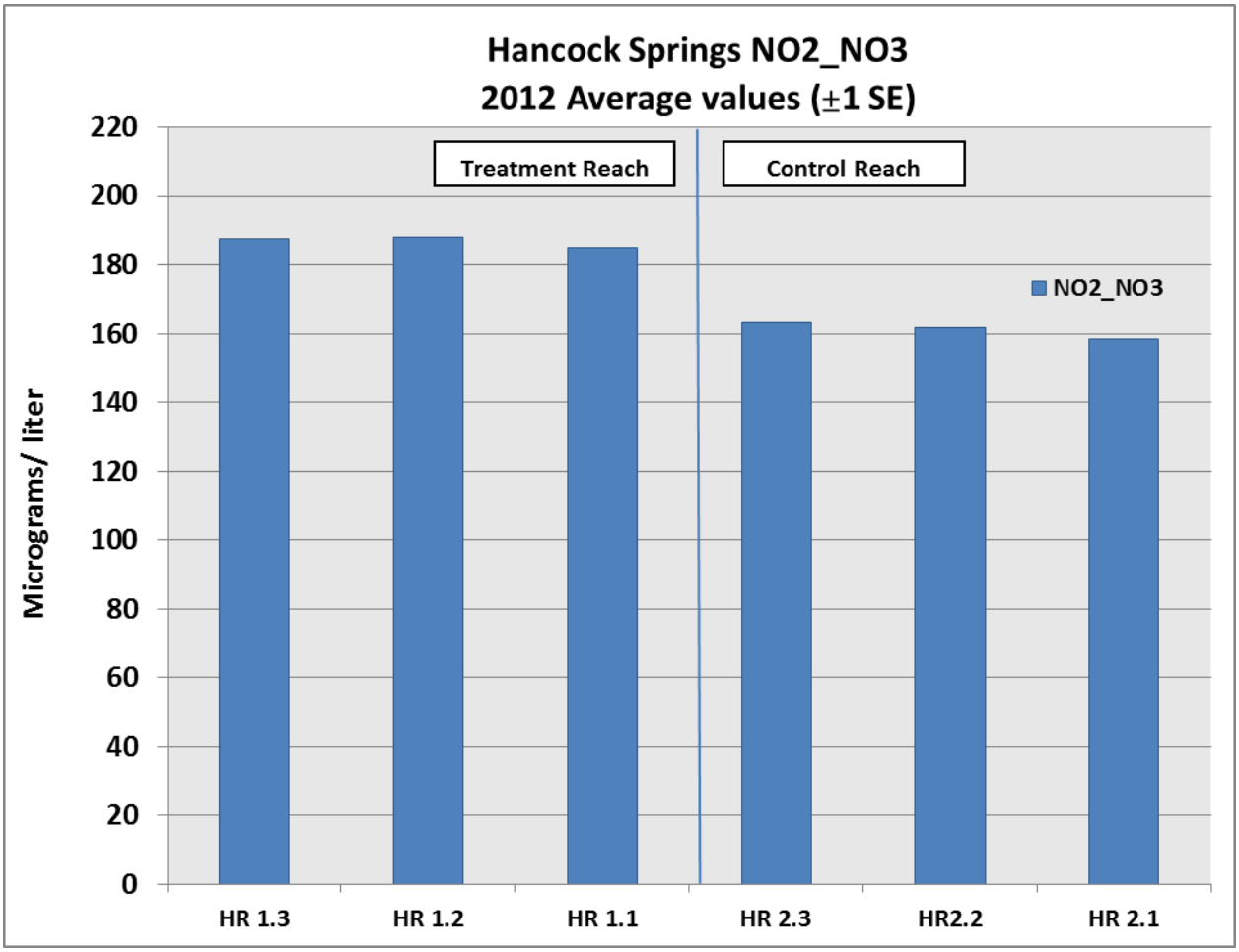


Figure 29. Mean nitrite (NO^2)+ nitrite (NO^3) concentrations in Hancock Springs, 2012.

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).

Table 13. 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%)

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.

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).

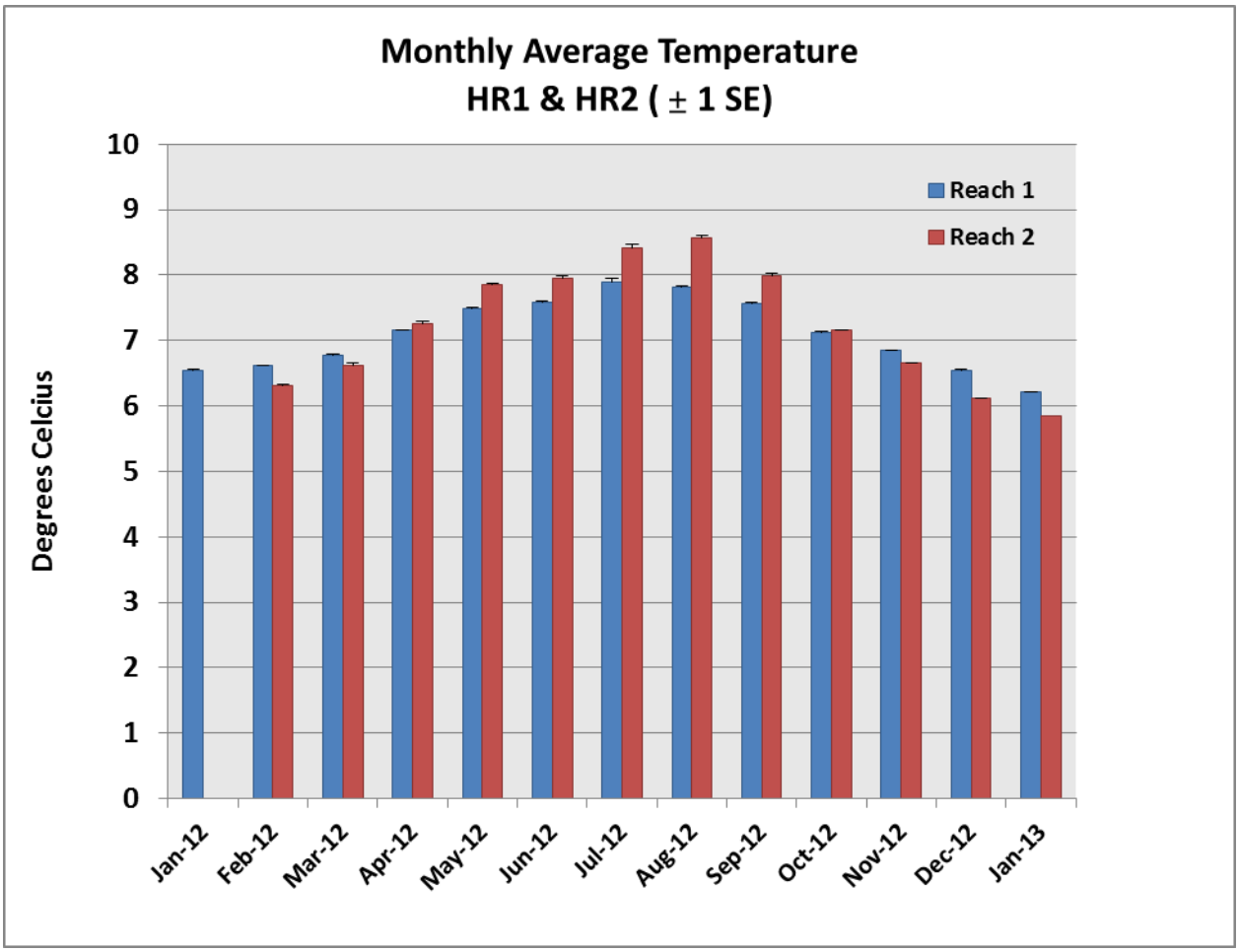


Figure 30. Mean monthly water temperature in Hancock Springs by reach from January 2012 through January 2013. Error bars represent one standard error.

Twisp River

Twisp River – Phase 1 (2008-2012)

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).

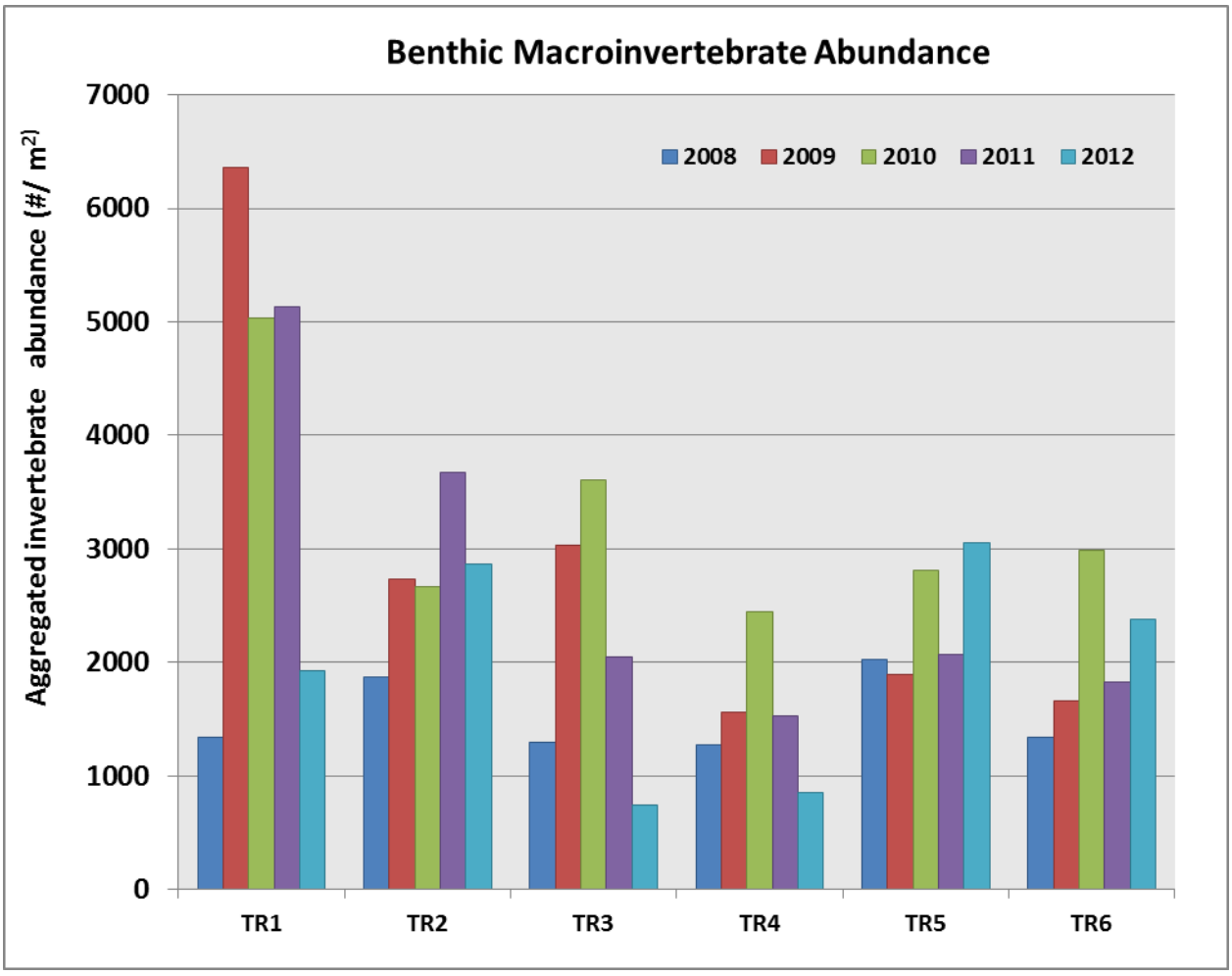


Figure 31. Abundance of aggregated benthic macroinvertebrate taxa (#/m^2) for the Twisp River, sites TR1 through TR6, 2008 through 2012.

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).

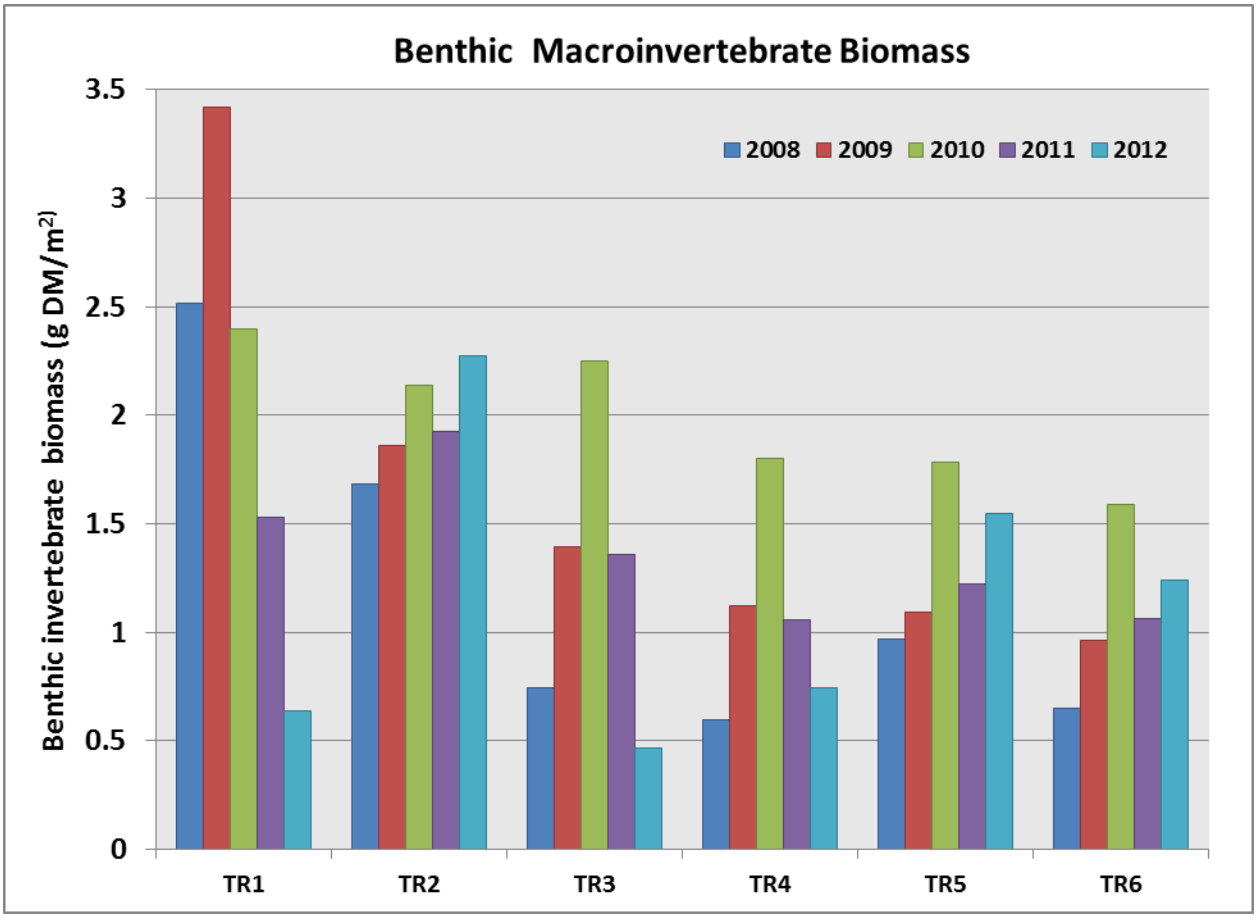


Figure 32. Benthic macroinvertebrate biomass (dry mass, g/m^2) for the Twisp River, sites TR1 through TR6, 2008 through 2012.

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).

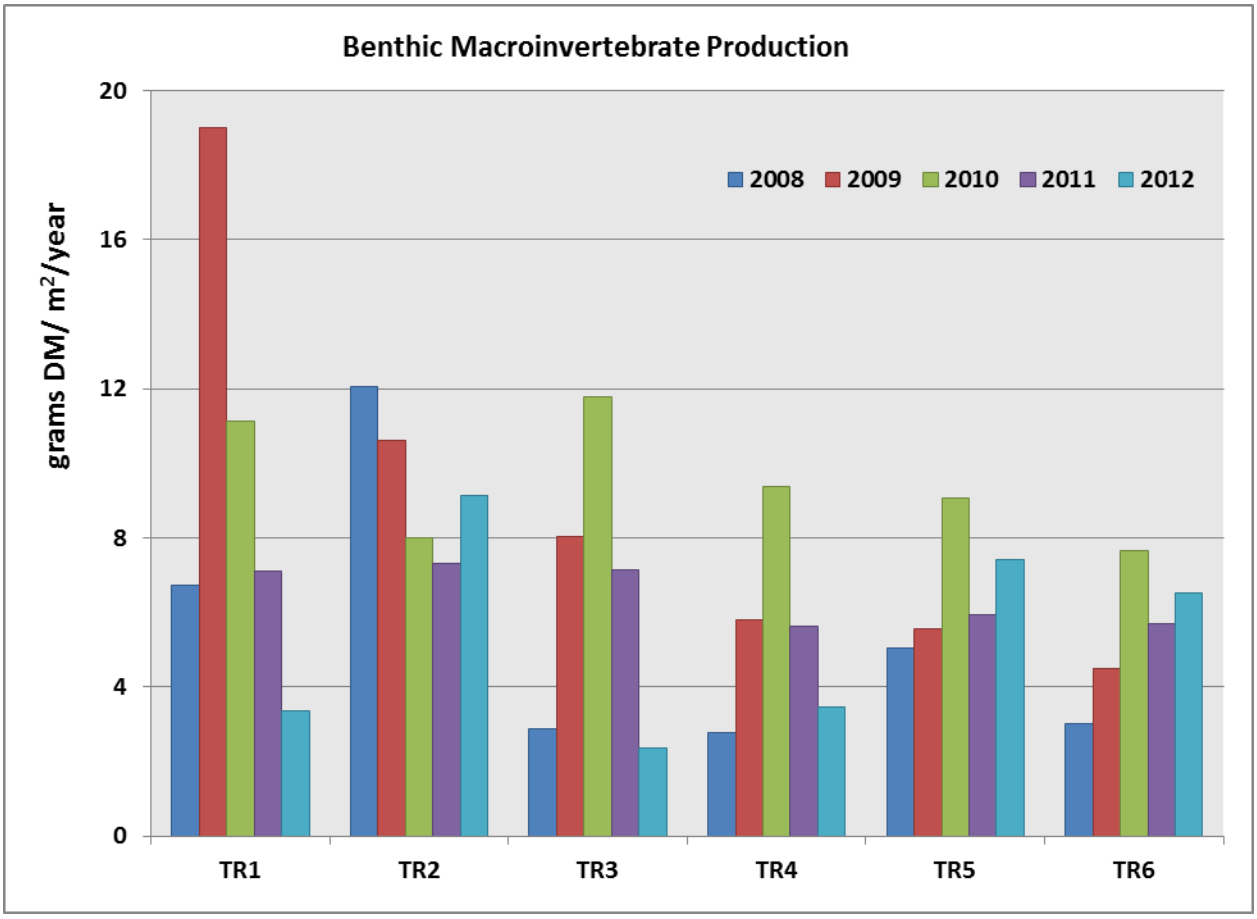


Figure 33. Benthic macroinvertebrate production (dry mass, g/m^2/yr) for the Twisp River, sites TR1 through TR6, 2008 through 2012.

Twisp River – Phase 2 (2012-2015)

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).

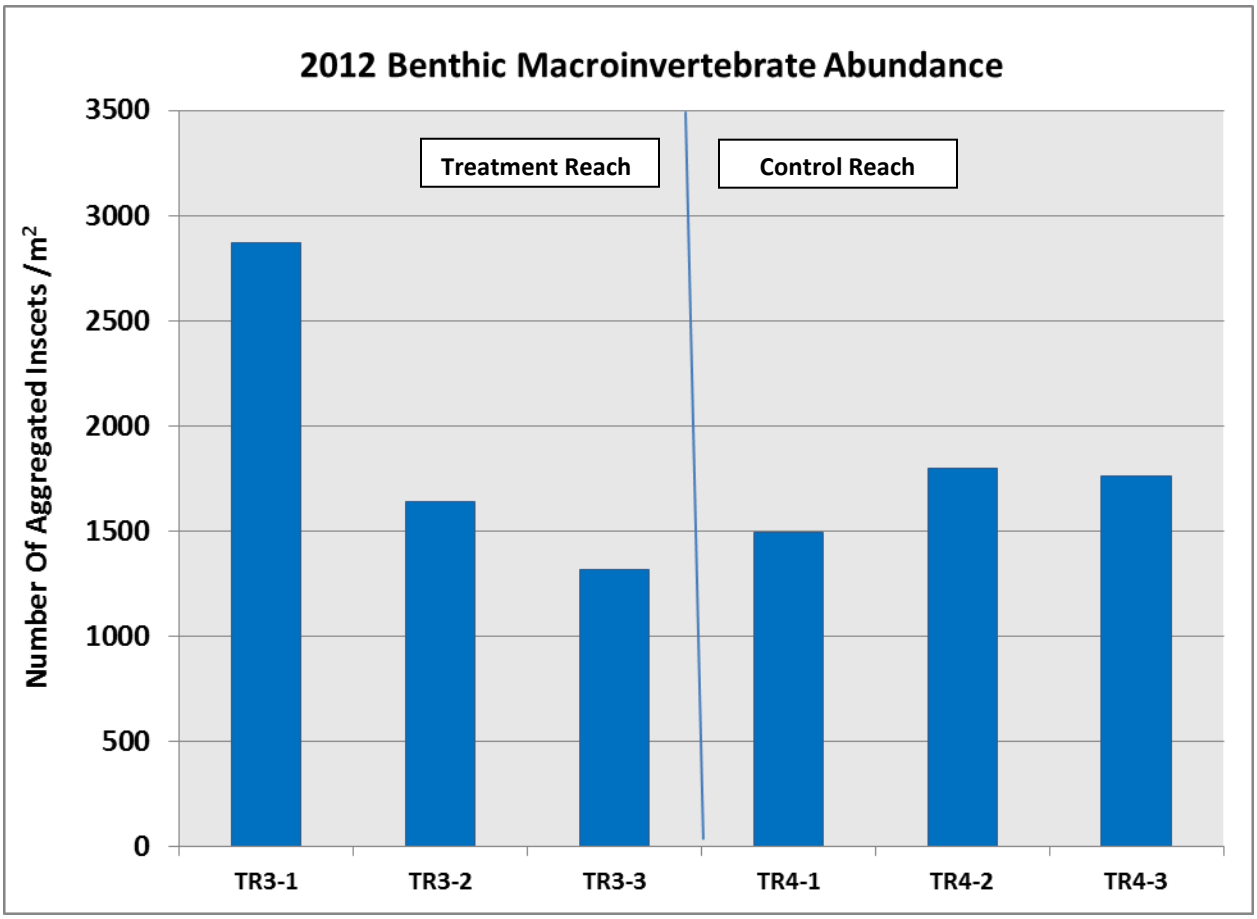


Figure 34. Benthic macroinvertebrate abundance (#/m^2) in the Twisp River for the control (TR4.1 through TR4.3) and treatment (TR4.1 through TR4.3) reaches during 2012.

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).

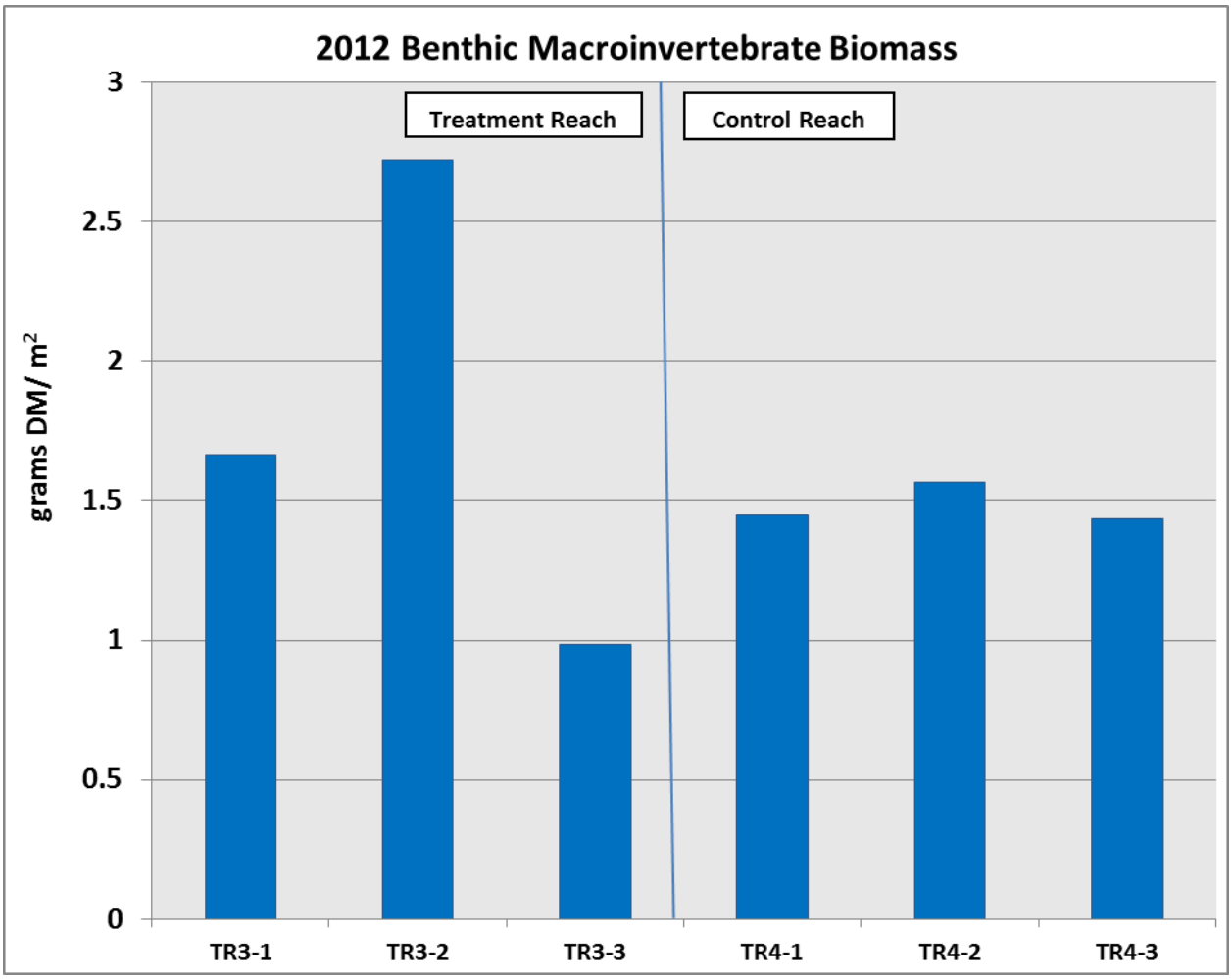


Figure 35. Benthic macroinvertebrate biomass (dry weight, g/m^2) in the Twisp River for the control (TR4.1 through TR4.3) and treatment (TR4.1 through TR4.3) reaches during 2012.

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).

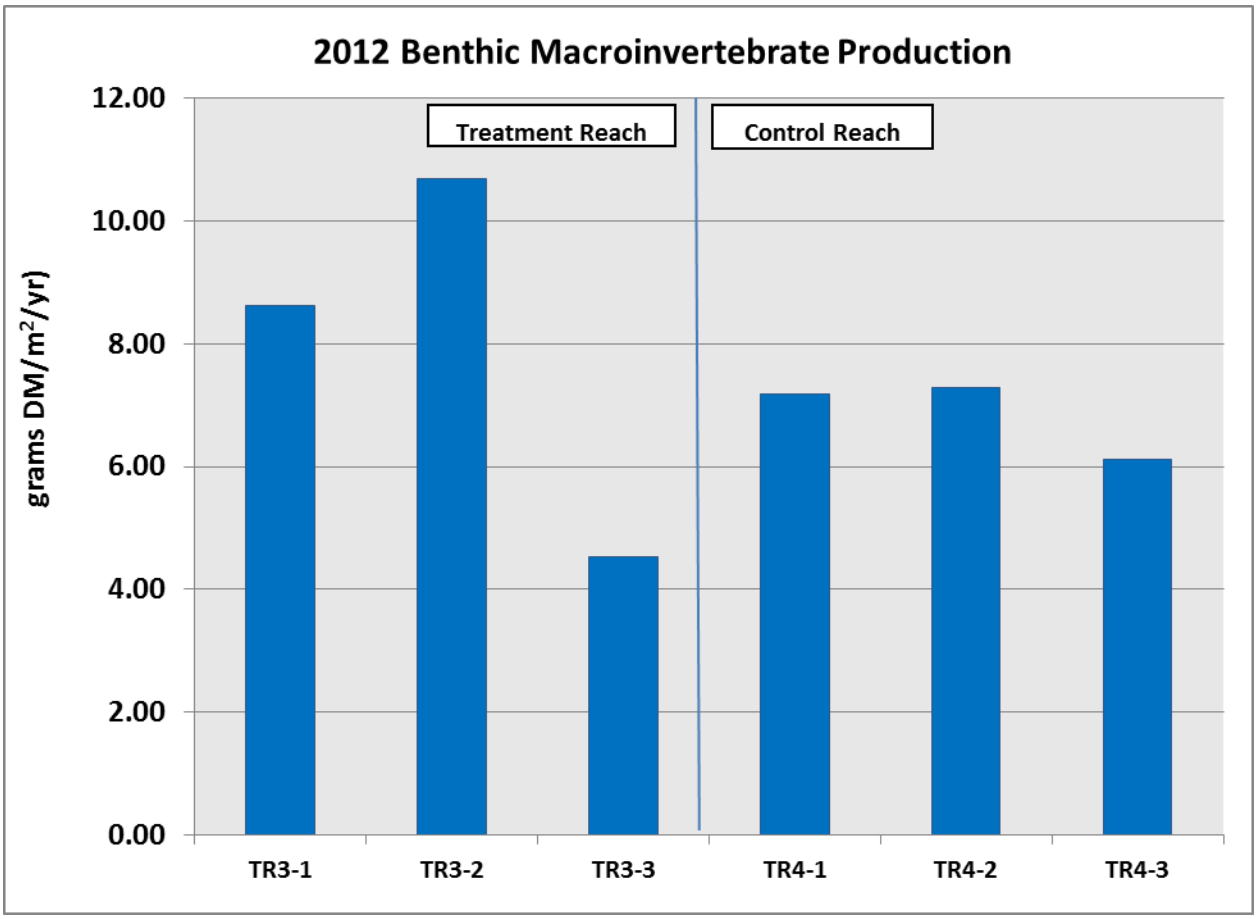


Figure 36. Benthic macroinvertebrate production (dry weight, g/m^2/yr) in the Twisp River for the control (TR4.1 through TR4.3) and treatment (TR4.1 through TR4.3) reaches during 2012.

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).

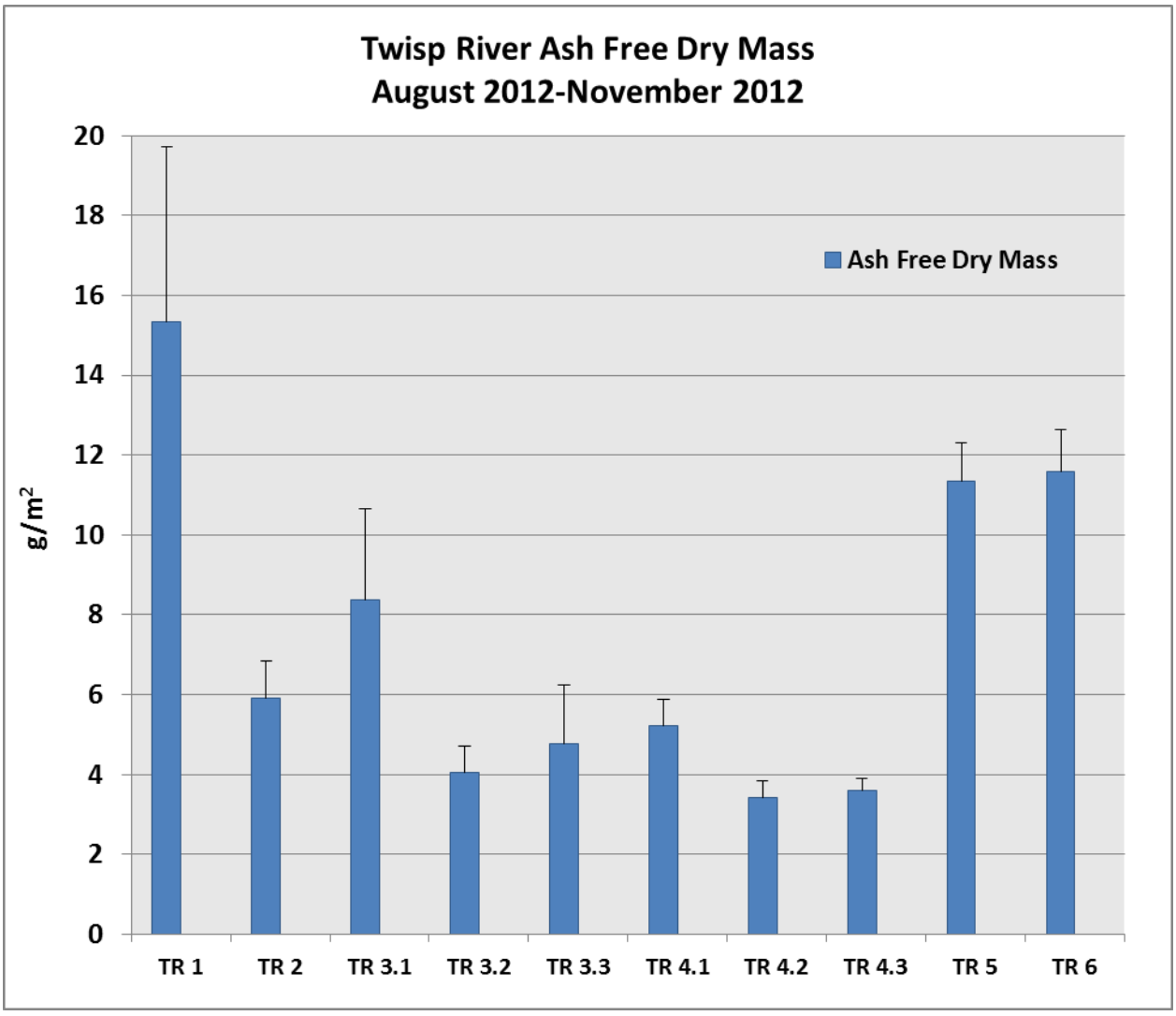


Figure 37. Ash free periphyton biomass (g/m^2) from the Twisp River, August through November, 2012. Error bars represent one standard error.

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).

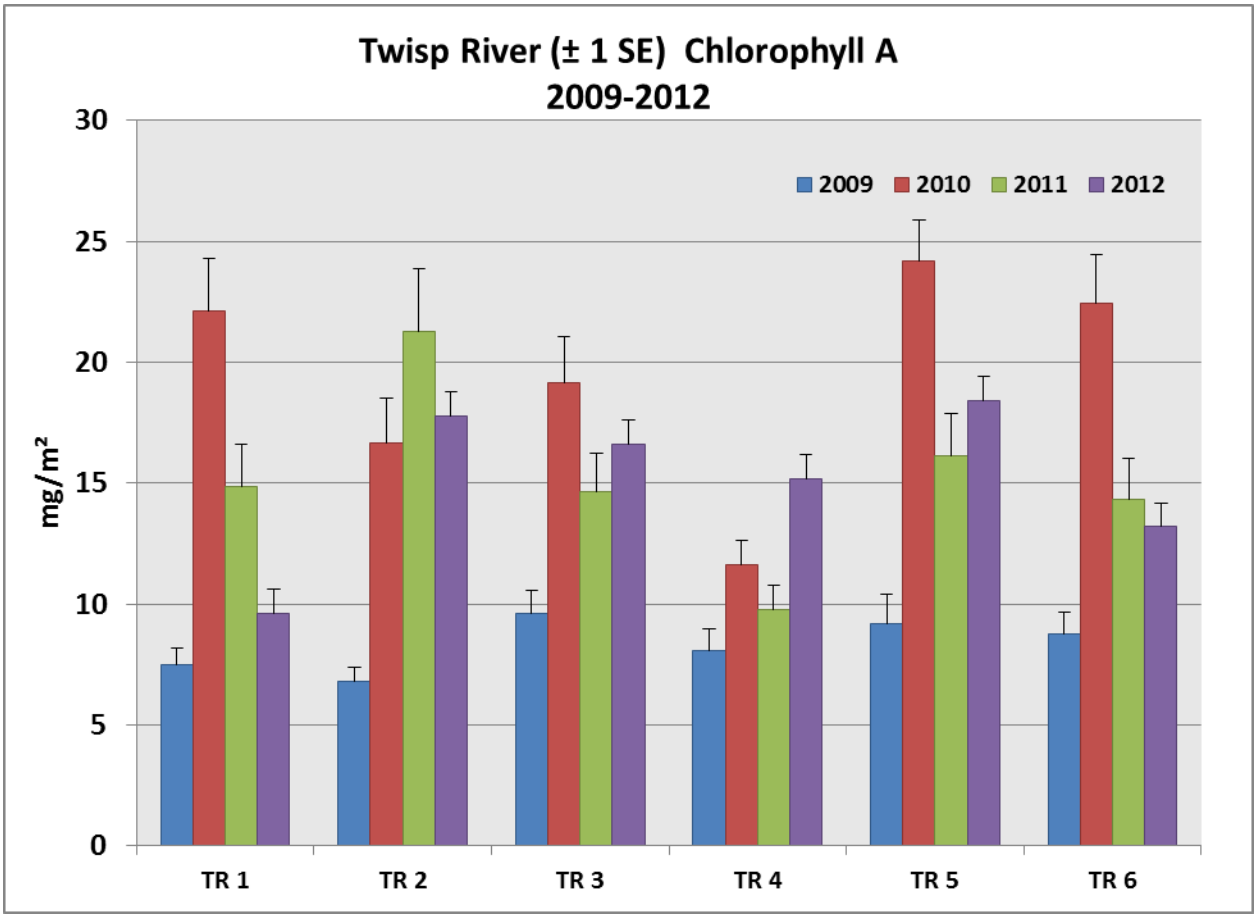


Figure 38. Chlorophyll a biomass (mg/m^2) in the Twisp River at sites TR1 through TR6 from 2009 through 2012. Error bars represent one standard error.

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).

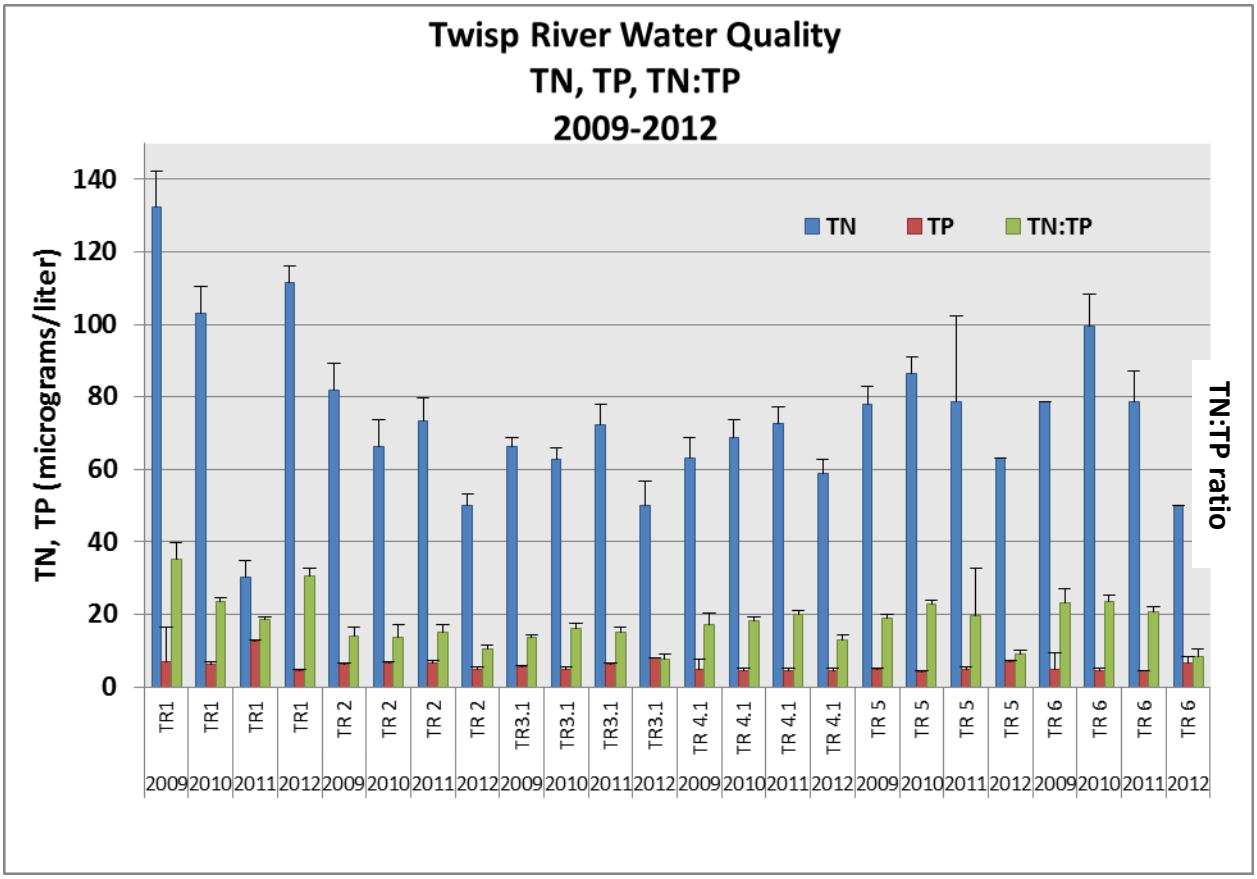


Figure 39. Total nitrogen (TN), total phosphorus (TP) and TN:TP ratio values for the Twisp River, 2009 through 2012. Error bars represent one standard error.

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).

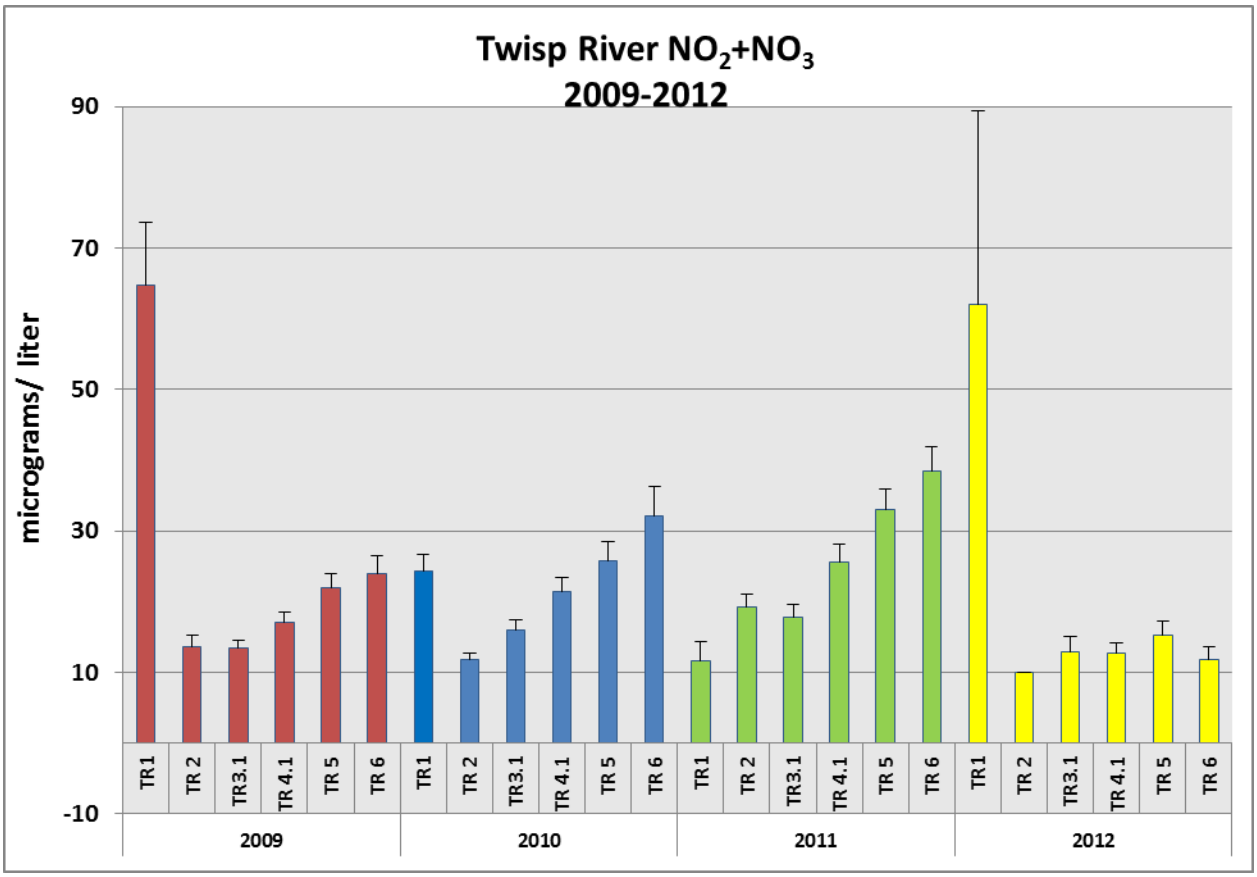


Figure 40. Nitrite (NO_2) + nitrate (NO_3) concentrations (microgram/L) in the Twisp River, 2009 through 2012. Error bars represent one standard error.

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).

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

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.

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).

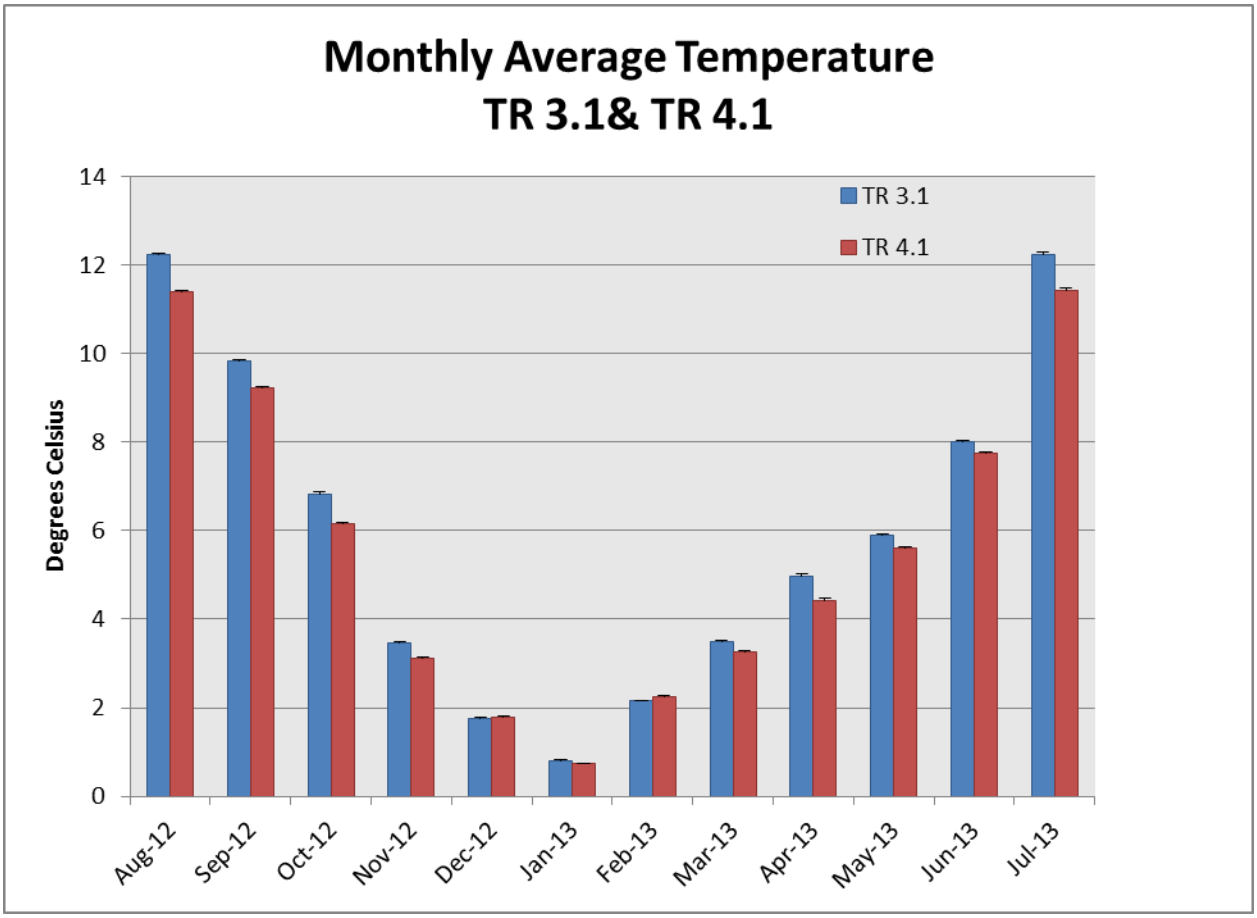


Figure 41. Mean water temperature in the Twisp River in the treatment (TR3.1) and control (TR4.1) reaches from August 2012 through July 2013. Error bars represent one standard error.

Discussion

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.

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.

Hancock Springs

Responses to physical habitat restoration

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.

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.

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.

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.

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

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.

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).

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.

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.

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.

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.

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.

Preparation for nutrient addition (Treatment 2)

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.

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.

Twisp River

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.

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 1: Aquatic Trophic Productivity Model

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The *Aquatic Trophic Productivity Model* (ATP Model) is a mechanism-based dynamic food web model, whereby fish production is explicitly tied to transfers of organic matter between different components of a simplified river food web (Figure A-1). The transfer and production of organic matter within and among different components of the food web is mediated by both in-stream physical habitat conditions (i.e., water temperature, background nutrient load, substrate, etc.), and the structure and composition of the adjacent riparian community (Figure A-1). The model predicts the temporal dynamics of periphyton biomass, detritus biomass, invertebrate biomass, and fish biomass; all of which are measured as part of the *Upper Columbia Natural Production Restoration Project*. The framework of the model is very similar to earlier stream ecosystem models (McIntire 1978; Power et al. 1995; D'Angelo et al. 1997), but in this case the model is being explicitly structured to explore the potential consequences of different mitigation and environmental change scenarios associated with anadromous salmonids.

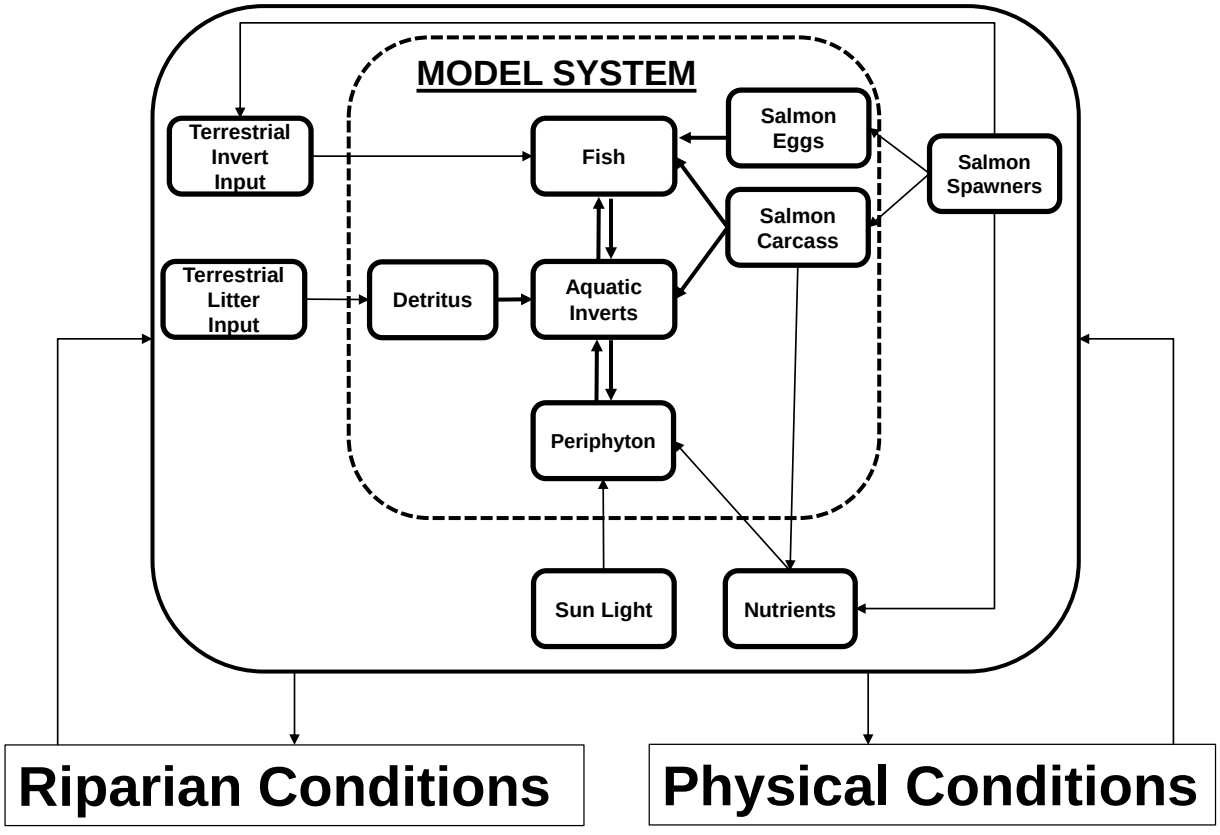

Figure A-1. A conceptual representation of the Trophic Productivity Model, illustrating: (1) the key consumer-resource interactions in the model system, (2) inputs of resources from outside the model system (e.g., terrestrial litter, salmon spawners), and (3) interactions and feed-backs with physical habitat and riparian vegetation.

In the Methow River this model is be used to evaluate the response of fishes and key ecosystem processes (e.g., primary and secondary production) to alternative salmon recovery options. In the context of Yakama Nation led experiments in Hancock Springs and the Twisp River, (the model will be used to simulate, *a priori*, the potential responses to both nutrient analog additions and brook trout removals. In turn, the results of such experiments will provide critical data sets, which will be used to calibrate and validate the ATP model, which can then be used to diagnose limitation and improve restoration efforts among Columbia Basin programs..

Data inputs necessary to run the ATP model include: daily water and air temperatures, discharge, photosynthetically active radiation, stream shading and riparian vegetation composition, dissolved nitrogen and phosphorus concentrations, water turbidity, salmon spawner density, substrate size, channel gradient, and simple metrics of channel and floodplain morphology. For study sites in Hancock Springs and the Twisp River, the model will be parameterized using data from CHaMP habitat surveys, as well as water nutrient and discharge data being collected as part of the project. These data provided by the Yakama Nation's Upper Columbia Nutrient Supplementation Project (BPA Project No. 2008-471-00) are critical to the development and validation of our ARP modeling efforts.

The parameterized model will be used to explore the potential consequences of the planned treatment scenarios outlined in the body of this report. However, prior to predicting potential treatment responses, pre-treatment simulations from the model will be compared to empirical periphyton, invertebrate, and fish data to validate model behavior. If necessary, formal calibration procedures will be used to "fine-tune" the model. The idea is to create a feedback loop between modeling and monitoring, whereby modeling is utilized to generate hypotheses, prioritize experiments, and determine data needs. Reciprocally, results/data from monitoring and experiments are used to parameterize, calibrate and, if necessary, modify the model structure. This feedback between modeling and monitoring should not only create a cycle of adaptive learning, but also is expected to increase the predictive capacity (value) of the model itself. Once appropriately calibrated and validated in Hancock Springs and the Twisp River, the model can then be used to explore the potential consequences of alternative salmon recovery scenarios at many other locations in the Columbia basin.

A unique aspect of this modeling approach is that it is designed to provide a mechanistic understanding of how fish populations might respond to mitigation treatments (e.g., nutrient augmentation, habitat restoration), and environmental changes (e.g., climate change, species invasions). Moreover, by explicitly incorporating key ecosystem processes into the modeling framework, such as primary production and organic matter dynamics, this modeling approach goes beyond the fish response to restoration. In other word, we can use this model (and our empirical data) to explore how the general health, or the "heart-beat" (Palmer and Febria 2012), of these ecosystems might respond to mitigation actions designed to recover anadromous salmonid populations in the Pcific Northwest.

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