Document ID #P142639

Upper Columbia Natural Production Restoration Project Research, Monitoring and Evaluation (RM&E) Annual Progress Report

Contents

Title page

Upper Columbia Nutrient Supplementation/Natural Production Restoration Project

2008-471-00

Report covers work performed under BPA contract # 56662 REL 55

Report was completed under BPA contract #56662 REL 55

Report covers work performed from: March, 2014 – February, 2015

Lucius Caldwell, Kristen Kirkby, and John Jorgensen, Yakama Nation Fisheries, Twisp, WA

Report Created: March 2015

“This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.”

Abstract

Low levels of natural production of anadromous Pacific salmonids (*Oncorhynchus* spp.) continue throughout the Columbia Basin despite significant ongoing restoration efforts. Research commonly reports limitations in food production and physical habitat complexity during early life stages of salmon in rivers and streams. Although nutrient experiments have been conducted in the region, few studies have quantified a fish-level production response to nutrient addition. The goal of this long-term project is to quantify foodweb responses to nutrient additions n an effort to improve the structure and functions of aquatic foodwebs that support natural production. Our specific aim is to understand the impact of particular restoration efforts aimed at improving the natural production of ESA-listed salmonids. Using a set of empirical and mechanism-based approaches, this study addresses several key inter-linked questions:

1. How does habitat complexity affect fish production and energy routing within foodwebs?
2. How does the interaction of added nutrients and habitat complexity affect fish production and energy routing through foodwebs?
3. How do non-native fish densities affect energy routing and subsequent production of native fish, before and after nutrient addition in simple and complex habitat conditions?
4. Can a mechanistic trophic production model accurately estimate observed responses to nutrients? And, if so, how can the model be applied to help improve restoration efforts in the Upper Columbia River Basin?

To meet these goals, the results presented within this report constitute “pre-” data that will be used to analyze the effects of a nutrient addition experiment in August 2015. The data collected thus far therefore represents baseline data that will be essential for elucidating trophic routing mechanisms connecting marine-derived nutrients with endemic fish species.

Introduction

Since the late 1980s, many nutrient addition studies have been published, covering a wide variety of research objectives, nutrient sources, experimental settings, stream order, and monitoring activities (e.g., Wipfli, Hudson *et al.* 1998, Marcarelli, Baxter *et al.* 2014). However, few studies to date have tracked the fate of nutrients and associated biological responses through foodwebs to fish (cf., Johnston, Perrin *et al.* 1990, Bilby, Fransen *et al.* 1998, Wipfli, Hudson *et al.* 2003). To address this information gap, the Upper Columbia Natural Production Restoration Project (UCNPRP) is currently planning a nutrient addition study in a tractable field setting, which will monitor key ecological attributes from basal resource production to fish production via the construction of quantitative energy flow webs. Flow webs can be used to characterize both species interactions and energy transfers associated with those interactions, and therefore quantify and visualize complex ecological responses to habitat and/or species changes (Benke and Wallace 1980, Benke 2011, Cross, Baxter *et al.* 2013). The goals of conducting this research are 1) to address why past salmon restoration efforts requiring restored natural production have not achieved recovery goals, and 2) to quantify and compartmentalize the interconnected responses to nutrient augmentation among various empirical pathways and trophic levels, including fish and all supporting trophic levels. This technique provides managers and implementation professionals with a powerful tool for identifying gaps in restoration strategies that can be addressed efficiently, in order to increase the efficacy of strategies aimed at increasing returns of endangered salmonids.

With these goals in mind, in 2013-14 the UCNPRP collected baseline "pre-treatment" data covering aspects of epilithic periphyton community structure and production, benthic and terrestrial macroinvertebrate community structure and production, and fish production at Hancock Springs Creek, NW of Winthrop, WA. Ultimately, these data will provide the foundation for energy flow web-based analyses describing the effects of nutrient additions in Hancock Springs Creek.

Methods

Located in the Methow River Subbasin of north central Washington (Fig. 1), Hancock Springs is a small (1.6 km-long) spring creek that is tributary to the mainstem Methow River. The mouth of Hancock Springs Creek forms a confluence at RKM 96 on the Methow River. The

study area within Hancock Springs Creek includes two 300 m reaches (Fig. 2). Reach 1 (upstream) was restored in 2011 and comprises a complex channel. Reach 2 (downstream) remains fairly unchanged and comprises a degraded, simple channel. For the purposes of current and future sampling, each reach has been broken into three sub-reach sites (i.e., 1-1, 1-2,…, 2-3). Channel conditions and riparian zones within both reaches of the study area were degraded following over 30 years of a dairy operation. Past grazing in the riparian zone skewed natural width- to-depth ratios, reduced habitat complexity, and contributed to a high percentage of fine sediment throughout the wetted area. Ungulate exclusion fencing and a conservation easement now prevent livestock use along the entire length of the stream. Reach 1 channel reconfiguration treatment was performed during 2011 in a coordinated effort by Yakama Nation biologists, 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 empirical intra- and inter-annual discharge information.

At six times during 2014 (March, May, June, August, October, and December), exhaustive field sampling efforts were undertaken to collect data on water chemistry, periphyton, aquatic invertebrates, fish populations and communities, and fish diets; at eight separate times terrestrial invertebrates were also collected (Table 1).

Water quality

On-site water quality analyses

A suite of water quality response variables were monitored on-site at Hancock Springs Creek. Onset ® TidbiT v2 data loggers were placed underwater in two locations in the stream, to record water temperature every 30 minutes continually throughout the year. Portable YSI™ EXO2 Water Quality Sonde Hydrolabs were placed underwater in two locations at Hancock Springs

and measured dissolved oxygen concentration (DO), pH, and conductivity, to provide data for a coordinated multi-agency metabolism project.

Field sample collection and laboratory-based water chemistry assays

In addition, water samples from Hancock Springs Creek were collected for off-site third-party analysis of a suite of water chemistry endpoints. Replicate samples were collected by dipping containers into the thalweg just below the surface at each site. All water quality samples were stored at 4ºC until being shipped overnight on ice to Aquatic Research, Inc. (Seattle, WA.), a division of IEH Analytical Laboratories, for analysis of concentrations of total phosphorous (TP), total dissolved phosphorous (TDP), soluble reactive phosphorous (SRP), combined nitrate and nitrite (NO_2^- + NO_3^-), ammonium (NH_4^+), and total nitrogen (TN). Reported limits of detection were 2.0 µg L^{-1} for TP and TDP, 1.0 µg L^{-1} for SRP, 10.0 µg L^{-1} for NO_2^- + NO_3^-, 5 µg L^{-1} for NH_4^+ and 5.0 µg L^{-1} for TN.

Epilithic biofilm (periphyton)

Field sample collection

Periphyton standing crop and community structure were analyzed in both study areas. Standing crop is expressed in terms of both chlorophyll-*a* (Chl-*a*) concentration (mg m^{-2}) and ash-free dry mass (AFDM, g m^{-2}). Chl-*a* concentration provides an estimate of autotrophic periphyton biomass, while AFDM provides an estimate of combined autotrophic and heterotrophic biofilm, the latter comprising bacterial and fungal taxa (Moulton, Kennen *et al.* 2002). Within each site, five (5) rocks were scraped clean of all biofilm into 300 mL stream water. Next, the mixture was homogenized by brief whisking, after which two 30 mL aliquots were drawn by vacuum through a pre-dried glass fiber filter, one each for determination of AFDM and Chl-*a* concentration.

Lab-based biofilm standing crop and chlorophyll-a concentration

Both aliquots of filtered periphyton slurry residue were wrapped in aluminum foil and stored on ice in the field. Upon returning to the lab, samples were stored at -20°F until further analysis. Samples were later trucked to University of Idaho (Moscow, ID), where they were analyzed by the Analytical Sciences Laboratory for Chl-*a* and AFDM using standard laboratory methods (American Public Health Association 2014).

Lab-based community characterization

From the same periphyton slurry generated by scraping rocks for periphyton biomass and Chl-*a* concentration, a third 20 mL aliquot of slurry was collected from a single rock within each site, mixed with Lugol's solution, and then stored in dark bottles under refrigeration until analysis. At regular intervals (2-4 months), samples were shipped to BSA Environmental Services, Inc. (Beachwood, OH), where periphyton slides were 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 were 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) was used to enumerate filtered periphyton samples. The magnification used depended on the size of dominant taxa and presence of particulates. Cell counts were performed at multiple magnifications to successfully identify and enumerate taxa with cell sizes that vary by several orders of magnitude. If a sample was dominated by cells or natural units below 10-20 µm, or when cells were fragile and difficult to identify, the majority of counting will be completed at 630X. The relative abundances of common algal taxa were estimated by random field counts. A minimum of 400 natural units (colonies, filaments, unicells) were enumerated to the lowest possible taxonomic level (in most cases, species) from each sample. In addition, an entire strip of the filter was 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 were quantified on a per milliliter basis. Bio-volumes were estimated using formulae for solid geometric shapes that most closely match the cell shape. Bio-volume calculations were based on measurements of 10 organisms per taxon for each sample where possible. Mean bio-volumes were then used to calculate the total biovolume contributed by each taxon to its representative sample.

Benthic and Terrestrial Macroinvertebrate Communities

Field sample collection

A Hess sampler (1,000µm mesh size) was used to sample benthic macroinvertebrates (BMI) from riffle and pool-tail habitat in Hancock Springs. Within each site, samples from three instances of a given habitat type (*i.e.*, pool, riffle) were pooled to best represent the diversity of conditions within each habitat type. Terrestrial macroinvertebrates (TMI) were sampled using

stream bank pan traps, consisting of 41 cm x 33 cm x 16 cm polyethylene plastic bins lashed to riparian vegetation via 4mm nylon cord. TMI pan traps were set on Monday and trapped invertebrates collected on Thursday, every 3 weeks from May to October. TMI pan traps were filled with 5-10 cm of stream water and 2-3 drops of phosphate-free, biodegradable dish soap (omitted in the case of samples for stable isotope analysis) to disrupt surface tension and promote retention of invertebrates touching down on the contained water.

Lab-based biomass and secondary production estimation

For estimating biomass and secondary production, BMI and TMI were rinsed, preserved in 70% ethanol, and then sent to Invertebrate Ecology, Inc. (Moscow, ID), where samples were sorted and identified to the lowest feasible taxonomic level, usually species. All macroinvertebrates were measured to the nearest mm in the lab. Individual biomass was estimated from body length, using established length-mass relationships for similar systems (Benke, Huryn *et al.* 1999). To facilitate accurate estimates of production for all macroinvertebrate orders present, including those with complex life histories or for which more than one generation may be present at any time in a given year (e.g., ephemeropteran mayflies), macroinvertebrate secondary production values were estimated using the non-cohort size-frequency method (Hamilton 1969, Hobson and Welch 1995, Benke and Huryn 2006), a removal-summation technique that iteratively sums apparent losses of biomass between adjacent size classes in the ontogenetic progression from egg to adult (Waters 1977). This method relies on a simple model that is informed by population density and individual biomass data, structured by size class, and estimates annual secondary production as:

$$P_u = \sum(\Delta n_{ij} \times \overline{w}_{ij} \times No.\,of\ size\ classes) \qquad (Eq.\ 1)$$

where:

P_u = uncorrected production estimate, in units of mass*area^{-1}*year^{-1}

$\sum$ represents the summation of data across all size classes, measured at a given time

Δn_{ij} = decrease in population density from size class *i* to size class *j*

$\overline{w}_{ij}$ = mean biomass, averaged across size class *i* and size class *j*, in units of mass*area^{-1}, and

No. of size classes is in units of year^{-1}.

Inclusion of the correction factor "*No. of size classes*" in Eq. 1 is based on the presumption that number of size classes present in a given sample reflects the number of cohorts present in that year. For invertebrate taxa with development times that differ substantially from one year, secondary production values will be corrected by a factor (F_c) based on the taxa-specific cohort production interval (CPI). CPI for a given taxon is the number of days required by an average individual of that taxon to develop from hatching to adulthood. From CPI, F_c is calculated as follows:

$$F_c = \frac{365}{CPI} \qquad (Eq.\ 2)$$

To correct P_u for insects with very short development times, the following equation will be used to generate a corrected production estimate (P_c):

$$P_c = P_u \times F_c \qquad (Eq.\ 3)$$

Beyond utility for quantifying the flow of energy through taxa and constructing foodwebs based on such quantifications, secondary production values can be used to infer additional information about systemic ecological processes (Benke 2010). For instance, the ratio of production (P) to biomass (B), essentially a weighted average of individual growth within a given taxon, provides a measure of annual turnover rate, sometimes termed the "turnover ratio" (Hynes and Coleman 1968). P:B ratios facilitate quantitative comparison of standardized energy flows through macroinvertebrate taxa between pre- and post-treatment periods.

Fish Community

Field sample collection

Standard upstream multiple pass depletion methods were employed, following equipment operational guidelines reported by Hankin and Reeves (1988). Abundance estimation techniques followed those described by Seber (1967) and Zippin (1958). Briefly, electrofishing passes were completed until adequate depletion is achieved (following Connolly 1996). Salmonid fishes were depleted to meet abundance estimates at a coefficient of variation (CV) < 12.5%, where sculpin

were depleted to meet abundance estimates at a CV < 25%. Electrofishing techniques are consistent with regionally accepted settings and protocols for sampling fish in small streams.

Abundance estimation

Abundance estimates for fish populations were generated using standard multiple pass depletion estimate techniques and the K-pass removal package (Ogle 2012) within Program R (R Core Team 2013).

Biomass estimation

For each effort, biomass (g m^{-2}) was estimated by adding a correction factor to the summed mass of fish captured during a single effort, according to the equation:

$$\hat{B}_j = (\sum m_{ij}) + [\overline{m}_j \times (\hat{A}_j - Catch_j)] \qquad (Eq.\ 4)$$

where:

$\hat{B}_j$= estimated biomass for species *j*

Σ represents the summation of all individuals within a given time period

m_{ij}= mass of fish *i* within species *j*

$\overline{m}_j$ = mean of individual fish masses for all captured fish of species *j*

$\hat{A}_j$= population abundance estimate for species *j*

$Catch_j$ = total number of captured fish of species *j*.

Next, $\hat{B}_j$ (g) was divided by habitat area (m^2) for each species within each site. Wet biomass was converted 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).

Organismal growth rate estimation

Specific growth rate, an approximation of instantaneous growth rate, was calculated to compare organismal growth patterns among individual fish within a species. Although accumulation of fish mass is often assumed to follow a logistic curve over an individual's lifetime, juvenile salmonids are generally in the "log-phase" of growth and tend to accumulate body mass rapidly and steadily, adopting an exponential growth curve described by the equation:

$$w = a \times e^{Gt} \qquad (Eq.\ 5)$$

where:

w = fish weight (mass)

a = initial size (at time 0)

G = instantaneous growth rate, and

t = duration of interest.

Eq. 5 is useful for predicting future fish size or population biomass given reliable organismal mass and growth rate data, but it is useless for inferring growth rate from observations. To do this, growth rate is generally assumed to be constant between observations and then inferred by comparing change in mass over an interval of time, i.e., substituting beginning and end mass and time values for the static placeholders in Eq. 1. Following this method, to express logarithmic growth rate over the interval from t_0 to t_1, given an initial mass w_0 and final mass w_1, we substitute, take the log of both sides, and rearrange Eq. 1 to yield the familiar equation for instantaneous growth rate after Ricker (1946, 1979):

$$G = \frac{\ln w_1 - \ln w_0}{t_1 - t_0} \qquad (Eq.\ 6)$$

where:

G = instantaneous growth rate over the time period from capture at time (*0*) to recapture at time (*1*):

t_0 = time (d) of first measurement

t_1 = time (d) of last measurement

w_0 = the weight (g) of an individual at time t_0, and

w_1 = the weight (g) of an individual at time t_1.

Eq. 6 can be used for calculating both individual growth rates, as a standalone organismal metric, or, by substituting population mean weight for individual weight (i.e., w_0 and w_1) to estimate mean growth rate for a population. As a note, the instantaneous growth rate assumes both constant and logarithmic growth, which may appear problematic. However, concern is unnecessary for two reasons: First, most fish sampled at Hancock Springs are juvenile and thus

can be reasonably presumed to be growing exponentially. Second, statistical comparisons of instantaneous growth rate are robust to violations of either or both assumptions, so long as time intervals of comparison are similar (Hayes, Bence *et al.* 2007). As a note, only *O. mykiss*, *O. tshawytscha*, and *S. fontinalis* were recaptured with sufficient frequency to calculate organismal growth rates.

Tertiary (fish) production estimation

Production of dominant salmonid fish species was estimated using the instantaneous growth rate method (Hayes, Bence *et al.* 2007), which estimates production as the product of population mean instantaneous growth rate and mean fish biomass:

$$P_{ij} = G_{ij} \times B_{ij} \qquad (Eq.\ 7)$$

where:

P_{ij} = production for a given species over the interval t_i to t_j

G_{ij} = instantaneous growth rate for a given species over the interval t_i to t_j (as calculated in Eq. 3), and

B_{ij} = mean species biomass over the interval t_i to t_j.

As a note, small salmonid population sizes preclude satisfactorily binning cohorts within each species to calculate cohort-specific production values. Were species to be subdivided by size class, the number of members of each cohort would preclude meaningful statistical comparisons. However, this is not problematic as growth rates may not vary widely across size classes within a species in this system.

Production of sculpin was estimated using the size-frequency method of Hynes and Coleman (1968). Readers interested in the calculations used to generate production estimates using this method are referred to Garman and Waters (1983).

Fish diets

Field sample collection

Fish diets were sampled by analyzing gut contents (Northington and Hershey 2006). Stomach content samples all species present at Hancock Springs were collected using gastric lavage to flush gut contents from live fish. This technique has been reported to remove up to 98.9% of gut

contents with little effect on subsequent survival and condition (Strange and Kennedy 1981, Waters, Kwak *et al.* 2004). Gut contents from five fish per species per site were collected; samples were distributed haphazardly among size classes within each species, resulting in representative coverage of all but the smallest sculpin (Fig. 3), which could not be sampled for logistical and fish health reasons.

Lab-based diet analysis

Samples were preserved in 70% ethanol before being sent to Invertebrate Ecology, Inc. (Moscow, ID) for taxonomic identification and biomass estimation. Fish gut contents were identified to lowest taxa logistically possible (species in most cases), and biomass values were calculated using a length-mass regression model (Benke, Huryn et al. 1999). In the case of fish presence in stomach samples, biomass was estimated from length-weight regressions developed using electro-fishing data (data not shown).

Results

Water Chemistry

During 2014, water sampled at Hancock Springs Creek exhibited what appears to be both seasonal and spatial variability (Fig.4); however, no clear trends are apparent. Mean values did not differ between samples collected from Reach 1 and those collected from Reach 2 (Fig. 5).

Periphyton

Over the course of 2013, periphyton rock scrapings from Hancock Springs Creek exhibited substantial variation in levels of both chlorophyll-*a* (Chl-*a*), and chlorophyll-*b* (Chl-*b*) across the two reaches, but much less variation within each reach and site (Fig. 6). When considering AFDM and Chl-*a*, differences between reaches are not significant (2-tailed *t*-test, $p>0.1$), but some trends are apparent (Fig. 7). Within Reach 1, Chl-*a* appears to increase during spring months, peak during summer, and decline during autumn; AFDM within Reach 1 does not exhibit any obvious trends. Within Reach 2, both AFDM and Chl-*a* appear to peak during spring months, and then decline over the summer and autumn months. When the two reaches are compared, some non-significant trends also emerge. During the spring months both AFDM and Chl-*a* were higher within Reach 2. During summer and autumn months, AFDM of eplithic

biofilm scrapings did not differ between the two reaches, but Chl-*a* was consistently slightly higher in Reach 1.

BMI

Annual BMI biomass and production values were fairly stable from 2012-2013 within each reach, and were consistently, though not significantly (2-tailed *t*-tests, $p \geq 0.05$), higher in the complex reach, compared to the simplified reach (Fig. 8).

Fish community

All fishes sampled at Hancock Springs Creek tended to exhibited higher abundance density in the complex reach compared to the simplified reach (Fig. 9). Fish abundance density estimates exhibited far less variation within each reach versus between the two reaches. Also, within each reach, species showed different trends regarding variability: Abundance density estimates for *O. mykiss*, *S. confluentus*, *S. fontinalis*, and *Cottus* spp. were all fairly stable over time within both reaches, while estimates for *O. tshawytscha* exhibited substantial seasonal variation in abundance, especially within Reach 1.

Based on recaptures of PIT-tagged fish, specific (instantaneous) growth rate for mass was higher in the complex reach (2-tailed *t*-tests, $p < 0.05$) compared to the simplified reach for *O. mykiss* and *S. fontinalis*, but not for *O. tshawytscha* (Fig. 10).

At each sampling effort during 2014, all fishes exhibited trends of greater biomass in the complex reach compared to the simplified reach (Fig. 11). *O. mykiss*, *O. tshawytscha*, *S. confluentus*, *S. fontinalis*, and *Cottus* spp. each exhibited greater biomass densities within the complex reach than in the simplified reach at every time-point, although significance level of the difference between reaches varied.

From October 2013 through October 2014, all fish species exhibited non-significantly greater (2-tailed *t*-tests, $p \geq 0.05$) production in the complex reach, compared to the simplified reach (Tables 2 & 3).

Fish diet

Fish diets, as assessed by gastric lavage and quantitative gut content analysis, showed a high degree of stability over the years 2012-2013, for most species collected. *O. mykiss* stomachs contained high proportions of chironomids, baetids, and hydroptilids in both 2012 and 2013 (Fig. 12). *O. tshawytscha* stomachs contained high proportions of chironomids, baetids, and tipulids in both 2012 and 2013 (Fig. 13). *S. confluentus* stomach contents exhibited greater variability than

other fishes sampled: cottid fishes were the only commonality among the top five prey items identified in both 2012 and 2013 (Fig. 14). *S. fontinalis* stomachs contained high proportions of limnephilids, baetids, hydroptilids, and chironomids in both 2012 and 2013 (Fig. 15). *Cottus* spp. stomach contents were sampled beginning in 2013, when stomach contents comprised primarily tipulids, baetids, chironomids, ephemerellids, and gammarids (Fig. 16).

Discussion/Conclusion

In the US, billions of dollars have been spent on stream habitat restoration (Rahr 2004-2015), much of which focuses on increasing channel complexity. In spite of these expenditures, ecological mechanisms underlying the impacts of such projects on targeted fish species remain largely unstudied, and monitoring efforts continue to be an afterthought in the design of restoration projects. In light of these knowledge gaps, the overall goal of this project is to quantify aquatic food-web changes due to habitat restoration efforts focused on increasing channel complexity in a small spring-creek tributary to the Methow River. Here, we report baseline ("pre") data that will serve as the foundation for future analyses of the effects and underlying trophic routing pathways that deliver marine derived nutrients (both natural and supplemental) to endemic fish species.

During 2014 water chemistry parameters at Hancock Springs Creek were moderately variable across both space and time, conforming to anecdotal and empirical expectations of chemical stability due to the spring-fed nature of this stream.

During 2013, periphyton standing crop biomass and Chl-*a* concentration from rock scrapings sampled at Hancock springs also exhibited substantial spatial variability, along with some moderate seasonal trends. However, samples from Reach 1 were more homogenous than those collected within Reach 2. Also, within each reach, different seasonal trends emerged, likely the effect of differences in riparian vegetation: Within Reach 1—which comprises a largely open floodplain and short, shrubby and non-woody vegetation—Chl-*a* concentration showed a moderately phasic trend, peaking in early summer, while periphyton biomass was less variable and peaked at mid-summer. Within Reach 2—which comprises a more forested floodplain including stands of hardwoods and other large trees—Chl-*a* and AFDM concentrations both peaked in mid-spring before leaf-out, then steadily declined over the summer and autumn seasons.

Between 2012 and 2013, aquatic insect annual production and mean biomass density showed little variation within each reach. However, there was a slight trend toward higher insect biomass density and annual production in Reach 1.

In 2014, fish populations in Reach 1 exhibited consistent trends of greater abundance and biomass densities, organismal growth rates, and annual production values, compared to fishes in Reach 2. Within Reach 1, Chinook salmon and bull trout exhibited seasonal patterns consistent with documented life history patterns and associated behaviors, while other fishes exhibited a relatively high degree of stability.

Among fish species sampled, diet compositions were highly consistent across years for all species except bull trout, which appear to exhibit a high degree of opportunism regarding prey items chosen.

These results form the foundation of future nutrient augmentation experiments that will be conducted at Hancock Springs Creek. Taken together, the moderate level of temporal and intra-reach spatial variability seen at Hancock Springs Creek suggests that this system is amenable to experimental study, and that treatment effects and underlying trophic routing mechanisms can be detected within this study zone.

Adaptive Management & Lessons Learned

Upon completion of the UCNPRP, results generated will be interpreted within a food-web context, to help inform project mangers and implementation professionals throughout the Columbia Basin, as to what are the underlying mechanisms linking restoration actions with response of listed fish species. With this tool in hand, it will possible for agencies and individuals alike to prioritize recovery actions based on an efficiency and efficacy, to develop highly targeted, area-specific approaches to solving conservation problems.

References

American Public Health Association (2014). *Standard Methods for the Examination of Water and Wastewater*, General Books, LLC.

Bellmore, J. R., C. V. Baxter, K. Martens and P. J. Connolly (2013). "The floodplain food web mosaic: a study of its importance to salmon and steelhead with implications for their recovery." *Ecological Applications* **23**(1): 189-207.

Benke, A. C. (2010). "Secondary production as part of bioenergetic theory-contributions from freshwater benthic science." *River Research and Applications* **26**(1): 36-44.

Benke, A. C. (2011). "Secondary Production, Quantitative Food Webs, and Trophic Position." *Nature Education Knowledge* **3**(10): 26-35.

Benke, A. C. and A. D. Huryn (2006). Chapter 29: Secondary production of macroinvertebrates. *Methods in Stream Ecology, 2nd Edition*. F. R. Hauer and G. A. Lamberti. Burlington, MA, Academic Press**:** 691-710.

Benke, A. C., A. D. Huryn, L. A. Smock and J. B. Wallace (1999). "Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the Southeastern United States." *Journal of the North American Benthological Society* **18**(3): 308-343.

Benke, A. C. and J. B. Wallace (1980). "Trophic basis of production among net-spinning caddisflies in a southern Appalachian stream." *Ecology* **61**(1): 108-118.

Bilby, R. E., B. R. Fransen, P. A. Bisson and J. K. Walter (1998). "Response of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) to the addition of salmon carcasses to two streams in southwestern Washington, U.S.A." *Canadian Journal of Fisheries and Aquatic Sciences* **55**(8): 1909-1918.

Connolly, P. J. (1996). *Resident Cutthroat Trout in the Central Coast Range of Oregon: Logging Effects, Habitat Associations, and Sampling Protocols*. PhD Thesis, Oregon State University.

Cross, W. F., C. V. Baxter, E. J. Rosi-Marshall, R. O. Hall Jr, T. A. Kennedy, K. C. Donner, H. A. Wellard Kelly, S. E. Seegert, K. E. Behn and M. D. Yard (2013). "Food-web dynamics in a large river discontinuum." *Ecological Monographs* **83**(3): 311-337.

Garman, G. C. and T. F. Waters (1983). "Use of the size–frequency (Hynes) method to estimate annual production of a stream fish population." *Canadian Journal of Fisheries and Aquatic Sciences* **40**(11): 2030-2034.

Hamilton, A. L. (1969). "On estimating annual production." *Limnology and Oceanography* **14**(5): 771-781.

Hankin, D. G. and G. H. Reeves (1988). "Estimating total fish abundance and total habitat area in small streams based on visual estimation methods." *Canadian Journal of Fisheries and Aquatic Sciences* **45**(5): 834-844.

Hayes, D. B., J. R. Bence, T. J. Kwak and B. E. Thompson (2007). Abundance, biomass, and production. *Analysis and interpretation of freshwater fisheries data*. C. S. Guy and M. L. Brown. Bethesda, MD, American Fisheries Society**:** 279-325.

Hobson, K. A. and H. E. Welch (1995). "Cannibalism and trophic structure in a high Arctic lake: insights from stable-isotope analysis." *Canadian Journal of Fisheries and Aquatic Sciences* **52**(6): 1195-1201.

Hynes, H. B. N. and M. J. Coleman (1968). "A simple method of assessing the annual production of stream benthos." *Limnology and Oceanography* **13**(4): 569-573.

Johnston, N. T., C. J. Perrin, P. A. Slaney and B. R. Ward (1990). "Increased juvenile salmonid growth by whole-river fertilization." *Canadian Journal of Fisheries and Aquatic Sciences* **47**(5): 862-872.

Marcarelli, A. M., C. V. Baxter and M. S. Wipfli (2014). "Nutrient additions to mitigate for loss of Pacific salmon: consequences for stream biofilm and nutrient dynamics." *Ecosphere* **5**(6): art69.

Moulton, S. R., II, J. G. Kennen, R. M. Goldstein and J. A. Hambrook (2002). Revised Protocols for Sampling Algal, Invertebrate, and Fish Communities as Part of the National Water-Quality Assessment Program. Reston, VA, USGS**:** 87 p.

Northington, R. M. and A. E. Hershey (2006). "Effects of stream restoration and wastewater treatment plant effluent on fish communities in urban streams." *Freshwater Biology* **51**(10): 1959-1973.

Ogle, D. (2012). fishR Vignette–Depletion methods for estimating abundance (R package).

R Core Team (2013). R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing.

Rahr, G. R., III. (2004-2015). "Why is salmon conservation important." Retrieved January 14, 2015, from http://www.wildsalmoncenter.org/about/whySalmon.php.

Ricker, W. E. (1946). "Production and utilization of fish populations." *Ecological Monographs* **16**(4): 373-391.

Ricker, W. E. (1979). Chapter 11: Growth Rates and Models. *Fish Physiology*. W. S. Hoar, D. J. Randall and J. R. Brett. New York, NY, Academic Press. **8:** 678-744.

Seber, G. A. F. and E. D. Le Cren (1967). "Estimating population parameters from catches large relative to the population." *Journal of Animal Ecology* **36**(3): 631-643.

Strange, C. D. and G. J. A. Kennedy (1981). "Stomach flushing of salmonids: A simple and effective technique for the removal of the stomach contents." *Aquaculture Research* **12**(1): 9-15.

Waters, D. S., T. J. Kwak, J. B. Arnott and W. E. Pine, III (2004). "Evaluation of stomach tubes and gastric lavage for sampling diets from blue catfish and flathead catfish." *North American Journal of Fisheries Management* **24**: 258-261.

Waters, T. F. (1977). "Secondary Production in Inland Waters." **10**: 91-164.

Wipfli, M. S., J. Hudson and J. Caouette (1998). "Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A." *Canadian Journal of Fisheries and Aquatic Sciences* **55**(6): 1503-1511.

Wipfli, M. S., J. P. Hudson, J. P. Caouette and D. T. Chaloner (2003). "Marine subsidies in freshwater ecosystems: Salmon carcasses increase the growth rates of stream-resident salmonids." *Transactions of the American Fisheries Society* **132**(2): 371-381.

Zippin, C. (1958). "The removal method of population estimation." *Journal of Wildlife Management* **22**(1): 82-90.

Appendices

A.1: Figures:

Trophic level	Sampling location	Sampling Type	Events per Year
Water Chemistry	6 sites, 2 replicates	Water column dips	6
Periphyton	6 sites, 2 replicates of 3 pooled samples per site	Scraping a known area	6
Benthic Invertebrates	6 sites, 3 replicates per habitat type (P, R) per site	Hess sampler	6
Terrestrial Invertebrates	6 sites, 2 replicates of 3 pooled samples per site	Pan traps	8
Fish community and diet	6 sites	E-Fishing, Gastric lavage	6

Table 1. Hancock Springs Creek sampling regime, during 2014.

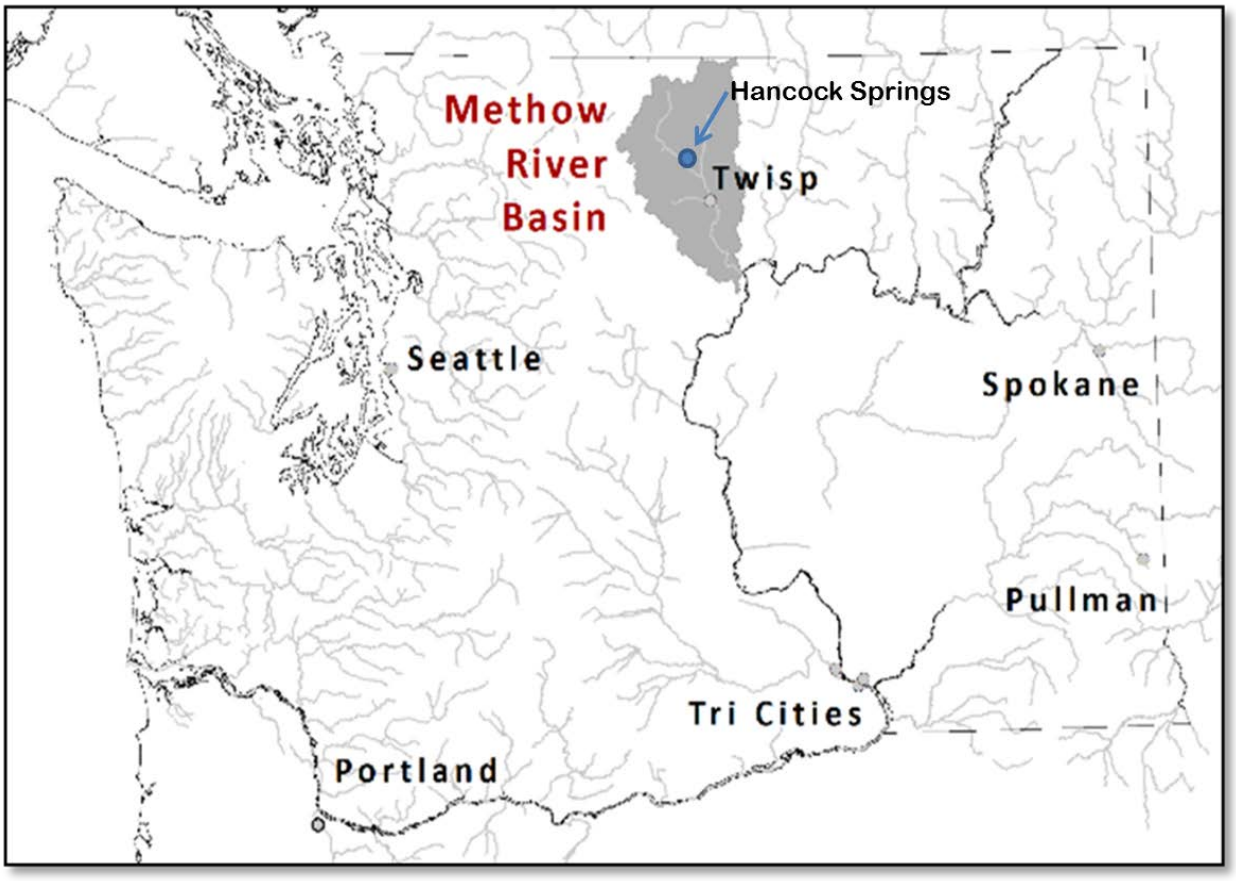

Figure 1. Location of Methow River Subbasin and Hancock Springs Creek, in North Central Washington.

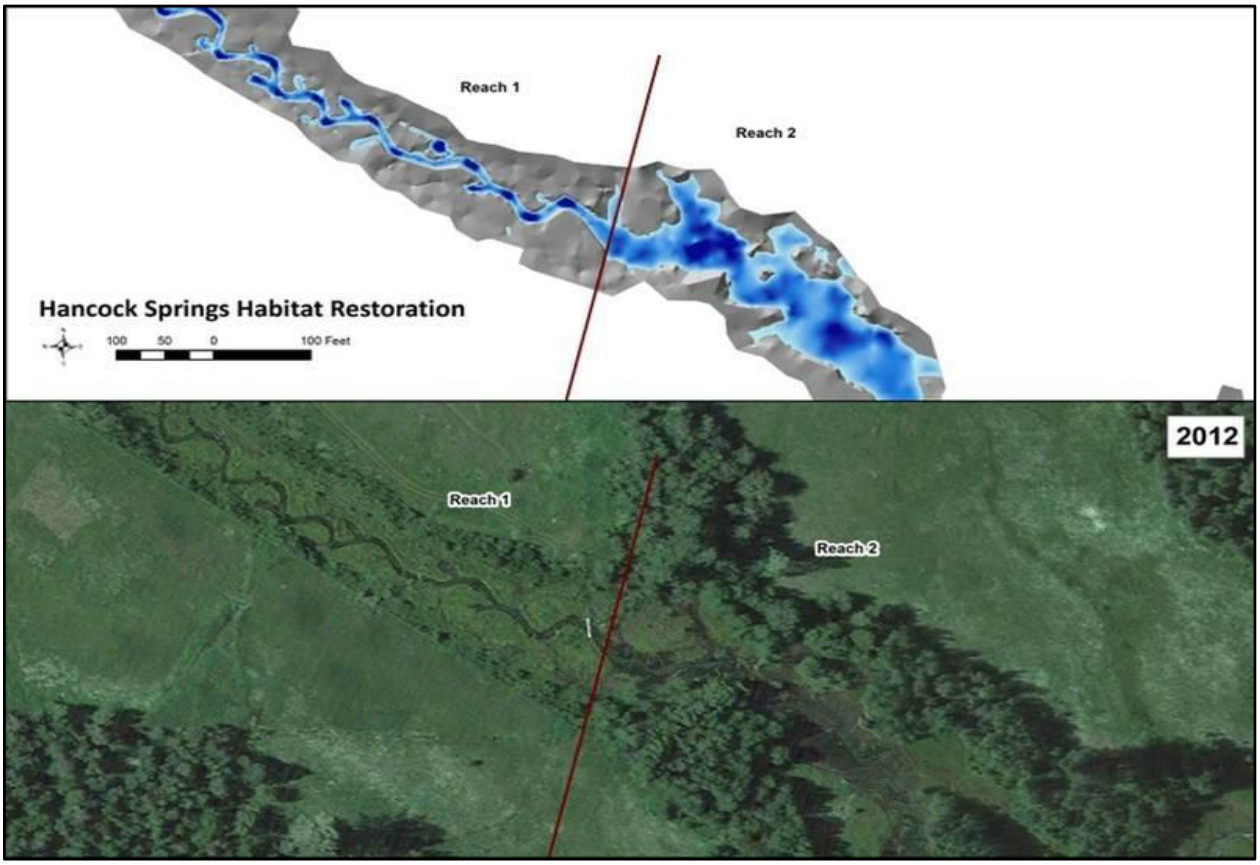

Figure 2. Digital elevation map and aerial photograph of Hancock Springs Creek subdivision into Reach 1 (upstream, complex channel) and Reach 2 (downstream, simplified channel).

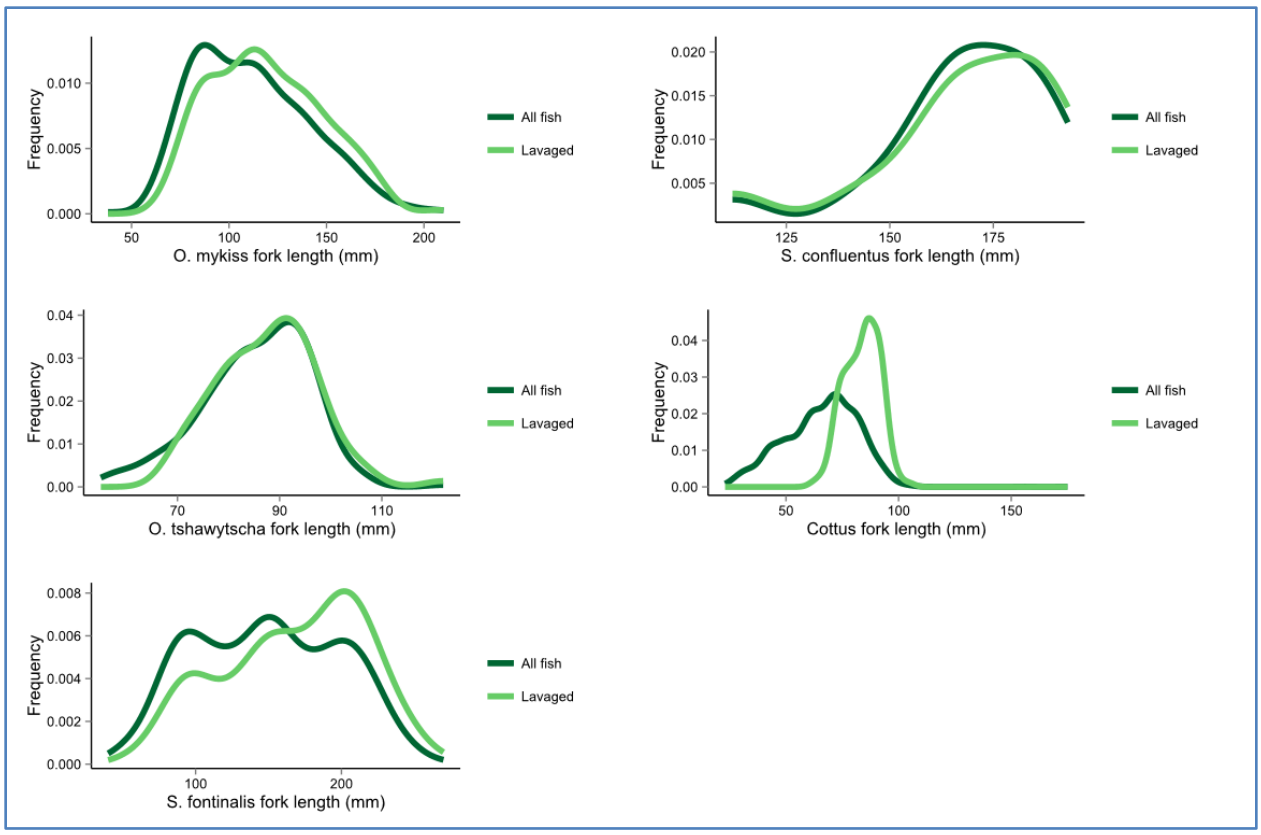

Figure 3. Smoothed curve fit of fork-length distribution for all fish sampled at Hancock Springs Creek during 2014 (dark green line), compared to subsampled individuals that provided gastric lavage samples for analysis of gut contents to estimate diet composition (light green line), separated by species.

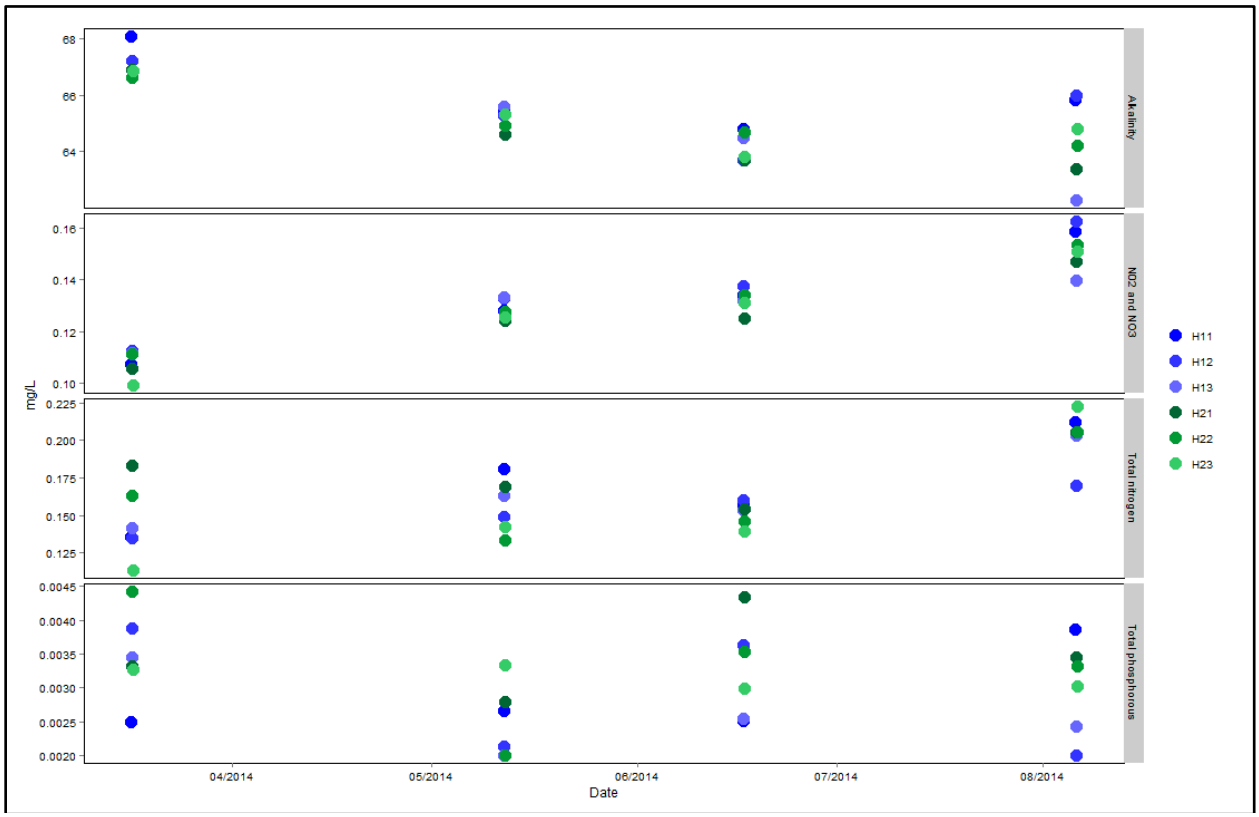

Figure 4. Water chemistry parameters from samples collected at Hancock Springs Creek during 2014. Abscissa for each point represents site value for that particular sampling effort.

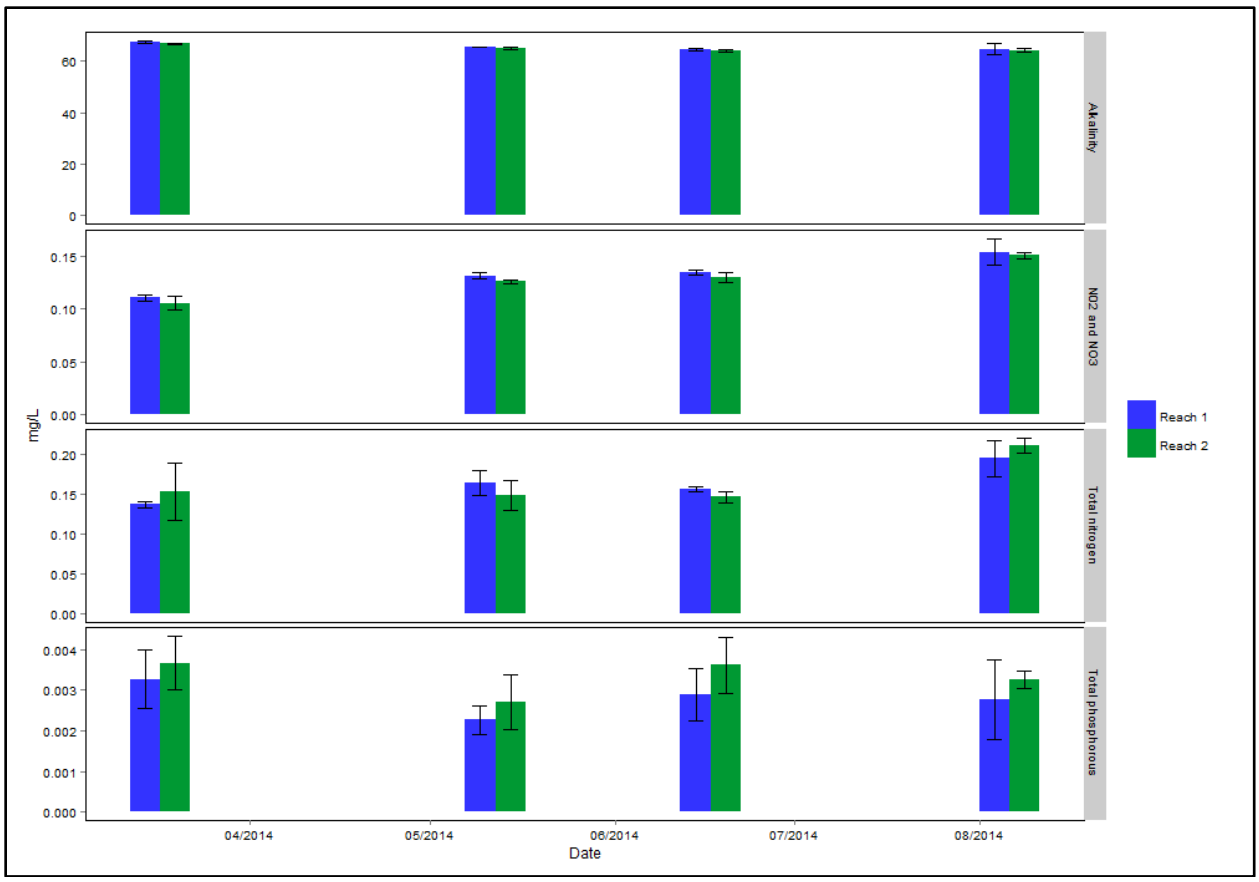

Figure 5. Water chemistry parameters from samples collected at Hancock Springs Creek during 2014. Bar heights represent arithmetic mean of sites within each reach for a particular sampling effort; error bars represent ± 1x SD. Reach 1 and Reach 2 mean values do not differ at any time point.

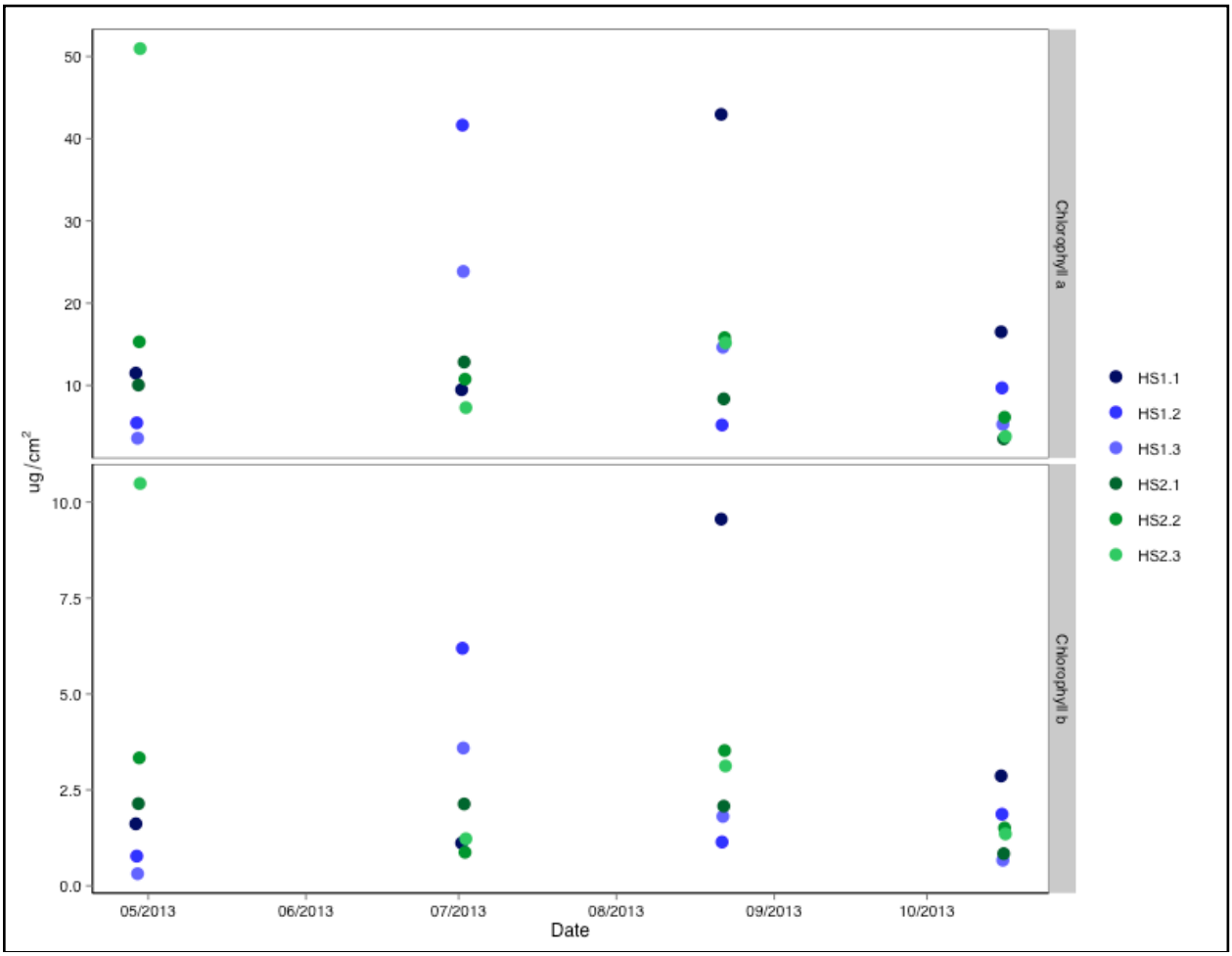

Figure 6. Chlorophyll *a* (Top Panel) and *b* (Bottom Panel) concentrations from rock scrapings at Hancock Springs Creek during 2013. HS1.1-3 represent sites within the upstream, complex reach; HS2.1-3 represent sites within the downstream, simplified reach.

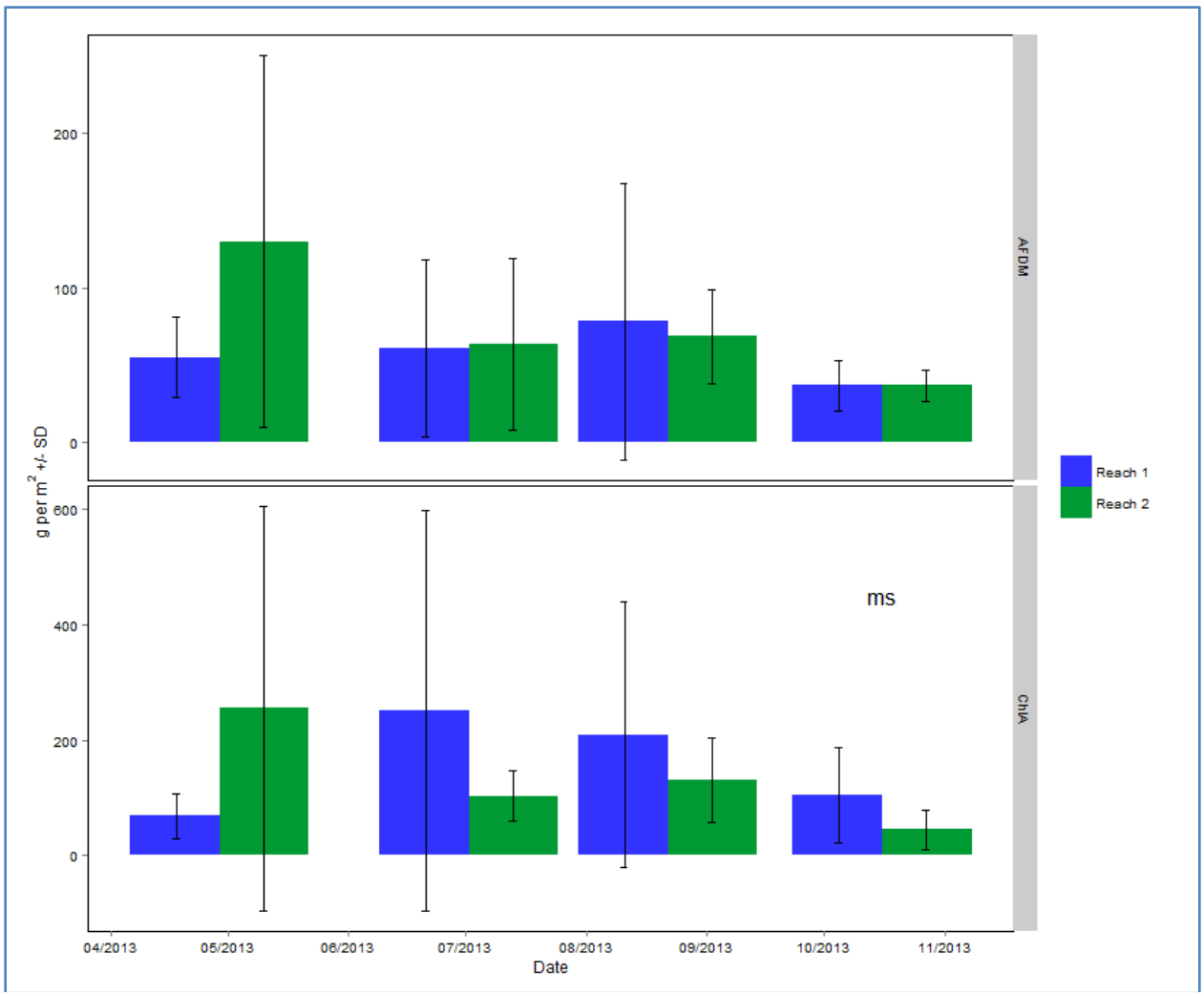

Figure 7. Ash-free dry mass (AFDM, top panel) and Chlorophyll *a* (bottom panel) concentrations from rock scrapings at Hancock Springs Creek during 2013. Bar heights represent arithmetic mean of sites within each reach for a particular sampling effort; error bars represent ± 1x SD; within each time-point, mean reach values differ marginally significantly (two-tailed *t*-test, p<0.1) when marked ''ms''.

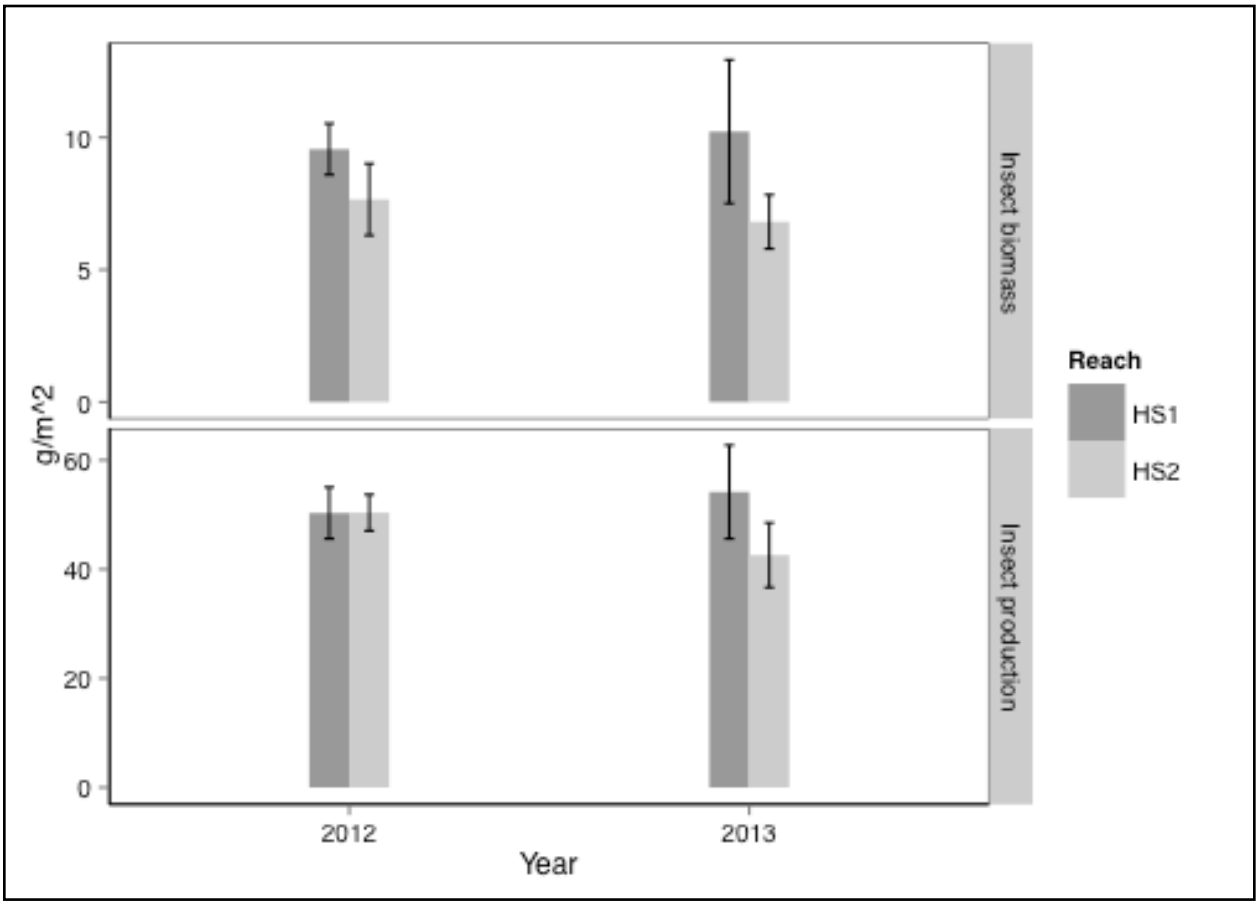

Figure 8. Benthic macroinvertebrate (BMI) estimated biomass density and annual production values at Hancock Springs Creek during 2012 and 2013. Bar height depicts arithmetic mean; error bars represent ± 1x SD. Reach 1 and Reach 2 mean values do not differ at any time point.

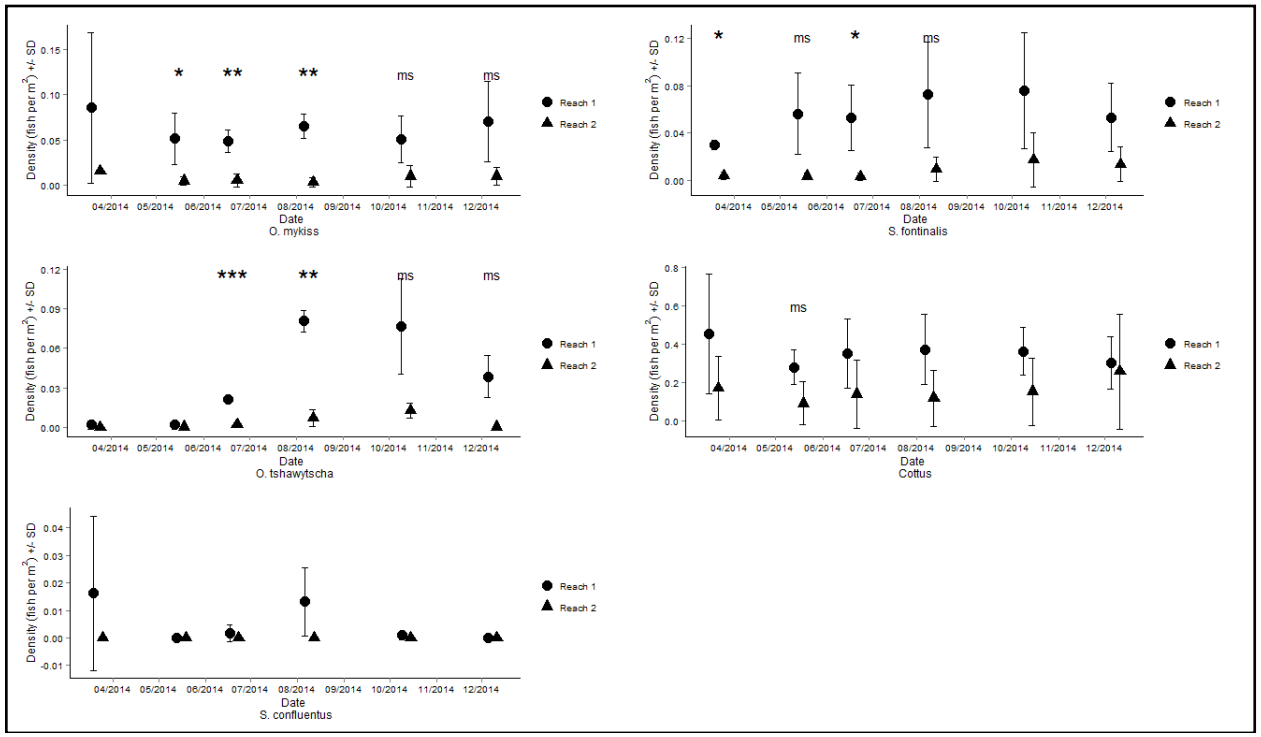

Figure 9. Estimated fish densities, expressed relative to stream channel benthic area, in Hancock Springs Creek, during 2014. Abscissas for each point represent arithmetic means of site values within each reach at that time-point; error bars represent ± 1x SD. For each species and within each time-point, mean reach values differ marginally significantly (two-tailed *t*-test, p<0.1) when marked ''ms'', significantly (two-tailed *t*-test, p<0.05) when marked ''*'', highly significantly (two-tailed *t*-test, p<0.01) when marked "", and very highly significantly (two-tailed *t*-test, p<0.001) when marked "***".**

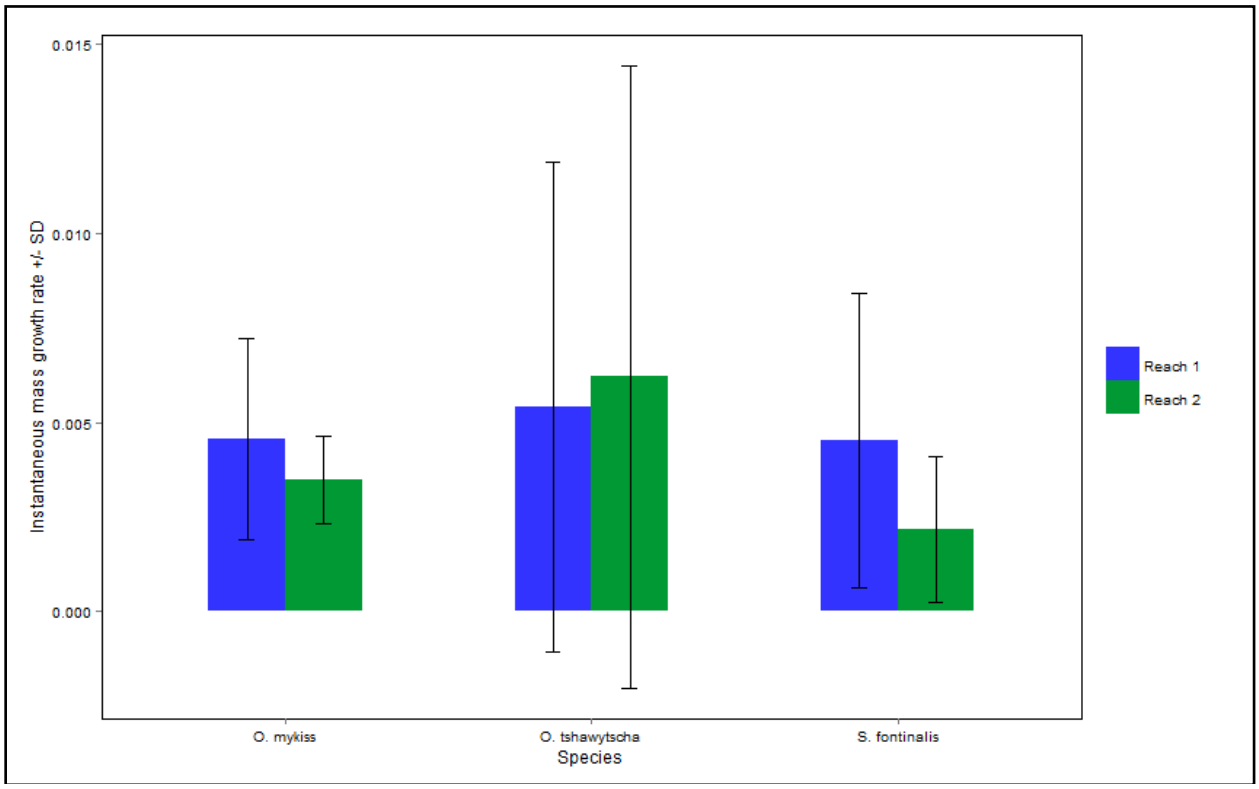

Figure 10. Instantaneous (specific) growth rate in mass, for *O. mykiss*, *Oncorhynchus tshawytscha*, and *S. fontinalis* re-captured at Hancock Springs Creek from October 2013 through October 2014. Bar height represents arithmetic mean of site values within each reach; error bars represent ± 1x SD. Reach 1 and Reach 2 mean values do not differ at any time point.

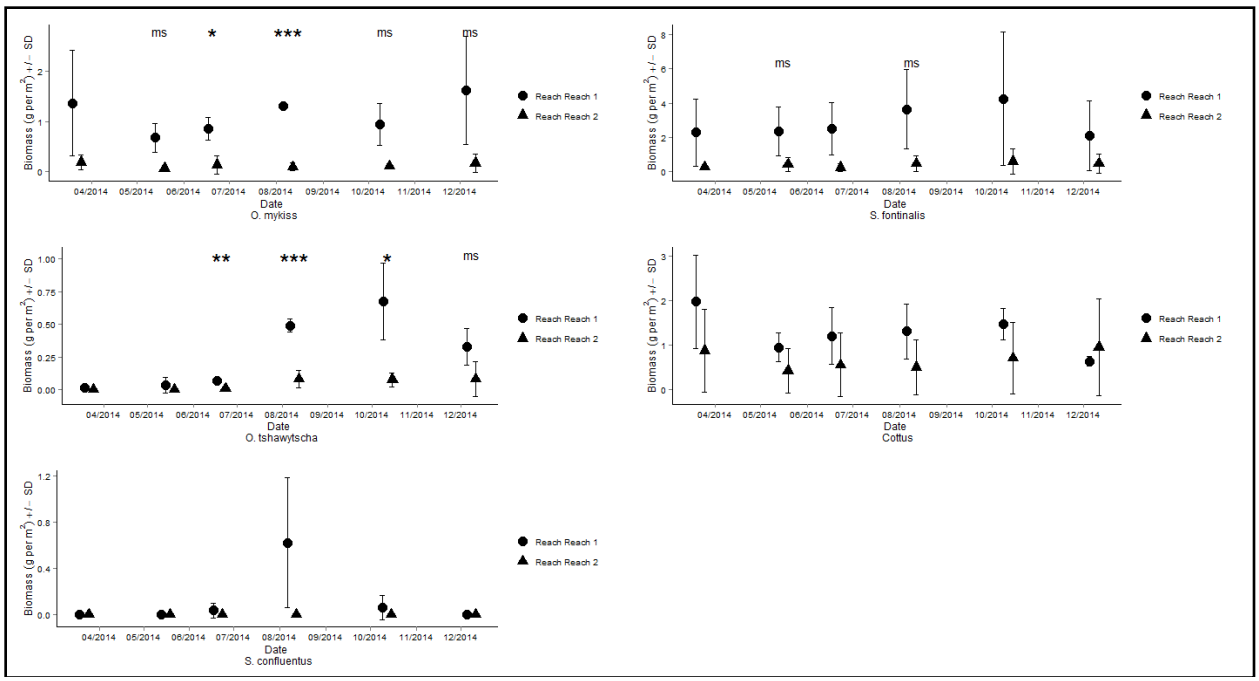

Figure 11. Estimated fish biomass, expressed relative to stream channel benthic area, in Hancock Springs Creek, during 2014. Abscissa for each point represents arithmetic mean of site values within each reach; error bars represent ± 1x SD. See Figure 8 for an explanation of symbols.

		Reach 1		Reach 2	
		Estimate	SD	Estimate	SD
S. fontinalis	A0	0.0294	0.0387	0.0243	0.0361
	A1	0.8346	0.2377	0.0769	0.0191
	A2	1.6910	0.9498	0.1966	0.2168
	Total	**2.5550**	**0.9798**	**0.2978**	**0.2206**
O. mykiss	A0	0.0208	0.4201	0.1113	0.0534
	A1	0.3284	0.4162	-0.0177	0.0345
	Total	**0.3492**	**0.5914**	**0.0012**	**0.0636**
O. tshawytscha	A0	0.3354	0.0619	0.0298	0.0295
	A1	0.0613	0.0233	0.0000	0.0000
	Total	**0.3966**	**0.0661**	**0.0012**	**0.0295**

Table 2. Mean and standard deviation values for annual production ($gm^{-2}y^{-1}$) for *S. fontinalis*, *O. mykiss*, and *Oncorhynchus tshawytscha*, showing breakdown by size class and summed total, calculated after (Hayes, Bence *et al.* 2007).

	Reach 1		Reach 2	
	Estimate	SD	Estimate	SD
Cottus spp.	0.1422	0.0304	0.0175	0.0082

Table 3. Mean and standard deviation values for annual production ($gm^{-2}y^{-1}$) for *Cottus* spp., calculated after Hynes and Coleman (1968), following modifications described by Garman and Waters (1983)

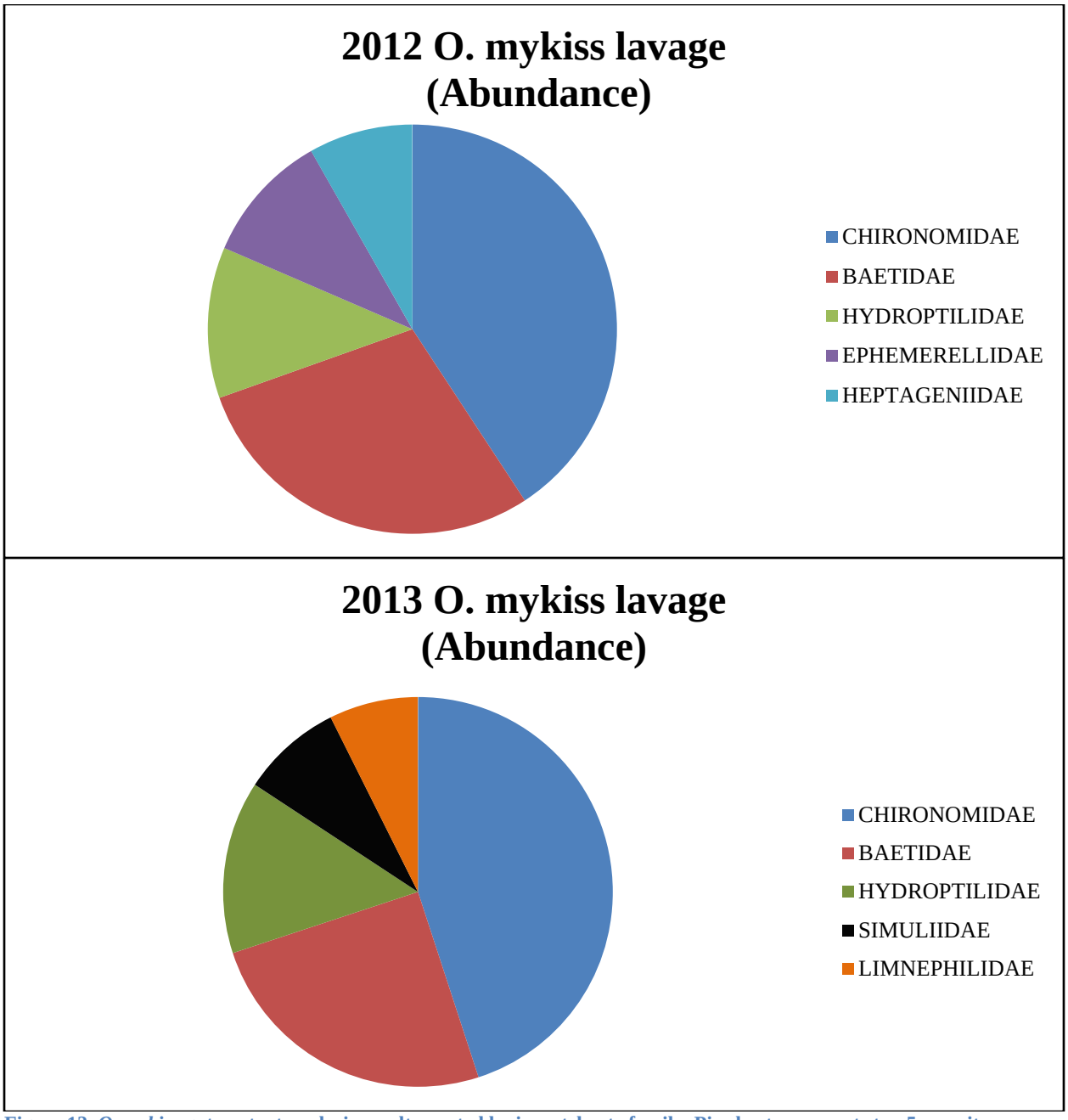

Figure 12. *O. mykiss* gut content analysis results, sorted by invertebrate family. Pie chart represents top 5 prey item families, as ranked by abundance in gastric lavage samples, from fish sampled at Hancock Springs Creek throughout the year.

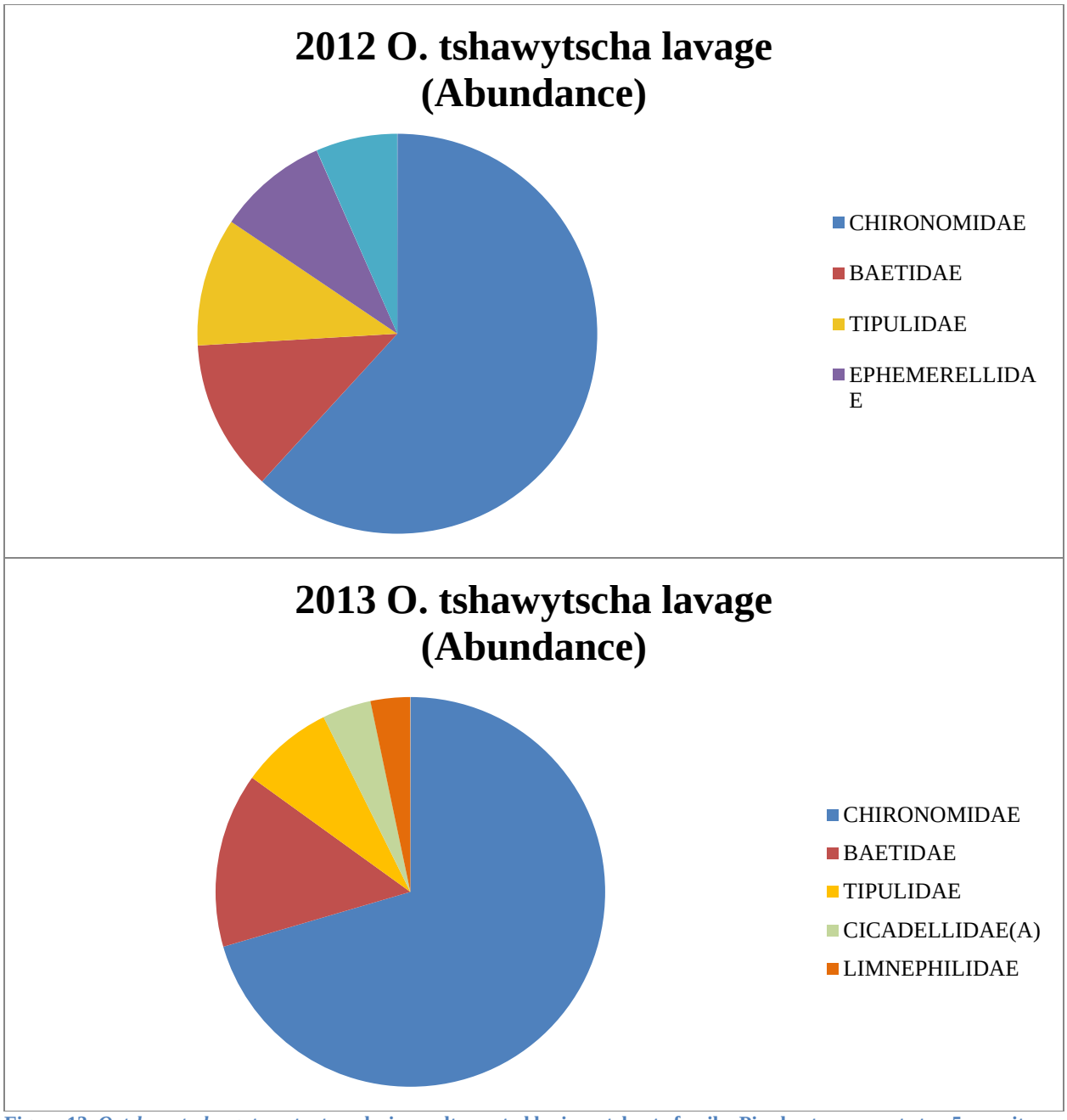

Figure 13. *O. tshawytscha* gut content analysis results, sorted by invertebrate family. Pie chart represents top 5 prey item families, as ranked by abundance in gastric lavage samples, from fish sampled at Hancock Springs Creek throughout the year.

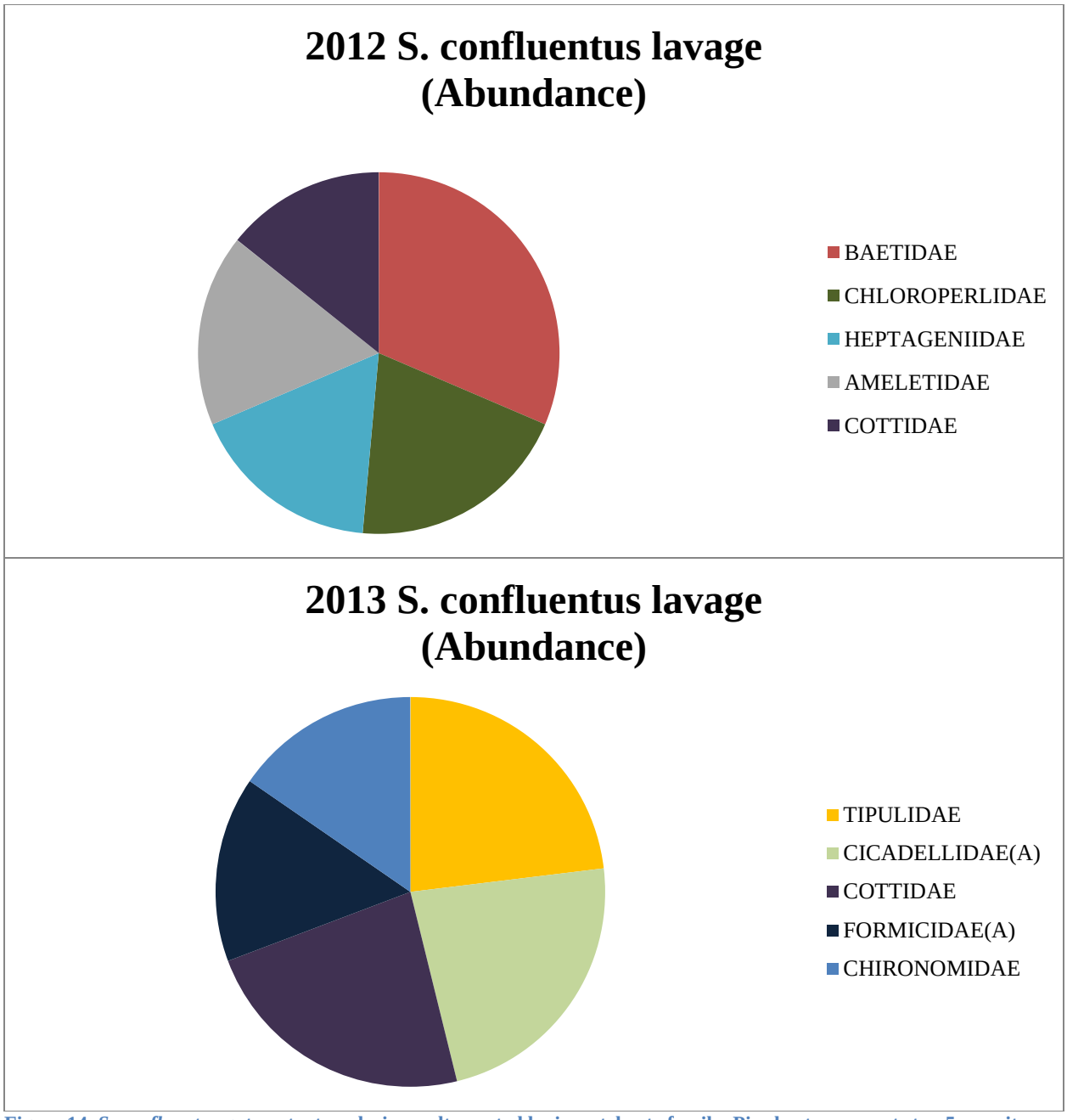

Figure 14. *S. confluentus* gut content analysis results, sorted by invertebrate family. Pie chart represents top 5 prey item families, as ranked by abundance in gastric lavage samples, from fish sampled at Hancock Springs Creek throughout the year.

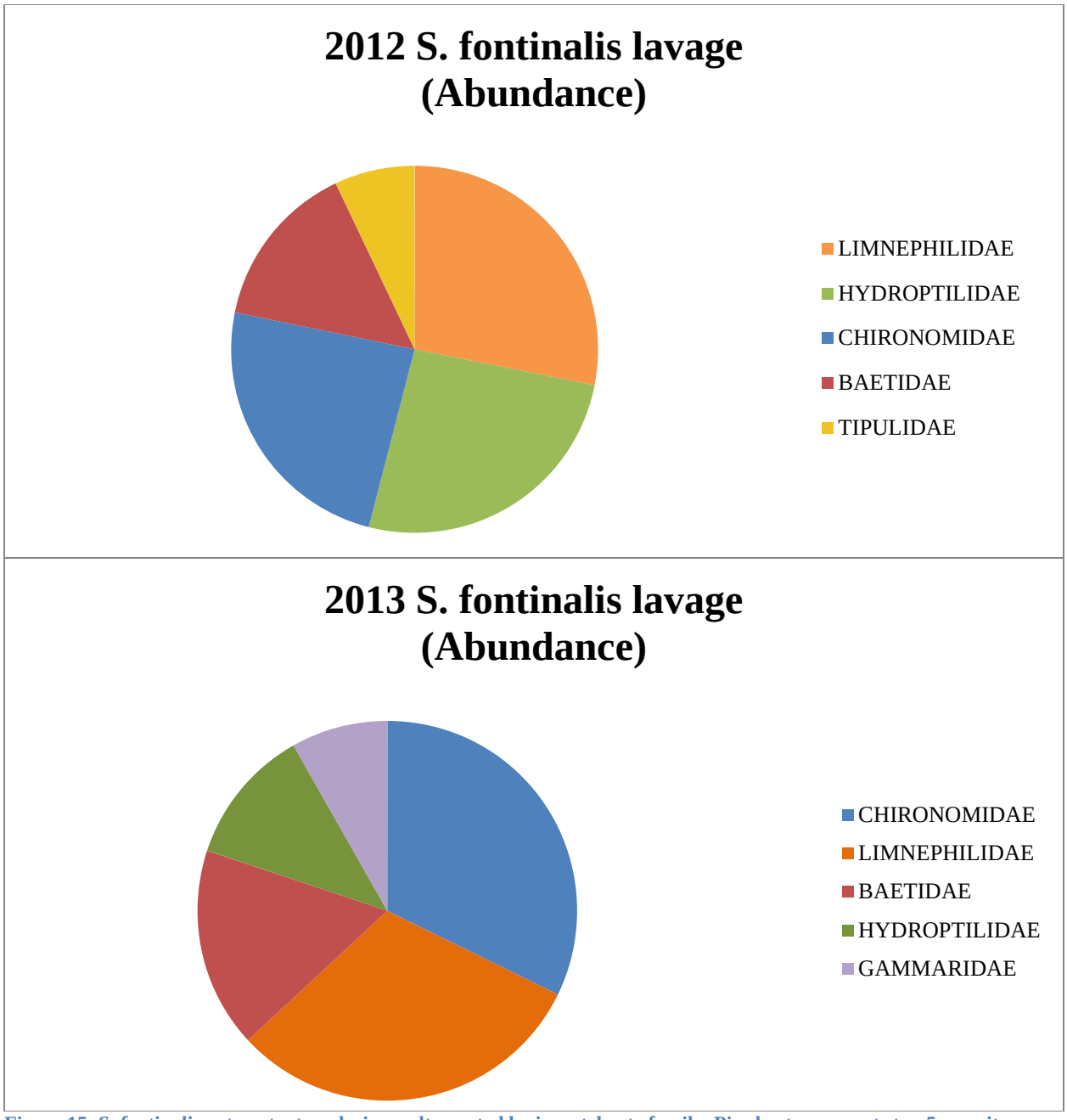

Figure 15. *S. fontinalis* gut content analysis results, sorted by invertebrate family. Pie chart represents top 5 prey item families, as ranked by abundance in gastric lavage samples, from fish sampled at Hancock Springs Creek throughout the year.

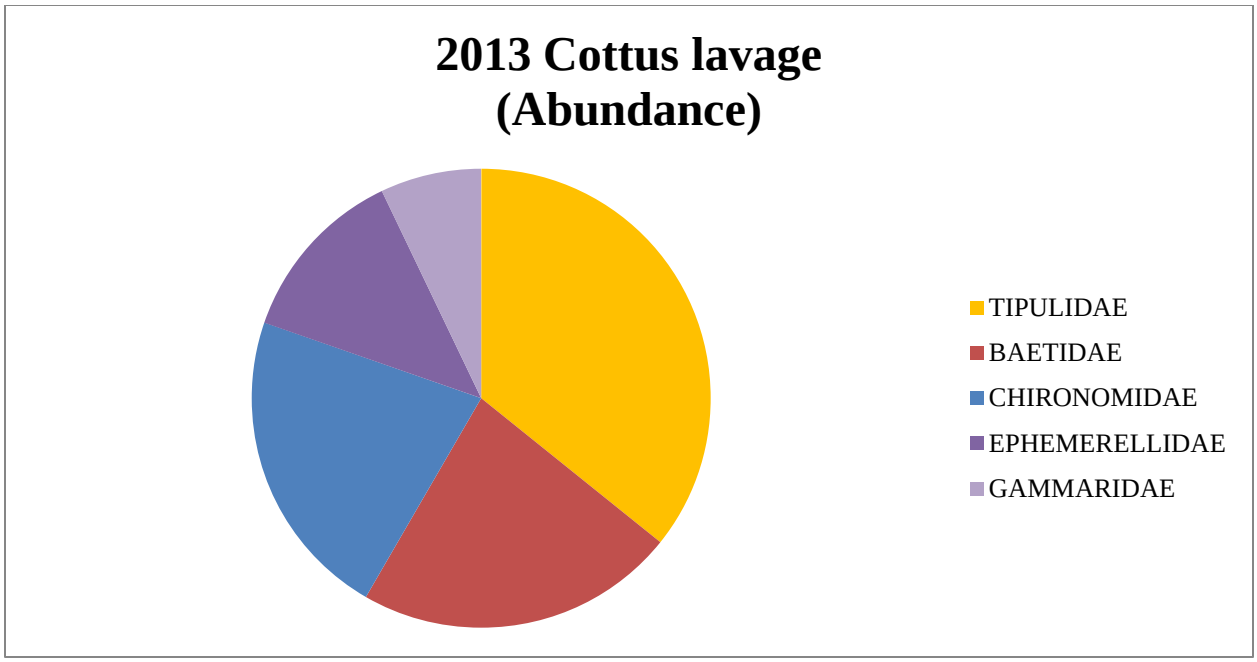

Figure 16. *Cottus* spp. gut content analysis results, sorted by invertebrate family. Pie chart represents top 5 prey item families, as ranked by abundance in gastric lavage samples, from fish sampled at Hancock Springs Creek throughout the year.