Juvenile Salmon Ecology in Tidal Freshwater Wetlands of the Lower Columbia River and Estuary: Synthesis of the Ecosystem Monitoring Program, Trends (2005– 2013) and Food Web Dynamics (2011-2013)

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Jina Sagar, Lower Columbia Estuary Partnership, Portland, Oregon

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Jina P. Sagar¹ Amy B. Borde² Lyndal L. Johnson³ Tawnya D. Peterson⁵ Joe A. Needoba⁵ Kate H. Macneale³ Matthew Schwartz¹ April Silva⁴ Catherine A. Corbett¹ Amanda C. Hanson¹ Valerie I. Cullinan² Shon A. Zimmerman² Ron M. Thom² Paul M. Chittaro³ O. Paul Olson³ Sean Y. Sol³ David J. Teel³ Gina M. Ylitalo³ Michelle A. Maier⁵ Claudia E. Taus z^5

Prepared by the Lower Columbia Estuary Partnership with funding from the Bonneville Power Administration.

Lower Columbia Estuary Partnership 811 SW Naito Parkway, Suite 410 Portland, OR 97204

 ¹ Lower Columbia Estuary Partnership
 ² Battelle-Pacific Northwest National Laboratories
 ³ Northwest Fisheries Science Center, NOAA-National Marine Fisheries Service

⁴ Columbia Estuary Study Task Force

⁵ Oregon Health and Sciences University

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List of Acronymns and Abbreviations

AGST	Creeping bentgrass (Agrostis stolonifera L.)
BB	Ilwaco-Baker Bay
BiOp	Biological Opinion
BPA	Bonneville Power Administration
CALY	Lyngby's sedge (Carex lyngbyei)
CASP	Carex (<i>Carex</i> spp.)
CCMP	Comprehensive Conservation and Management Plan
CDOM	Colored dissolved organic matter
CEERP	Columbia Estuary Ecosystem Restoration Program
CF	Condition factor
CLM	Cunningham Lake
CREST	Columbia River Estuary Study Taskforce
CS	Campbell Slough
CV	Coefficients of variation
DIC	Dissolved inorganic carbon
ELPA	Common spikerush (Eleocharis palustris)
EM	Emergent marsh
EMP	Ecosystem Monitoring Program
ESU	Evolutionary Significant Unit
EV	Emergent vegetation
FCRPS	Federal Columbia River Power System
FLM	Franz Lake
GLM	Generalized linear model

GPP	Gross Primary Production
LCRE	Lower Columbia River and Estuary
LIOC	Western lilaeopsis (Lilaeopsis occidentalis)
MRPP	Multi-response permutation procedure
NDS	Nutrient-diffusing substrate
NEM	Net ecosystem metabolism
NEP	National Estuary Programs
NOAA	National Oceanic and Atmospheric Administration
NMFS	National Marine Fisheries Service
NMS	Nonmetric multidimensional scaling
NPCC	Northwest Power and Conservation Council
NWR	National Wildlife Refuge
OESA	Water parsley (Oenanthe sarmentosa)
OHSU	Oregon Health Sciences University
OW	Open water
PAD	Potential annual detritus
PHAR	Reed canarygrass (Phalaris arundinacea)
PNNL	Pacific Northwest National Laboratory
POAM	Water ladysthum (Polygonum amphibium)
Rkm	River kilometer
RM	River mile
SALA	Wapato (Sagittaria latifolia)
SAV	Submerged aquatic vegetation
SCTA	Softstem bulrush (Schoenoplectus tabernaemontani)
SEV	Sum exceedance value

SR	Secret River
TOC	Total organic content
USACE	United States Army Corps of Engineers
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
UW	University of Washington
WHC	Whites Island
WI	Welch Island

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1 Executive Summary

Context

The Ecosystem Monitoring Program (EMP) is managed by the Lower Columbia Estuary Partnership (Estuary Partnership) and is an integrated status and trends program for the lower Columbia River and Estuary (LCRE). As part of the National Estuary Program, the Estuary Partnership works with regional partners to develop and implement a Comprehensive Conservation and Management Plan (CCMP), which identifies key actions needed to restore and protect the *biological integrity* of the lower river. Ecosystem monitoring is a key element of the Estuary Partnership's CCMP. The CCMP specifically calls for sustained long-term monitoring to understand ecological conditions in the river and to evaluate the impacts of management actions (e.g., habitat restoration) over time. The EMP was created to serve as this long-term monitoring program. The EMP is designed to provide an inventory of the different types of habitats within the lower river, track trends in the overall condition of these habitats and the estuary ecosystem, provide a suite of reference sites for use as end points in the region's habitat restoration actions, and place findings from management actions into context with the larger ecosystem. Monitoring minimally disturbed habitats each year under the EMP can provide a stronger understanding of the natural range of variability of ecological structure and processes in these habitats, and provide a fundamental understanding of processes and relationships, such as the aquatic food web. This information is key in identifying the conditions resource managers hope to realize with restoration and other management activities and provides a cost-effective method for evaluating the success of these efforts.

The EMP is funded by the Northwest Power and Conservation Council/Bonneville Power Administration (NPCC/BPA). NPCC/BPA funding for this program focuses on those actions addressing BPA's Columbia Estuary Ecosystem Restoration Program (CEERP) goal of improving habitat opportunity, capacity and realized function for aquatic organisms, specifically salmonids. Funding of the EMP also provides leverage for the Estuary Partnership to expand efforts beyond CEERP (for example, toxic contaminants and multi-species recovery) for a comprehensive, integrated and collaborative ecosystem based program. The EMP is designed to monitor the status and trends of ecosystem condition, which provides information for the NPCC's High Level Indicators for the estuary subbasin. It also addresses strategies listed within the Draft Columbia River Basin Monitoring, Evaluation, Research, Reporting and Data Access Framework (draft MERR Framework) and Mainstem Lower Columbia River and Columbia River Estuary Subbasin Plan.

A primary goal of the EMP is to provide a better understanding of those conditions in the lower river that improve (or reduce) survival and performance of aquatic organisms. The EMP is designed to annually collect a suite of ecological metrics that indicate the quantity, quality and availability of estuarine and tidal freshwater floodplain habitats, focusing on those typically used by juvenile salmonids for rearing, as well as metrics that indicate the benefit juvenile salmon derive from those habitats. Metrics collected under the EMP include:

- Salmonid occurrence, composition, growth, diet, condition and residency;
- Habitat structure, including physical, biological and chemical properties of habitats;

- Food web characteristics, including composition, rates and contribution to salmon diets of primary and secondary production within floodplain habitats and within the mainstem lower river; and
- Biogeochemistry of tidal freshwater region of the lower river for comparison to the biogeochemistry of floodplain habitats and lower estuary section.

This synthesis report focuses analyses on two main components of the EMP: *variability* in the status and trends data for the habitat structure, habitat hydrology, fish and fish prey components collected between 2005 and 2013 and a *synthesis of food web dynamics* in the LCRE collected between 2011 to 2013. In 2013, a synthesis report was completed that provided an analysis of all EMP data collected between 2005 and 2010 (Sagar et al. 2013; available on the Estuary Partnership website: http://www.estuarypartnership.org/resource/juvenile-salmon-ecology-tidal-freshwater-wetlands-lower-columbia-river-estuary-synthesis). Please see that report for trends analyses by site and across sites for each EMP metric. Additionally, our annual reports to the BPA (available from our website) provide updates on these trends analyses.

This synthesis report builds on the 2013 synthesis by focusing on identifying the variability in metrics and by providing results on food web and abiotic conditions. A major impetus of this synthesis was to gain a better understanding of the "range" possible for each EMP metric by determining the variability in our datasets. If we find that some metrics are more "predictable", we could adjust the collection frequency of that metric to reduce costs or focus efforts on other metrics or sites that we understand less. Similarly, we can adjust the EMP sampling design, frequency of collection for metrics that are strongly correlated with other metrics. For example, if results demonstrate that vegetation cover is directly related to duration of inundation during the growing season, we could focus our sampling on water surface elevation during that time period, a much easier, less expensive metric to collect. Once we improve our understanding of how lower Columbia River habitats function ecologically, we can predict how the system varies in response to different conditions so that we can adjust sampling methodology to improve the efficiency of the monitoring program in the future.

Additionally, food web and abiotic condition metrics were not collected under the EMP until 2010, and therefore are not included within the 2013 synthesis. We also added two fixed sites in Reaches A and B to the EMP in 2011 and 2012. Results from these sites were not included in the first synthesis report and are included in this report.

Approach

The EMP is a collaborative effort between the Estuary Partnership, Pacific Northwest National Laboratories (PNNL), National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA NMFS), U.S. Geological Survey (USGS), Oregon Health Sciences University (OHSU) and Columbia River Estuary Study Taskforce (CREST). The EMP partnership has collected data on habitat structure (vegetation community, water surface elevation, channel morphology, sediment grain size and total organic content [TOC], sediment accretion, and site profiles; by PNNL), fish use (fish community, salmon metrics and diet, contaminants, genetics and stock information), macroinvertebrate prey availability, and water temperature at the time of fish sampling (by NOAA NMFS), abiotic site conditions (water temperature, specific conductance, pH, dissolved oxygen, and depth; by USGS), food web dynamics (composition and rates of primary and secondary production; conditions directly

influencing production, such as nutrients, light availability; stable isotope analysis of salmon tissue, diet; prey by OHSU and USGS), and biogeochemistry in the tidal freshwater section of the lower river by OHSU.

The study area includes the tidally influenced reaches of the lower river, extending from the mouth of the estuary to Bonneville Dam. Sample sites are minimally disturbed, tidally-influenced freshwater emergent wetlands representative of the eight hydrogeomorphic reaches (reaches A-H; reach A is at the mouth of the river with reach letters ascending with river kilometer) across the study area. The Estuary Partnership and its monitoring partners have focused on providing an inventory of juvenile salmon habitats (or "status") across the lower river stratifying by hydrogeomorphic reach and including a growing number of fixed sites for inter-annual variability (or "trends"). Each year prior to 2012, three to four "status" sites, in a previously unsampled reach, were selected along with the continued sampling of a growing number of "trend" sites. Ilwaco Slough in Baker Bay (2011-2013); Secret River in Gray's Bay, Welch Island in the Julia Butler Hansen National Wildlife Refuge (NWR; 2012-2013); Whites Island (2009, 2011-2013); Campbell Slough in the Ridgefield NWR (2005–2013); and Franz Lake Slough in the Franz Lake NWR (2008-2009 and 2011-2013) are the six trend sites currently sampled under the program. Habitat structure and hydrology data collection began in 2005, fish data collection began in 2007, fish prey data collection began in 2008, and abiotic conditions and food web data have been collected since 2011. Sampling generally occurs March through September (during the primary outmigration period for many juvenile salmonid stocks), except in 2012-2013, where fish sampling expanded to a full-year schedule in an effort to capture juvenile salmon that may utilize the lower river for rearing during late winter and early spring (e.g., unmarked juvenile Chinook salmon and chum salmon, Oncorhynchus keta). Emergent marsh vegetation is measured at low and high elevation locations, up to the zone transitioning to woody vegetation within each EMP site. Vegetation is sampled twice per year, once in the summer at peak biomass to identify dominant species present in the vegetation community and again in winter, after vegetation die-off to compare plant biomass between the two seasons to determine detrital contributions to the system. Fish and food web metrics are sampled monthly to detect temporal changes that may result from variable biotic and abiotic conditions, such as stock-specific migration patterns, water temperature, or river hydrology. Biogeochemistry is collected at hourly intervals at two in situ sensor locations, River Mile 53 and River Mile 122, with monthly grab samples for plankton composition and standing stock.

Results

Variability

Habitat structure and hydrology

Site similarity is a comparison of an individual site's conditions to itself over time and a comparison to conditions at other sites. It is an analysis of variability that is useful for highlighting trends in vegetation over time and space as a result of an environmental variable (e.g., annual changes in hydrology). In the LCRE, the average site similarity of EMP sites, based on vegetation cover, decreased significantly with an increasing number of years the site was monitored. This is due, in part, to the longer data records (nine years) from upper river sites (rkm 145 and 149), where the signal of seasonal and inter-annual hydrologic variability is much stronger (Jay et al. in review). This hydrologic variability has a direct effect on the vegetation cover in up-river locations, as shown by our previously conducted trend analyses (Sagar et al. 2013), which demonstrated that in higher water years, the increase in inundation (as measured by the sum

exceedance value [SEV], representing the amount of water over a site in a given time period) results in decreased vegetation cover. Thus, the decrease in site similarity with time is primarily due to hydrologic variability between years rather than a gradual vegetation community shift over time.

At the lower river sites, the trends in cover over time are more stable, with subtle changes between years. Among these sites, the lowest similarity in cover between years was observed at the low marsh site at Secret River. The reason for the difference between the cover measured at this site in 2008 and that measured in 2012 and 2013 is not clear. The difference could be related to variation in winter flooding from the local watersheds as opposed to Columbia River hydrology. The site is one of the lowest elevation sites we have surveyed in the estuary, located at the emergent vegetation elevation threshold, and perhaps slight variations in hydrology and sediment effect these sites more dramatically than higher elevation sites.

Spatial variability among tidal marsh sites in the LCRE is dependent on location of the sites relative to each other and relative to the hydrologic gradient of the river. We found that site similarity based on vegetation cover decreases with increasing distance between sites. Similarity was generally highest within individual Emergent Marsh zones. These are five zones along the estuarine tidal freshwater gradient that demarcate different vegetation communities and different hydrology and resulting inundation patterns (see Jay et al. in review, modified from Borde et al. 2012). The five zones were established using data from the EMP, Reference Site and Cumulative Effects of Restoration (USACE) studies. Higher variability occurred in some Emergent Marsh zones indicating the need for additional spatial sampling to identify if additional zones are warranted (e.g., tributaries vs. mainstem). As a result of this work, future habitat monitoring efforts could focus on sampling and comparing sites within Emergent Marsh zones rather than using the eight hydrogeomorphic reaches to stratify vegetation sampling.

Tidal marsh vegetation provides refuge habitat for juvenile fish and macrodetritus contributions to the lower river ecosystem, enhancing habitat capacity for juvenile salmonids. Annual fluctuations in hydrology drive inundation patterns and the spatial and temporal variability of vegetation characteristics, particularly in the river-dominated upper reaches. High water years have led to reduced vegetation cover and decreased site similarity in these sites over time; however, our understanding of these upper reaches remains limited by a small number of sites (n=2) and a minimal number of sampling years (two of which were higher than average water years). Continued long term sampling would cover a greater range of annual hydrologic conditions at the trend sites and across Emergent Marsh zones, creating a better understanding of the range of natural variability that exists for vegetation characteristics across the lower river. This improved understanding would be invaluable for improving the success of our habitat restoration activities in these reaches and for evaluating the effectiveness of regional habitat restoration efforts in general.

Fish

Our analyses show a high degree of variability in many of the fish metrics we sample under the EMP. Some of this variability may be a result of differences in catch composition among beach seine sets. Additionally, our understanding of variability by month or by season is limited by the restricted amount of sampling we have carried out in the late fall and winter months (November, December; only in 2012 and 2013). However, with the level of sampling conducted to date, we are able to detect seasonal and spatial trends in many of the variables we measured, including:

- **Fish community characteristics**. We observed increased species richness, species diversity, and percentages of non-native fish species and fish that are potential predators of salmon in catches during the summer months. Species diversity, species richness, percentages of non-native species and percentages of predators also tended to be higher in reaches F-H, farther from the river mouth and closest to areas of greatest human disturbance.
- Juvenile salmon spatial patterns. In the lower river reaches B and C, we observed higher proportions for unmarked Chinook salmon (*Oncorhynchus tshawytscha*) from Lower Columbia Evolutionary Significant Unit (ESU) stocks, including high proportions of salmon fry. In middle to upper river reaches E-H, proportions of marked fish in catches were highest and, especially in Reach H, coho salmon (*Oncorhynchus kisutch*) were more abundant. Chinook salmon from interior Columbia River stocks were also found more frequently in the upper reaches.
- Juvenile salmon seasonal patterns. Chinook and chum salmon were found in greatest numbers in spring but coho salmon density did not show a clear seasonal pattern. Genetic stock composition also shifted seasonally, with higher proportions of interior Columbia River stocks appearing later in the sampling season. Marked and unmarked Chinook salmon also showed distinct patterns of seasonal occurrence: marked fish were present primarily from May through July, while unmarked juvenile Chinook salmon were found for most of the sampling season with the exception of late summer and fall months (August-October). Unmarked juvenile Chinook salmon showed significant increases in body size and condition as the sampling season progressed, while marked fish were more uniform in size.

Genetic stock was an important source of variability for some fish metrics, including timing, lipid content, growth rate, and fish size. However, the number of fish belonging to non-Lower Columbia River ESU stocks (i.e., Willamette River basin and interior Columbia River basin stocks) was relatively small, making it difficult to identify significant trends.

For the majority of fish metrics, location within the lower river explained more of the variability than the timing of sampling (month). Generally, power analyses indicate that in spite of variability among beach seine sets and sampling events, we have conducted sufficient sampling to establish the existence of seasonal and spatial trends in fish community and salmon occurrence parameters at the EMP sites with longer term data. However, at the newer trend sites (those in Reaches A and B), using only EMP data, additional years of sampling would be necessary to identify if trends exist, and to determine whether there are differences between the fish community and salmon metrics in these two reaches versus other sections of the lower river. Reaches A and B are more influenced by salinity and tidal exchange than other hydrogeomorphic reaches, providing an important opportunity to capture any spatial changes along the longitudinal habitat gradient of the lower river, and whether differences in fish metrics correlate to differences in habitat capacity along the estuarine tidal freshwater gradient. In addition, long term sampling in Reaches A and B will continue to expand on past fisheries work conducted in these lower reaches (Bottom et al. 2005, 2008, 2011; Roegner et al. 2010, 2012), forming a more comprehensive annual dataset.

Results from fish data collection efforts in the lower Columbia River inform regional salmon recovery efforts about limiting factors affecting juvenile salmon survival, measures of fitness (e.g., condition, growth), habitat use, and residency, and how these factors differ across space and time. Through the EMP we are elucidating the benefits lower Columbia River habitats provide to the different salmonid stocks by gaining a better understanding of how juvenile salmonids use the lower river (spatially and temporally) as well as some of the sources of variability in the fish community, such as genetic stock and location of capture. However, salmon habitat use in the LCRE during fall and winter is still relatively unknown. Also, as a result of their low numbers, the extent that non-lower Columbia River ESU stocks use emergent wetland habitats in the lower river during their out-migration period and the condition and growth characteristics of fish belonging to these stocks are not well known. Year-round sampling could capture habitat use and condition for a variety of salmonid stocks that may be present in the lower river during the fall and winter seasons, and continued sampling will improve our understanding of non-lower Columbia River ESU stock behavior, condition, and growth. Prior to the EMP, fish sampling was limited to just the lowest river reaches; thus, continued long term fish data collection, in combination with habitat and food web data, across all reaches (particularly the upper reaches) will benefit our understanding of the opportunity, capacity and realized function of juvenile salmon habitat throughout the LCRE.

Synthesis of Food Web Dynamics

Declines in Columbia River basin salmonid populations, relative to historical times, are due to a variety of factors including alterations to salmonid food webs. Over the past century there has been a concomitant decrease in vascular plant detritus inputs, which have traditionally fueled salmonid prey populations, and an increase in fluvial phytoplankton contributions to organic matter production in the LCRE. The influence of this ecological shift on salmonid prey populations is poorly understood. A central focus for the EMP has been to determine what the important food web components are for juvenile salmon by investigating the factors that influence prey densities, species composition, and seasonality.

Since 2011, activities of the EMP have focused on the determination of factors that influence the availability of salmon prey (and their prey) and on assessing how this availability translates to prey consumption patterns and ultimately fish condition. The spatial and temporal observations initiated through this program aim to identify the underlying ecological processes that influence prey population structure and availability in a spatially and temporally variable environment. Discerning key processes in an environment bearing high inherent variability can only be served through consistent, long-term observations. The EMP is the first program to study the full suite of salmon food web metrics, including composition and rates of primary and secondary production, as well as the contribution of these two trophic levels to salmon diets, over space and time in the lower river. This synthesis report presents the first three years' worth of food web data collected under the program, and we are only beginning to learn how the ecosystem functions at the site level. Continued annual collection of these metrics at the trend sites will provide a clearer picture of how the food web varies with annually fluctuating hydrological and climatic conditions. Ultimately, once we capture what is influencing site-specific food web dynamics, we hope to extrapolate to other sites in the river and eventually to the landscape scale. The incorporation of continuous in situ sensors to monitor water quality at two mainstem sites in the lower Columbia (River

Mile 53 in 2011, and River Mile 122 in 2013) has been very useful for providing larger scale context for our observations.

Detrital export - Detritus from emergent wetland plants historically provided the basis of the juvenile salmonid food web in the lower Columbia River. However, large scale conversion and diking of habitats for urban, industrial and agricultural development has greatly reduced vascular plant macrodetritus inputs into the system. Additionally, construction of the hydropower system on the Columbia River resulted in greater planktonic production and possibly a switch in the base of the food web from macrodetrital to plankton sources which could favor pelagic-feeding species (e.g., American shad; Alosa sapidissima) over epibenthic-feeding species like salmon (see Bottom et al. 2005 for discussion). Data collected as part of the EMP on emergent wetland plant biomass production is improving our understanding of the macrodetritus contribution from these emergent wetlands in the LCRE. Data from six sites along the estuarine gradient, collected over a three-year period, provide some insight to patterns and trends in biomass production and the resulting detritus contribution. Three wetland strata were sampled as part of this study: high marsh, low marsh, and submerged aquatic vegetation (SAV). At sampling sites, high marsh had the greatest plant biomass (average of 929 g/m^2), compared to low marsh and SAV (average of 249 g/m^2 and 42 g/m^2 , respectively) and the four lower river sites had greater biomass than the two upper river sites (high marsh average of 1162 g/m² and 426 g/m², respectively). This spatial trend was likely due to the effects of two consecutive high water years and the upper sites having greater cover of reed canarygrass (*Phalaris arundinacea*), which reduced the vegetation cover and biomass production in the upper river sites during all three years of the study.

By evaluating the plant biomass remaining in the winter, we are able to estimate the amount of plant biomass that has been contributed to the detrital based food web between summer and winter. Detrital biomass is important not only for ocean-type salmonids (e.g., fall Chinook salmon and chum salmon) but also for stream-type salmonids such as steelhead (*Oncorhynchus mykiss*) through export to the mainstem and to habitats further downstream. Similar to production, the greatest detrital contribution is from the high marsh strata. In general, the detrital contribution was greater in the lower river sites than the upper river sites; however high variability and limited sampling period (2012 and 2013) in the lower river make these results inconclusive. Some trends in detrital contribution were seen at the species level in this analysis, with higher detrital contribution from Lyngbye's sedge (*Carex lyngbyei*; average = 1,021 g/m²) than from reed canarygrass (average = 291 g/m²). This is likely due to a couple of reasons: 1) there are differences in the breakdown between the two species, with reed canarygrass having a greater amount of standing stock that remains between years, and 2) reed canarygrass which had a much greater frequency of occurrence in the two upper river sites than in the four lower river sites, was more affected by the high water and therefore did not produce as much biomass in the years of this study.

Salmon prey availability and preference - Based on six years of EMP salmon prey availability data, availability of macroinvertebrates in and amongst emergent vegetation is significantly higher compared to open water areas within sites. This distinction suggests a critical role for emergent wetlands in providing desirable prey to support juvenile salmon populations. Based on stomach contents of captured juvenile Chinook salmon, the EMP has demonstrated that juvenile salmon show a clear preference for insects of the Order Diptera, particularly juvenile non-biting midges (Family Chironomidae), with crustacean amphipods (*Corophium* spp.) constituting the second most abundant food source found in juvenile salmon diets. Electivity values indicate that juvenile Chinook salmon consumed dipterans and amphipods at a rate

higher than would be expected given their abundance in the habitats sampled. Amphipods were often highly selected for by juvenile salmon at sites in the lower reaches especially, but they appear to have been avoided at sites upstream of Reach E where dipterans were the preferred prey. Other food sources such as cladoceran and copepod crustaceans were of secondary importance and only when their ambient abundances were high. Hymenoptera were often rare but, when present, were selected at a high rate, at certain sites. The data collectively suggest that although juvenile salmon display flexibility in their diets, they exhibit strong preferences for certain taxa.

Base of Salmon Food Web - A food web model that relates stable isotope signatures (¹³C and ¹⁵N) of organic matter from different sources across primary and secondary trophic levels to those found in salmon tissues was used to predict the most important food sources for juvenile salmon and their prey. The data revealed that fluvial phytoplankton constitute an important part of the diet of juvenile salmon prey, particularly among the non-biting midges (Family Chironomidae, Order Diptera) in the spring (May) before shifting to a mixed isotopic signature of both phytoplankton and vegetation in the summer months (June and July). In contrast, stable isotope signatures for vegetation were associated with amphipod prey throughout the sampling season, suggesting that amphipods rely more heavily on macrodetritus for growth. These preliminary results (2011 - 2012 only) provide important spatial and temporal caveats to the traditional theory that macrodetritus forms the base of juvenile salmon food web in the lower Columbia River. However, it is not known whether chironomids opportunistically forage on phytoplankton when it is available in the spring (even though they may otherwise consume macrodetritus) or if chironomids are selecting for phytoplankton due to a limited availability of macrodetritus in the upper river. The analysis of additional data collected in 2013-2014 will help clarify the roles of the different organic matter sources across sites and temporally within the spring-summer period of use at these shallow off-channel areas.

Preliminary results from the stable isotope food web model suggest that both macrodetritus and phytoplankton are important food sources for invertebrate prey populations of juvenile salmon. The stable isotope data indicate that fluvial phytoplankton are important for chironomids during the spring and early summer, which coincides with the highest chlorophyll a levels measured using the in situ sensor platforms located in the mainstem at River Mile 53 and River Mile 122. The sensor data reveal the recurrence of an annual phytoplankton bloom (evidenced by spikes in chlorophyll a) as well as smaller blooms that occur when growth conditions are favorable. The largest blooms occur in the spring, before the freshet, and in early summer once discharge and turbidity have decreased. In contrast, phytoplankton biomass is low during winter and during episodic storm events that produced water conditions unsuitable for rapid plankton growth. Based on microscopic analysis, phytoplankton assemblages were invariably dominated by diatoms (Class Bacillariophyceae), although contributions by cyanobacteria and green algae increased between spring and summer, especially at the Campbell Slough site. Diatoms are commonly known to be lipid-rich, fast-growing organisms that are important prey for secondary producers (including salmonid prey) in aquatic habitats worldwide. Since phytoplankton are microorganisms, which divide exponentially, their biomass can increase rapidly when conditions are favorable. In turn, zooplankton grazers, such as crustacean copepods and cladocerans (secondary producers), likely respond to phytoplankton blooms by increasing their grazing rates and population sizes following bloom events, which could in turn result in increased competition for salmon prey food sources. These dynamics are likely important for determining juvenile salmon food availability and quality, although we have little data to address this significant gap in knowledge.

Although little information is available to assess the influence of zooplankton grazing on phytoplankton populations, dissolved oxygen data from the in situ sensors demonstrate that growth rates of phytoplankton remain high during periods when phytoplankton biomass is diminished, a pattern that can be explained if phytoplankton are being consumed at a faster rate than their biomass can accumulate. We have documented that phytoplankton are important in the diet of dipterans but the competition for this resource (i.e., phytoplankton) by zooplankton is not known. We note that although zooplankton taxa such as cladoceran and copepod crustaceans were not a preferred food source for juvenile salmon, they could play a role as competitors for juvenile salmon prey (i.e., dipterans and amphipods) for fluvial organic matter from phytoplankton. This makes tracking their populations important for determining potential organic matter availability (and limitations) for salmonid food webs.

The Columbia River phytoplankton populations were dominated in the spring by large, colony-forming diatoms, the primary species being *Asterionella formosa*. Large colonial diatoms are generally thought to escape consumption by zooplankton (and reduce efficiency of organic matter entry into food webs) because of their large size. However, we have documented – in part through the EMP – that this species is prone to heavy parasitism by aquatic fungi ("chytrids"). The infection of large diatoms by chytrid fungi ultimately repackages organic matter from large, inedible diatoms to smaller zoospores, which are broadly considered to be highly nutritious for large and small zooplankton species. Thus, parasitism of dominant primary producers (i.e., diatoms such as *A. Formosa*) could, in fact, increase carbon consumption efficiency and transfer of nutrients to higher trophic levels. In addition, parasitism of diatoms by chytrid fungi may also introduce competition between zooplankton and preferred salmon prey (i.e., dipterans and amphipods) for organic matter from fluvial phytoplankton, although this has yet to be tested directly. The process of nutrient transfer from infected host cells (i.e., diatoms) to zooplankton via chytrid zoospores is termed the 'mycoloop' (Kagami et al. 2014). The mycoloop process is currently not well understood, although it represents a potentially important and overlooked contributor to aquatic food webs. An overview of food web interactions within LCRE emergent wetlands is shown in Figure 1.

Mainstem conditions - Our primary production results document that the mainstem lower Columbia River functions similarly to freshwater tributaries or lakes in that it is phosphorous limited and has high seasonal chlorophyll *a* concentrations (over 40 mg m⁻³), potentially a sign of eutrophic conditions, while the "true estuary" or area of tidal exchange is nitrogen limited. The lower Columbia River is net autotrophic (photosynthesis exceeds respiration) during spring and summer months, while it is heterotrophic (opposite pattern, respiration exceeds photosynthesis) in winter. Additionally, cyanobacteria were documented in several locations in summer months, which could be a concern given that cyanotoxins have been shown to accumulate in zooplankton and the tissues of some fish species.

The mainstem observations indicate that water temperatures above the recognized threshold for suitable salmon habitat (19°C; Bottom et al. 2011) occur each summer in the mainstem portion of the river. Temperatures above 19°C occurred during more days in 2013 than in the previous two years, a result that is correlated to lower discharge levels associated with the spring freshet as compared to 2011 and 2012. Given that the temperature between the two mainstem monitoring sites is similar at all times of the year and that there is no gradient in temperature with water depth, we conclude that the observed periods when temperature surpasses 19°C represent the *lowest* temperatures in the mainstem lower Columbia River for these time periods. Therefore, if salmonids are seeking cold water refuges during this time, they would

need to move off from the mainstem Columbia to localized regions of cold water input, perhaps from tributaries, deltas, or underground springs.

Exploratory Multivariate Analyses - Multivariate analysis results confirmed many of the spatial and temporal patterns observed in the individual food web analyses for primary production, vegetation, macroinvertebrate abundance, and fish abundance. The variability in multiple aspects of the food web at this point, however, makes it difficult to detect fine scale associations between biotic and abiotic elements in multivariate analyses. With only three years of food web data available at this time, our ability to detect patterns and trends from the multivariate analysis remains limited; thus, future annual food web sampling may help elucidate potential trends in the data and provide improved predictions of how food web data spatially and temporally vary.

Shallow, emergent wetlands provide rearing and refugia habitat for juvenile salmon, productive habitat for salmon prey, and areas of deposition for phytoplankton. The salmonid food web in the lower Columbia River is complex, with seasonal and spatial factors that appear to influence habitat quality and prey dynamics. Plant biomass data show reduced macrodetritus contributions from upper reaches, potentially due to reduced vegetative cover and increased presence of reed canarygrass at these sites. Study of the phytoplankton community revealed a dominance of pelagic diatoms in the lower river and identified seasonal fluctuations in abundance in both emergent wetlands and in the river mainstem. Stable isotope analyses determined that both phytoplankton and plant detritus are important food sources for chironomids and amphipods (two preferred prey items for juvenile Chinook salmon), depending on location and time of year. The increased importance of phytoplankton in the diets of salmon prey, in addition to reduced vegetative detritus in the upper reaches, supports the concept of a shift from a historic detritus fueled food web to one where pelagic phytoplankton also play a key role. Zooplankton are also believed to be important components of the salmon food web and continuing to determine composition and abundances at EMP sites will further our understanding of their role in the food web as salmon prey and as phytoplankton grazers (or competitors for salmon prey).

Although we have learned a great deal about the structure and function of the salmonid food web in emergent marsh habitats of the lower river, many knowledge gaps remain. The limited number of sampling years and variability in the data have made plant biomass and macrodetrital export results inconclusive at this time, particularly in the upper reaches. Continued annual sampling will benefit future analyses and provide for a wider range of conditions typical of the Columbia River basin. Whether prey preferences and prey availability affect fish physiological condition and survival is still unclear. Additional study linking fish growth and condition with diet can help us better understand prey availability, resulting from habitat condition that is tied to juvenile salmon performance within the lower Columbia River. Phytoplankton abundance affects water quality (e.g., dissolved oxygen and pH levels) and provides food for salmon prey, but plankton communities and abundances are spatially and temporally variable. Understanding annual patterns at trend sites can allow us to better predict how varying hydrologic and climatic conditions may ultimately affect salmon and salmon prey. Continued food web sampling, such as studying the relationship between phytoplankton community, zooplankton grazing, and zooplankton competition with salmon for food sources, will contribute to understanding salmon food availability and salmon survival. The EMP food web sampling focuses on emergent marsh habitats and has not evaluated diet or origin of the stomach contents of fish caught within the mainstem or tributary locations. An expansion of our food web sampling to juvenile salmon caught within the

mainstem and in tributaries would provide key information on how estuary habitats benefit those fish that spend less time in the lower Columbia than juvenile Chinook (e.g., steelhead and sockeye). In addition, monitoring mainstem conditions is useful for identifying declining conditions (e.g., warming water temperatures, eutrophication) and whether regional actions are effective in mitigating these.

The EMP is unique in that a broad range of ecological data are collected at relatively undisturbed sites throughout the LCRE over extended time periods and form the most comprehensive dataset of reference site conditions in the region. This dataset is vitally important for use in allowing regional managers to understand how these sites function, how they benefit juvenile salmon and how temporal and spatial changes may affect various aspects within the juvenile salmon life history. The data are key for comparing restored sites to EMP sites, placing them along a trajectory of recovery from disturbance and what we should expect at the restored sites for food web, fish use and habitat characteristics.

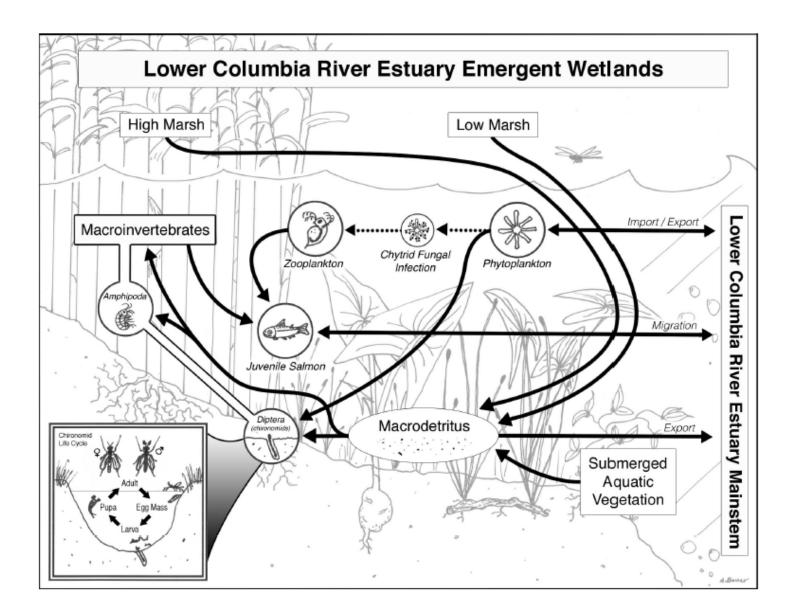


Figure 1. Conceptual model of food web interactions within Lower Columbia River estuary emergent wetlands.

Policy Implications

Shallow water emergent wetlands have been shown in this study to provide not just habitat for juvenile salmonids, but cover and complexity for salmon prey and an area of deposition for phytoplankton that concentrates this resource close to salmon prey. Preliminary analyses indicate that wetland macrodetritus and fluvial phytoplankton may both be important energetic sources for invertebrates that are selected prey items for juvenile Chinook salmon, although their importance may vary across sites. Even at sites where phytoplankton appeared to be the most important direct food source for invertebrate prey, emergent vegetation provided important habitat for those invertebrates. Protection of these relatively undisturbed habitats therefore provides benefits for multiple aspects of the salmonid food web and restoration practitioners should consider designing habitat restoration actions with the goal of attaining similar conditions as those found at EMP sites. Further study to better understand how wetland characteristics (e.g., native vs. non-native vegetation cover) interact with salmon prey would help to understand which functional aspects to restore for salmon.

Preliminary data indicate a higher contribution of macrodetritus to wetlands from a native wetland species, Lyngbye's sedge, compared with the contribution of the invasive non-native species reed canarygrass. Restoration of wetlands in the upper reaches of the river, with a focus on native species establishment, could potentially increase the amount of macrodetritus production in this area. Further work to elucidate differences in macroinvertebrate and macrodetritus availability and production between these two vegetation types in the LCRE is planned for 2014 in the EMP.

Wetland biomass productivity data from this study can be used to estimate macrodetritus contribution from LCRE restoration areas. These data would be very useful for making more specific estimates of macrodetritus production in restored areas, looking at the cumulative effects of restoration on macrodetritus production in the LCRE and providing benchmarks for biomass production at restoration sites.

Fish occurrence data highlight potential effects of human activities, even in the relatively undisturbed habitats sampled as part of the EMP. A prevalence of non-native fish species, high summer water temperatures in both shallow water habitats and the mainstem, and dominance of marked hatchery fish were all found in these relatively 'undisturbed' sites. Chemical contaminants, not covered in detail in this document but covered in the earlier synthesis (Sagar et al. 2013) and annual reports, are also a concern. If these anthropogenic effects are found in the least disturbed sites in the LCRE, the impacts to salmon would be greater when considering the estuary as a whole, including more disturbed sites.

The fish data also indicate differences among genetic stocks in factors such as migration timing, size range, growth rate, and lipid content. The differences in stocks should be taken into account when evaluating the influence of habitat condition on salmon performance. For example, habitat restoration designs should ensure site hydrology and inundation patterns (water level and flood duration) correspond to migration timing of the stocks and life stages intended to benefit most from the restoration action.

Hydrology in the LCRE, particularly the spring freshet, has a strong influence on both wetland vegetation cover and pelagic phytoplankton (prey of salmon prey) abundance and species composition. The regulation of the magnitude, frequency and duration of flood events would directly impact these two aspects of the salmonid food web. For example, in this study, spring freshet flows in the upper river reaches (farthest away from salt water influence), have been shown to reduce vegetation cover, important

habitat for salmon prey, and to reduce plankton standing stocks. The prevalence of phytoplankton as an important food source for salmon preferred prey (dipterans) also diminishes post-freshet as contributions from vegetation detritus increase. While very high flows tend to reduce the availability of plankton to fuel growth of salmon prev in the lower Columbia, it is likely that during periods of low flow in the summer months, the spill from Bonneville Dam provides a source of organic matter in the form of phytoplankton biomass to downstream sites. Since construction of the dams, a combination of reduced suspended sediment (i.e., greater water clarity) and longer water residence time (i.e., reduced flow velocities) in the reservoirs has created conditions that stimulate phytoplankton growth upstream of the dams, providing a possible source of plankton downstream of the dams through spill. We hypothesize that monitoring conditions in the impoundment behind Bonneville Dam would provide a much better understanding of how river discharge patterns influence the amount of phytoplankton-based organic matter available to salmon prey downstream and whether the reservoirs are a partial source of this organic matter. This could be accomplished by installing continuous monitoring sensors (similar to the EMP platform currently monitoring mainstem conditions at RM 122) behind the dam and retrieving periodic samples from the reservoir for analysis of planktonic assemblages. The results would provide a host of implications for river management and reinforce the importance of gaining a better understanding of the temporal and spatial variability of food web resources.

Finally, high seasonal chlorophyll *a* values in the mainstem lower Columbia documented through this study and CMOP should be further examined to determine if eutrophication (nutrient enrichment) problems exist in the lower river, and if so, the sources and loadings of nutrients. Preliminary results document cyanobacteria species within two trend sites (Campbell Slough and Franz Lake) and (to a lesser extent) in the mainstem lower Columbia near Beaver Army Terminal (River Mile 53), which provides support for this concern.

2 Introduction

2.1 Summary

The Lower Columbia River Ecosystem Monitoring Program (EMP) encompasses the study area of the Lower Columbia Estuary Partnership (Estuary Partnership) and includes all tidally influenced areas of the mainstem and tributaries from Bonneville Dam to the plume. Tidal influence is defined as historical tidal influence, relative to post-dam construction in the 1930s. The Columbia River historically supported diverse and abundant populations of fish and wildlife and is thought to have been one of the largest historical producers of Pacific salmonids in the world (Netboy 1980). Anthropogenic changes since the 1860s and the construction of the hydropower system have significantly reduced the quantity and quality of habitat available to fish and wildlife species. Contributing factors include altered timing, magnitude, duration, frequency, and rate of change in river flows; degraded water quality and increased toxic, chemical contaminants; introduction of invasive exotic species; and altered food web dynamics. Ecosystem-based restoration of the Lower Columbia River and Estuary (LCRE) has become a regional priority to aid in the recovery of the historical productivity and diversity of fish and wildlife.

The lower Columbia River is designated an "estuary of national significance" by the U.S. Environmental Protection Agency (USEPA), making it one of 28 National Estuary Programs (NEPs) under Section 320 of the Clean Water Act. The Estuary Partnership was created in 1995 by the governors of Washington and Oregon and the USEPA to coordinate regional partners in protecting and restoring the LCRE ecosystem. Each NEP works with regional stakeholders, such as local, state, tribal and federal governments; industry; citizens; non-profit organizations; and academia, to 1) identify issues facing the ecosystem of that estuary, 2) to determine goals and quantifiable objectives to address the issues, 3) create a Comprehensive Conservation and Management Plan (CCMP) outlining these as well as specific steps to reach the goals and objectives, 4) develop a long-term monitoring plan to track progress and ecosystem condition and 5) maintain a "management conference" of stakeholders. Each NEP relies heavily on its partners to implement the actions and meet the goals within the shared CCMP.

The EMP is an integrated status and trends program for the LCRE. The main objectives of this program are to track trends in the overall condition of the lower river, provide a suite of reference sites for use as end points in our restoration actions, and place results of other research and monitoring programs into the context of the larger ecosystem. The EMP is funded by the Northwest Power and Conservation Council/Bonneville Power Administration (NPCC/BPA) under the Fish and Wildlife Program. A primary goal for their funding is to collect key information on ecological conditions for a range of habitats in the lower river characteristic of those used by outmigrating juvenile salmon and provide information toward implementation of the 2008 Federal Columbia River Power System (FCRPS) Biological Opinion (BiOp; NMFS 2008). Specifically, NPCC/BPA funding for this program focuses on addressing BPA's Columbia Estuary Ecosystem Restoration Program (CEERP) goal of improving habitat opportunity, capacity and realized function for aquatic organisms, specifically salmonids.

Recent research has documented that Chinook salmon, especially subyearlings, and other salmon such as chum (Oncorhynchus keta) and lower Columbia River coho (O. kisutch), to a lesser degree, can rear extensively in shallow water and vegetated habitats within the estuary, including tidal channels, tributary confluences, and nearshore areas (e.g., Bottom et al. 2005; Fresh et al. 2005, 2006; Good et al. 2005; Roegner et al. 2008; Casillas 2009). Subyearling migrants that enter the estuary as fry or fingerlings, or "ocean-type" salmon, exhibit a wide range of residence periods depending on the species, from days to weeks (chum salmon) to several months (Chinook salmon; Thorpe 1994). Juvenile salmon may occur in the estuary all year, as different species, size classes, and life history types continually move downstream and enter tidal waters from multiple upstream sources (Bottom et al. 2005). Peak estuarine migration periods vary among and within species, suggesting that different life history strategies may provide a mechanism for partitioning limited estuarine habitats (Myers and Horton 1982, as cited in Bottom et al. 2005). In the Columbia River estuary, subyearling Chinook salmon are most abundant from May through September, but are present all year (McCabe et al. 1986 and Rich 1920 as cited in Bottom et al. 2005). The recent United States Army Corps of Engineers (USACE) Columbia Estuary Ecosystem Restoration Program (CEERP) Synthesis Memo (Thom et al. 2013) provides a recent synopsis of our current understanding of salmonid migratory and habitat use patterns:

1. Six species of salmonids use shallow water and wetland habitats within the lower river, including peripheral bays and backwater sloughs: Chinook salmon, coho salmon, chum salmon, sockeye salmon (*O. nerka*), steelhead and coastal cutthroat trout (*O. clarkii*) with Chinook, chum and coho salmon found in higher abundances.

- 2. The various (ESUs) display variations in juvenile life history characteristics, including in the timing and pathways of their migrations.
- 3. Chinook and coho salmon exhibit yearling and subyearling life-history types, while chum salmon are primarily captured as fry migrants.
- 4. Yearling Chinook salmon, coho salmon and steelhead primarily use main channel migratory pathways during spring (Dawley et al. 1986; Magie et al. 2008; Weitkamp et al. 2012, as cited in Thom et al. 2013), and larger smolted subyearling Chinook salmon also tend to migrate rapidly through the lower river (Dawley et al. 1986; Harnish et al. 2012, as cited in Thom et al. 2013). However, a portion of these larger fish are also found in shallow water habitats (Poirier et al. 2009a, b; Bottom et al. 2011; Sather et al. 2011; Roegner et al. 2012, as cited in Thom et al. 2013).
- 5. Smaller subyearling Chinook and chum salmon make substantial use of shallow tidal habitats, and subyearling coho salmon are often abundant in the lower sections of tributaries (Poirier et al. 2009a, b; Roegner et al. 2010; Sagar et al. 2011; Sagar et al. 2012, as cited in Thom et al. 2013).

National Marine Fisheries Service (NMFS) recommends that the LCRE contributes to the viability and persistence of all anadromous salmonid populations within the Columbia River Basin in the following ways: 1) the amount of estuarine habitat that is accessible affects the abundance and productivity of a population; 2) the distribution, connectivity, number, sizes, and shapes of estuarine habitats affect both the life history diversity and the spatial structure of a population; and 3) attributes of estuarine habitats (e.g., temperature and salinity regimes, food web interactions) affect diversity and productivity of populations (Fresh et al. 2005). Diverse habitats and the expression of life history strategies based on use of these habitats are directly linked to salmon population viability (i.e., persistence) over long time scales (McElhany et al. 2000). Hence, changes to the estuarine ecosystem such as degradation and loss of estuarine habitat, can directly alter salmonid population viability.

2.2 Ecosystem Monitoring Program Background

As an NEP, the Estuary Partnership works with regional partners (local, state, federal, and tribal governments, industry, citizens, not-for-profits, and academia) to develop and implement a Comprehensive Conservation and Management Plan (CCMP). Ecosystem monitoring is a key element of the Estuary Partnership's CCMP. Action 28 of our CCMP calls for the Estuary Partnership, with its partners, to implement sustained long-term monitoring to understand conditions in the river and to evaluate the trends and impacts of management actions over time (Estuary Partnership 1999). The EMP was originally designed to address the CCMP actions, and where possible address partner goals, such as Reasonable and Prudent Alternatives (RPAs) 161, 163, and 198 of the 2000 Biological Opinion for the Federal Columbia River Power System, and RPAs 58, 59, 60, and 61 of the 2008 Biological Opinion.

When the EMP was created in 2004, most previous research in the lower river had occurred in a small section of the lower reaches, close to the river mouth in Reaches A and B. There was a considerable lack of research and monitoring within the tidal freshwater section of the lower river, resulting in little basic understanding of habitats, fish use and food web dynamics in this region. The Estuary Partnership and its partners developed a list of questions for which there was little current information and which were fundamental to understanding how the estuary functions and its condition with respect to natural or

historic conditions. These questions formed the basis of the EMP sampling design. Initially we focused on one specific habitat type (emergent wetlands that are relatively undisturbed) and habitat structure fish use metrics. Over time, additional metrics were added to the sampling design including food web and abiotic conditions. Subsequent to 2005, the Estuary Partnership and its monitoring partners have focused on providing an inventory of juvenile salmon habitats (or "status") across the lower river stratifying by hydrogeomorphic reach (A–H) and including a growing number of fixed sites for inter-annual variability (or "trends"). Each year prior to 2012, three to four "status" sites, in a previously unsampled reach, were selected along with the continued sampling of a growing number of "trend sites."

The focus of the EMP has been on minimally disturbed tidally influenced emergent wetland sites. These habitats serve as targets for regional restoration efforts, and this program provides baseline information for understanding the success of the regional habitat restoration program and can be used in comparison for results of action effectiveness monitoring in the LCRE. The results of this program provide essential information on ambient environmental conditions and yield insight into the cumulative effects of existing and new management actions and anthropogenic impacts.

For a full description of the current and past EMP monitoring design see the 2013 synthesis on the Estuary Partnership's website (Sagar et al. 2013; <u>http://www.estuarypartnership.org/resource/juvenile-salmon-ecology-tidal-freshwater-wetlands-lower-columbia-river-estuary-synthesis</u>). While the EMP sampling design does not constitute a rotational panel design as originally envisioned by the Estuary Partnership (2004), the current approach is appropriate for an observational study characterizing the condition of selected habitats in the LCRE. Recognizing this limitation, the goals and objectives of the project have been refined to reflect this focus on undisturbed emergent wetlands and their role as juvenile salmonid habitat. Additionally, since this monitoring effort concentrates on a specific habitat type and is not based on a probabilistic design, results cannot be inferred to all tidally influenced wetlands within a reach or at the estuary scale. Although there are no randomly selected sites in this program, selected sites have been re-sampled over time to provide information on trends in undisturbed emergent wetlands.

To address the initial knowledge gaps in the LCRE and the Estuary Partnership's and regional partner's goals, the EMP objectives between 2005 and 2013 were to track the status and trends of ecosystem condition to inform decisions for the purpose of conserving and restoring the LCRE through:

- 1. A comprehensive assessment of status (spatial variation) and trends (temporal variation) of habitat, fish, food web, and abiotic conditions in the lower river, focusing on shallow water and vegetated habitats used extensively by juvenile salmonids for rearing and refugia;
- 2. A coordinated effort to gather baseline data about estuarine resources (from Johnson et al. 2004);
- 3. A determination of the variety of salmon life histories currently expressed in the estuary and habitats that support them (from Bottom et al. 2005); and
- 4. A better understanding of salmon-habitat associations to improve predictions of habitat opportunity and restoration strategies (from Bottom et al. 2005).

To address these goals, the EMP partnership has collected data on habitat structure (vegetation community, water surface elevation, channel morphology, sediment grain size and total organic content [TOC], sediment accretion, and site profiles; by PNNL), fish use (fish community, salmon metrics and diet; by National Oceanic and Atmospheric Administration [NOAA], NMFS), abiotic site conditions (water temperature, specific conductance, pH, dissolved oxygen, and depth; by USGS), macroinvertebrate

prey availability and water temperature at the time of fish sampling (by NOAA, National Marine Fisheries Service or NMFS), primary and secondary production (by USGS and OHSU) and mainstem conditions (by OHSU).

The first EMP synthesis was completed in 2013 and was an analysis of data collected between 2005 and 2010 (available on the Estuary Partnership's website:

http://www.estuarypartnership.org/resource/juvenile-salmon-ecology-tidal-freshwater-wetlands-lowercolumbia-river-estuary-synthesis). Food web data and abiotic conditions, however, were not collected until 2010 so a second synthesis was initiated in order to evaluate initial findings and inter-annual variability in those data. Also not included in the first synthesis are trend sites in Reaches A and B that were added in 2011 and 2012. General trends in the habitat structure and hydrology and fish and fish prey components are not the focus of this synthesis for three reasons: 1) only a few years and sites have been added since the last synthesis, 2) we were interested in gaining a better understanding of the variability in datasets to understand the "range" of natural conditions for each metric and 3) we were interested in the variability of datasets in order to inform adaptive management of the EMP sampling design/frequency. General trends for habitat structure/hydrology, fish, fish prey, abiotic conditions, mainstem conditions and food web dynamics are described in greater detail in the BPA annual report for 2013 (Sagar et al. 2014). This synthesis report therefore evaluates two main components of the EMP: *variability* in the status and trends data for the habitat structure and hydrology and fish and fish prey components (between 2005 and 2013) and a *synthesis of food web dynamics* in the LCRE (2011 to 2013).

2.3 LCRE Knowledge Gaps

2.3.1 Variability

Salmon species occupy the upper trophic levels in the Columbia River system, and therefore, the particular threats to their survival and reproduction can come from a variety of sources. Added to this complexity is the fact that they spend parts of their life history in fresh water, estuarine water, and oceanic water. Poor survival could thus arise from stressors occurring at any one of several life stages or types of habitat. One of the central questions of the EMP is: do the habitats of the LCRE appear to meet the needs of outmigrating juvenile salmonids for growth and survival? However, before we can answer this question, we must first understand enough about fish use of these environments in space and time, and the spatial and temporal variability in estuarine habitat characteristics to predict whether habitat conditions meet the needs of migrating juvenile salmonids.

Effective ecosystem management requires knowledge of changes (particularly detrimental changes) that occur in the ecosystem, and the factors that lead to those changes. The ultimate goal of status and trends monitoring is to track the status of a resource over time but also to allow researchers and managers the ability to distinguish between variability associated with natural conditions and from any changes and variability that may result from human impacts. The creation and maintenance of long-term datasets have irreplaceable value for documenting the history of change (long-term trends) within important resource populations, for evaluating the potential significance of human activities on natural resources, and for visualizing and formulating testable hypotheses about the interactions among species, between species and their environment, and the mechanisms for these interactions.

The EMP has been collecting and analyzing data on spatial and temporal patterns in vegetation and hydrology since 2005, fish/ fish prey since 2007/2008 and abiotic and food web conditions since 2011.

Analysis of these datasets allows us to understand the intra- and inter-annual variability of these metrics, which can then help us design a program that is efficient and effective at monitoring conditions and changes in the lower Columbia River over time. With this analysis, we are interested in better understanding the variability in our datasets to: 1) understand the "range" of conditions for each metric to assess our ability to detect change, and 2) inform adaptive management of the EMP sampling design (e.g., metrics, frequency) to more efficiently and cost effectively capture this "range". Once we understand the range of conditions that exist, with continued monitoring in the future, we can begin to tease out the variability resulting from human versus natural causes. Information gained from data collected on the spatial and temporal variability of estuarine habitats and fish use in these habitats will assist in answering the question of whether current habitat conditions meet the growth and survival requirements of outmigrating juvenile salmonids, which can subsequently inform management and habitat restoration actions in the region.

2.3.2 Synthesis of Food Web Dynamics

Fundamental changes in ecological characteristics of the lower Columbia River food web occurred following the installation of the present-day hydropower system and the loss of wetland habitats due to diking and agricultural conversion. Several studies (Thomas 1983; Allen 1999; Garono 2003; Estuary Partnership 2012) noted losses of approximately 70% of vegetated tidal wetlands and 55% of forested uplands for the study area since the mid-1800s. Bottom et al. (2005) concluded that loss of estuarine habitat has reduced rearing opportunities for subyearling Chinook salmon (*Oncorhynchus tshawytscha*). In particular, removal and isolation of wetland and shallow water habitats and flow regulation by dams are thought to have contributed to the loss of important habitats for Chinook salmon and potentially reduced the diversity of salmon life histories in the estuary. It is thought that a reduction in the diversity of life histories in the estuary may undermine the resilience of populations to changing environmental conditions that may accompany climate change or changes in land use practices (Healy 1991; Thorpe 1994).

Additionally, losses in wetland area have resulted in an estimated reduction of 84% of macrodetritus that historically supported river and estuarine food webs (Sherwood et al. 1990). Factors contributing to the loss of macrodetritus input include wetland habitat loss from conversion and development; decreased plant biomass due to the higher dominance of reed canarygrass which has a lower macrodetritus contribution; and decreases in downstream transport of macrodetritus due to reduction of floodplain area inundation. With the reduction of macrodetritus inputs to the system, the organic matter driving estuarine production is currently thought to be derived in large part from fluvial phytoplankton (~58%; Small et al. 1990). The construction of dams on the lower Columbia River has converted a high-turbidity, detritus-driven river ecosystem to a much 'greener' river, where pelagic primary production (i.e., fluvial phytoplankton) has increased as a result of a reduced sediment load and longer water residence time behind the dams (Sullivan et al. 2001). Thus, the LCRE food web is dramatically different today than it has been historically, with a reduction in production associated with emergent marshes and an increase in the contribution by fluvial phytoplankton (Simenstad et al. 1990; Bottom et al. 2005).

The consequences of these shifts in organic matter compartmentalization have undoubtedly impacted food web dynamics; however, the full effects of the change are not yet clear. Outstanding questions remain: (1) *How has seasonality, amount, and availability of organic matter production changed?* (2) *What are the major drivers of organic matter production and supply in today's system?* (3) *How has a shift in organic*

matter availability changed or altered food web structure (in terms of trophic pathways and temporal dynamics)? (4) Given that the contribution by fluvial phytoplankton to organic matter production has increased in the present system relative to pre-dam conditions, how can this information be used to better manage the system and our restoration actions to achieve suitable habitat characteristics for juvenile salmon?

Phytoplankton and zooplankton are important components of the diet of salmon prey, including the diets of chironomids (dipteran insects of the family Chironomidae) and benthic amphipods [Corophium spp. (Lott 2004), Americorophium spp. (Bottom et al. 2008)], which together, these two groups of macroinvertebrates comprise ~90% of the diet of juvenile Chinook salmon in the Columbia River estuary (Lott 2004). Recently, isotopic data showed that phytoplankton are also an important component of the food web supporting salmon, with fluvial phytoplankton accounting for up to 60% of organic matter assimilated by chironomids and 40% of that assimilated by benthic amphipods in March-April (Maier and Simenstad 2009). A seasonal shift toward a greater contribution to vascular plant detritus and benthic diatoms as the dominant organic matter sources supporting chironomids was observed in June and July, respectively (Maier and Simenstad 2009). Maier and Simenstad (2009) also found that the benthic amphipod Corophium salmonis consumed high proportions of fluvial phytoplankton in March-April (although vascular plant sources still dominated during that time period), while between June and August the dominant organic matter source was almost entirely vascular plant detritus. These patterns could demonstrate a seasonal reliance on phytoplankton resulting from the lack of vascular plant detritus in the early spring at the start of the growing season, or from a preference for phytoplankton when they are abundant.

Although they have important implications for the restoration of critical salmon habitats, the major factors that modulate primary and secondary productivity and species composition of plankton are poorly known, both in the mainstem lower Columbia River and among the braided channels and sloughs. Quantifying the contemporary contribution of plankton and detritus to the food web (versus the historic contribution) is thus essential for defining the status, function, and health of the lower Columbia River ecosystem. The EMP, carried out through the Estuary Partnership seeks to fill this critical gap in our knowledge of food web structure and its spatiotemporal dynamics to more strongly link assessments of physical habitat opportunity with evaluations of habitat capacity. Understanding these links will yield a more holistic picture of environmental factors influencing juvenile salmon condition and occurrence within the estuary, and whether estuarine habitats are supporting the needs of juvenile salmon and other aquatic organisms.

3 Methods

3.1 Sampling Design

The LCRE extends from the plume of the Columbia River upstream to the Bonneville Dam at river kilometer (rkm) 235. As part of the sampling design for the EMP, the Estuary Partnership, University of Washington (UW), and USGS developed a hydrogeomorphic classification scheme, the Columbia River Estuary Ecosystem Classification (Classification; <u>http://www.estuarypartnership.org/columbia-river-estuary-ecosystem-classification</u>), for the LCRE. Based on classification schemes developed for other estuarine ecosystems and concepts of ecosystem geography (Bailey 1996), the Classification has six hierarchical levels (see Simenstad et al. 2011):

- 1. Ecosystem Province (based on USEPA Ecoregion Level II)
- 2. Ecoregion (based on USEPA Ecoregion Level III)
- 3. Hydrogeomorphic Reach (based on modified USEPA Ecoregion Levels III and IV)
- 4. Ecosystem Complex (based on Primary Cover Class and geomorphic setting within each hydrogeomorphic reach)
- 5. Geomorphic Catenae
- 6. Primary Cover Class (based on cover data from Landsat or other remote sensing data sets)

The Estuary Partnership and monitoring partners use a multi-scaled stratification sampling design for the habitat monitoring component of the EMP using Level 3 Hydrogeomorphic Reaches. The Classification divides the LCRE into major hydrogeomorphic transitions, yielding eight reaches, each with unique characteristics and physical processes (Figure 2; Simenstad et al. 2011). These Reach boundaries are based on the USEPA's Level IV Ecoregions, which were modified to include important parameters such as salinity intrusion, maximum tide level, upstream extent of current reversal, geology, and major tributaries.

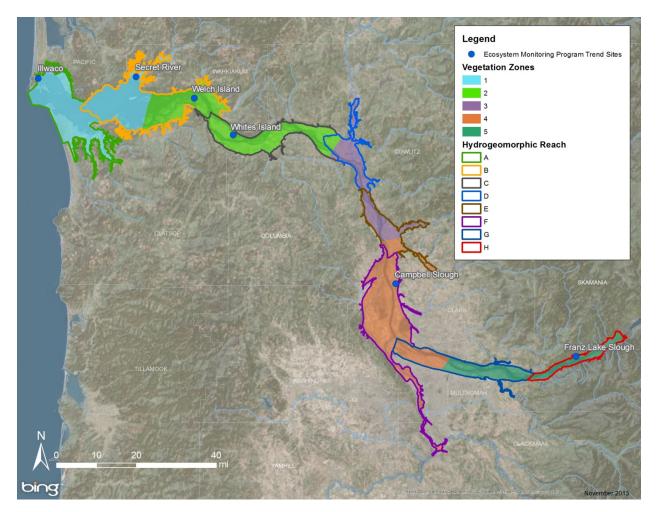


Figure 2. LCRE with hydrogeomorphic reaches (A–H) and emergent marsh zones (1-5) outlined and specified by color.

The Estuary Partnership and its monitoring partners have focused on providing an inventory of salmon habitats (or "status") across the lower river stratifying by hydrogeomorphic reach (A–H) and including a growing number of fixed sites for inter-annual variability (or "trends"). Appendix 1 shows the specific sites that have been monitored since the beginning of the EMP and the type of sampling associated with the site. Habitat structure and hydrology data began to be collected in 2005, fish data collection began in 2007, fish prey data collection began in 2007, and water quality data collection has been conducted at a small number of sites, dependent on funding levels. Maps in Appendix 2 show the overlap in sampling components for the trend sites.

The focus of the EMP between 2005 and 2013 has been on minimally disturbed tidally influenced emergent wetland sites. Each year prior to 2012, three to four "status" sites, in a previously unsampled reach, were selected along with the continued sampling of a growing number of "fixed sites." The six trend sites include Ilwaco in Baker Bay (2010-2013), Secret River in Grays Bay (2012-2013), Welch Island in the Julia Butler Hansen Wildlife Refuge (2012-2013), Whites Island (2009, 2010); Campbell Slough in the Ridgefield National Wildlife Refuge (2005–2010), and Franz Lake (2008, 2009). Cunningham Lake is an additional trend site used to collect information on habitat structure and hydrology as supplemental, comparison data for the Campbell Slough site, due to the irregular presence of cows at Campbell Slough that may influence vegetation biomass. In 2012 and 2013, due to budget changes, the EMP scope included only the six trend sites, and no status sites. For a full description of EMP methods please see www.monitoringmethods.org.

3.2 Site Selection

To characterize juvenile salmon habitat across the spatial extent of the LCRE, the EMP sampled one previously unsampled reach per year. Prior to a site visit, the potential areas were evaluated using GIS layers including current imagery, Light Detection and Ranging (LiDAR) digital elevation models, and historical maps from the late 1800s. Using these sources of information, the potential sites were narrowed to those that appeared relatively undisturbed and hydrologically connected to the mainstem of the Columbia River. A set of potential sites was then assessed in the field, and a final set of sites was agreed upon by all monitoring partners (PNNL, NOAA, USGS, OHSU and the Estuary Partnership). The final habitat criteria used to select monitoring sites were:

- 1. The site's wetland vegetation is classified as "emergent" in the National Wetland Inventory (NWI; available at http://www.fws.gov/wetlands/index.html/).
- 2. The site has tidal connectivity with the mainstem Columbia River.
- 3. The site's wetland is minimally disturbed (e.g., no diking, active grazing, tide-gates, or modifying flow regime present at the site).
- 4. The area of wetland is greater than five acres.
- 5. Wetlands at the site are shallow water.
- 6. The site is mainstem fringing or off-channel habitat.
- 7. The site is not located near immediate stressors or disturbances like industry, grazers, or recreational use.
- 8. Site sediments are generally smaller particle sizes, which are characteristic of lower-energy systems and more likely to support emergent marsh habitats than habitats with larger particle sizes.

Additional logistical criteria included:

- 9. Slough channels are present at the site to facilitate the collection of cross-section and fish data.
- 10. The site is fishable by beach seine or similar gear type.
- 11. The site is accessible for sampling purposes with landowner permission.

The final criteria for site selection were based on funding levels, the desire for data comparability with previously collected data, and other reasons outlined above.

3.3 Variability in the LCRE

3.3.1 Habitat Structure and Hydrology

3.3.1.1 Vegetation Cover and Data Analysis

For vegetation cover sampling methods please see <u>www.monitoringmethods.org</u>. Spatial variability among tidal marsh sites (51 sites, including "status, "trend" and other minimally disturbed tidally influenced emergent wetlands) in the LCRE was focused on vegetation cover. Other habitat metrics measured as part of the EMP did not have enough data to adequately test the variability in the same way the vegetation data were analyzed. A similarity analysis was conducted using the Bray-Curtis similarity on the average absolute cover of all plants that were observed in at least one site over the years sampled. Percent cover was transformed to the arcsine square root.

Temporal similarity was evaluated by calculating the similarity between years for each trend site. Spatial similarity for sites observed for greater than one year was evaluated by calculating the average similarity over years for each pair of sites. Descriptive statistics, box plots and line plots were used to visualize the temporal and spatial similarity. Regression analysis was conducted on the average similarity between years as a function of the number of years separating the observations (span) and on the average similarity over years between sites as a function of the distance between sites. A general linear model (GLM) with main effects of Start Year and Site and with the span of years between observations (e.g., 2008 to 2010 equals a span of two years) fit as a covariate.

Spatial similarity was evaluated using all sites that have been observed in the LCRE (51 sites). The average pairwise similarity for sites observed within the same year and emergent marsh (EM) zone was calculated based on the following zone delineation:

EM Zone	River Kilometer (rkm)
1	0-40
2	41-89
3	90-136
4	137-181
5	182-235

A GLM was used to assess the main effects of EM zone and Year with the maximum extent of the distance between sites observed that year as a covariate. Bar graphs of the least square means for a given effect (with the other effects including the covariate removed) were used to assess the similarity visually.

3.3.2 Fish

3.3.2.1 Fish Sampling

Between 2007 and 2013, NOAA-Fisheries conducted surveys to monitor juvenile Chinook salmon habitat occurrence at Hardy Slough, Franz Lake, Pierce Island, and Sand Island in Reach H; Lemon Island, Reed Island, and Washougal Wetland in Reach G; Campbell Slough, near Ridgefield National Wildlife Refuge, in Reach F; Sandy Island, Burke Island, Deer Island, and Goat Island in Reach E; Wallace Island, Ryan Island, Lord-Walker Island, Whites Island, and Bradwood Slough in Reach C; Secret River and Welch Island in Reach B; and Ilwaco Slough in Reach A (Figure 2). The location and year that each of the sites were sampled are shown in Appendix 3. Trend sites were sampled monthly in multiple years to monitor temporal trends: Franz Lake (sampled in 2008, 2009, and 2011-2013); Campbell Slough (sampled from 2007-2013); Whites Island (sampled from 2009-2013).

Key variables monitored included indicators of:

- fish community characteristics (fish community composition, fish species diversity and fish species richness, proportion of non-native species present [as proportion of species collected and as proportion of catch]); and proportions of potential salmon predators in catch:
- indicators of salmon habitat occurrence (salmonid species composition; proportions of marked and unmarked fish present);
- Chinook salmon stock composition;
- salmon species density;
- salmon life history diversity; and
- indicators of salmon health (lipid content, condition factor, growth as estimated from otolith examination, and concentrations of chemical contaminants).

In this report we present a variability analysis of the fish parameters measured as part of the EMP to identify those factors which contributed the most to their variability, and to determine for which parameters sufficient data exist to understand trends and relationships, and those for which additional sampling would be required. For a full description of EMP fish methods please see www.monitoringmethods.org.

3.3.2.2 Fish Metrics Data Analysis Methods

In order to assess the variability in fish metrics from 2007-2013 we looked at the dataset from three different perspectives:

1) We graphically characterized general spatial and temporal trends for all metrics

2) We conducted a stepwise regression to look at sources of variation in the fish data (between month, site and reach) and a power analysis to determine how much more sampling would be needed to detect significant differences

3) For select metrics we used descriptive statistics to look at the variability between months and reaches for multiple years of data to see where to target sampling in the future (based on variability, meaning those sites with high variability, we should sample further to detect change).

Multiple regression, analysis of variance (ANOVA), and Chi-square analysis were used to examine the effects of sampling season, site, and reach, and sampling year on the variables monitored as part of the EMP. Analyses were conducted with the JMP statistical package. For those variables that showed no significant relationship with site, reach, season, or year, a power analysis was conducted to determine the degree of sampling that would be necessary to detect significant variation for these variables.

3.3.3 Fish Prey

3.3.3.1 Fish Prey Sampling

3.3.3.1.1 Open water and emergent vegetation

For the invertebrate prey sampling, the objective was to collect aquatic invertebrate samples and identify the taxonomic composition and abundance of salmonid prey available at sites when juvenile salmonids were collected. These data could then be compared with the taxonomic composition of prey found in stomach contents of fish collected concurrently.

We quantified the density, diversity and size of invertebrate prey available to juvenile Chinook salmon across 18 sites between 2008 and 2013, focusing on months during which salmon were abundant (generally when n > 5; Appendix 3). We typically collected two emergent vegetation (EV) samples and two open water (OW) samples per site per month (concurrent with two beach seine collections), but occasionally one or three of each were collected depending on field conditions (Appendix 3). For a full description of EMP fish and fish prey methods please see www.monitoringmethods.org.

Taxonomists at Rhithron and Associates (Missoula, MT) processed all invertebrate samples. For tow samples, processing included sorting, identifying, counting, measuring and, for 2012 and 2013 samples only, weighing up to 500 invertebrates per sample. If a sample contained more than 500 individuals, it was subsampled and total counts were estimated based on the proportion that was processed. Invertebrates were identified to the lowest possible taxonomic level (typically species or genus), except for the Chironomidae (Diptera) that were identified to family and Oligochaetes that were identified to subclass. For samples collected in 2012 and 2013, all individuals per sample were composited by family and life stage (e.g., Chironomidae larvae) and each composite was weighed (blotted wet weight to nearest 0.0001 g).

3.3.3.2 Fish Prey Data Analyses

To assess general patterns, we grouped most taxa by order for statistical analyses. To explore how prey densities varied by habitat (EV vs. OW), site, year and month, we used these factors and all sample data in a stepwise regression. Because the sampling design and distribution of samples was not balanced, we also used paired mean values of prey densities from EV and OW samples collected during the same sampling event to evaluate if there were differences between the densities of prey caught in the two habitat types. In addition, we used these paired samples to determine if there was a strong correlation between prey availability in the EV and OW habitats at a site. A strong correlation may suggest connectivity between habitats within a site (e.g., the EV habitat may be a source of prey for the adjacent OW habitat) and/or that prey availability is determined by site conditions and not by smaller scale habitat conditions. A weak correlation between prey availability in EV and OW habitats may suggest there is little connectivity among adjacent habitats within a site and/or prey availability is strongly determined by

conditions at the habitat scale. Paired samples $(n_{pairs} = 60)$ consisted of mean values from the emergent vegetation samples and mean values from the open water samples collected concurrently at a site.

To address whether the local extent of emergent vegetation was correlated with the availability of invertebrate prey, we recorded the presence and estimated the percent cover of bare ground, dead vegetation, live grass, and live "other" vegetation present along the 10 m transect. We did this using five, 0.5 x 0.5 m quadrats placed every 2 m along each transect, and visually estimated the percent cover of each type. This was done for 71 transects that were sampled between 2010 and 2012. The mean percent cover by type for each transect was used in regression analyses.

3.4 Food Web

3.4.1 Primary and Secondary Production

3.4.1.1 Phytoplankton abundance

In order to estimate the abundance of aquatic primary producers with the potential to carry out carbon fixation in the LCRE, samples were collected from four to six fixed sites, depending on the analysis (Figure 2). Chlorophyll a, an estimate of algal biomass, was determined at four sites (2011-2013), while the numerical abundance of microscopic algae was determined at four sites (2011) or six sites (2012, 2013) using light microscopy. In addition, chlorophyll a associated with attached algae, or periphyton, was determined at four fixed sites (2011-2013). Briefly, five to 10 one liter water samples were collected and combined into a sample churn and homogenized. The churn was subsampled (250 - 750 mL) and the sub-sample was filtered through 47 mm GF/F filters in the field using gentle vacuum pressure (<100 mm Hg). The filters were placed in petri dishes and kept in a cooler on ice during transportation back to the laboratory. Once in the laboratory, they were stored at -20°C pending analysis using a Thermo Electron spectrophotometer. For the periphyton samples, a representative amount of material was scraped from natural substrates (rocks, plants, wood), rinsed with deionized water, and a subsample was filtered as described above for the chlorophyll a samples. Chlorophyll a was extracted from each filter or biomass aliquot in 90% acetone for 20-24 h and the concentration of extracted chlorophyll a was determined spectrophotometrically (Standard Methods for the Examination of Water and Wastewater 1998). The limit of detection for the method is approximately 1 mg chl m^{-3} .

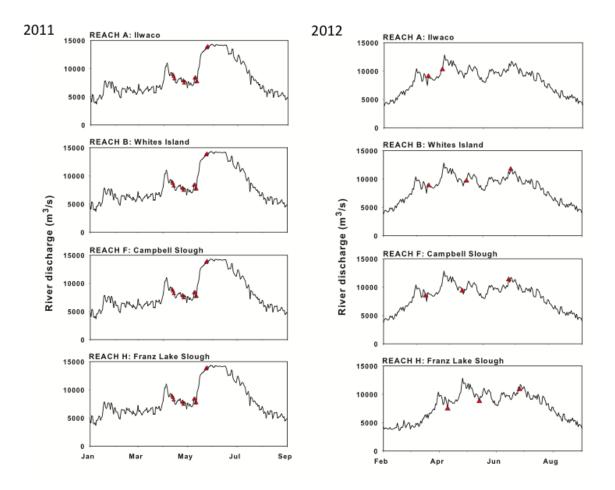


Figure 3. Dates (2011, 2012) where samples for the characterization of primary and secondary production were collected (red triangles) indicated on line plots of river discharge volume at Bonneville Dam (m³ s⁻¹) showing the distribution of sampling events in relation to flow variability.

3.4.1.2 Primary productivity of phytoplankton and periphyton

3.4.1.2.1 Phytoplankton species composition

To determine species composition and abundance of the various taxa comprising the phytoplankton and microzooplankton communities, whole water samples were collected for identification and enumeration of phytoplankton from the sites identified above. For each sample, 100 mL was collected in duplicate and preserved immediately with Lugol's iodine (final concentration ~1%). The samples were placed in a cooler with ice packs and transported back to the laboratory for processing and subsequently stored in the dark at room temperature. For identification and enumeration, 10-25 mL sub-samples were concentrated using the Utermohl settling method (Utermohl 1958). Briefly, each 100 mL whole water sample was gently inverted ~100 times and poured into a settling chamber and left for 24 h. After 24 h, the supernatant was discarded and the concentrated cells were enumerated using an inverted light microscope (Leica DMIL). At least 400 cells per sample were enumerated in at least five fields of view. Observations were made at 200x or 400x magnification, with an additional scan performed at 100x magnification to capture rare cells in a broader scan of the slide. An error estimate was derived by performing a sub-set of duplicate counts. The estimated error in abundance was < 5% at the class level, and ~10% for genus-level

counts. The concentrated material was then transferred to small (7 mL) sampling vials for archiving and more detailed examination of acid-cleaned material. The archived samples were not examined during this part of the study; however, a detailed survey of the diatom populations could provide a highly informative snapshot of phytoplankton biodiversity at key time points relative to seasonal habitat gradients.

3.4.1.3 Rates of primary production

The assimilation of the radio-tracer, carbon-14 in the form of sodium bicarbonate (NaH¹⁴CO₃), into particulate organic matter (POM) was measured in order to determine rates of primary production (organic matter production through the process of photosynthesis; Wetzel and Likens 1991) in 2 – 4 hour incubations in 250 mL bottles held just below the water surface at four fixed sites in 2011: Ilwaco, Whites Island, Campbell Slough, and Franz Lake Slough. Following incubation, phytoplankton were concentrated onto 47 mm GF/F filters and kept in the dark on ice during transportation back to the laboratory where they were stored at -20°C in plastic 20 mL scintillation vials pending analysis. After the filters were thawed and dried overnight, they were fumed with 0.25 mL of 1 N HCl for 24 h to degas non-specific ¹⁴C as ¹⁴CO₂. A 10 mL scintillation cocktail was added to each vial and the samples were run on a Beckman Liquid Scintillation Counter. Two ¹⁴C uptake experiments (using triplicate bottles) were performed at each site, except for at Campbell Slough, where limited site access in 2011 only allowed for one experiment per time point. The results were corrected for dark carbon assimilation (measurements from darkened bottles were subtracted) and for non-specific retention of ¹⁴C on the filters in the absence of phytoplankton (time zero blanks).

In 2012, phytoplankton productivity was determined using the ¹⁴C uptake approach described above, except that the stable isotope ¹³C was used as a tracer in place of ¹⁴C (Hama et al. 1983). The samples were spiked with a solution of ¹³C-labeled sodium bicarbonate (NaH¹³CO₃, with 99% C as ¹³C) at a concentration of ~8% of the average ambient dissolved inorganic carbon (DIC) across the four sites based on calculations from 2011. This ¹³C spike is in the range of rates used in other studies (Hama et al. 1983; Kanda et al. 1985; Hashimoto et al. 2005). In the field, the samples were processed as described above for ¹⁴C. However, in the laboratory, after thawing and fuming the filters overnight with 1 N HCl, the filters were pelletized into tin capsules and analyzed using an Isotope Ratio Mass Spectrometers interface to an Elemental Analyzer to yield the mass of organic carbon and its isotopic enrichment relative to the standard, Pee Dee Belemnite.

Nutrient-diffusing substrate (NDS) periphytometers were used to estimate periphyton productivity. Micro-NDS periphytometers, as described by Wise et al. (2009), were used to estimate periphyton accrual three times during both 2011 and 2012. Periphyton productivity was measured as the accumulation of chlorophyll *a* on submerged 21 mm glass-fiber filters over a two-week period. Rates are reported as the average of four unscreened replicates (in mg chlorophyll *a* m⁻²). For each deployment, eight 40 mL glass vials were filled with different treatment solutions: (1) Control (deionized water); (2) nitrogen addition (350 μ M sodium nitrate); (3) phosphate addition (100 μ M sodium hydrogen phosphate); and (4) combined nitrogen and phosphorus addition (350 μ M NaNO₃ +100 μ M Na₂HPO₄). The control treatment was used to determine the ambient rate of periphyton production, while the nutrient addition treatments were designed to assess potential nutrient limitation or co-limitation of peiphyton growth. The vials were capped with a 0.45 μ m nylon barrier membrane filter and a glass-fiber filter, with the latter serving as the artificial substrate for periphyton growth. Half of the replicates within each treatment were covered with 18 x 14 mesh fiberglass window screens to exclude grazers, which could potentially limit periphyton

accrual and bias production rates. If significant differences in periphyton accrual between screened and unscreened filters were observed, then only the screened values were used for the productivity calculations.

Experiments were run starting the weeks of April 11, May 9, and June 20, 2011, with some sites having no data due to difficulties accessing the sites (e.g., Campbell Slough in April 2011). In May 2011, periphytometer experiments were deployed at Campbell Slough, but could not be retrieved for four weeks due to high water. Those filters were too degraded to be analyzed, so 2011 data from Campbell Slough are only available from the June deployment.

3.4.1.4 Zooplankton abundance

Secondary productivity (the rate of growth of consumers of primary production) was not measured directly, but was estimated from the abundance of zooplankton. Due to the lower abundance of zooplankton compared to the smaller phytoplankton, zooplankton samples were first concentrated through the use of a 80 μ m nylon mesh net with a mouth diameter of 0.5 m and a length of 2 m. The samples were collected from near the surface of the water (<1 m). When possible, the net was fully submerged under the water and was dragged back and forth through the water for ~5 min or over a distance of 100 m. A flow meter (General Oceanics Inc., Model 2030R) was mounted to the net's bridle to provide an estimate of the volume flowing through the net. Unfortunately, the flow meter was not available until the late-May sampling dates in 2011; therefore, abundances of zooplankton were estimated for the dates sampled in April and early May 2011 based on the distance covered during the tows. Estimates of volume were made based on an approximation of the distance covered during the tow, multiplied by the volume of a cylinder, according to: total volume = (π D² h)*distance, where r = radius and D = diameter of the net opening. Further details regarding sampling and calculations can be found in Sagar et al. (2012). Samples were identified to species or class.

3.4.1.5 Benthic invertebrates

To characterize the benthic macroinvertebrate assemblage, coring sites were selected to correspond to locations directly adjacent those where the fish community was assessed. The core samples were collected at low tide from exposed sediments. The samples were collected using a 2 inch diameter PVC pipe. One end was inserted approximately four inches into the sediment of the channel at or near low tide, and a rubber stopper was placed on the other end of the pipe creating a vacuum suction used to contain the sample in the pipe while it was removed from the substrate. The samples were rinsed through a 500 µm mesh sieve using deionized water and preserved in individual plastic jars with 95% Ethanol. Rose bengal, an inert stain, was applied to facilitate sorting invertebrates from other debris in the sample. Samples were identified to order, family, genus, etc.

3.4.1.6 Data Analysis

Differences observed between planktonic assemblage biomass (either phytoplankton or zooplankton) over space and time were tested for statistical significance according to a Two-Way Analysis of Variance (ANOVA) with alpha set to 0.05 using SigmaStat (v.11). Post hoc multiple comparisons tests (Holm-Sidak) were performed to identify the factors driving statistically significant differences observed in the Two-Way ANOVAs. When the data were not normally distributed (assessed using a Kolmogorov-Smirnov test), non-parametric tests were performed, including Wilcoxon Rank Sum test to assess between-group differences. Regression statistics were generated using a Pearson correlation test.

3.4.2 Vegetation

3.4.2.1 Sampling Methods

From 2011 to 2013 above ground biomass was sampled to estimate the primary productivity at the six trend sites. Samples were collected in the summer during July or August during peak biomass and again in February during the winter low biomass period. For the emergent marsh biomass sampling, a 1 m square plot was randomly placed along the established vegetation transect, but off-set 2 m from the transect to ensure that the biomass plots did not intersect the vegetation percent cover plots. In 2012 and 2013, the biomass was randomly sampled within distinct vegetation strata to 1) more clearly associate the samples with vegetation type and 2) to reduce the variability between samples within strata. Within the 1 m square biomass plot, a 0.1 m^2 quadrat was placed in a randomly selected corner and all rooted vegetation, live or dead, was removed using shears. Each sample was placed in a uniquely numbered bag, and held in a cooler for the remainder of the sampling trip. For the submerged aquatic vegetation (SAV) plots, similar methods were employed with the exception of the placement of the plots. Either existing transects were extended past the baseline or new transects were created to reach the main slough. In some instances, an existing transect intersected the slough and an SAV plot was randomly placed along it. Depending on the width of the channel, either one or two SAV plots were randomly placed along each transect. Vegetation species were recorded in field notebooks along with the corresponding biomass sample number.

In the laboratory, the biomass samples were stored in a cold room until processing could begin. The samples were then individually rinsed of all non-organic material, obvious root material was removed, and for the winter samples live and dead material was separated. Pre-weighed pieces of tinfoil were used to secure the individual biomass samples, a wet weight was then measured, and the samples were placed in an oven set at 90°C for three to four days. When the samples were deemed completely dry, a second weight was then measured for each sample, and entered either into a datasheet or directly into a spreadsheet software program.

3.4.2.2 Data Analysis Methods

The dry weight of biomass samples collected from a given year, season (summer and winter), site, and strata were averaged. There were three refinements of strata at each site that considered the spatial and vegetative characteristics of the marsh at increasingly finer levels of resolution but also at increasing levels of uncertainty. The coarsest strata were at the marsh landscape level and only considered the general elevation (high marsh, low marsh, and submerged aquatic vegetation [SAV]). The middle resolution strata considered groupings of vegetative species and elevation in the vicinity of the biomass sample, and the finest resolution strata considered the dominant species at the location of the sample. The finest resolution strata are the dominant species based on the notes of what was collected in the biomass sample. Each refinement of the strata altered the number of strata and thus the number of samples used to calculate the mean dry weight for a given year, season, and site. Thus, the number of replicates for testing effects of year, location (rkm or ecological zone), and strata decreased as the strata increased in resolution. At a minimum, descriptive statistics for each strata were calculated by site, season, and year.

For the marsh level strata (high marsh, low marsh, and SAV) analysis was conducted on the average dry weight observed for each season and for the difference between seasons (summer minus winter). The

average dry weight produced in the summer, remaining in the winter, and exported was compared using a generalized linear model (GLM) with main effects of year and strata, the interaction of year and strata, and site location (rkm) as a covariate. A second GLM included the main effects of ecological zone and strata, and the interaction of zone and strata was used when the interaction of year and strata and the main effect of year were not found to be significant. Tukey's pairwise comparisons were used to compare the means of each level within strata. When the interaction term could not be estimated, only the main effects were used in the GLM and data plots were used to assess the magnitude of the potential interaction. Data were log10 transformed to reduce within class heterogeneity.

For the middle resolution strata (grouping of vegetation and elevation) analysis was conducted on the average dry weight observed for each season. The average dry weight produced in the summer and remaining in the winter was compared using a GLM with a main effect of strata and a site location (rkm) as a covariate. Data were log10 transformed to reduce within class heterogeneity. No further analysis beyond descriptive statistics was conducted for the species level strata.

3.4.3 Mainstem Conditions

3.4.3.1 Sampling Methods

The Center for Coastal Margin Observation and Prediction (CMOP) at the OHSU operates two in situ water quality monitoring platforms in the mainstem Columbia River that provide baseline water quality measurements in support of the EMP. The first platform, funded by the National Science Foundation, was installed in July 2009 at River Mile (RM) 53 (in Reach C) and is physically located on a USGS Dolphin piling (46 11.070 N, 123 11.246 W). A second platform, funded by the EMP was installed in August 2012 at RM 122 (in Reach G) and is physically located on the outer-most floating dock at the Port of Camas-Washougal (45 34.618 N, 122 22.783 W). Each instrument platform consists of a physical structure, sensors, sensor control, power supply and distribution, and wireless communication. Data transmitted from the sensors are available within 1-2 hours of collection. Raw data can be downloaded from a dedicated webpage (http://columbia.loboviz.com/) and also can be accessed as part of the CMOP observation network from the CMOP server (http://www.stccmop.org/datamart/observation_network). In addition to collecting spatial and temporal resolution of basic water quality and biogeochemical observations for the mainstem Columbia River, an additional outcome of this effort is to provide daily estimates of other useful parameters for assessing ecosystem conditions and relevant biogeochemical processes in the Columbia River watershed. One such product is flux calculations for various inorganic or organic components such as nitrate or phytoplankton biomass. Knowledge of flux of nutrients and organic matter for a large river is important for a variety of applications, including assessment of pollution, indications of eutrophication, and quantification of loading to the coastal zone, where many important ecological processes may be affected. An additional data analysis product is the assessment of Net Ecosystem Metabolism, which provides a daily measure of the gross primary production and aerobic respiration occurring in the river as measured by hourly changes in dissolved oxygen.

The instrument platform at RM-122 ran continuously from September 2012 through December 2013. Regular maintenance visits were conducted by OHSU personnel. The instruments were removed in December 2013 for factory maintenance, including new calibrations.

Common instruments/sensors to both platforms are provided in Table 1. Sensors are configured to collect a sample and telemeter the data every hour. In addition to the parameters listed in Table 1, the RM-122

station is designed to operate a WET Labs Cycle- PO_4 to measure dissolved ortho-phosphate concentration. This measurement is a wet chemistry analysis and therefore this instrument has reagent limitations, which restricts its operation to a reduced schedule (three consecutive measurements daily).

Company	Sensor	Parameters
Satlantic	LOBO	Power distribution
		Sensor control Wireless communication
		Data management
Satlantic	SUNA Nitrate	Nitrate Concentration
WET	ECO-CDS	Colored Dissolved Organic Matter (CDOM)
Labs		
WET	WQM Water Quality	Conductivity, Temperature, Dissolved Oxygen, Turbidity,
Labs	Monitor	Chlorophyll a Concentration

Table 1. Description of the components on the in situ sensor platforms located at River Mile 53 (RM-53, Beaver Army Terminal near Quincy, OR) and River Mile 122 (RM-122, Port of Camas-Washougal, WA).

The sensors on both platforms are designed to operate autonomously, at high temporal resolution (hourly), and over long periods between maintenance (estimated at three months, although sensors are typically maintained at shorter intervals). Maintenance trips include cleaning of all sensors and surfaces and performing any other needed maintenance (Table 2). Additionally, water samples are routinely collected for laboratory analysis of nutrient and chlorophyll concentrations.

Table 2. Maintenance and water	grab sample dates between	September 2012 and December 2013.
	0	

RM-53	RM-122	
9/5/2012	9/5/2012	
12/4/2012 12:00	12/10/2012	
1/8/2013 11:00	1/16/2013	
2/12/2013 11:15	2/7/2013	
3/26/2013 10:30	3/27/2013	
4/23/2013 11:35	4/17/2013	
5/21/2013 10:05	5/29/2013	
6/18/2013 11:30	6/27/2013	
8/20/2013 11:00	7/15/2013	
12/4/2013 11:00	8/6/2013	
	8/14/2013	
	9/3/2013	
	12/15/2013	

Initial sensor calibration was performed by the manufacturer (listed in Table 1). During periodic sensor maintenance samples are collected for quality control. At RM-53, nutrients and chlorophyll *a* samples are returned to OHSU laboratory and analyzed using established laboratory techniques. Chlorophyll *a*

measurements are used to correct the in situ fluorometer measurements. The discrete sample data and the corresponding sensor data for nitrate and chlorophyll *a* are shown in Figure 4.

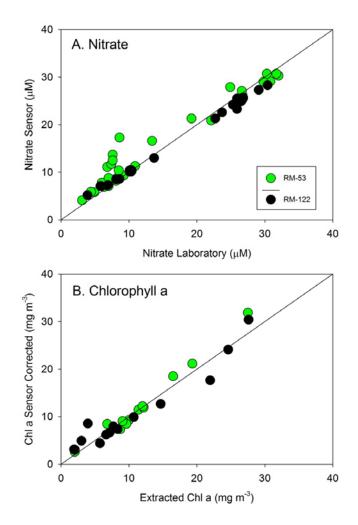


Figure 4. QC data. A) Nitrate concentration measured by the Satlantic SUNA sensor at the two platform locations (y-axis) compared with discreet sample collection and laboratory analysis (x-axis). Also shown is the 1:1 relationship (solid line). B) Chlorophyll *a* measured by the Wet Labs WQM-FLNTU (y-axis) and by sample collection and laboratory analysis (x-axis). Samples were collected during 2012-2013 during the dates listed in Table 2.

3.4.3.2 Determination of biogeochemical fluxes

Measurements of flux of inorganic and organic material can be achieved by multiplying the daily average concentration (computed from the hourly measurements) and the daily river discharge to determine a daily flux. The resulting high resolution flux measurements are useful to observe variations in the system associated with episodic events such as storm runoff and to monitor changes associated with seasonal shifts in climate, and thus are compared to river discharge and other measured parameters to observe correlations in the time series data. Columbia River discharge is measured at RM-53 by the USGS (http://waterdata.usgs.gov/usa/nwis/uv?site_no=14246900) and at Bonneville Dam by BPA. The

difference between discharges at the two sites (Figure 5) reflects inputs from the tributaries of the lower Columbia River, including the Willamette, Cowlitz, and many smaller rivers.

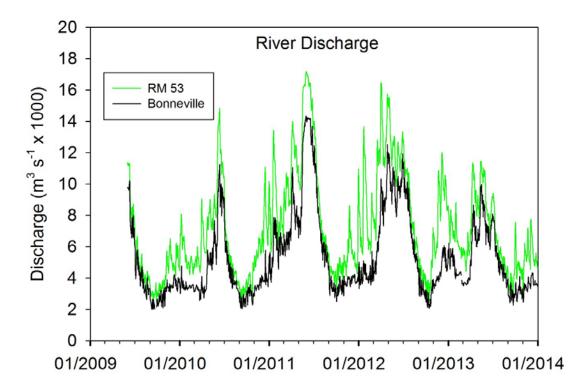


Figure 5. Columbia River daily discharge ($m^3 d^{-1}$) measured at RM-53 (green line) and Bonneville Dam (black line) during the period July 2009-December 2013. The difference in discharge between the two sites represents the input from tributaries in the Lower Columbia River.

3.4.4 Trophic pathways

3.4.4.1 Stable Isotope Analysis

The ratios of carbon and nitrogen stable isotopes in tissues of consumers reflect the stable isotope ratios of their food sources (Neill and Cornwell 1992; France 1995), and therefore, can be useful to determine major food sources, provided that the food sources have distinct isotopic ratios. Stable isotope analysis of carbon and nitrogen was used to determine the relative importance of algae and wetland plants to the food web supporting juvenile salmonids. Stable isotope analysis of carbon and nitrogen was used in determining trophic position of food sources.

Most carbon atoms have 12 neutrons (12 C), but approximately 1% of carbon atoms have 13 neutrons (13 C). Similarly, most nitrogen atoms have 14 neutrons (14 N), while 0.36% have 15 neutrons (15 N). Lighter isotopes are metabolized preferentially to heavier isotopes, so consumers at higher trophic levels (higher in the food web) become enriched in the heavier isotopes. Therefore, the ratios of heavy to light isotopes (15 N/ 14 N and 13 C/ 12 C) in the tissues of food sources, plus a small compensation for the metabolic loss of light isotopes ("trophic fractionation"), are reflected in the tissues of consumers. Typically, with an increase of one trophic level (i.e., from a plant to an herbivore or an herbivore to a carnivore), the 15 N/ 14 N

ratio increases by 2.2 to 3.4 parts per thousand ("permil"; ‰), so stable isotope analysis of nitrogen is useful in determining trophic position. The ${}^{13}C/{}^{12}C$ ratio usually changes by less than 1‰, making stable isotope analysis of carbon useful for determining inputs of primary producers when the different primary producers analyzed have distinct stable isotope ratios.

The stable isotope ratios of carbon and nitrogen were measured from juvenile Chinook salmon (*Oncorhynchus tshawytscha*) tissues and several potential food sources to provide information on the food web supporting juvenile salmonids (Table 3). Juvenile salmonids were collected by NOAA Fisheries staff using a beach seine (see Section 3.3.2). Isotopic signatures of more metabolically active tissues such as liver, mucus, or blood turn over more quickly than those of muscle, otoliths, or scales, so they are good media with which to examine relatively recent dietary sources (Phillips and Eldridge 2006; Michener and Kaufman 2007; Church et al. 2009; Buchheister and Latour 2010). Starting in 2012, epidermal mucus was collected from a subset of juvenile salmonids from which muscle samples were also collected to test the suitability of mucus for this analysis. Epidermal mucus was collected from individual juvenile salmonids as described by Church et al. (2009) and composited in order to meet the minimum sample mass requirements for the analysis. In 2013, muscle, mucus, and liver samples were collected.

Aquatic invertebrates were collected by USGS staff in open water and in emergent vegetation at the water's margin using opportunistic sampling. The aquatic midge *Chironomidae* and the amphipod *Corophium spp.* were selected because they have been found to be preferred food sources for juvenile salmonids in the lower Columbia River (this study; Maier and Simenstad 2009). Most invertebrate samples were found attached to submerged portions of vegetation. Those invertebrates were collected by rinsing the exterior of the vegetation with deionized water and manually removing the invertebrates from the rinse water using clean forceps. Invertebrate samples were then rinsed with deionized water to remove algae or other external particulate matter. Salmon and aquatic invertebrate samples were frozen for later processing.

	Fish	Invertebrates			
Marked Chinook salmon	Unmarked Chinook salmon	Chironomidae	Corophium spp.		
Chironomidae	Chironomidae	Particulate organic matter (POM)	Particulate organic matter (POM)		
Corophium spp.	Corophium spp.	Periphyton	Periphyton		
Hatchery food		Vegetation	Vegetation		

Table 3. Potential food sources for fish and invertebrate consumers.

Samples of terrestrial and emergent vegetation and aquatic macrophyte species were collected from representative areas within each site (Table 4; Sagar et al. 2013). Vegetation samples were rinsed at least five times in deionized water to remove external material, such as invertebrates and periphyton, and were kept frozen for later processing. Samples of particulate organic matter (POM) and periphyton were filtered onto 25 mm glass-fiber GF/F filters and were kept frozen for later processing.

Franz Lake Slough	Campbell Slough	Whites Island	Ilwaco
Polygonum amphibium	Eleocharis palustris	Alisma triviale	Carex lyngbyei
	Phalaris arundinacea	Elodea canadensis	Cladophora columbiana
	Sagittaria latifolia	Equisetum fluviatile	Eleocharis parvula
		Iris pseudacorus	Fucus distichus
		Myriophyllum spicatum	Lilaeopsis occidentalis
		Phalaris arundinacea	Schoenoplectus americanus
		Potentilla anserina	Ulva lactuca
		Potamogeton richardsonii	Zannichellia palustris
		Sagittaria latifolia	
		Typha angustifolia	

Table 4. Vegetation and macroalgal species collected for stable isotope analysis 2010–2012, by site.

Frozen filters, salmon tissue, invertebrate, and plant material were freeze dried using a lyophilizer. Freeze-dried plants of the same species from the same sample date were composited and ground using a clean coffee grinder. Freeze-dried invertebrate bodies of the same taxa from the same collection site and date were composited, ground using a clean glass mortar and pestle, and subsampled when enough material was present. Otherwise, whole bodies of all individuals of the same taxa from the same site were composited into a single sample. Skinned muscle tissue samples from individual juvenile salmonids were analyzed separately; muscle tissue samples from different bodies were not composited. Epidermal mucus samples were composited from multiple juvenile salmonid bodies in order to have sufficient sample mass for analysis.

Stable isotope ratios of carbon (${}^{13}C/{}^{12}C$; "delta ${}^{13}C$ "; " $\delta^{13}C$ ") and nitrogen (${}^{15}N/{}^{14}N$; " $\delta^{15}N$ ") were measured at the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The atomic ratios of the heavy isotope to the light isotope were measured, compared to universal standards, and reported in permil (‰) units. Vienna PeeDee Belemnite and air were used as the standards for carbon and nitrogen, respectively.

Samples were submitted to the lab in duplicate or triplicate, depending on the amount of sample material available. Sample data were not included in the analysis if replicates did not meet reproducibility standards. Sample data with acceptable reproducibility were averaged; therefore, individual data points in this analysis represent the average isotopic ratios of two to three subsamples.

Additional whole bodies of juvenile salmon collected for this project in 2010 by NOAA Fisheries staff were analyzed for stable isotope ratios of carbon and nitrogen at the NOAA Northwest Fisheries Science Center (Sagar et al. 2013). Those data were included in some analyses (where noted) for comparison.

3.4.4.2 Data analysis

Summaries of stable isotope ratios

3.4.4.2.1.1 Comparisons of means

Analysis of similarity (ANOSIM) in the vegan package of R statistical software was used on ranked data to test for significant differences in isotope ratios among groups (e.g., marked vs. unmarked salmon; different types of organic matter; samples from different sites; Gotelli and Ellison 2004; R Development Core Team 2012). The ANOSIM produces two statistical values, R and p. R values range from -1 to 1 and measure the strength of difference between two or more groups (Clarke 1993). An R value of 0 indicates that there is no difference between groups (that the groupings are not meaningful), while an R value of 1 is the strongest difference between groups. Negative R values are generally less common, with R values of -1 indicating larger differences within samples than among groups (Chapman and Underwood 1999). The p values represent the statistical significance of differences. Here, the threshold for significant differences was p < 0.05.

3.4.4.2.2 SIAR models

The mixing model SIAR (R Core Development Team 2009; Parnell et al. 2010) was used to estimate the proportions of potential food sources into consumer diets. The SIAR model is a Bayesian mixing model that accounts for the variability within different types of food sources and allows for the use of many more food sources than isotopes, which are some of the shortcomings of earlier mixing models (Parnell et al. 2010). Using stable isotope ratios from consumers and their potential food sources, the SIAR model uses Markov chain Monte Carlo simulation to estimate plausible proportions from each food source into the consumer diet many thousands of times and returns a distribution of probable estimates from those simulations (Parnell et al. 2010). Although SIAR has been used across multiple trophic levels in other studies, it is better suited for estimating between two trophic levels, so it was used in this manner for this analysis. Results of the model can be plotted on proportion density plots, which show the distributions of plausible proportion estimates for each food source in the diet of a consumer. The estimated dietary proportion is on the X axis and the density of those estimates is plotted on the Y axis. On the plots, higher values on the X axis indicate higher dietary proportion. Distributions that plot narrowly represent more model certainty than those that plot with a wider distribution along the X axis.

For this analysis, the SIAR model was run for juvenile Chinook salmon as consumers and invertebrate prey species and hatchery food as potential food sources. It was also run using two types of invertebrates that are known to be major food sources for juvenile Chinook salmon in the lower Columbia River and Estuary, the aquatic midge *Chironomidae* and the amphipod *Corophium spp*. (Sagar et al. 2013; Maier and Simenstad 2009). For the model runs using invertebrates as consumers, multiple types of organic matter collected from four sites were used as potential food sources for those invertebrates. Organic matter sources were broadly grouped as vegetation, periphyton, and POM (a proxy for phytoplankton), but more refined groupings were also used in the analyses in order to explore which groupings yielded the most informative results. The organic matter type groupings explored were ecologically meaningful divisions, such as brackish vs. freshwater, terrestrial vegetation vs. macrophyhtes, and saltmarsh vs. freshwater wetland vegetation. To account for fractionation effects, trophic enrichment factors of 0.4 $\pm 1.3\%$ for δ^{13} C and $3.4\pm 1.0\%$ for δ^{15} N were used in the model (Post 2002).

3.4.5 Interdisciplinary Relationships

The goal of the multivariate analysis was to examine how different abiotic factors and/or species abundance at different levels of the food web are associated with other selected abiotic factors or species abundances at the same site, month, or year. EMP data collected between 2011 and 2013 at multiple sites were used in the multivariate analysis and represent the full complement of food web data (food web data collection began in 2011; Appendix 1). Different non-parametric multivariate techniques were used to identify relationships between sites, species, and environmental gradients (Kruskal 1964; Mather 1976). To perform the nonparametric multivariate analysis, it is necessary to have two matrices, a species matrix and an environmental matrix, and for both matrices to have the same site, month, and year represented; therefore, depending on the analysis, the number of sites will differ. PC-ORD (McCune and Mefford 2011) was used to run the analysis.

Higher salinity and regular tidal inundation are unique environmental characteristics at the Ilwaco site, in contrast to the other sites used in the analysis. We hypothesized these strong environmental gradients could be masking gradients at other sites; therefore, the same analyses for each multivariate analysis were run with Ilwaco excluded.

3.4.5.1 Water Temperature Analysis

From 2011-2012, water temperature data were collected by PNNL and USGS at Ilwaco, Whites Island, Campbell Slough, and Franz Lake Slough trend sites. At sites with measurements in the same month and in the same year, water temperature data were averaged by month and year. A two-sided t-test was used to compare the mean monthly water temperature from the same trend site and year between the PNNL and USGS datasets to determine if there was a significant difference between measured values.

3.4.5.2 Multi-response Permutation Procedures

Multi-response permutation procedure (MRPP) is a nonparametric procedure for testing the hypothesis of no difference between two or more groups of entities (McCune and Mefford 2011). Site, month, and year were used to determine group membership. MRPP was performed to determine if sites are similar or different from other sites included in the analysis related to site measurements. Sorensen distance and natural weighting were used for this test.

3.4.5.3 Nonmetric multidimensional Scaling

The EMP data are characterized as non-normal, discontinuous, and variable in scale of which nonmetric multidimensional scaling (NMS) is well suited to analyze (McCune and Grace 2002). Prior to NMS analyses, bivariate scatter plots were constructed to determine if strong relationships were evident between species and environmental factors. It was determined there were no strong linear relationships between species and environmental factors and the bivariate scatter plots were characterized by non-normal distributions. For all NMS analyses, a random starting configuration was used with 250 runs performed with the real data. The number of dimensions assessed for the analysis was determined by a Monte Carlo randomization test (250 runs) to determine the number of significant axes with a low stress solution. In order to determine which environmental gradients are related to species abundance, environmental wariables were overlaid on the ordination. The angle and length of vectors from the environmental matrix indicate the direction and strength of the relationship with species and sample units (McCune and Grace 2002).

4 **Results**

4.1 Variability in the LCRE

4.1.1 Habitat Structure

Overall patterns and variability in habitat structure are observable temporally and spatially at sites monitored as part of the EMP. In general, vegetation cover is higher and more consistent between years at lower estuary sites than at the upper estuary sites where cover tended to be lower and more variable between years (Figure 6). Species composition varies in the estuary shifting from sites dominated by Lyngby's sedge (*Carex lyngbyei*) at and below rkm 53 to sites dominated by reed canarygrass (*Phalaris arundinacea*) at and above rkm 72. The number of species also changes longitudinally, with the highest number of species observed at the sites located at rkm 53 and 72 (Welch and Whites, respectively). A similarity analysis using average percent cover of wetland vegetation was conducted to elucidate temporal and spatial variability between sites and over time.

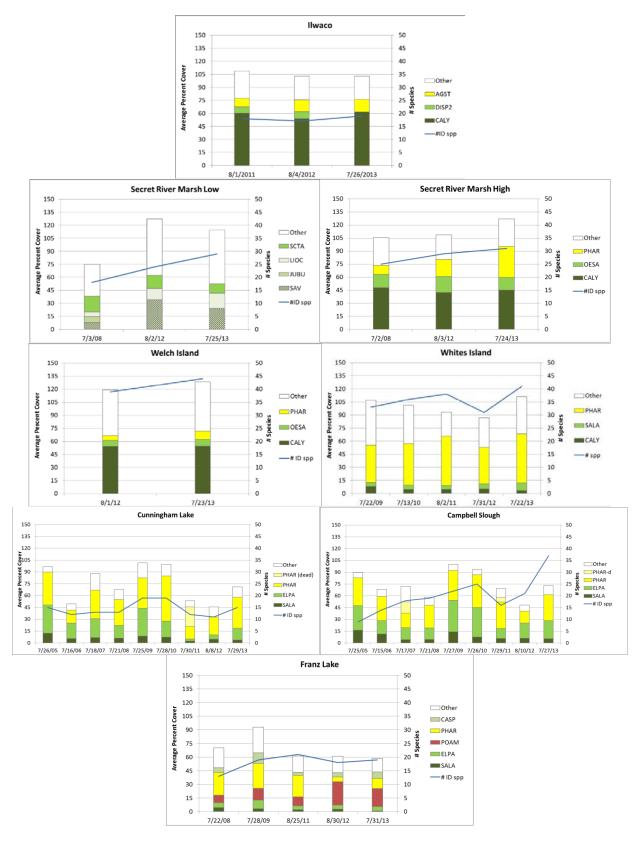


Figure 6. Average percent cover and number of identified species at the trend sites for all years monitored. Sites are ordered from nearest the mouth (top) to nearest the dam (bottom).

4.1.1.1 Temporal Similarity

4.1.1.1.1 Vegetation cover

In general, the average similarity between years at the trend sites was the greatest at BBM and WI2 (Figure 7 and Table 5) at 82 percent (n = 3) and 85 percent (n = 2), respectively. The lowest average similarity was at Cunningham Lake (CLM) with 72 percent similarity (n = 36); however, the lowest single comparison was 53.1 percent also at Cunningham Lake (CLM) between 2005 and 2011. In general, as the span between years increases, the pairwise similarity for a given site decreases (Figure 8 left). Comparisons to the first year observations also shows a similar response, that is, the similarity increases as the starting year gets closer to the current year (Figure 8, right). Thus, for those trend sites observed over a greater number of years, the average similarity decreased significantly with an increasing number of years between observations (Figure 9; Regression; p = 0.001).

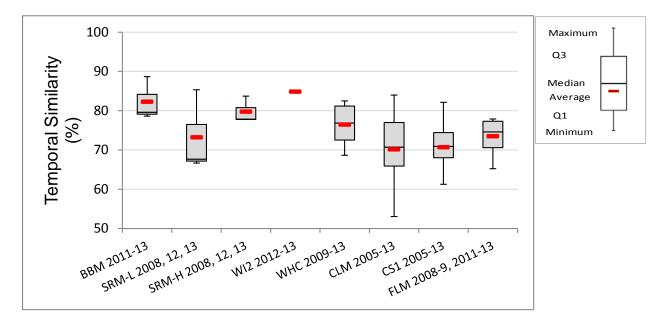


Figure 7. Pairwise similarity (%) of vegetation percent cover over time for each trend site (BBM=Ilwaco Slough (Baker Bay), SRM=Secret River, WI2=Welch Island, WHC=Whites Island, CLM=Cunningham Lake, CS1=Campbell Slough, FLM=Franz Lake).

Table 5. Descriptive statistics of the site similarity with itself over time. The number of comparisons (*n*) is based on the number of years a site was monitored, so CLM for example was monitored 9 years and 36 year comparisons could be made. BBM=Ilwaco Slough (Baker Bay), SRM=Secret River, WI2=Welch Island, WHC=Whites Island, CLM=Cunningham Lake, CS1=Campbell Slough, FLM=Franz Lake.

Site	п	Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BBM	3	82.3	5.5	78.6	78.6	79.6	88.7	88.7
CLM	36	70.2	7.9	53.1	64.9	70.8	77.1	84.0
CS1	36	70.7	5.0	61.3	67.5	70.9	74.5	82.1
FLM	10	73.5	4.4	65.3	70.2	74.6	77.5	77.9
SRM-H	3	79.8	3.4	77.8	77.8	77.8	83.7	83.7
SRM-L	3	73.2	10.5	66.7	66.7	67.7	85.3	85.3
WHC	10	76.5	5.1	68.7	71.5	76.9	81.8	82.5
WI2	1	84.9	*	84.9	*	84.9	*	84.9

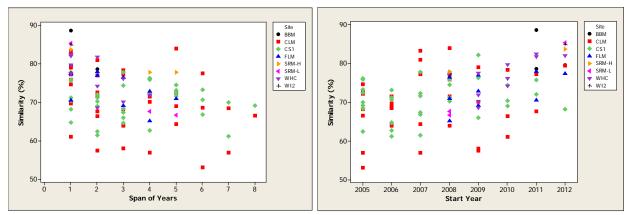


Figure 8. Pairwise similarity (%) as a function of the span in the number of years observations were taken (left) and as a function of the first year observations were taken for each trend site (right). BBM=Ilwaco Slough (Baker Bay), SRM=Secret River, WI2=Welch Island, WHC=Whites Island, CLM=Cunningham Lake, CS1=Campbell Slough, FLM=Franz Lake.

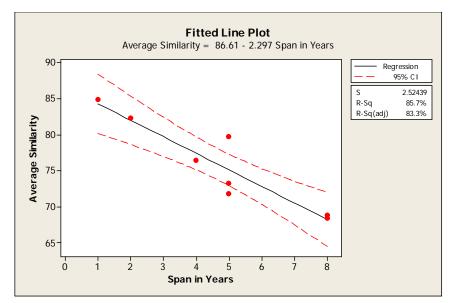


Figure 9. Average pairwise similarity (%) as a function of the span in the number of years observations were taken.

Individual years as well as the natural variability within a given site can also affect the similarity. The pairwise similarity between years for sites Cunningham Lake (CLM; Rkm 145) and Campbell Slough (CS1; Rkm 149), each with 36 pairwise observations, was plotted (Figure 10 top). The variability in pairwise similarity between years was greater at Cunningham Lake (CLM; CV = 11%) than at Campbell Slough (CS1; CV = 7%). The similarity between years at Cunningham Lake (CLM) was least between 2005 and 2011 (53%; a low water year and a high water year) while at Campbell Slough (CS1) the similarity was least between 2006 and 2013 and between 2007 and 2009 (61% for each pair; perhaps due to disturbance from cow grazing and flooding). Even though trend sites Whites Island (WHC) and Franz Lake (FLM) are 149 km apart, both had a similar pattern and variability in the pairwise similarity between years (CV = 6%; Figure 10 bottom).

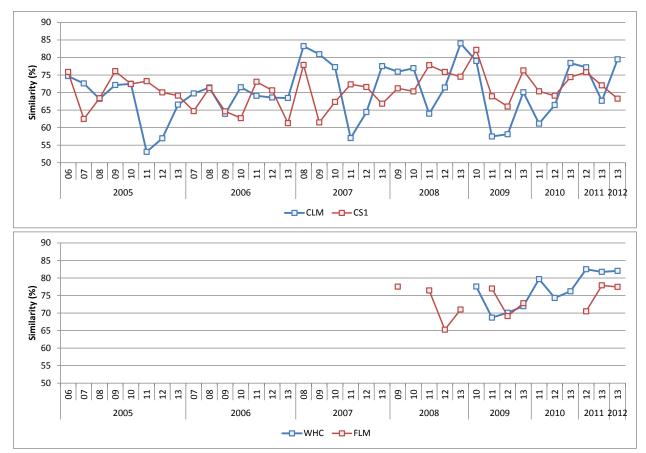


Figure 10. Pairwise similarity (%) between each pair of years for trend sites Cunningham Lake (CLM) and Campbell Slough (CS1), 4 km apart (top) and for Whites Island (WHC) and Franz Lake (FLM), 149 km apart (bottom).

The similarity between sites can also be evaluated as a function of the distance between sites (Figure 11). All sites with greater than one year of observations were used in this analysis. However, the regression was only conducted using sites with at least three years of observations. The trend site Secret River low marsh (SRM-L) and site Secret River high marsh (SRM-H) are low and high marsh observations and although the pairwise similarities are included in the regressions for all other sites, a regression analysis was not conducted on these data. The average over years of the similarity between trend sites tends to decrease with the distance between sites; only significant slopes for trend sites Ilwaco Slough (BBM), Cunningham Lake (CLM) and Campbell Slough (CS1), and Franz Lake (FLM; p < 0.01) are shown in the plot. Cunningham Lake (CLM) and Campbell Slough (CS1) are highly correlated and are only 4 rkm apart. The slopes for Cunningham Lake (CLM), Campbell Slough (CS1), and Franz Lake (FLM) are not significantly different (p = 0.185), however a nonparametric runs test using the residuals from the common slope model goodness of fit for Franz Lake (FLM) was rejected (p < 0.01). Thus, only a common slope and intercept linear model were fit using Cunningham Lake (CLM) and Campbell Slough (CS1).

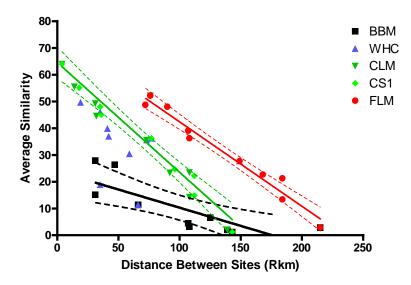


Figure 11. Regression of the average similarity over years between sites as a function of the distance between sites. BBM=Ilwaco Slough (Baker Bay), WHC=Whites Island, CLM=Cunningham Lake, CS1=Campbell Slough, FLM=Franz Lake.

4.1.1.2 Spatial Similarity

4.1.1.2.1 Vegetation Cover

The species assemblages differ somewhat between all sites in the estuary. The minimum similarity was very low ($\leq 10\%$) for all sites indicating the dissimilarity between sites, however 35 of the 51 sites included in the analysis have a similarity of 50% or greater to at least one site and four sites have a similarity to each other between 65% and 70% (Table 6).

Table 6. Descriptive statistics of the percent similarity of each site compared to all other marsh sites used in the analysis (51 sites). The similarity for sites with multiple years was averaged for comparison to other sites. Sites are listed in Appendix 1.

Site	Year	rkm	EM zone	Mean	Median	Stdev	Q1	Q3	Min	Max
BBM	Multiple	6	EM1	9.62	4.51	11.85	1.94	12.76	0.00	52.49
CHM	2009	12	EM1	9.38	4.04	11.47	1.88	12.59	0.00	52.49
TBB	2008	12	EM1	8.48	2.40	13.06	0.61	9.48	0.00	47.18
FCB	2008	19	EM1	23.23	23.42	7.84	18.85	27.71	3.58	38.59
LCM	2009	20	EM1	22.35	20.77	11.62	16.99	26.16	0.49	54.21
CSM	2007	23	EM1	8.84	7.65	6.06	5.07	13.04	0.00	24.05
GIM	2009	23	EM1	15.67	12.45	10.72	8.31	18.69	0.00	42.00
HIB	2009	23	EM1	26.59	27.30	7.80	20.41	30.71	9.70	54.21
SRMH	Multiple	37	EM1	24.44	22.40	11.37	16.36	28.94	9.29	64.44
SRML	Multiple	37	EM1	15.91	15.20	5.89	13.35	18.39	2.93	35.21
MSC	2009	39	EM1	8.68	7.70	7.23	2.47	12.56	0.00	31.33

Site	Year	rkm	EM zone	Mean	Median	Stdev	Q1	Q3	Min	Max
KIB	2008	41	EM2	27.23	28.71	9.35	23.60	32.10	2.85	45.26
WI2	Multiple	53	EM2	26.50	23.46	11.91	19.28	32.76	7.90	64.44
WIM	2008	53	EM2	21.84	19.03	9.18	15.57	27.59	5.76	52.85
RIM	2009	61	EM2	26.77	25.38	11.19	18.44	32.19	7.39	58.04
JIC	2010	71	EM2	32.65	33.56	10.21	23.61	39.61	10.25	60.31
WHC	Multiple	72	EM2	28.58	30.14	12.46	18.80	35.28	6.23	58.04
WAC	2010	77	EM2	29.34	29.95	11.22	21.34	36.34	7.70	60.31
CRM	2009	80	EM2	27.80	29.06	12.48	20.01	37.58	0.00	51.54
GUC	2009	89	EM2	22.84	22.10	9.95	16.49	30.57	2.37	45.26
LI1	2009	99	EM2	25.38	28.75	9.95	20.89	32.25	0.65	42.45
LI2	2009	100	EM3	25.96	26.64	10.67	20.21	33.09	0.00	44.84
DIB	2005	104	EM3	32.18	33.34	12.55	24.40	42.36	2.48	56.35
CI1	Multiple	113	EM3	31.06	32.65	11.70	25.51	39.15	2.62	59.37
CI2	Multiple	114	EM3	31.86	32.10	12.99	26.37	41.46	1.56	59.37
SI1	2007	121	EM3	33.81	34.96	16.27	23.06	44.06	0.00	69.89
SI2	2007	123	EM3	32.10	36.00	14.57	24.26	41.30	0.00	65.82
MIM	2007	129	EM3	30.87	32.07	11.41	25.71	39.21	3.44	47.17
BIM	2011	131	EM3	29.34	30.43	14.55	18.66	38.52	1.14	59.73
GIC	Multiple	131	EM3	32.22	32.12	14.05	24.14	41.35	1.73	62.34
DIC	2011	132	EM3	32.51	32.82	15.87	23.92	43.01	0.78	62.34
NNI	2007	136	EM3	30.82	33.40	12.19	22.99	37.83	2.97	59.92
SBM	2010	143	EM4	34.54	33.56	17.15	24.70	44.07	0.00	69.89
CLM	Multiple	145	EM4	33.66	33.90	16.78	23.39	44.25	0.71	66.44
CS1	Multiple	149	EM4	33.84	35.78	16.50	24.37	44.96	0.59	65.62
SCM	2005	154	EM4	33.37	34.85	16.87	24.18	44.13	0.00	66.44
WRC	2006	175	EM4	22.43	21.97	10.15	15.64	29.59	1.76	39.45
GOM	2012	181	EM4	16.09	17.06	8.01	10.48	21.70	0.00	37.13
MIC	2006	190	EM5	28.49	30.77	13.38	20.27	37.43	0.52	61.27
OWR	2012	195	EM5	26.42	28.80	13.07	16.19	36.54	0.00	50.97
WRM	2010	195	EM5	21.88	22.25	11.74	13.50	29.70	0.82	45.57
SRD	2006	196	EM5	28.77	31.10	13.71	18.61	38.23	0.00	53.43
OSM	2007	198	EM5	25.82	28.45	11.30	19.29	33.48	1.07	46.94
GAM	2008	200	EM5	25.41	26.12	13.96	14.71	34.68	0.00	57.21
CIC	2006	201	EM5	24.16	23.82	12.74	16.90	33.06	0.00	61.27
RIC	2007	201	EM5	28.80	29.88	12.94	21.13	35.76	0.00	59.92
RI2	2012	204	EM5	24.26	23.95	13.03	15.32	35.11	0.00	54.41
SIM	2008	211	EM5	30.40	32.22	14.78	19.82	41.96	0.00	55.28
FLM	Multiple	221	EM5	28.68	28.55	13.39	22.73	36.64	0.86	54.47
PIM	2008	228	EM5	25.33	25.87	11.88	17.40	33.60	0.00	52.43
HC	2008	230	EM5	16.44	16.84	10.85	7.91	22.76	0.00	43.68

The similarity was generally highest between sites in the same emergent marsh (EM) zone (EM1 – EM5; Table 7). A few sites are exceptions and have low similarity to all other sites, including those in the same EM zone. These sites include Government/Lemon Island (GOM) and Miller Sands (MSC), both very sandy, high current, flow-through sites; Cooperage Slough (CSM), a very high marsh site located in the Youngs River approximately 10 km from the mainstem; and Hamilton Creek (HC), a marsh site with a perennial flowing stream running through it located in the very fluvial portion of the estuary at rkm 230.

By averaging the similarity within an EM zone, then comparing that to all other sites within that zone and to all other sites in each of the other zones, a pattern emerges regarding the similarity of sites within and between zones (Figure 12). The highest similarity occurs between the sites within the same EM zone (e.g., the similarity of Avg EM2 [red bar in Figure 12] is highest when compared to the other sites within EM zone 2). The highest similarity within an EM zone occurs between sites within EM zones 3 and 4 and lowest similarity occurs between sites within EM zone 1. The similarity between sites decreases with distance, with the sites in EM zone 1 least similar to the sites in all other zones.

Table 7. Percent similarity for each site compared to the average site similarity in each EM zone. For sites sampled multiple years, the average similarity over years for each pair of sites was used.

Year	Rkm	EM zone	Avg EM1	Avg EM2	Avg EM3	Avg EM4	Avg EM5
Multiple	6	EM1	25.9	12.71	4.09	2.28	2.83
2009	12	EM1	24.2	12.27	5.12	2.25	2.68
2008	12	EM1	27.29	11.08	1.54	0.92	1.40
2008	19	EM1	21.42	29.07	23.98	20.03	20.97
2009	20	EM1	32.50	27.25	21.26	16.81	14.24
2007	23	EM1	11.38	10.97	7.71	5.01	7.99
2009	23	EM1	28.48	21.44	10.60	6.24	10.00
2009	23	EM1	29.70	29.78	27.54	22.79	22.69
Multiple	37	EM1	27.18	37.37	23.01	20.87	15.25
Multiple	37	EM1	15.53	18.14	18.94	13.03	13.26
2009	39	EM1	10.81	15.62	8.68	5.23	3.30
2008	41	EM2	19.97	35.33	30.70	27.08	24.91
Multiple	53	EM2	29.88	41.37	24.94	20.74	17.33
2008	53	EM2	23.89	34.01	20.73	17.18	14.77
2009	61	EM2	22.55	43.30	27.50	24.20	19.45
2010	71	EM2	27.70	43.94	38.35	31.65	24.67
Multiple	72	EM2	20.85	43.83	32.71	30.12	20.34
2010	77	EM2	20.71	42.62	35.52	30.28	21.80
2009	80	EM2	13.10	40.91	35.74	31.89	22.54
2009	89	EM2	14.49	31.91	27.39	20.63	20.79
2009	99	EM2	12.04	30.36	32.41	26.23	26.89
2009	100	EM3	11.84	29.28	34.00	32.54	26.14
2005	104	EM3	16.24	31.81	42.48	41.87	33.55
Multiple	113	EM3	16.33	34.98	41.12	37.24	29.94
Multiple	114	EM3	15.77	31.49	45.11	39.64	31.98
	Multiple 2009 2008 2008 2009 2007 2009 2009 Multiple 2009 2008 Multiple 2008 2009 2010 Multiple 2010 2009 2010 2009 2009 2009 2009 2009	Multiple6200912200819200920200723200923200923200923Multiple37200939200939200961200961201071Multiple7220107720098020099920091002009104Multiple113	Multiple 6 EM1 2009 12 EM1 2008 12 EM1 2008 19 EM1 2008 19 EM1 2009 20 EM1 2009 20 EM1 2009 23 EM1 Multiple 37 EM1 2009 39 EM1 2009 39 EM2 2008 53 EM2 2008 53 EM2 2009 61 EM2 2010 71 EM2 2010 77 EM2 2010 77 EM2 2009 80 EM2 2009 89 EM2 2009 99 EM2 2009 99 EM3 2005 104 EM3 2005 104	Multiple 6 EM1 25.9 2009 12 EM1 24.2 2008 12 EM1 27.29 2008 19 EM1 21.42 2009 20 EM1 32.50 2007 23 EM1 11.38 2009 23 EM1 28.48 2009 23 EM1 29.70 Multiple 37 EM1 27.18 Multiple 37 EM1 15.53 2009 39 EM1 10.81 2008 41 EM2 19.97 Multiple 53 EM2 29.88 2008 53 EM2 23.89 2009 61 EM2 27.70 Multiple 72 EM2 20.85 2010 71 EM2 20.71 2009 80 EM2 13.10 2009 89 EM2 14.49 2009 99	Multiple 6 EM1 25.9 12.71 2009 12 EM1 24.2 12.71 2008 12 EM1 24.2 12.27 2008 19 EM1 27.29 11.08 2009 20 EM1 32.50 27.25 2007 23 EM1 11.38 10.97 2009 23 EM1 29.70 29.78 Multiple 37 EM1 27.18 37.37 Multiple 37 EM1 15.53 18.14 2009 39 EM1 10.81 15.62 2008 41 EM2 19.97 35.33 Multiple 53 EM2 23.89 34.01 2008 53 EM2 23.89 34.01 2009 61 EM2 22.55 43.30 2010 71 EM2 20.71 42.62 2009 80 EM2 13.10 40.91	Multiple 6 EM1 25.9 12.71 4.09 2009 12 EM1 24.2 12.71 4.09 2008 12 EM1 24.2 12.27 5.12 2008 12 EM1 27.29 11.08 1.54 2008 19 EM1 21.42 29.07 23.98 2009 20 EM1 32.50 27.25 21.26 2007 23 EM1 11.38 10.97 7.71 2009 23 EM1 29.70 29.78 27.54 Multiple 37 EM1 27.18 37.37 23.01 Multiple 37 EM1 15.53 18.14 18.94 2009 39 EM1 10.81 15.62 8.68 2008 41 EM2 19.97 35.33 30.70 Multiple 53 EM2 23.89 34.01 20.73 2008 53 EM2	Multiple 6 EM1 25.9 12.71 4.09 2.28 2009 12 EM1 24.2 12.27 5.12 2.25 2008 12 EM1 27.29 11.08 1.54 0.92 2008 19 EM1 21.42 29.07 23.98 20.03 2009 20 EM1 32.50 27.25 21.26 16.81 2007 23 EM1 11.38 10.97 7.71 5.01 2009 23 EM1 28.48 21.44 10.60 6.24 2009 23 EM1 27.18 37.37 23.01 20.87 Multiple 37 EM1 15.53 18.14 18.94 13.03 2009 39 EM1 10.81 15.62 8.68 5.23 2008 41 EM2 19.97 35.33 30.70 27.08 Multiple 53 EM2 29.88 41.37 24

Site	Year	Rkm	EM zone	Avg EM1	Avg EM2	Avg EM3	Avg EM4	Avg EM5
SI1	2007	121	EM3	12.74	29.46	48.10	49.25	36.85
SI2	2007	123	EM3	13.11	32.12	43.08	44.95	33.76
MIM	2007	129	EM3	14.55	33.61	39.24	34.47	34.48
BIM	2011	131	EM3	11.14	28.94	41.01	42.74	29.90
GIC	Multiple	131	EM3	13.95	27.19	43.09	45.41	37.11
DIC	2011	132	EM3	12.25	27.80	44.85	47.38	36.91
NNI	2007	136	EM3	14.56	29.94	38.43	32.14	38.81
SBM	2010	143	EM4	12.60	32.50	49.85	50.50	35.59
CLM	Multiple	145	EM4	11.52	29.37	47.90	51.21	36.90
CS1	Multiple	149	EM4	11.18	29.93	47.42	49.82	38.38
SCM	2005	154	EM4	11.38	29.27	47.62	50.07	36.66
WRC	2006	175	EM4	9.65	21.68	30.78	32.05	23.05
GOM	2012	181	EM4	6.63	13.23	20.58	18.21	21.69
MIC	2006	190	EM5	12.26	24.52	35.44	31.73	38.69
OWR	2012	195	EM5	9.26	19.55	35.00	36.34	35.03
WRM	2010	195	EM5	9.05	18.43	29.40	33.64	23.74
SRD	2006	196	EM5	10.81	25.22	38.37	36.99	35.28
OSM	2007	198	EM5	9.88	24.63	32.54	27.62	34.35
GAM	2008	200	EM5	9.11	20.73	33.41	28.51	35.36
CIC	2006	201	EM5	8.69	18.80	31.53	29.45	33.41
RIC	2007	201	EM5	14.75	24.14	35.98	28.67	39.05
RI2	2012	204	EM5	9.84	19.01	32.14	31.73	30.90
SIM	2008	211	EM5	12.41	22.27	40.38	38.48	40.47
FLM	Multiple	221	EM5	10.88	24.01	38.86	40.47	33.64
PIM	2008	228	EM5	11.44	20.72	31.46	28.51	34.70
HC	2008	230	EM5	7.05	15.48	22.09	24.43	16.67

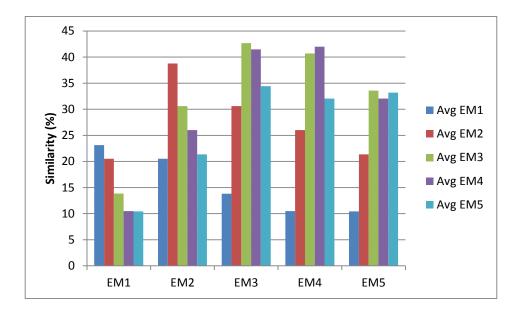


Figure 12. Average similarity of each pair of sites within and between emergent marsh (EM) zones ignoring the year sampled.

A general linear model (GLM) was used to evaluate significance of site similarity between years and between EM zones. For sites within the same year and same EM zone, the average percent similarity between sites decreases significantly as the distance between sites increased (Regression; p < 0.001; Figure 13). Thus, the maximum distance between sites within the same zone and observed within the same year was used as a covariate in the GLM. The average similarity between years with the effect of zone removed was not significant (p = 0.527), however 2007 and 2011 were the least similar (Figure 14, top). The average similarity between EM zones with the effect of year removed was significant (p = 0.015) with the similarity between sites within EM1 less than between sites in EM4 (Figure 14, bottom).

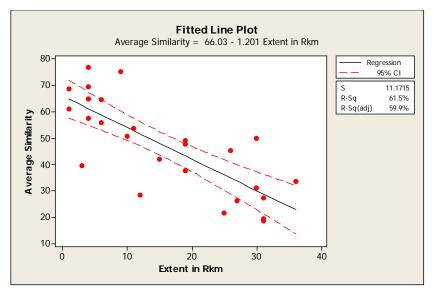


Figure 13. Regression of the average similarity between sites within the same zone and observed within the same year as a function of the distance between sites.

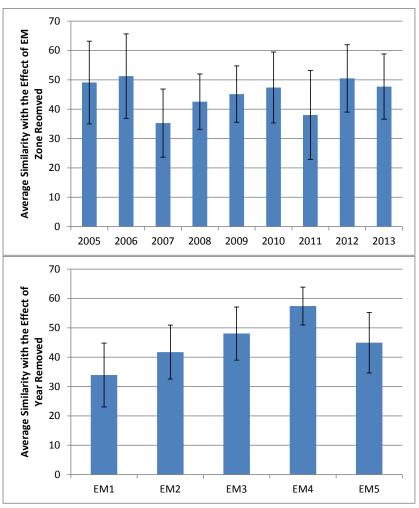


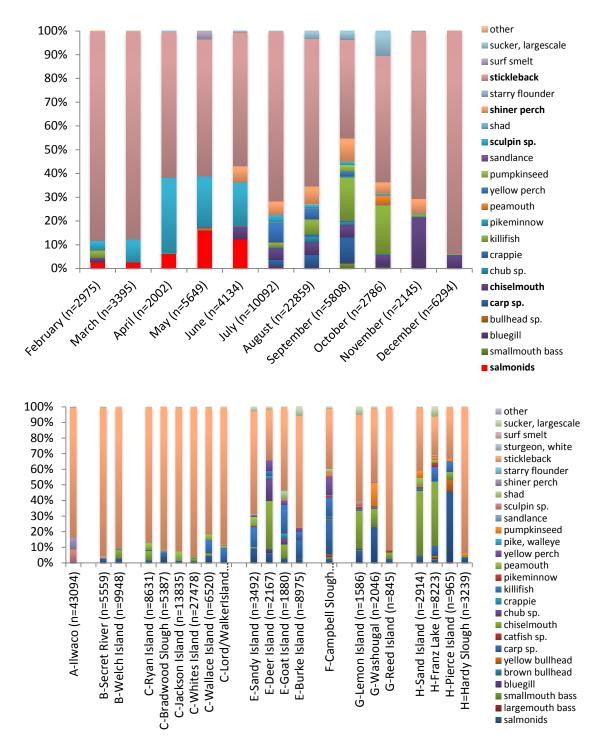
Figure 14. Bar charts of the least square means from the GLM with main effects of year (top) and emergent marsh (EM) zone (bottom).

4.1.2 Fish Variability

4.1.2.1 General spatial and temporal trends

4.1.2.1.1 Fish Community Characteristics

The EMP sampling sites supported a diverse range of fish species, with community composition changing by sampling season and site location (Figure 15). In late winter and spring (February through June), salmonids, sculpins [staghorn sculpin (*Leptocottus armatus*) in Reach A and other sculpin species (*Cottus spp.*) in other reaches], and stickleback (*Gasterosteus aculeatus*) were the predominant species. From July through October, although stickleback were still present in large numbers, salmonids were largely absent from catches, and species such as killifish (*Fundulus diaphanous*), chiselmouth (*Acrocheilus alutaceus*), carp (*Cyprinidae* spp.), and shiner perch (*Cymatogaster aggregata*) were more abundant. In November and December, many of the species common during the summer months were no longer observed, with stickleback and chiselmouth being the dominant species. Fish community composition also tended to vary



from reach to reach (Figure 15). In lower river reaches, stickleback was the dominant species, in some places making up the majority of the catch.

Figure 15. Fish community composition by sampling month and sampling site.

Species diversity. Species diversity tended to increase with distance from the mouth of the river, from a mean of 0.34 to 0.51 in Reaches A-C, to a mean of 0.94 in Reach H (Figure 16). Also, species diversity tended to be highest in the spring and summer, with lower diversity during the fall and winter months (Figure 16).

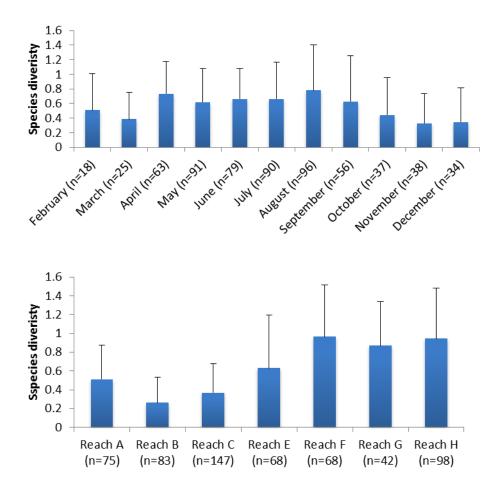


Figure 16. Fish species diversity by month and reach.

Species richness. Species richness (mean per set) was lowest at sites in Reaches A-C, increased to a peak of 6.6 at Campbell Slough in Reach F, and then declined slightly in Reach G and H (Figure 17). Also, species richness tended to be highest in the summer, with lower values in the spring, fall and winter months (Figure 17).

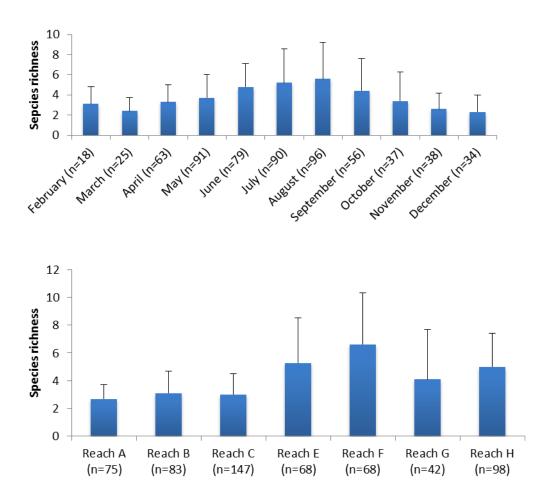


Figure 17. Fish species richness by month and reach.

Percentage of non-native species. The percentage of non-native species in catches was lowest at sites in Reaches A-C, increased to a peak at Campbell Slough (a mean value of 38% per beach seine set) in Reach F, and then declined slightly in Reach G and H (Figure 18). Also, the percentage of non-native species in the catch tended to be highest in summer, with a maximum value of 28% (mean per set) in September. The percentage of non-native species in catches was lower in the spring, fall, and winter months with mean values per set in the 3-5% range (Figure 18).

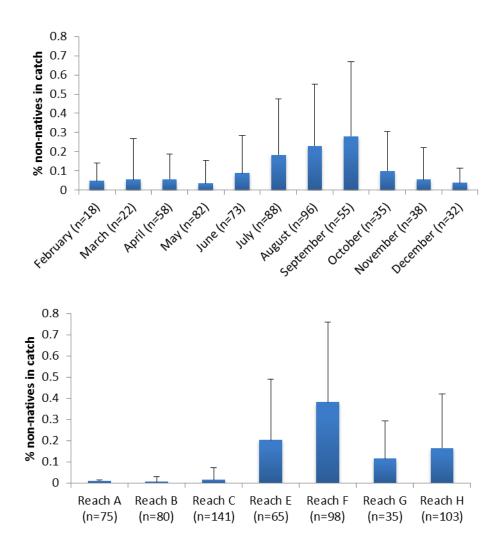


Figure 18. Percentage of non-native fish species in catch by month and reach.

Percentage of piscivorous predators. The percentage of predatory fish was lowest at sites in Reaches A-C, and then increased upstream, with highest values occurring at Franz Lake in Reach H (Figure 19). Sites with highest percentages of predatory fish in catches included Campbell Slough (Reach F), Washougal Wetland (Reach G), and Sand Island (Reach H) and Franz Lake (Reach H). The percentage of predatory fish in the catches tended to be highest in summer, with a maximum mean values in July, August and September ranging from 1.7 to 3.6% of catch per set. The percentage of predatory species in catches was lower in the spring, fall and winter months with mean values per set of less than 1%.

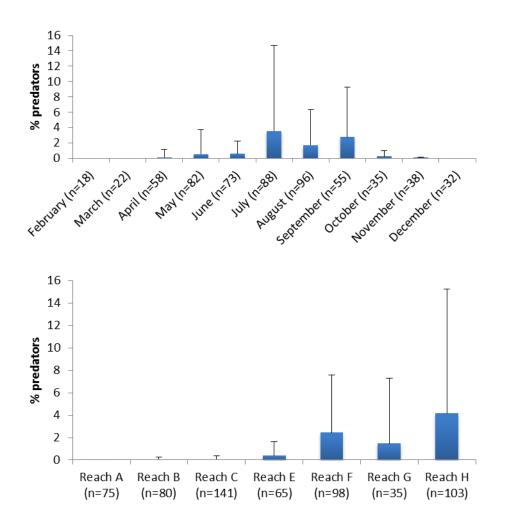


Figure 19. Percentage of fish that are potential salmon predators in catch by month and reach.

4.1.2.1.2 Juvenile Salmon Species Composition

Salmon species collected at the EMP sampling sites included mostly Chinook salmon, coho salmon, and chum salmon. Chinook salmon were the most abundant species, accounting for 80% of all salmon collected, with coho salmon accounting for 11% and chum salmon for 9% of the catch. Only a very small number of other salmon species were collected, including sockeye salmon, steelhead trout, and other trout species. Salmon species composition (Figure 20) and percentage marked/unmarked (Figure 21) tended to remain consistent across years at the trend sites.

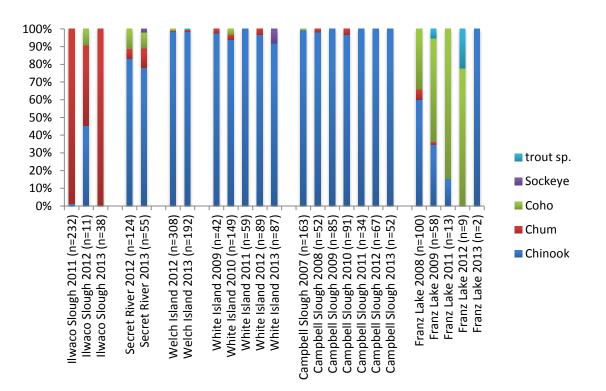


Figure 20. Salmonid catch composition by year at the EMP trend sites. N indicates the number of total number of salmon and trout caught at that site and year.

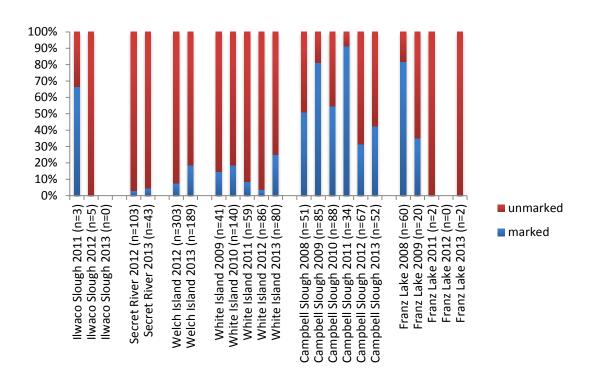


Figure 21. Percentage of Chinook salmon in catches that were marked and unmarked by sampling year at the EMP trend sites. N indicates the number of total number of Chinook salmon caught at that site and year.

Salmon species diversity. Salmon diversity was highest in April and May, and generally lower in other months, although salmon diversity was also quite high in December. Salmon diversity also showed highest values at sites in Reach H (Figure 22), although some relatively high values were also observed at sites in Reach B and C. Salmon species diversity remained consistently higher at two of the trend sites (Franz Lake and Secret River; Figure 22).

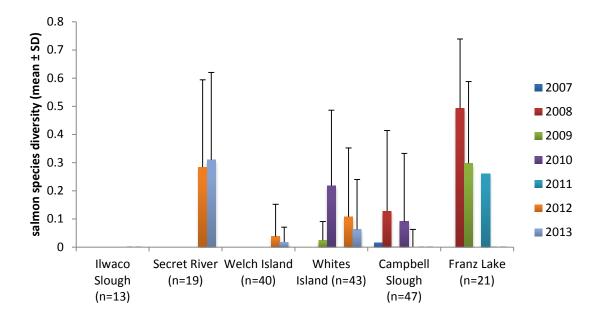


Figure 22. Salmon species diversity (mean \pm SD) by sampling year at the EMP trends sites. N indicates the total number of beach seine sets at that site; means represent the average of density values calculated for the individual beach seine sets.

Seasonal Salmon Density

Overall, Chinook salmon were the most abundant salmon found at EMP sites, with an average density over the sampling season of 24 unmarked fish and six marked fish per 1,000 m². Coho salmon, especially unmarked coho, were generally less abundant than Chinook salmon, with an average density over the sampling season of 5.8 unmarked fish and 4.5 marked fish per 1,000 m². Densities of chum (all unmarked) were still lower, with an average of two fish per 1,000 m². Density of all three salmon species was most influenced by site and then year (Figure 23 to Figure 25), where a particular year did not show the same trend across sites.

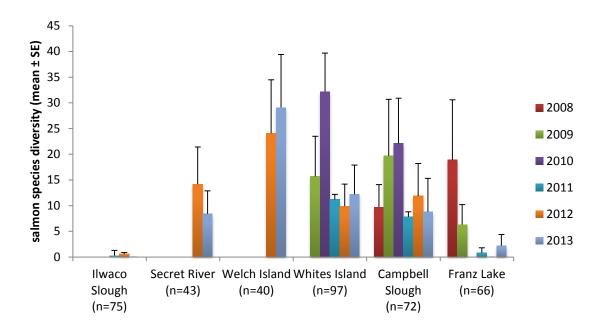


Figure 23. Density (fish per 1,000 m²) of Chinook salmon (mean \pm SE) by sampling year at the EMP trends sites. N indicates the total number of beach seine sets at that site; means represent the average of density values calculated for the individual beach seine sets.

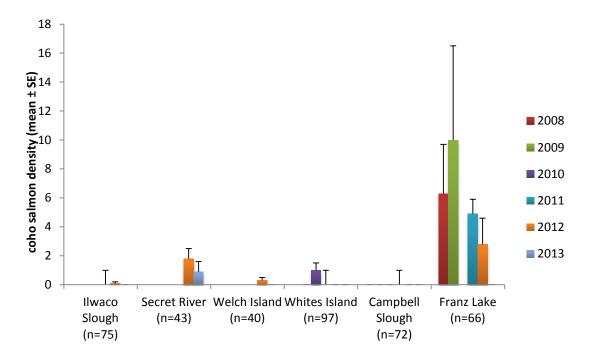


Figure 24. Density (fish per 1,000 m²) of coho salmon (mean \pm SE) by sampling year at the EMP trends sites. N indicates the total number of beach seine sets at that site; means represent the average of density values calculated for the individual beach seine sets.

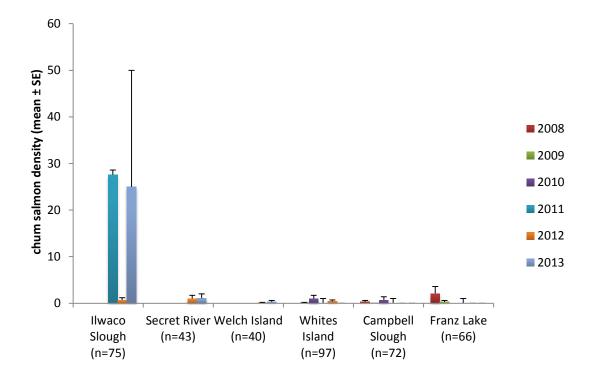
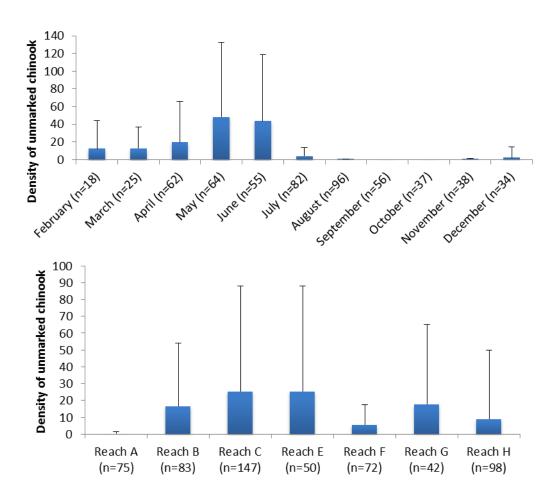


Figure 25. Density (fish per 1,000 m²) of chum salmon (mean \pm SE) by sampling year at the EMP trends sites. N indicates the total number of beach seine sets at that site; means represent the average of density values calculated for the individual beach seine sets.

Chinook Salmon. Chinook salmon were present from April through July or August, with peak densities in May and June (Figure 26). Densities declined to very low numbers by August, and no unmarked Chinook salmon were observed in September or October; however, low densities were present in November and December. Densities of unmarked Chinook salmon were generally highest at sites in Reaches B, C, and E, with particularly high densities at Bradwood Slough and Jackson Island in Reach C. Lowest densities were at Ilwaco Slough in Reach A and Campbell Slough in Reach F. Similar to unmarked Chinook salmon, marked Chinook salmon were most abundant in May and June. Low densities were observed in February, April, and July. No marked Chinook salmon were captured from August through December. Densities of marked Chinook salmon tended to be higher in reaches E-G.

Coho Salmon. Especially high densities of unmarked coho salmon were observed at Bradwood Slough and Jackson Island in Reach C, and at Hardy Slough and Pierce Island in Reach H (Figure 27). We observed a tendency for high densities of unmarked coho in Reaches G and H. Unmarked coho were present at low densities throughout the sampling season, although highest densities were observed in August and May. While low densities of marked coho were present during most of the sampling season, high densities were seen only in May (Figure 28). Highest densities of marked coho were observed at Washougal Wetland in Reach G and Pierce Island and Sand Island in Reach H.



Chum Salmon. The highest chum salmon density was found in Reach A at Ilwaco Slough in March and April (Figure 29).

Figure 26. Mean (\pm SE) density of unmarked Chinook salmon by month and reach. N indicates the total number of beach seine sets at that site; means represent the average of density values calculated for the individual beach seine sets.

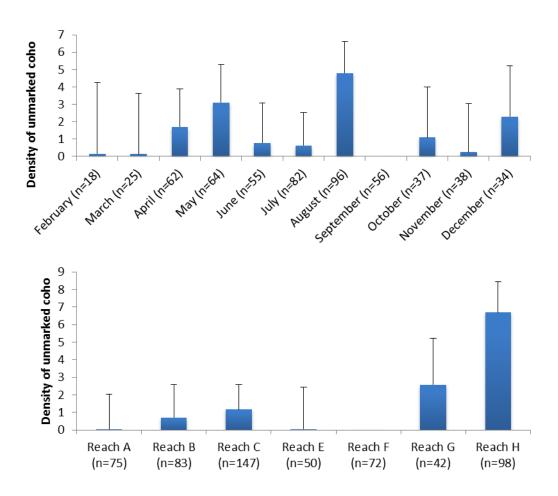


Figure 27. Mean (\pm SE) density of unmarked coho salmon by month and reach. N indicates the total number of beach seine sets at that site; means represent the average of density values calculated for the individual beach seine sets.

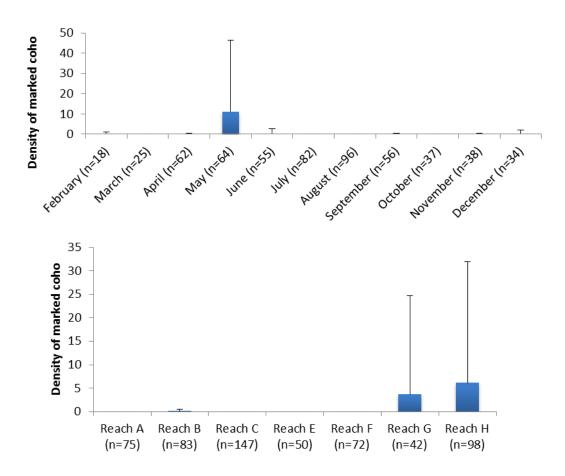


Figure 28. Mean (\pm SE) density of marked coho salmon by month and reach. N indicates the total number of beach seine sets at that site; means represent the average of density values calculated for the individual beach seine sets.

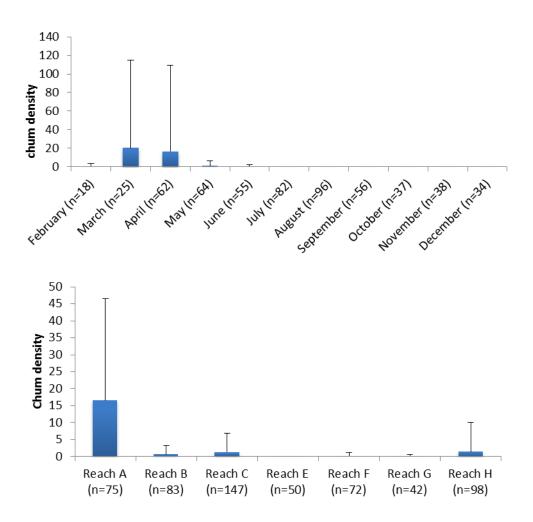


Figure 29. Mean (\pm SE) density of chum salmon by month and reach. N indicates the total number of beach seine sets at that site; means represent the average of density values calculated for the individual beach seine sets.

4.1.2.2 Chinook Salmon Stock Composition

Stock composition. Juvenile Chinook salmon from stocks originating throughout the Columbia River Basin were present at EMP sites (Figure 30). West Cascades fall Chinook salmon and Spring Creek Group Chinook salmon were the predominant stocks, with West Cascades fall Chinook salmon tending to be more prevalent in Reaches A-E, and Spring Creek Fall Chinook salmon becoming more common in Reaches F-H (Figure 31). Fish from a number of other stocks were also present, particularly Upper Columbia summer/fall Chinook salmon, but also a smaller number of Snake and Deschutes River fall Chinook salmon, Upper Willamette spring Chinook salmon, West Cascades spring Chinook salmon, and fish with genetic similarity to Rogue River Chinook salmon. While a variety of stocks were present in all reaches sampled, fish from stocks not part of the Lower Columbia River ESU (interior basin and Willamette River stocks) tended to be more prevalent at sites in Reach F and above. Stock composition also varied by sampling month (Figure 32). The proportions of West Cascades fall Chinook salmon and Spring Creek Group fall Chinook salmon remained fairly constant from February through June, but West Cascades fall tended to increase while Spring Creek Group fall decreased during the summer months. Upper Columbia summer/fall Chinook salmon also became more prominent in June and July. Interestingly, the small number of Chinook salmon caught in November and December were primarily Upper Willamette spring and West Cascade spring Chinook salmon.

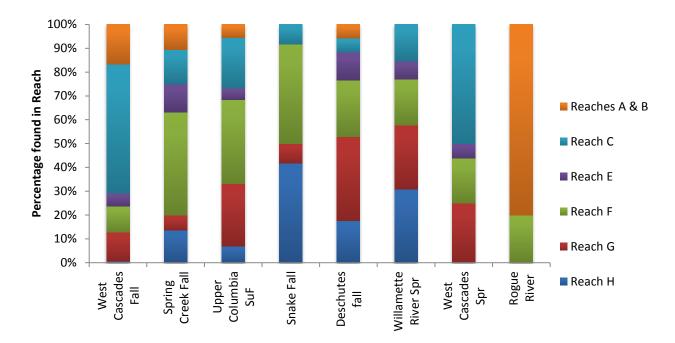


Figure 30. Percentages of Chinook salmon stocks by reach.

Stock diversity. Sites with higher stock diversity values included Lord/Walker Island, Campbell Slough, and Pierce Island; although, of these only Campbell Slough had a substantial number of sampling events. Patterns of stock diversity were most clearly represented by reach, with highest values in Reach F and lowest values in Reaches A-C (Figure 33).

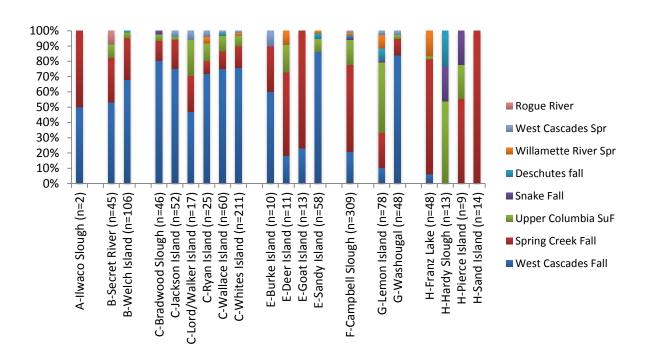


Figure 31. Percentages of Chinook salmon stocks by sampling site. N indicates the total number of beach seine sets at that site.

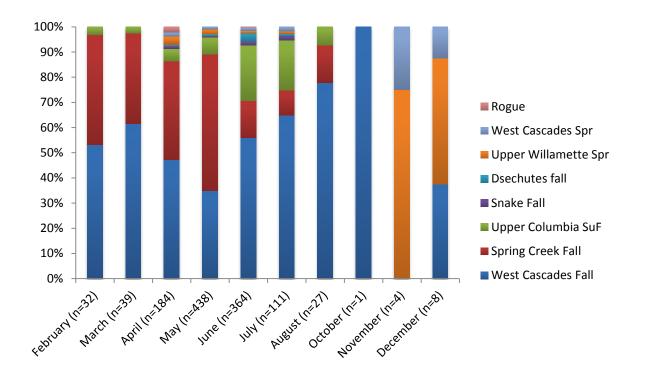


Figure 32. Percentages of stocks in unmarked and marked Chinook salmon by sampling month. N indicates the total number of beach seine sets in that month.

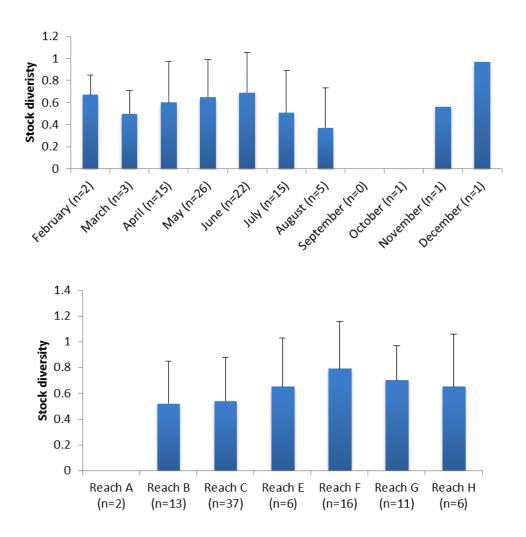


Figure 33. Mean (\pm SE) Chinook salmon stock diversity by month and reach. N indicates the total number of beach seine sets in that month or reach.

Percentage of non-Lower Columbia River ESU stocks. The percentages of non-LCR stocks (i.e., Willamette River basin and interior Columbia River basin stocks) were highest at Lemon Island in Reach G and Hardy Slough in Reach H, and no fish from non-LCR stocks were found at Ilwaco Slough, Goat Island, Burke Island, or Sand Island. The percentage of non-LCR stocks tended to increase with increasing distance upstream, and was highest in Reaches F-H, and low in reaches A-C (Figure 34). Numbers of non-lower Columbia River ESU stocks were too low to see clear temporal trends (Figure 35).

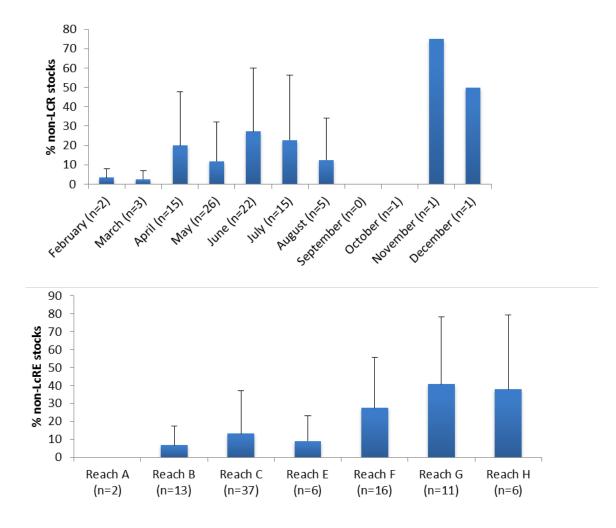


Figure 34. Mean (\pm SE) percentage of non-Lower Columbia River ESU stocks by month and reach. N indicates the total number of beach seine sets in that month or reach.

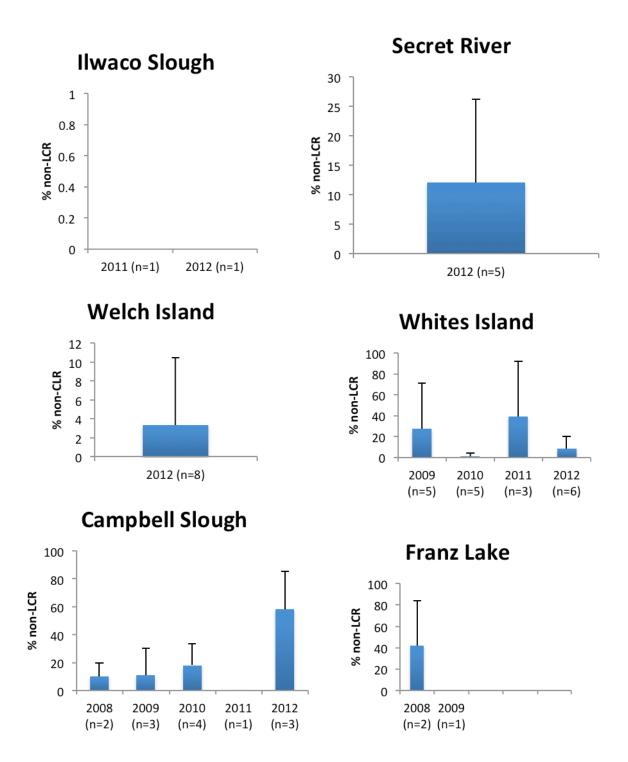


Figure 35. Mean (\pm SE) percentage of non-Lower Columbia River ESU stocks by year at EMP trend sites. N indicates the total number of beach seine sets in that year.

4.1.2.3 Size distribution, condition factor, and lipid content

Chinook salmon length. Marked vs. unmarked origin, month, site or reach, and stock all contributed to variability in Chinook salmon length. Marked fish overall were larger than unmarked fish, with an average length of 81 ± 11 mm as compared to 58 ± 15 mm for unmarked fish. The size range of marked fish was also relatively narrow, ranging from 60 to 150 mm, with 80% of fish between 70 and 90 mm in length. In contrast, unmarked fish ranged in size from 32 to 127 mm fork length, with 90% of fish being less than 80 mm in length. Among unmarked fish, there was a very clear increase in size from an average of 41 mm in February to 105 mm in December. This pattern was not found in marked fish (data not shown), whose mean length of marked fish varied little over the sampling season (p = 0.0898), with values within 79 – 86 mm range over the April to August sampling season when marked fish were present. The size of unmarked Chinook salmon also varied by site and reach. Average fish length was greatest at Lemon Island in Reach G and Campbell Slough in Reach F, and least at Franz Lake in Reach F, and Washougal Wetland in Reach G. By reach, fish of the largest average size were found in Reach F, and the smallest in Reaches B and H (Figure 36). The mean size of fish from most stocks was similar, with the exception of Willamette River spring Chinook salmon, whose length was significantly greater than other stocks (Figure 36). Some slight annual differences in length existed at trend sites between years (Figure 37).

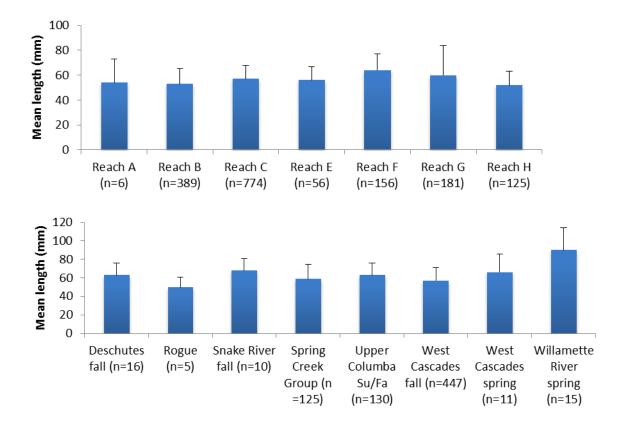


Figure 36. Mean length of unmarked Chinook salmon by reach and stock.

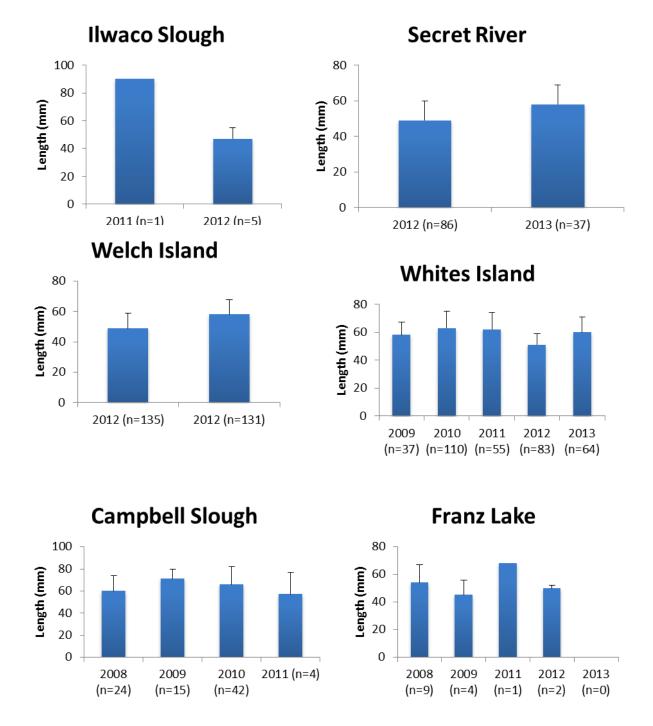


Figure 37. Mean length of unmarked Chinook salmon by year at EMP trend sites.

Condition factor of Chinook salmon. Among unmarked fish, condition factor (CF) increased steadily from February through August (Figure 38). Chinook salmon were not present in September or October, but in November and December CF values were lower when fish were present again (November and December CF values were comparable to March and April values). This pattern was less evident among marked fish,

which were present only from April through August and showed less variability in CF. Among reaches, highest values of CF for unmarked Chinook salmon were seen in Reaches H and F, and lowest values in A, B, and G. Among sites, highest values of CF were seen at Hardy Slough and Pierce Island in Reach H, Wallace Island West in Reach C, and lowest values at Washougal and Reed Island in Reach G, and Franz Lake in Reach H. Marked fish (data not shown) showed a somewhat different pattern, tending to have higher CF at sites in Reaches B and C. Figure 39 shows CF values for trend sites across sampling years.

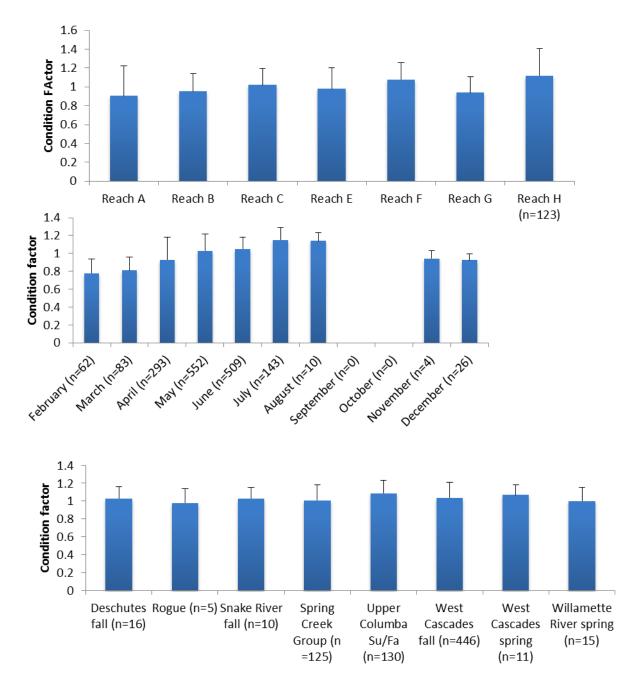


Figure 38. Condition factor of unmarked Chinook salmon by reach, month, and stock.

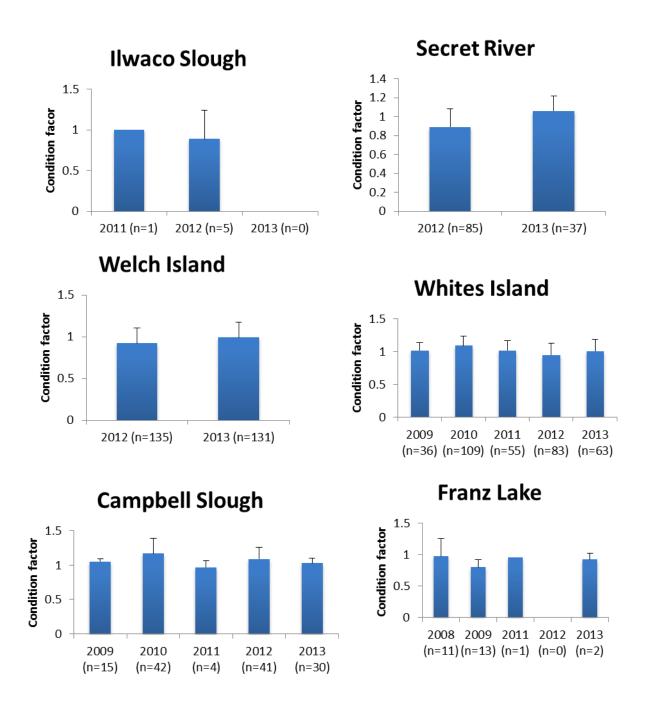


Figure 39. Condition factor of unmarked Chinook salmon by year at EMP trend sites.

Lipid content of Chinook salmon. Sources of variability for lipid content were more difficult to evaluate than for fish length or CF, because of the limited number of samples. At this point we have data only for 2007-2011, so there are no data yet for Reaches A, B, or G. Data are also lacking for fall and winter months, in part because too few Chinook salmon are collected at these times to provide sufficient tissue for lipid analyses. While overall the mean lipid content (% lipid) of marked and unmarked fish was very similar (marked, 1.59 ± 0.73 ; unmarked, 1.56 ± 0.56), the two groups of fish showed somewhat different spatial and seasonal patterns in lipid content (Figure 40). Among the marked fish, lipid content tended to be higher in samples from fish at sites in Reach H (i.e., Franz Lake and Sand Island) than fish from sites in other reaches. Additionally, lipid content of marked fish from Deer and Goat Islands in Reach E was relatively low. These patterns were much less apparent among unmarked fish. Similarly, among marked fish, lipid content was significantly higher in samples of marked fish collected in April and lower in samples collected in August, but seasonal patterns were less distinct among unmarked fish. Generally, lipid content did not vary greatly across stocks, and was similar in marked and unmarked fish of the same stock. Willamette River spring Chinook salmon were an exception to this trend, with substantially higher lipid levels in marked than in unmarked fish of this stock. Unmarked Deschutes River fall Chinook salmon also had relatively high lipid levels in comparison to other stocks, but this observation was based on one sample only. Figure 41 shows lipid content by year at each trend sites where Chinook salmon were collected.

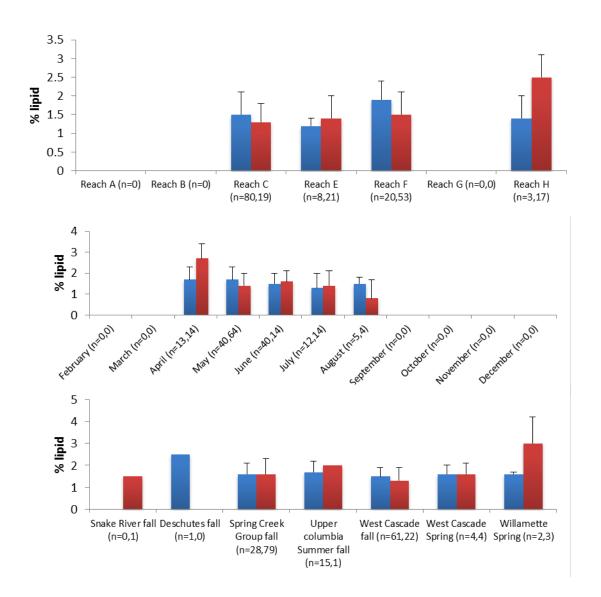
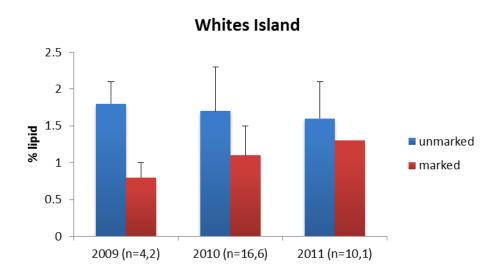
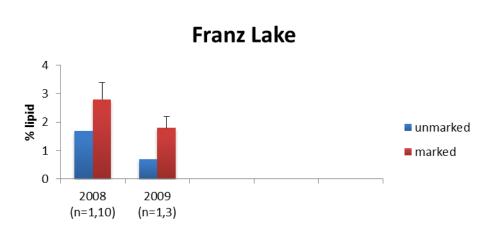
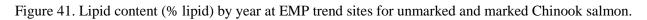


Figure 40. Lipid content (%) of unmarked and marked Chinook salmon by reach, month, and stock. Blue bars represent unmarked Chinook salmon and red bars represent marked Chinook salmon.



Campbell Slough 3 2.5 2 pidil 1.5 unmarked 1 marked 0.5 0 2007 2008 2009 2011 2010 (n=7,8) (n=1,9) (n=1,18) (n=10,11) (n=1,7)





4.1.2.4 Sources of Variability

On the whole, site and reach variables explained more variability than month for each of the fish metrics (see Appendix 4 for tables). Power analyses, in some cases, indicate that in spite of variability among beach seine sets and among sampling events, we have conducted sufficient sampling to establish the existence of seasonal and spatial trends in fish community and salmon occurrence parameters at the EMP sites. At newer trend sites, however, more sampling would be needed to determine whether significant temporal differences exist.

4.1.2.5 Descriptive statistics

Variability between months and reaches ranged widely (see Appendix 5 for tables). Coefficients of variation (CV) were often over 100% and in those instances the standard deviations were greater than the mean. Of note however, some of the means were so low (e.g., potential salmon predators) that the high CV represents a small difference in means. Additionally, the high variability in certain reaches may be due to the high variability between sites (see Sources of Variability section 4.1.2.4) within a reach.

4.1.3 Fish Prey

4.1.3.1 Prey Availability

The abundance of prey varied across sampling years by site, month, and habitat (Figure 42 to Figure 46). Exploratory analyses indicate "habitat" (emergent vegetation [EV] vs. open water [OW]) consistently explains more of the variation in the number of preferred prey (Diptera) captured during each sampling event (stepwise regression, "habitat" entered first, $R^2 = 0.49$; "site" explained an additional 9% and "month" an additional 1% of the variation). Differences between invertebrate densities found in the two habitat types were also quite clear in the paired EV and OW analyses (p < 0.0001 all invertebrates; p < 0.0001 for Diptera); however, there was little correlation between the abundance of preferred prey from EV and OW habitats collected during the same sampling event (n = 60 pairs; r = 0.28 for all invertebrates, r = 0.29 for Diptera, r = 0.24 for amphipods). These patterns indicate there is more variation in preferred prey within a site on a local scale (i.e., within the area surveyed by each beach seine) than there is systematically among sites or the times in which they are sampled.

For taxa less associated with the benthos (Cladocerans and Copepods), there was a higher correlation between densities found in the EV and OW habitats (for Cladocerans r = 0.55, for Copepods r = 0.44). Although densities of these pelagic taxa were generally at least 10x greater in the EV habitats than in the OW habitats, the higher correlation in densities of these taxa between habitat types within a site suggests conditions that affect their distributions may operate at the site level and/or there may be more movement of taxa between these habitat types.

The densities and biomass of invertebrates were consistently higher in the EV habitats compared to the OW habitats (Figure 42), and this was especially true for preferred prey items (see prey selection results below). Overall, densities in EVs were 20.3x those of OWs, and for Diptera, Amphipods and Hemiptera, densities in EVs were 17.4x, 5.2x, and 155.1x greater than those in OWs, respectively. Interestingly, even for taxa that are pelagic and typically abundant in open water habitats, densities were generally greater in EVs compared to OWs. For instance, for Copepods and Cladocerans, mean densities in EVs were 11.2x and 25.3x greater than in OWs, respectively. These comparisons are even more dramatic when considering biomass. Comparing wet weights of invertebrates caught in the 2012 and 2013 tows, invertebrate biomass was 105.6x greater per meter towed in the EV habitats than in OW habitats. For

Diptera, Amphipods and Hemiptera, the EV:OW ratios of biomass were 23.2:1, 13.7:1, and 252.8:1, respectively. As with densities, biomass of Copepods and Cladocerans was also greater in the EV habitats compared to the OW habitats (254.5x and 131.5x, respectively).

Generally, prey densities and prey biomass increased in the emergent vegetation habitat throughout the growing season (Figure 43, Figure 44, Figure 45), suggesting there may be a relationship with plant growth and invertebrate abundance. Indeed, the percent cover of live vegetation (grass as well as other vegetation) was significantly and positively correlated to the density of Diptera across emergent vegetation transects (p = 0.03). However, little variation in invertebrate density was explained by this measure of cover ($r^2 = 0.06$), and there were no other significant relationship with other measures of cover or for the total mean number of invertebrates. Using a stepwise regression for the densities of Diptera, adding "percent live vegetation" to the model after "month" and "site" did not improve the fit of the model ($\Delta AICc < 2$). Therefore, although there was a significant and positive relationship between Diptera abundance and percent live emergent vegetation, the large amount of scatter suggests the relationship is weak and other environmental factors with similar seasonal patterns, such as air temperature, phytoplankton biomass, etc. may play a role.

Although we sampled only two EMP sites in similar months over four years (Campbell Slough and Whites Island), it appears "year" and "month" are not as useful in explaining variation compared to "site". For the mean abundance of all invertebrates, there was no consistent difference among years or during the months that we sampled (Figure 46; ANOVA: site p = 0.03, year p = 0.95, month p = 0.69).

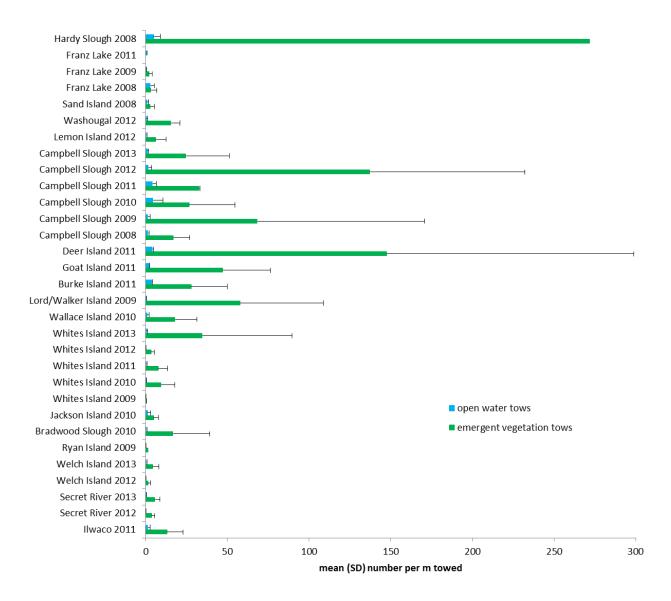


Figure 42. Mean (SD) number of invertebrates captured at each site by year. Note the extreme variation within and among sites and the difference between abundances captured in the emergent vegetation tows and the open water tows. (Also note, a second EV sample from Hardy Slough was not presented here; it had an estimated 1506 individuals per m towed).

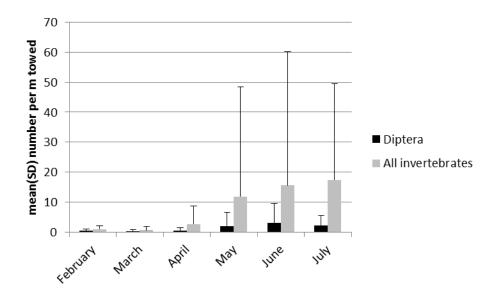


Figure 43. The mean (SD) number of all invertebrates and Diptera by month, for *all* collections from all sites samples during 2008-2013.

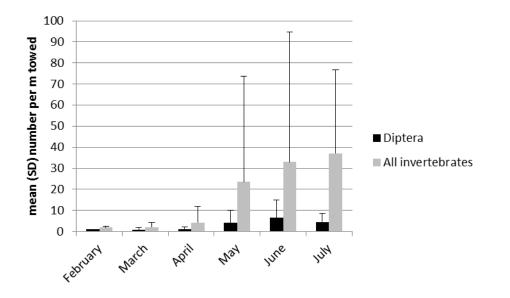


Figure 44. The mean (SD) number of all invertebrates and only Diptera by month, for only the *emergent vegetation* samples collected during 2008-2013.

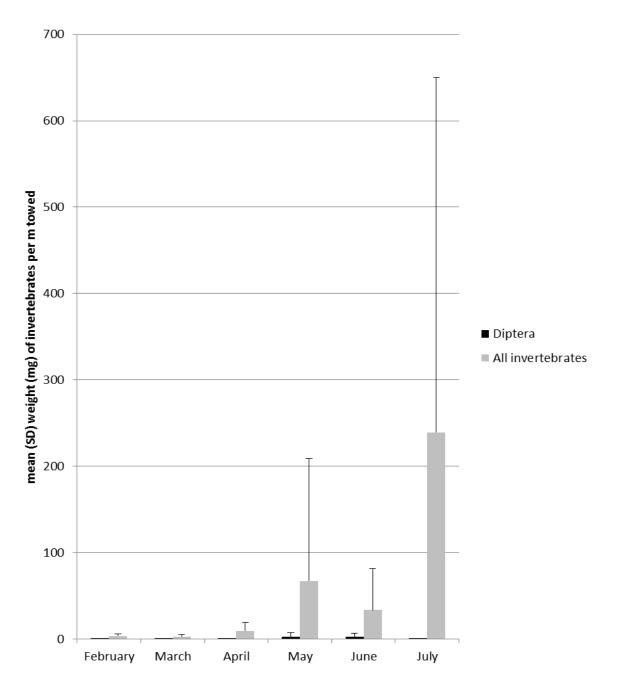


Figure 45. The mean (SD) wet weight of all invertebrates and only Diptera by month, for only the emergent vegetation samples collected during 2012 and 2013.

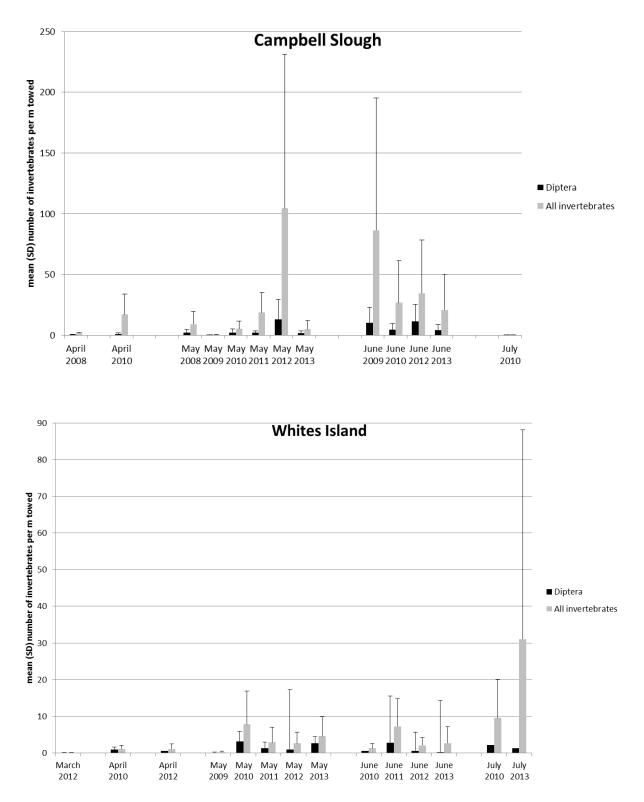


Figure 46. The mean (SD) number of invertebrates captured across both emergent vegetation and open water habitats during April-July from all years that were sampled at Campbell Slough and Whites Island. Note that tow samples were not collected at Campbell Slough in June 2011.

4.1.3.2 Chinook salmon diets and prey selectivity

The juvenile Chinook salmon stomachs contained on average 23.3 (SD 41.1) invertebrates and weighed on average 32.4 mg (SD 53.1, wet weight). Diptera were by far the most represented order in the diets (Table 8) at most sites (Figure 47). Diptera were on average 64.8% of all diet items by count (n_{total} invertebrates in stomachs = 24,048; $n_{total diptera in stomachs} = 15,585$), and 46.0% of the biomass in the stomachs at the time of collection (wet weight of all invertebrates in all stomachs = 33.44 g, wet weight of all diptera in all stomachs = 15.37g). On average, amphipods are the second most abundant taxa consumed (by weight and by count). The ranks of other taxa in the diets vary somewhat because of the differences in the relative sizes of the invertebrates (Table 8). When comparing the proportions of the diets by count and by weight, larger bodied invertebrates (e.g., Amphipods, Hemiptera, Trichoptera) made up a larger portion of the diets by weight than by count. Likewise, some smaller invertebrates (e.g., Cladocerans, Copepods, Nemata) made up a slightly larger portion of the diet by counts than by weight (Table 8). In addition, some of the differences in proportions between the weights and counts are due to the inclusion of "unknown" in the weights estimates. Invertebrates without intact heads are not included in the counts, but are included in biomass measures as "unknown". Despite the slight differences in the estimates based on counts compared weights, it was clear from both measures that Dipterans and Amphipods are among the most important prey items consumed.

The variation in diet composition is less than the variation seen in the tow samples, but the variation in the diets was often still quite high (Table 8, Figure 48). For example, even for abundant prey types such as Diptera and Amphipoda, the variation in the percent of the diets made up by those two taxa can be greater than the mean values themselves (Figure 48). Likewise, the composition of prey in the diets of fish caught at the same sites across multiple years (Figure 48) and months (Figure 49) suggests there can be a wide variety of prey consumed within and across the times sampled. Summary statistics and graphs suggest the abundance of Dipteran in the diets varies predominantly by site and then by month sampled. It appears very little of the variation in Diptera in the diets is explained by year. However, because the Diptera abundance and % Diptera cannot be transformed to meet the assumptions of most parametric statistical analyses, we cannot determine which factors are most useful in explaining differences in diets among sites, years and months sampled.

Table 8. Mean % (SD) of juvenile Chinook salmon diets by taxa, averaged over all fish captured between 2008-2013 (n = 1033). Invertebrates that could not be identified or lacked a head were not counted, but were likely included in the "unknown" composite when weighed. The percent of the total mass of the average stomach that was "unknown" was 8.19%.

	mean %			mean %		
	diets by	SD % diets	rank by	diets by	SD % diets	rank by
taxa	weight	by weight	weight	counts	by counts	count
Diptera	48.97	41.62	1	64.12	36.67	1
Amphipoda	16.74	33.06	2	12.52	26.47	2
unknow n	8.19	23.73	3			NA
Hemiptera	4.37	14.89	4	3.60	11.83	5
Trichoptera	2.82	12.80	5	1.53	6.97	6
Cladocera	2.68	13.33	6	5.37	20.21	3
Nemata	2.58	13.63	7	3.96	15.89	4
fish	1.44	11.56	8	1.19	9.74	7
Coleoptera	1.15	7.37	9	0.92	5.18	11
Hymenoptera	0.91	7.02	10	1.12	6.95	8
Ephemeroptera	0.88	6.29	11	0.80	5.65	12
Araneae	0.70	5.25	12	0.93	5.58	10
Odonata	0.62	6.08	13	0.34	3.88	16
Mysida	0.59	6.97	14	0.36	4.60	15
Oligochaeta	0.45	6.11	15	0.14	1.88	20
Collembola	0.29	2.63	16	0.70	4.63	13
lsopoda	0.29	2.70	17	0.25	2.31	17
Ostracoda	0.27	4.74	18	0.40	5.22	14
Megaloptera	0.23	3.50	19	0.04	0.70	25
Copepoda	0.20	1.81	20	1.00	8.21	9
Lepidoptera	0.17	2.80	21	0.12	0.98	21
Psocoptera	0.10	1.39	22	0.16	1.39	18
Plecoptera	0.06	1.64	23	0.05	0.75	24
Bivalvia	0.05	1.09	24	0.03	0.71	26
Acari	0.04	1.20	25	0.06	1.12	23
Chilopoda	0.03	1.08	26	0.11	3.26	22
Clitellata	0.02	0.80	27	0.00	0.12	28
Thysanoptera	0.01	0.24	28	0.15	2.37	19
Gastropoda	0.01	0.30	29	0.01	0.41	27

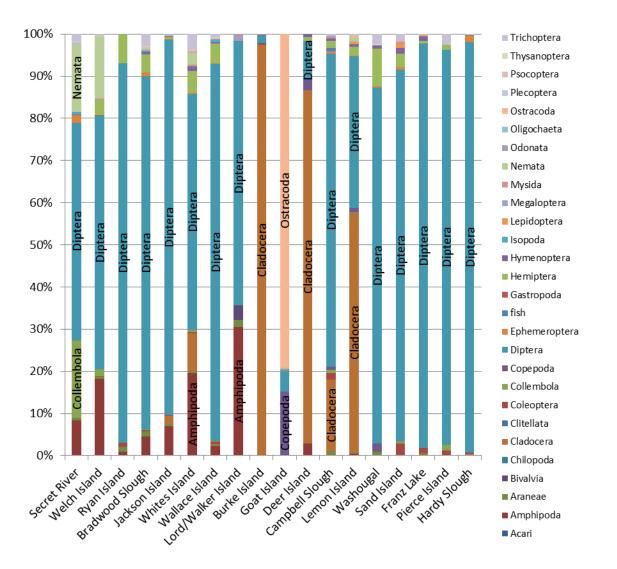


Figure 47. Mean composition of diets by site (using abundance values). Diptera and some other taxa are noted to orient readers.

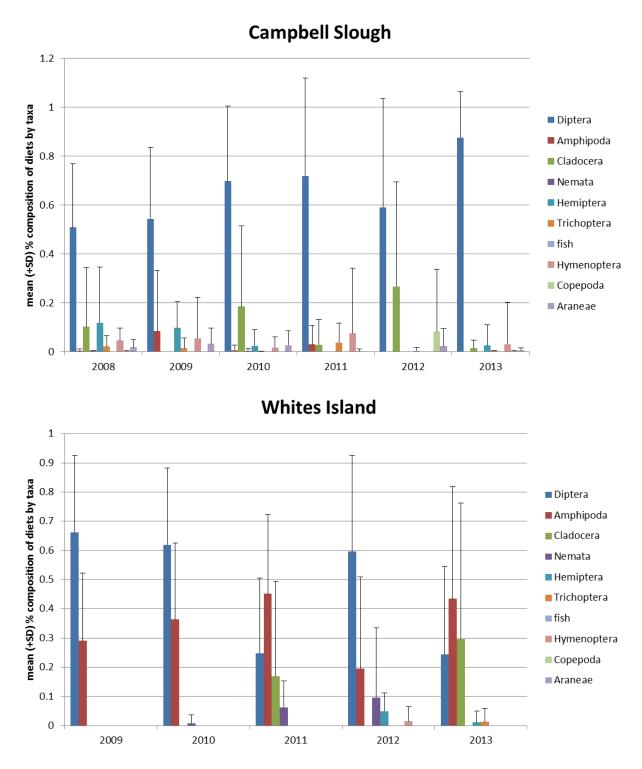


Figure 48. Mean (SD) percent composition (by abundance) of prey in juvenile Chinook salmon stomachs collected from Campbell Slough and Whites Island each year in May. The 10 orders of prey presented here include on average over 95% of the consumed invertebrate prey for all fish, not just fish from these sampling events. Because not all orders are shown, the bars may not sum to 100%.

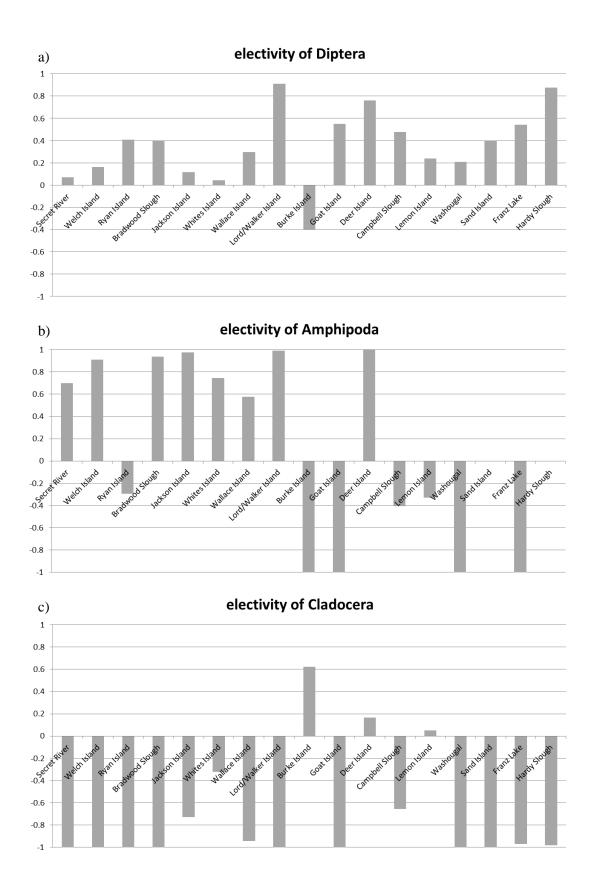
Welch Island 1.2 mean (+SD) % composition of diets by taxa 1 Diptera Amphipoda 0.8 Cladocera Nemata Hemiptera 0.6 Trichoptera fish 0.4 Hymenoptera Copepoda 0.2 Araneae 0 March June July Mav

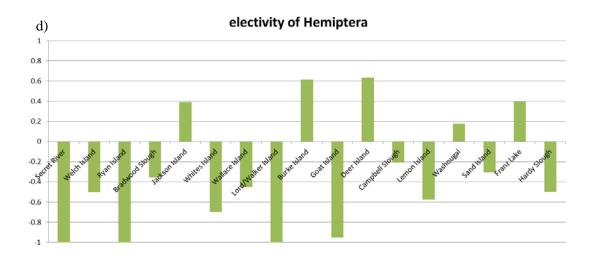
Figure 49. Mean (SD) percent composition (by abundance) of prey in juvenile Chinook salmon stomachs collected from Welch Island in each month sampled in 2013. The 10 orders of prey presented here include on average over 95% of the consumed invertebrate prey for all fish, not just fish from these sampling events. Because not all orders are shown, the bars may not sum to 100%.

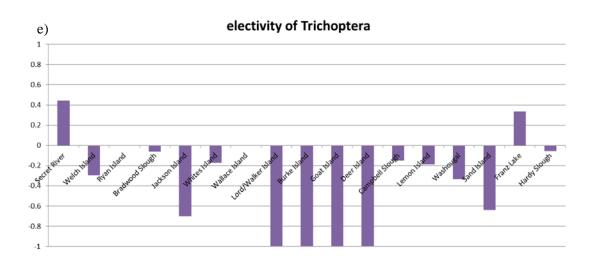
Electivity values indicate juvenile Chinook salmon consumed Amphipods and Dipterans at a rate higher than would be expected at some sites given the abundance of those taxa in the habitats sampled (Table 9, Figure 50). Amphipods were highly selected especially at sites in the lower reaches, but appear to have been avoided at sites upstream of Deer Island (Figure 50, Table 9). Hymenoptera were often rare but, when present, were selected at a high rate, especially at Whites and Wallace Islands (Table 9, Figure 50f). Other prey taxa were generally consumed at or below levels that would be expected given their abundances. The Ivlev's electivity value can appear skewed when abundances are low, and extreme values of -1 and 1 should be compared with actual counts. For example, if there were one Trichoptera larvae collected in a tow and none found in diets, the value would be -1. Likewise, if there had been 10,000 collected in tows and none in the diets, the value still would have been -1.

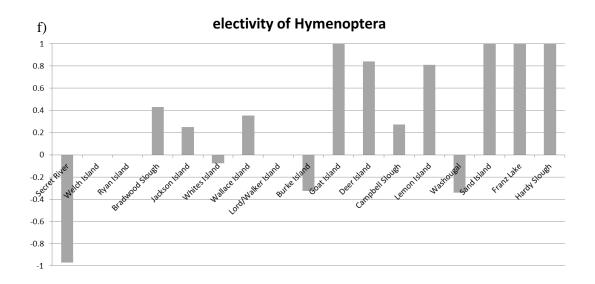
site	year	month	Diptera	Amphipoda	Cladocera	Hemiptera	Trichoptera	lymenoptera	Copepoda
		February	0.14	1.00		-1.00			
Secret River	2012	April	0.22	0.75		-1.00	0.94	-1.00	-1.00
		June	0.16	-0.13	-1.00	-1.00	-0.32	-0.88	
		May	-0.18	0.91	-1.00	-1.00		-1.00	-1.00
	2013	June	0.01	0.97		-1.00	0.72	-1.00	
		March	0.50	0.76	-1.00	1.00	0.72	1.00	1.00
		April	0.18	1.00	-1.00	-1.00			-1.00
	2012	May	-0.44	1.00	-1.00	-0.28	-1.00		-1.00
Welch Island –		· · ·			1.00				1.00
		June	0.40	0.62	-1.00	-0.51	-0.78		-1.00
		March	0.07	1.00	-1.00	-1.00	-0.03		-1.00
	2013	May	-0.07	0.89	-1.00	0.56	-1.00	-1.00	-1.00
		June	0.33	1.00		-0.88	0.03		
		July	0.31	1.00	-1.00	-0.41	1.00	1.00	-1.00
Ryan Island	2009	May	0.41	-0.29	-1.00	-1.00			
, Bradwood Slough		April	0.60	0.89		0.08			
	2040	May	0.23	1.00		-0.45	-0.95	1.00	
	2010	June	0.31	1.00	-1.00	-0.52		0.18	
		July	0.45	0.86	-1.00	-0.55	0.83	0.12	-1.00
			0.07	1.00	-0.48	-0.55	-1.00	0.12	-1.00
Jackson Island	2010	April				1.00		0.50	
	2010	May	0.06	0.95	-0.98	1.00	-0.11	-0.50	-1.00
		June	0.22			-0.22	-1.00	1.00	
	2009	May	0.51	0.63		-1.00			
		April	-0.06	1.00	0.91	-1.00			-1.00
	2010	May	0.21	0.97	-1.00	-1.00	-1.00	-1.00	-1.00
	2010	June	-0.09	0.97	-1.00	-1.00	0.45	-0.16	-1.00
		July	0.34	0.42	-1.00	-0.12	1.00	0.64	-1.00
		May	-0.20	0.57	0.57	-1.00	-1.00		-1.00
Whites Island	2011	June	-0.48	0.92	-1.00	-0.86	1.00	1.00	-1.00
			0.09	0.80	-1.00	-0.00		1.00	
		March				1.00	1.00	1.00	-1.00
	2012	April	-0.42	0.83	-0.30	-1.00	-1.00	-1.00	-1.00
		May	0.34	0.72		1.00	-1.00	1.00	-1.00
		June	0.45	0.48	-0.41	-0.74	-0.10		-1.00
	2013	May	-0.38	0.71	1.00	-0.69	0.11	-1.00	-1.00
	2015	June	0.27	0.66	-0.30	-0.98	1.00		-1.00
		April	0.31	0.90	-0.77	-1.00			-1.00
Wallace Island	2010	May	0.16	-0.17	-1.00	-0.64	-1.00	0.52	-1.00
		June	0.27	1.00	-1.00	-0.72			
		July	0.43	0.57	-1.00	0.55	1.00	0.19	-1.00
Land Attall and also al	2000							0.19	
Lord/Walker Island	2009	May	0.91	0.99	-1.00	-1.00	-1.00		-1.00
Burke Island	2011	May	-0.40	-1.00	0.62	0.61	-1.00	-0.32	-0.99
Goat Island	2011	May	0.55	-1.00	-1.00	-0.95	-1.00	1.00	-0.18
Deer Island	2011	May	0.76	1.00	0.16	0.63	-1.00	0.84	-0.72
Campbell Slough	2008	April	0.59		-0.32				-1.00
		May	0.38	-0.74	-0.20	0.56	0.92	1.00	-1.00
		May	0.16	0.30	-1.00	1.00	1.00	1.00	-1.00
	2009	June	0.54		-1.00	-0.55	-1.00	1.00	-1.00
		April	0.73		-1.00	0.27	1.00	1.00	-1.00
				0.70			0.02	0.52	
	2010	May	0.22	0.70	0.44	-0.73	-0.83	0.52	-1.00
		June	0.71	-1.00	-1.00	-0.50		1.00	-1.00
		July	0.34		-1.00	0.50		-1.00	-1.00
	2011	May	0.66	-0.12	-0.83	-1.00	0.85	0.96	-0.99
	2012	May	0.57	-1.00	-0.24	-1.00	-0.43	-1.00	-0.12
	2012	June	0.50		-1.00	-0.53	-1.00	0.27	-1.00
		May	0.31		-0.37	-0.31	-0.86	0.25	-0.53
	2013	June	0.51	-1.00	-1.00	-0.15	0.02	-1.00	-1.00
Lemon Island	1	March	0.46	1.00	2.00	-1.00	0.02	1.00	2.00
	2012	April	-0.09	-1.00	0.50	-0.29	-1.00		-0.73
		May	-0.20	0.35	0.58	-0.83	-0.56		-0.79
		June	0.79		-0.92	-0.17	1.00	0.81	-1.00
		April	0.36		-1.00	-1.00	-1.00	-0.65	0.55
	2012	May	0.10	-1.00	-1.00	0.79	-1.00	-1.00	-1.00
Washougal		June	0.17		-1.00	0.74	1.00	0.63	-1.00
Washougal						-0.31	-0.64	1.00	-1.00
	2008	April	0.40		-1.00	-0.51		1.00	
Washougal Sand Island	2008	April April		-1 00					
Sand Island		April	0.31	-1.00	-1.00	1.00	1.00	1.00	-1.00
	2008 2008			-1.00 -1.00					

Table 9. Ivlev's electivity values for Chinook salmon by site and by the month and year sampled.









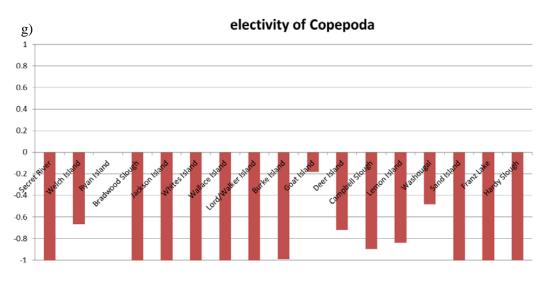


Figure 50. Ivlev's electivity values for juvenile Chinook salmon collected from sites sampled in 2008-2013.

4.1.1 Abiotic Conditions

4.1.1.1 Site Conditions

The four trend sites that were monitored for water quality typically had abiotic conditions that became less suitable for juvenile salmon during the course of the annual monitoring periods (April-July; see Appendix 6). The sites were typically cool and well oxygenated in April and May and became warm and less-oxygenated by July. Annual variation in Columbia River flows and weather had perceptible effects on abiotic conditions at the sites. High Columbia River flows in 2011 and 2012 had a larger impact on the two upstream sites (Franz Lake Slough and Campbell Slough) than at the downstream sites (Whites Island and Ilwaco), which experience stronger tidal flushing. The slower-flushing upstream sites had conditions that were more suitable for instream primary production, which affected pH and dissolved oxygen patterns. Among the sites, Whites Island consistently had the most suitable abiotic conditions. This site is close to the mainstem of the Columbia River, does not receive water from other water bodies, and experiences moderate tidal flushing.

4.1.1.2 Mainstem Conditions

4.1.1.2.1 River Mile 53 (Beaver Army Terminal, Reach C)

At the mainstem site at RM-53 where an in situ sensor platform provides continuous long-term data, the number of days showing temperatures that exceed the threshold of 19°C is given for the years 2009, 2010, 2012, and 2013 (Bottom et al. 2011, Table 10). During high water years in 2010 and 2012, the number of days exceeding this threshold was smaller than for the years 2009 and 2013, which were similar to each other.

The mainstem sensor data at RM-53 (Figure 51) clearly show that river discharge in 2013 had a relatively large winter flux compared to the previous three years and the discharge associated with the spring freshet

did not reach levels as high as 2011 or 2012. Chlorophyll *a* levels during the spring bloom reached values larger than in the two previous years and comparable to the high values measured in 2010. Summer chlorophyll *a* levels were similar to previous years in that there was less chlorophyll *a* overall than during spring, before the freshet. Turbidity, colored dissolved organic matter (CDOM) and nitrate displayed seasonal variability typical of high winter values associated with episodic events and low summer values associated with lower discharge, although inter-annual variability is evident. Temperature displayed seasonal variability consistent with previous years, including low temperatures during winter and high temperatures during summer. The number of days during 2013 that the mainstem river was above various thresholds for habitat quality are shown in Table 10. During 2013, there were more days above 19°C than in 2010 or 2012, but not 2009.

Table 10. Number of days with temperatures >19°C at River Mile 53 (Beaver Army Terminal near Quincy, OR).

Temperature Range	2009	2010	2012	2013
Range 19-21 °C	70	49	53	67
Above $> 21^{\circ}$ C	11	2	2	14
Total > 19°C	82	51	55	81

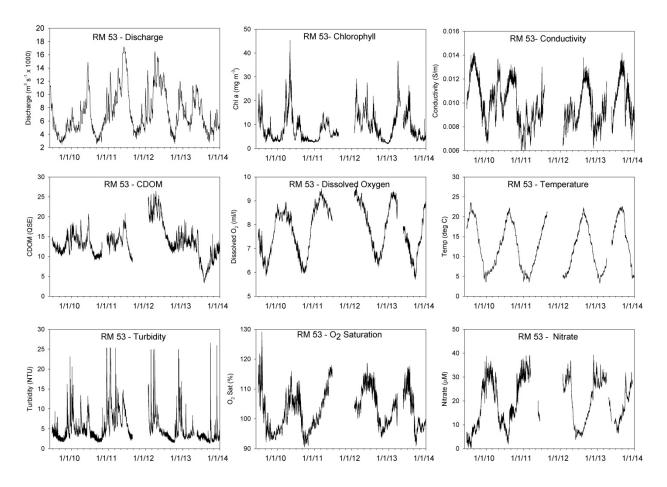


Figure 51. Time series of biogeochemical parameters measured hourly at RM-53 (Beaver Army Terminal near Quincy, OR) for the period July 2009 – December 2013.

4.1.1.2.2 River Mile 122 (Port of Camas-Washougal, Reach G)

The data for all measured parameters at RM-122 and river discharge are shown in Figure 52. River discharge was highest during the freshet, with winter fluxes lower than those measured at RM-53. Chlorophyll *a* levels reached 40 μ g L⁻¹ during the spring bloom and were lowest during winter months. CDOM levels were highest during the onset of the spring freshet and lowest during summer months. Dissolved oxygen changes were driven by temperature at the seasonal scale, and by biological productivity and possible super-saturation from spillage over dams at shorter time scales. Dissolved oxygen was never lower than 6 ml L⁻¹. Turbidity and nitrate displayed a seasonal cycle similar to RM-53, with highest concentrations measured during winter with lowest concentrations measured during summer.

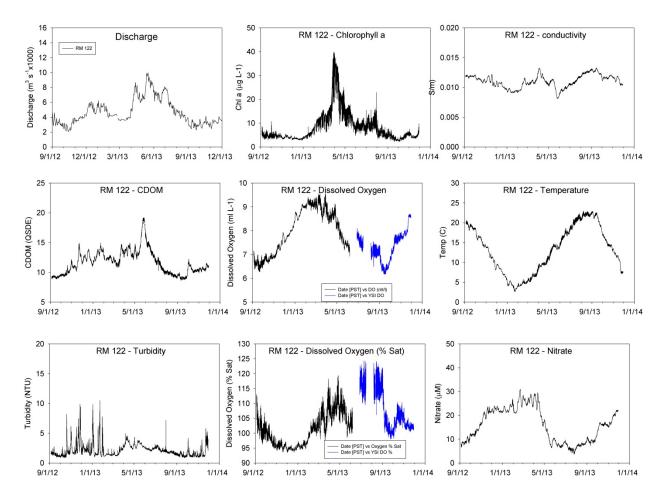
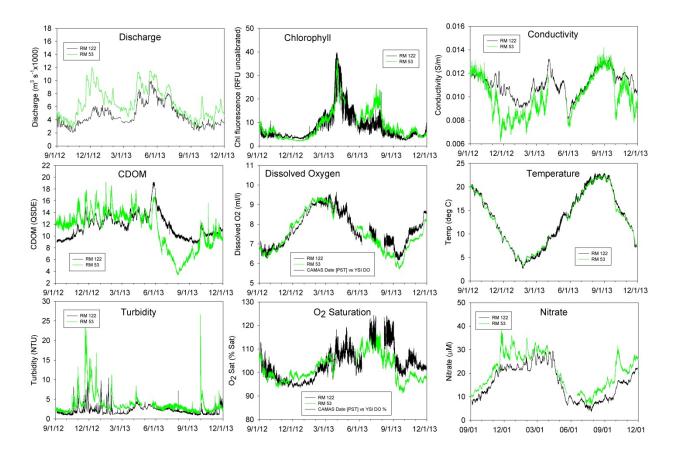


Figure 52. Time series of biogeochemical parameters measured hourly at RM-122 for the period September 2012 – December 2013.

4.1.1.2.3 Comparison of RM-122 to RM-53 (influence of the Willamette River on Columbia River biogeochemistry)

A comparison of biogeochemical parameters from the two sites (Figure 53) shows that river discharge was higher at RM-53 at all times as a result of tributary inputs downstream of RM-122, including the Willamette River. During winter months, the high discharge measured at RM-53 was not observed at Bonneville Dam. During freshet, discharge was high at both locations indicating the large flux from the mainstem Columbia River. During summer, discharge was nearly equal for several months indicating the low contribution from tributaries during this time. Chlorophyll *a* levels were very similar at both sites, including during the spring bloom. One exception was during summer, when RM-53 showed higher levels during August. Conductivity was comparable at both locations; however, during the winter, conductivity was lower at RM-53, correlating with the higher discharge observed during this period. CDOM levels were higher at RM-53 during winter and lower following the spring freshet. Dissolved oxygen and temperature showed very similar trends at the seasonal scale, and the measurements were very similar in magnitude throughout the year. Turbidity and nitrate tended to be higher at RM-53,



especially during the winter period when discharge was high; however, in summer both metrics had similar variability and similar concentrations.

Figure 53. Comparison of RM53 and RM122 for the time period September 2012 – December 2013.

Nitrate flux was higher at RM-53 during all times of the year (Figure 54), consistent with high turbidity and higher river discharge. The highest nitrate fluxes were observed between December – February when discharge was high and associated with regional rainfall events. A similar pattern is observed for turbidity and CDOM during the winter period. However in spring and summer the turbidity and CDOM fluxes are more similar between the two sites, especially after the spring freshet.

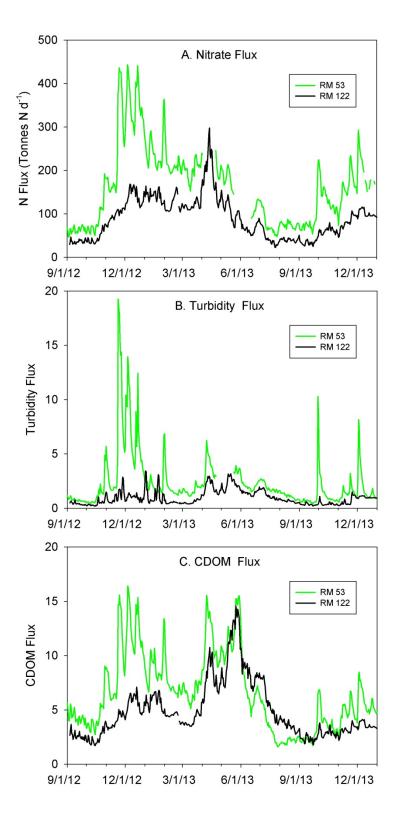


Figure 54. Flux calculations for nitrate, turbidity, and CDOM at RM-53 and at RM-122 calculated from sensor measurements and river discharge.

Phosphate ranged from below detection $(0.05 \ \mu\text{M})$ in winter to approximately 0.4 μM in late summer (Figure 55). Based on these concentrations, the N:P ratio can be calculated for the nitrate and phosphate measurements. Phytoplankton typically require an N:P ratio of 16 for nutritional requirements, therefore nearly all year long phytoplankton are potentially limited in growth by phosphate concentration.

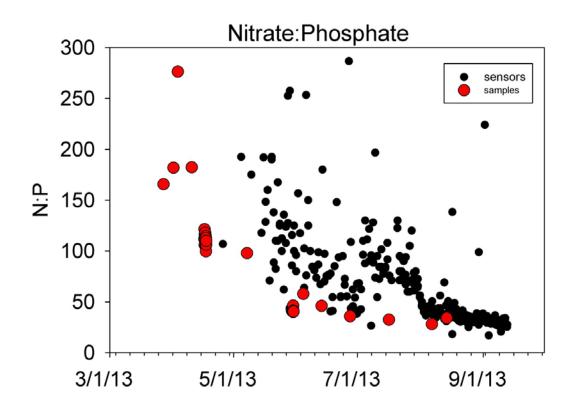


Figure 55. N:P at RM-122 using measurements made by grab samples and from in situ sensors.

4.2 Food Web Synthesis

4.2.1 Net Productivity (growth rate of phytoplankton and periphyton and plant biomass)

4.2.1.1 Temporal Patterns

4.2.1.1.1 Primary Production

4.2.1.1.1.1 Quantity

4.2.1.1.1.1.1 Pelagic fraction

The amounts of primary production associated with pelagic primary producers (phytoplankton) and attached microalgae (periphyton) were estimated from chlorophyll *a* concentrations determined spectrophotometrically (phytoplankton and periphyton) as well as from cell counts (phytoplankton). Other contributors to system primary production include submerged and emergent plants, which were not

evaluated in this section. When all the data were considered together, chlorophyll *a* concentration associated with the phytoplankton fraction of the primary producers was highly variable across sites, particularly in April. A three-way analysis of variance (ANOVA) on Site, Year, and Month of sampling revealed that there were no statistical differences in chlorophyll *a* among sites, among years, or among months sampled (p = 0.436 for site; p = 0.131 for year, and p = 0.620 for month). However, overall variability in the biomass estimated by chlorophyll *a* concentration was highest in April (Figure 56). The lack of differences could be partially attributable to sample aliasing (i.e., monthly averaging damps out real differences that might exist in the data).

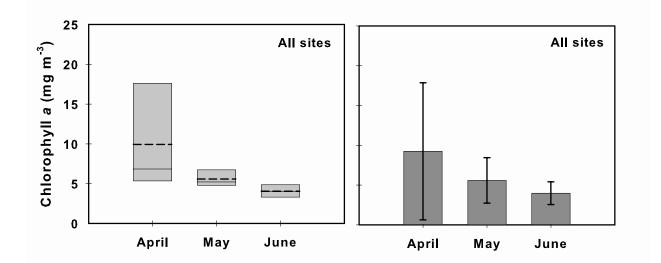


Figure 56. Variability in chlorophyll *a* concentration (in mg m⁻³) in phytoplankton in the LCRE between 2011 and 2013 at four fixed sites: Ilwaco (Reach A), Whites Island (Reach C), Campbell Slough (Reach F), and Franz Lake Slough (Reach H). Values obtained for each of the months of April, May, and June after averaging across space (all sites included) and time (all years included) are shown. *Left*: box plots showing the range, median (thin line), and mean (bold dotted line) values for observations collected in each of the three months. *Right*: averages and standard deviations for the same data.

At Ilwaco, levels of chlorophyll *a* associated with phytoplankton tended to be low, with the exception of one observation in April 2011, which skewed the mean (Figure 57). Without the one high value, the average chlorophyll *a* concentration across all time points was 3.8 ± 1.2 mg chl m⁻³. When comparing median values across the three years of observation, there was little difference in chlorophyll *a* concentration, with the overall median concentration found to be 4.2 mg chl m⁻³. Chlorophyll *a* concentrations showed similar median values across years at Whites Island, with greater variability observed in 2012 compared to 2011 and 2013. At Campbell Slough the variability in the data were similar in 2012 and 2013 (with too few observations in 2011 to produce a meaningful assessment), with slightly lower mean and median values observed in 2013 compared to 2012 (Figure 57).

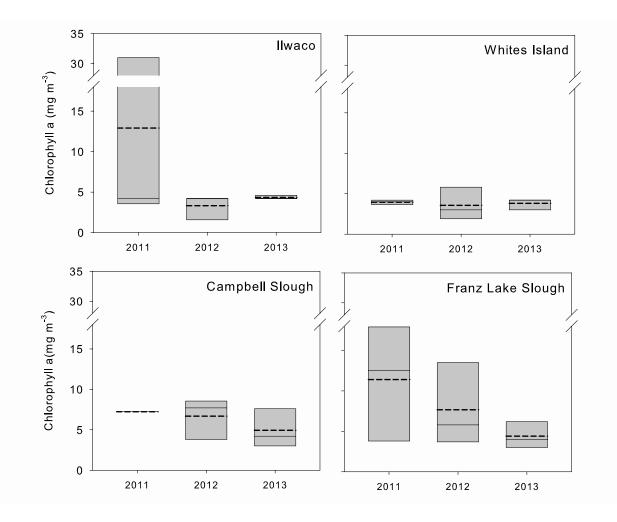


Figure 57. Within-site variability between 2011 and 2013 of phytoplankton chlorophyll *a* concentration (mg m⁻³) for Ilwaco (Reach A), Whites Island (Reach C), Campbell Slough (Reach F), and Franz Lake Slough (Reach H). Box plots show the range, median (thin line), and mean (bold dotted line) values for observations.

Diatom variability was greatest in April across all sites and all years (Figure 58). There was no significant difference between the abundance of diatoms, the dominant phytoplankton class, at Ilwaco between 2011 and 2012 (p < 0.001; Two-Way ANOVA). In both cases, abundances of phytoplankton (diatoms and total phytoplankton) were lower at Ilwaco than at the other EMP sites (Figure 59). The total abundance of diatoms at the other sites was higher in 2011 compared to 2012 (p < 0.001; Figure 59). However, the small number of observations at Campbell Slough in 2011 precludes any determination of temporal differences between years.

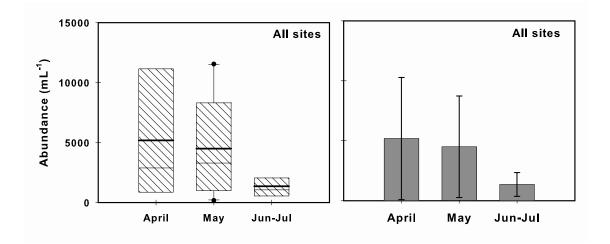


Figure 58. Variability in diatom (Class Bacillariophyceae) abundances (cells mL⁻¹) in the LCRE in 2011-2012 at EMP fixed sites. Values shown represent those obtained for each of the months of April, May, and June after averaging across space (all sites included) and time (all years included). *Left*: box plots showing the range, median (thin line), and mean (bold line) values for observations collected in each of the three months. *Right*: averages and standard deviations for the same data.

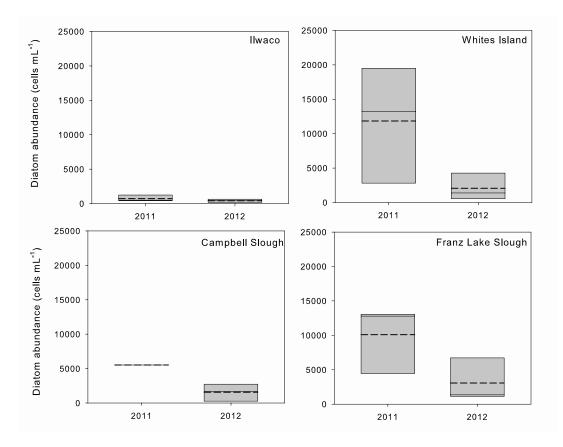


Figure 59. Variability in diatom abundances (cells mL⁻¹) in the LCRE in 2011-2013 at four EMP fixed sites. Values shown in the box plots represent those obtained from each of the months of April, May, and June in 2011, 2012, and 2013. Shown are the range (box), median (thin line), and mean (bold line) values.

There was a significant negative relationship (r = -0.83, p = 0.043) between river discharge at Bonneville Dam and the abundance of diatoms at Franz Lake Slough and Campbell Slough in 2012 (Figure 60). A weak positive relationship was observed between the abundance of diatoms at Campbell Slough and discharge from the Willamette River, but it was not significant (data not shown). Additional data would help to determine if this relationship might in fact be real.

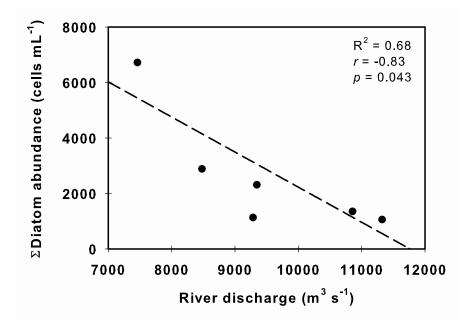


Figure 60. The sum of abundances of the observed diatom taxa plotted against Columbia River discharge $(m^3 s^{-1})$ reported at Bonneville Dam during the year 2012. Only abundances from Campbell Slough (Reach F) and Franz Lake (Reach H) were included in this analysis. A Pearson Product Moment Correlation analysis revealed that the negative relationship (r = -0.83) was significant (p = 0.043) at these two sites. Significant relationships between Bonneville discharge and phytoplankton abundance were not found at the other sites examined.

4.2.1.1.1.1.2 Periphyton

Periphyton abundance (from chlorophyll *a* concentrations) was determined at four fixed EMP sites in 2012 (Ilwaco, Whites Island, Campbell Slough, and Franz Lake Slough, Figure 61).

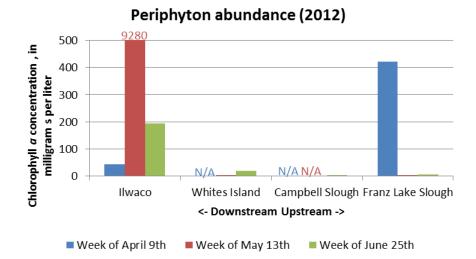


Figure 61. Periphyton abundance measured as chlorophyll *a* concentration at all four water-quality monitoring sites in 2012 [N/A, no sample collected].

4.2.1.1.1.1.3 Vegetation

The above ground biomass estimates for the emergent wetland vegetation found in the low and high marsh strata are provided in Table 11 and in the SAV strata in Table 12. Temporal trends are difficult to discern because of limited sampling over the three year time period (2011 – 2013). Comparisons between all three years are limited to three sites for the high marsh strata, one site for the low marsh strata, and two sites for the SAV strata. These limited data can be used to make broad generalizations that should be interpreted with caution given the limited number of comparisons. Within the high marsh strata, summer biomass was the lowest in 2011, except at the Whites Island site, where it was lowest in 2012. The low marsh strata have too few observations among sites and between years to be meaningful. The results from the two sites with samples from the SAV strata in all three years indicate a decline over the 3-year period, however two other sites measured in the last two years increased over that time period.

Table 11. Above ground biomass of emergent wetland vegetation from high marsh and low marsh strata. The difference in biomass for each year is the summer biomass minus the winter biomass in g/m^2 and is an estimate of the amount of biomass contributed to the system each year. Sites are ordered by distance from the mouth of the river.

						В	iomas	s Dry Wt. (g/	m ²)				
		S	ummer 2011		Winter 2012	Difference	Su	mmer 2012	W	inter 2013	Difference	Sı	ımmer 2013
Site	Strata	n	Avg Dry Wt. (SD)	n	Avg Dry Wt. (SD)		n	Avg Dry Wt. (SD)	n	Avg Dry Wt. (SD)		n	Avg Dry Wt. (SD)
Ilwaco (BBM)	high marsh	7	976.2 (420.8)	7	384.7 (133.4)	591.5	10	1175.0 (256.7)	10	254.0 (135.4)	921.0	10	1140.9 (429.4)
Secret River (SRM)	high marsh						5	1443.3 (148.2)	5	194.5 (209.5)	1248.5	9	1061.7 (385.8)
Welch Island (WI2)	high marsh						5	1141.5 (322.5)	9	272.2 (122.2)	869.3	9	1360.9 (647.0)
Whites Island (WHC)	high marsh	6	1152.3 (844.4)	5	517.1 (326.9)	635.2	8	739.2 (623.4)	8	346.4 (257.6)	392.8	9	1358.7 (833.5)
Campbell Slough (CS1)	high marsh	3	410.1 (356.0)	4	100.8 (63.9)	309.3						6	433.8 (66.6)
Franz Lake (FLM)	high marsh	8	203.2 (151.9)	12	245.4 (114.3)	-42.2	7	671.5 (557.3)	5	104.5 (106.7)	567.0	9	433.7 (317.4)
Ilwaco (BBM)	low marsh	1	24.2 (NA)										i
Secret River (SRM)	low marsh						5	265.1 (71.4)	5	15.2 (14.9)	249.9	9	175.0 (124.2)
Welch Island (WI2)	low marsh						4	401.3 (362.2)					
Whites Island (WHC)	low marsh	2	87.7 (88.8)	3	5.6 (6.4)	79.0	3	114.0 (101.8)	3	10.0 (14.6)	104.0	6	162.7 (126.2)
Campbell Slough (CS1)	low marsh	5	277.6 (150.9)	4	3.3 (4.5)	274.3						11	56.3 (37.5)
Franz Lake (FLM)	low marsh		``´´	1	66.2 (NA)				2	30.5 (23.7)			

SD = Standard Deviation

NA = Not Applicable

Table 12. Above ground biomass of submerged aquatic vegetation (SAV). The difference in biomass for each year is the summer biomass minus the winter biomass in g/m^2 and is an estimate of the amount of biomass contributed to the system each year. Sites are ordered by distance from the mouth of the river.

		Biomass Dry Wt. (g/m ²)											
		S	Summer 2011		Winter 2012	Difference		Summer 2012		Winter 2013	Difference	S	Summer 2013
Site	Strata	n	Avg Dry Wt. (SD)	n	Avg Dry Wt. (SD)		n	Avg Dry Wt. (SD)	n	Avg Dry Wt. (SD)		n	Avg Dry Wt. (SD)
Ilwaco (BBM)	SAV	4	81.8 (91.3)	4	0.0 (0.0)	81.8	6	28.5 (38.1)	6	0.1 (0.1)	28.4	6	14.5 (30.1)
Secret River (SRM)	SAV						6	30.1 (11.8)	6	2.4 (5.1)	27.7	6	94.4 (77.2)
Welch Island (WI2)	SAV						4	97.7 (62.2)	4	6.2 (4.2)	91.5	6	173.2 (197.6)
Whites Island (WHC)	SAV	8	49.3 (65.0)	8	0.4 (0.5)	48.9	6	35.8 (75.8)	6	0.3 (0.6)	35.5	6	11.2 (19.6)
Campbell Slough (CS1)	SAV	8	0.4 (0.8)	8	0.0 (0.0)	0.4						6	9.3 (22.8)
Franz Lake (FLM)	SAV						6	4.0 (9.7)	6	0.0 (0.0)	4.0	6	0.2 (0.4)

4.2.1.1.1.2 Productivity Rates

4.2.1.1.1.2.1 <u>Pelagic primary production (phytoplankton)</u>

Rates of primary production were determined for two components of the primary producers: fluvial (=pelagic) phytoplankton and periphyton (=phytobenthos, or a complex mixture of autotrophic and heterotrophic microbes and detritus attached to a submerged substrate). In both cases, tracers of carbon uptake were used: in 2011, the radioisotope ¹⁴C was used (as NaH¹⁴CO3), while in 2012 and 2013, the stable isotope, ¹³C, was used (as NaH¹³CO3). Daily rates of net carbon fixation (net primary production) were estimated from 2-4 h incubations of samples held in situ at a depth just below the water surface.

Through investigations carried out as part of the EMP, several nutrient addition experiments were performed to assess the degree to which pelagic primary production might be limited by low levels of one or more dissolved nutrient components. During a period of sunny conditions in summer 2012, orthophosphate addition experiments were performed on bottle samples collected from the Port of Camas-Washougal (RM-122). The results clearly show that the addition of dissolved ortho-phosphate stimulated the growth of phytoplankton (Figure 62). Of the phytoplankton species that responded to the addition, diatoms dominated the enhanced biomass. Interestingly, the taxa that responded included several small centric diatoms and not the dominant taxa, which included *Asterionella formosa* and *Aulacoseira granulata*. This suggests that during certain times of year, species successions may be controlled by nutrient availability (Maier et al. unpublished data). This is important since the size of diatoms likely plays a key role in food web dynamics. Large diatoms are often considered to be 'inedible' because when they are present in colonies, they are difficult to process for zooplankton herbivores. On the other hand, small taxa are readily grazed upon and therefore create a tighter coupling between growth and grazing at the base of aquatic food webs.

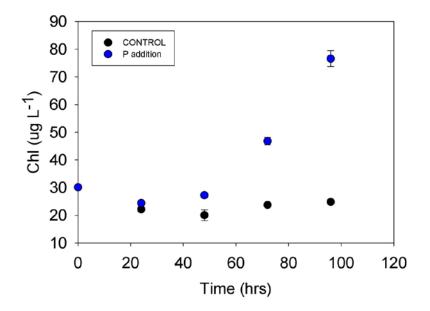


Figure 62. Phosphorus addition grow-out experiment conducted at RM-122.

4.2.1.1.1.2.2 Periphyton productivity

While significant spatial differences in primary productivity associated with periphyton were observed in this study (Two-Way ANOVA, p = 0.003; see section below), with ambient periphyton productivity increasing at sites moving downstream in 2011-2013, no significant temporal differences were found (Two-Way ANOVA, p = 0.975; Figure 63). Seasonal patterns in periphyton productivity varied across sites and years. Intra-annual differences in periphyton production were not significant between 2011 and 2012 (Figure 64).

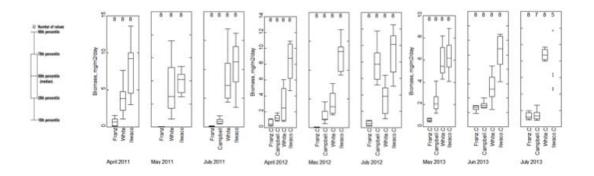


Figure 63. Boxplot summaries of periphyton production rates at four fixed sites 2011-2013. Biomass production is measured in milligrams of chlorophyll *a* per square meter, per day.

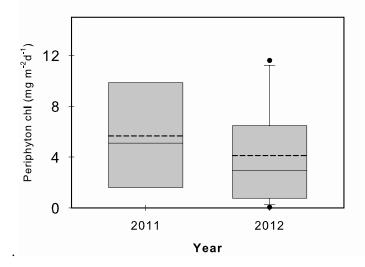


Figure 64. Periphyton primary productivity in 2011 and 2012 (all sites combined). The median is shown as a horizontal solid line while the mean is indicated as a dashed and bold line. Outliers are indicated by the circles above and below error bars. Intra-annual differences between 2011 and 2012 were not significant.

4.2.1.1.1.2.3 Net ecosystem metabolism

Oxygen is produced through the process of photosynthesis and it is removed through aerobic respiration. High-resolution measurements of dissolved oxygen can therefore provide insights into the balance between production and consumption of organic matter. The total amount of organic matter produced through primary productivity is termed Gross Primary Production (GPP), which is the sum of changes in dissolved oxygen concentration observed during daylight hours. Respiration is calculated from the sum of changes in oxygen occurring during darkness when oxygen is no longer produced. The difference between the two is termed net ecosystem metabolism or NEM.

Dissolved oxygen concentrations are determined hourly at two sites in the mainstem, RM-53 in Reach C and at RM-122 in Reach G. The data show temporal changes in both gross primary productivity and respiration (Figure 65), with a larger range in values associated with GPP. The system can be thought of as net autotrophic (photosynthesis exceeds respiration) during the spring and summer months, while in the winter the system is net heterotrophic (Figure 65).

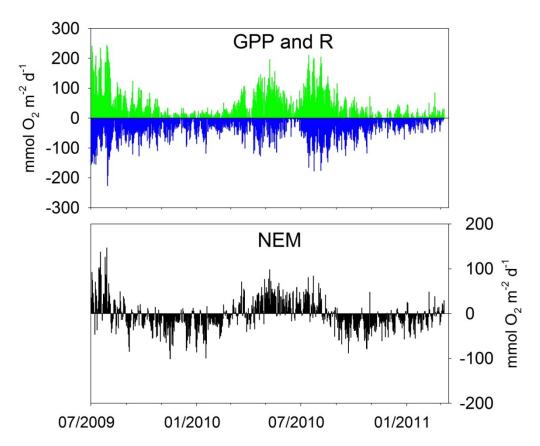


Figure 65. Daily calculated Gross Primary Productivity (GPP), aerobic cellular respiration (R), and net ecosystem metabolism (NEM) calculated at RM-53 for the two-year period July 2009- June 2011. Parameters were determined from hourly oxygen measurements, wind speed, and water velocity.

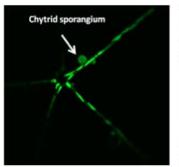
4.2.1.1.1.2.4 Annual Detrital Contribution

Summer peak biomass is an estimate of the annual primary production at the site (MacDonald 1984). When the remaining winter standing stock is subtracted from this value, an estimate of the potential annual detritus (PAD) contribution of detritus can be calculated. This value is presented in Table 11 and Table 12 as the difference between summer and winter biomass values. Since only two years of data are available at this time, limited temporal patterns are discernable regarding the PAD contribution of the marshes. At the Ilwaco (rkm 6) and Franz Lake (rkm 221) sites, PAD from the high marsh strata increased between years, while at the Whites Island (rkm72) there was a decrease between years.

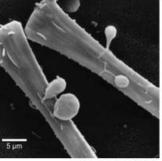
4.2.1.1.1.3 Species Composition

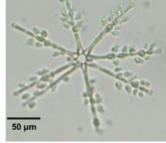
4.2.1.1.1.3.1 Pelagic phytoplankton

Results from this investigation clearly show that diatoms, Class Bacillariophyceae, dominate the phytoplankton assemblages at most sites throughout the year. Among the diatoms, repeatable succession patterns were observed between 2011 and 2012, with large, colonial diatoms dominating in the early months of spring, giving way to smaller, centric diatoms later in the spring and into the summer as well as green algae and flagellates. During this study we observed a remarkably high concentration of parasites of the dominant diatom species (Figure 66) present during spring blooms in the system (also indicated by elevated chlorophyll *a* concentrations in the mainstem time series). However, the percent contribution of diatoms was lower during the summer months compared to the spring and freshet time periods (Figure 67). In particular, the proportional contribution of green algae and flagellates was greatest during the summer at Campbell Slough and to some degree at Franz Lake Slough. In addition, there was a substantial contribution by cyanobacteria during the summer months, but due to funding constraints samples from these months were not quantitatively evaluated.



Columbia River A. formosa





Van Donk, 1983 Columbia

Columbia River A. formosa

Figure 66. Evidence for parasitism of *Asterionella formosa*, the dominant phytoplankton species during spring blooms in the Columbia River. *Left*, chitin-specific stain showing the attachment of a sporangium of the zoosporic fungal ("chytrid") species, *Zygorhizidium planktonicum* (confirmed through analysis of the DNA sequence of the Internal Transcribed Spacer 1 of the rRNA gene). *Middle*, Scanning Electron Microscope image of sporangium attachment in a similar infection reported from Western Europe. *Right*; Light microscope image showing heavily parasitized colony of *A. formosa*.

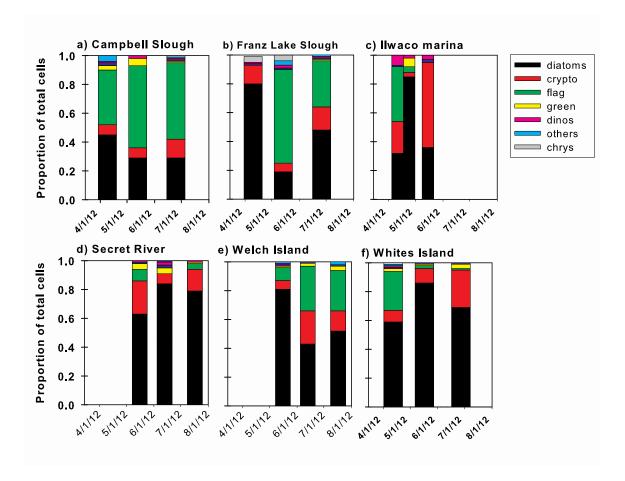


Figure 67. Time series of proportional abundances of phytoplankton taxa at the six EMP sites in 2012.

A time series of observations from the mainstem Columbia River at river mile 53 (Beaver Army Terminal) reveals successional patterns among the dominant diatom species (Figure 68). Repeatable spring blooms of the diatom, *Asterionella formosa*, a cosmopolitan colony-forming diatom are observed each year. Interestingly, we have discovered rampant parasitism of this diatom during bloom events (Maier and Peterson, submitted). The level of parasitism can reach up to ~55% of total *A. formosa* cells observed at Beaver Army Terminal (Figure 69) and calculations suggest that this lethal parasitism may divert between 15-20% of the organic carbon fixed in diatom biomass toward zooplankton or to pelagic microbes (Maier et al. 2012; Maier et al. 2013; Maier et al. in preparation).

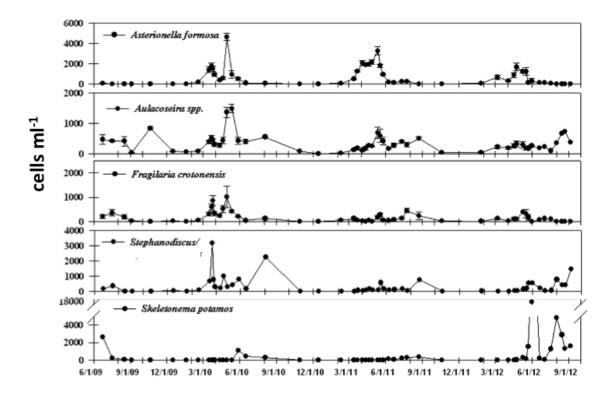


Figure 68. Time series of diatom (Class Bacillariophyceae) counts at River Mile 53 (Beaver Army Terminal near Quincy, OR) from June 2009 – September 2012.

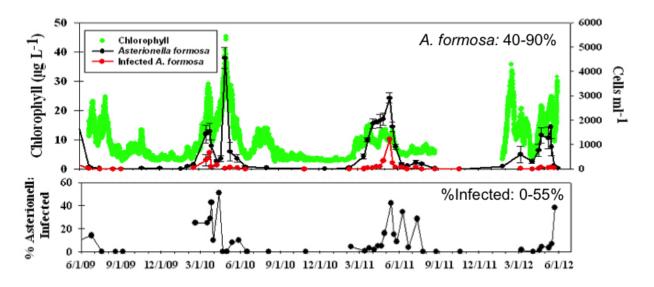
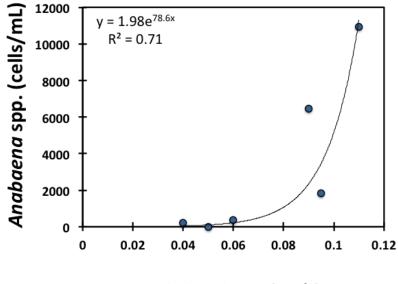


Figure 69. *Top*: Time series of chlorophyll *a* (from in situ sensor package at River Mile 53, Beaver Army Terminal, Reach C) and cell abundances of *Asterionella formosa* (black line). Red line shows number of *A. formosa* cells infected by *Z. planktonicum* (zoosporic fungi). *Bottom*: Percent of *A. formosa* cells infected with the chytrid parasite.

Although few counts of phytoplankton were made during the summer months due to financial constraints, observations indicated that toxigenic cyanobacteria (*Anabaena spp.*) were present at high abundances during July, particularly at Campbell Slough (data not shown). Some preliminary data suggest that cyanobacteria abundances were positively correlated with total dissolved phosphorus levels (Figure 70). Cyanotoxins can accumulate in zooplankton (Ferrao-Filho and Kozlowsky-Suzuki 2011), upon which many invertebrates feed. Cyanotoxins have been shown to accumulate within the tissues of some fish species, mainly in the liver, but also in muscle tissue, and in the gills (Cazenave et al. 2005). Relevant to the present study, it is alarming that some studies have reported higher mortality of *Chaoborus* (midge larvae) after preying on the cladoceran zooplankter, *Daphnia*, fed toxic cyanobacteria *Microcystis* than that fed non-toxic algae. This suggests that *Daphnia* was able to transfer toxins from *Microcystis* to *Chaoborus* (Laurén-Määtä et al. 1995).



Total phosphorus (mg/L)

Figure 70. Abundances of the potentially toxic cyanobacteria *Anabaena spp*. at Campbell Slough (Reach F) in summer 2012 versus the concentration of total dissolved phosphorus at the same site.

4.2.1.1.1.3.2 Vegetation biomass

The samples for the vegetation component of the primary production were taken from three primary marsh strata: high marsh, low marsh, and submerged aquatic vegetation (SAV), with 66 percent of the samples further categorized into species specific strata (Table 13). In general, the species comprising the vegetation biomass samples are the dominant species found in the LCRE. Vegetation species assemblages at the six study sites are described in detail in the annual reports from this study (e.g., Sagar et al. 2013). Although dominant species were noted in many the samples, frequently the samples were a mix of more than one species. The samples in the mixed categories were either a mix of many species, with no

dominant, or the dominant species were not indicated at the time of sampling. The high percentage of non-specific SAV samples is due to the fact that many of the samples contained no vegetation due to the patchy nature of the SAV in many of the channels.

			Percent of Samples Containing Dominant
Dominant Species	Common Name	Species Code	Species
	Emergent Speci		
Carex lyngbyei	Lyngby sedge	CALY	23
Eleocharis palustris	common spike rush	ELPA	5
Phalaris arundinacea	reed canarygrass	PHAR	17
Polygonum amphibium	water smartweed	POAM	3
Sagittaria latifolia	wapato	SALA	6
	Submerged Aquatic Spe	ecies (SAV)	
Elodea spp.	waterweed	ELSP	4
Potamogeton richardsonii	Richardson's pondweed	PORI	4
Zannichellia palustris	Horned pondweed	ZAPA	5
	Non-specific Categ	gories	
High marsh			5
Low marsh			6
SAV			24

Table 13. Dominant species in the vegetation biomass samples. The remaining samples that were not categorized by dominant species are lumped into the non-specific categories at the bottom.

In general, the species composition of the biomass samples did not change between years (2011, 2012, and 2013). There appeared to be an increase in the occurrence of reed canarygrass in the samples from Whites Island in 2013; however, it is difficult to distinguish this shift since many of the samples contained a mix of reed canarygrass and other species. At the Franz Lake site there was a decrease in samples containing only reed canarygrass after 2011 and a corresponding increase in samples containing only *Polygonum amphibium* in 2012 and 2013. This shift is consistent with the change in species composition observed in the cover at the site (see Section 4.1.1 above).

4.2.1.1.2 Secondary Production

4.2.1.1.2.1 Quantity

The data from different sites were grouped together to yield a sense of overall variability in zooplankton over the three years sampled (2011, 2012, 2013). The mean zooplankton abundances when all sites were considered together were remarkably similar among the three years studied, with large error bars around the mean (representing the standard deviation; Figure 71). Differences between years when the data were

organized according to site are also shown (Figure 72). The large error bars indicate high variability, but the observed patterns were similar between years.

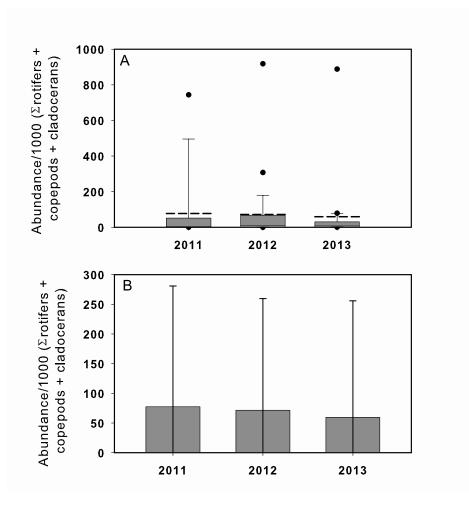


Figure 71. Temporal variability in zooplankton abundance (sum of the three dominant taxa, the rotifers, copepods, and cladocerans). A) Boxplots showing the range, standard deviation, mean (dashed line), and outliers. B) Mean and standard deviation calculated from all EMP fixed sites during the three years of the study.

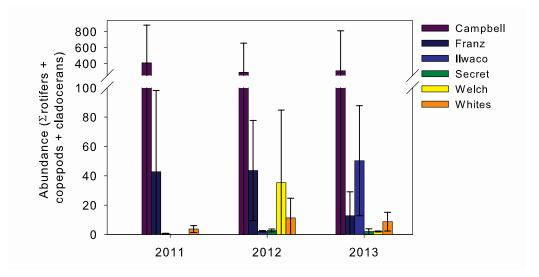


Figure 72. Abundance of the dominant zooplankton taxa at the six fixed sites in 2011, 2012, and 2013. Data were not collected at the Secret River and Welch Island sites in 2011.

4.2.1.1.2.2 Species composition

Changes in the composition of the zooplankton assemblages followed the seasonal hydrology of the river, with higher proportions of rotifers (Phylum Rotifera) present prior to and during the freshet and an increase in the relative proportions of copepods and cladocerans later in the season (e.g., Figure 73). Throughout the river reaches (excluding the salinity influenced Ilwaco site), rotifers numerically dominated the zooplankton assemblages prior to the peak of the spring freshet in 2011, 2012, and 2013. After water levels subsided, crustaceans (copepods and cladocerans) dominated the assemblages.

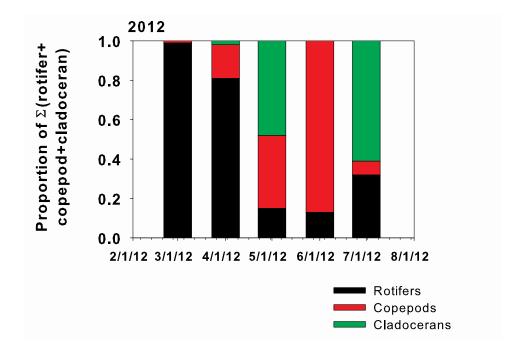


Figure 73. Temporal changes in proportional abundance of the most common zooplankton taxa: rotifers (Phylum Rotifera), copepods (Order Copepoda), and cladocerans (Order Cladocera) at Campbell Slough (Reach F), where zooplankton abundances were the highest. Data from 2012 are shown.

4.2.1.2 Spatial Patterns

4.2.1.2.1 Primary Production

4.2.1.2.1.1 Quantity

4.2.1.2.1.1.1 Pelagic primary production

Data from 2013 were not available in time for this report; therefore, only 2011 and 2012 data are included here. In 2011, the total abundance of diatoms (all sampling time points included, averaged by month), the dominant primary producers, was significantly lower at Ilwaco compared to the other EMP fixed sites. There was a significant interaction between site and year with respect to diatom abundances (p = 0.036, Two-Way ANOVA). Abundances at Ilwaco were significantly lower than the other sites in 2011, and statistically similar abundances were observed in 2011 and 2012 at this site. Diatom abundances at the other sites did not differ from each other within a given year (either 2011 or 2012, p > 0.05), but they did differ significantly between years at Whites Island, Campbell Slough, and Franz Lake Slough.

In 2012, periphyton abundance (as measured by chlorophyll *a* concentrations) was highest at Ilwaco, except in early April, when it was highest at Franz Lake Slough. By far, the highest chlorophyll *a* concentrations measured during 2012 were at Ilwaco in May, when filamentous periphyton was very abundant. The absence of filamentous periphyton at the other sites explains the large difference between the May sample at Ilwaco and even the highest concentrations measured at other sites. No samples were collected in April at Whites Island or in April or May at Campbell Slough due to the absence of substrate that could be sampled. Other than the overall higher chlorophyll *a* concentrations of periphyton at Ilwaco,

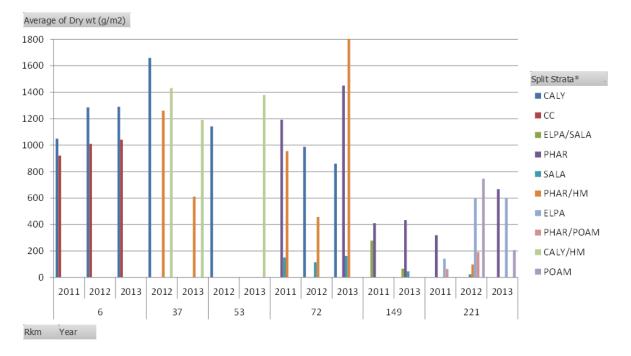
there were no clear temporal or spatial patterns in periphyton abundance among the fixed EMP sites in 2012.

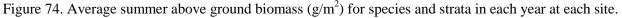
4.2.1.2.1.1.2 Vegetation

The above ground biomass estimates for the emergent wetland vegetation found in the low and high marsh strata are provided in Table 11 and for submerged aquatic vegetation in Table 12. Summer, wetland biomass was found to be positively correlated with elevation (r = 0.60, p < 0.01) and negatively correlated with rkm (r = -0.34, p = 0.04) and SEV (r = -0.57, p < 0.01). The greatest biomass was collected from the high marsh, with statistically significant differences between each of the three marsh strata ($r^2 = 73\%$, p < 0.01). At sites sampled, high marsh had the greatest plant biomass (average of 929 g/m²), compared to low marsh and SAV (average of 249 g/m² and 42 g/m², respectively)⁻

The highest summer emergent wetland biomass estimate was from Secret River in the high marsh during 2012 (1443 g/m²) and the lowest estimate was from the low marsh at Campbell Slough in 2013 (56.3 g/m²). A single sample from a low marsh depression at the Ilwaco site in 2011 was even less (24 g/m²), however since this was not a targeted strata (not high marsh, low marsh or SAV) no replicates were sampled. The submerged aquatic vegetation summer biomass was consistently less than either the low or high marsh; however the estimates were occasionally higher than those from the low marsh. The highest SAV biomass estimate was at Welch Island in 2013 (173 g/m²) and the lowest at Franz Lake in 2013 (0.2 g/m²).

For the years sampled, summer biomass estimates in all strata decreased with increasing rkm (Figure 74). The four lower river sites had greater biomass than the two upper river sites (high marsh average of 1162 g/m² and 426 g/m², respectively). The biomass estimates were found to be statistically significantly different between the lower estuary sites (zones 1 and 2) and the upper estuary sites (zones 4 and 5); no sites were sampled in the middle part of the estuary (zone 3; Figure 75) (r² = 77%, p < 0.01).





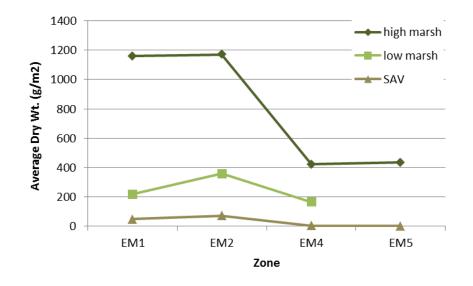


Figure 75. Average emergent vegetation and submerged aquatic vegetation (SAV) biomass for emergent marsh zones of the LCRE. Zones are defined as follows: EM1 = 0-40 rkm, EM2 = 41-89 rkm, EM3 = 90-136 rkm, EM4 = 137-181 rkm, EM5 = 182-235 rkm (Jay et al. in review, modified from Borde et al. 2012).

4.2.1.2.1.2 Rates

4.2.1.2.1.2.1 <u>Pelagic primary productivity (phytoplankton)</u>

Phytoplankton primary productivity (amount of organic matter fixed per unit time) was determined during the weeks of May 9 and June 20, 2011. In both May and June, rates of primary production attributable to phytoplankton were highest at Franz Lake Slough and decreased along a downstream gradient (Figure 76). No experiment was run in June at Campbell Slough. It should be noted that these uptake rates were not normalized to biomass, which means that the specific rates of primary production cannot be compared directly.

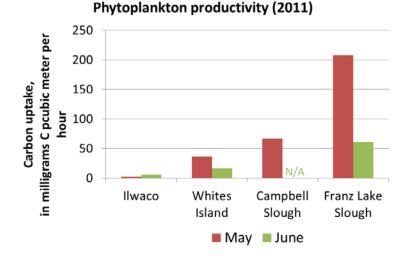


Figure 76. Phytoplankton productivity measured at the four water-quality monitoring sites in 2011 [N/A, no experiment]

4.2.1.2.1.2.2 Periphyton

Overall, the lowest periphyton productivity was measured at Franz Lake Slough, although the number of samples was small due to difficulties in accessing the site in 2011. In both years, there was an apparent increasing gradient in periphyton production in the downstream direction (Figure 77). The only significant differences, however, in rates of periphyton biomass production were noted for Ilwaco vs. Franz Lake Slough (p = 0.003); no significant differences were observed between years or for any other comparison of sites (Figure 78). More data are needed to more accurately determine if actual differences failed to be detected between other sites due to the small number of observations and resulting weak statistical power.

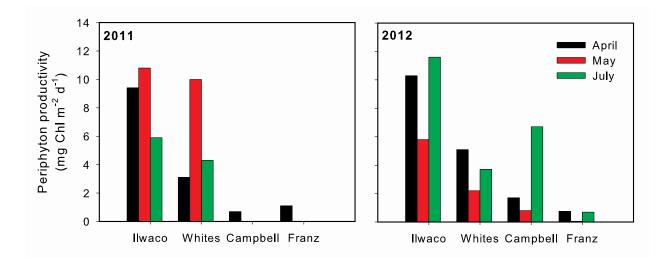


Figure 77. Periphyton productivity determined using micro-NDS periphytometers in the months of April, May, and July 2011 (*left*) and 2012 (*right*).

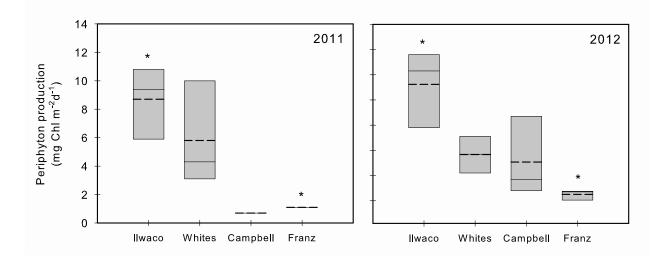


Figure 78. Between-site variability (averaging across time points sampled) at four of the fixed EMP sites in 2011 (*left*) and 2012 (*right*): Ilwaco (Reach A), Whites Island (Reach C), Campbell Slough (Reach F), and Franz Lake Slough (Reach H). Asterisks indicate significant differences (Two-Way ANOVA on site and year, p < 0.05) between periphyton biomass production at Ilwaco vs. Franz Lake Slough. Dashed lines indicate the mean, solid lines show the median. Boxes outline the range of observations. No other differences were found to be significant based on this dataset.

4.2.1.2.1.2.3 Annual Detrital Contribution

Similar spatial patterns to those observed for summer biomass are true regarding the potential annual detrital (PAD) contribution (summer – winter biomass) as well. In general, the PAD contribution was greater in the lower river sites and less in the upper river sites, with an increase observed at the Franz Lake site in 2012 (Table 11). A similar pattern is also apparent when individual species PAD is evaluated

(Table 14), however these results are confounded by the spatial distribution of Lyngby's sedge, in that the species only occurs in the lower portions of the river and is therefore not affected by high water flooding effects. The two most common species in the samples were Lyngby's sedge and reed canarygrass and therefore they have the greatest number of PAD contribution estimates. The average PAD contribution for Lyngby's sedge across all sites where it was measured was 1021 g/m², while reed canarygrass was 291 g/m².

	Average Summer/Winter Difference in Biomass (g/m ²)									
Species/Strata	BBM	SRM	WI2	WHC	CS1	FLM				
Carex lyngbyei (CALY)	839.8	1557.4	776.4	909.1						
Carex lyngbyei/ Agrostis stolonifera	656.2									
Polygonum amphibium						472.5				
<i>Phalaris arundinacea /</i> High Marsh				254.4						
Phalaris arundinacea / Polygonum amphibium						-184.5				
Phalaris arundinacea				488.8	309.4	74.5				
Low Marsh		260.1								
Schoenoplectus tabernaemontani		287.2								
Eleocharis palustris					482.8	75.2				
Eleocharis palustris/ Sagittaria latifolia					222.2					
Sagittaria latifolia				124.4						

Table 14. Average difference between summer and winter above ground biomass (g/m^2) for a given species or strata at each site. Sites are ordered by distance from the mouth of the River.

4.2.1.2.1.3 Species Composition

4.2.1.2.1.3.1 Pelagic

Phytoplankton species composition was determined less frequently than zooplankton species composition. Nevertheless, the time series illustrates that throughout the system, diatoms dominate the fluvial phytoplankton assemblages. There were considerable between-site differences in the contribution of other taxa, including green algae and cyanobacteria, some of which were not evaluated rigorously in this analysis. For example, there was an increase in the presence of potentially toxic cyanobacteria species in Campbell Slough during the summer months of all sampling years. We found post-freshet increases in the proportional contribution by flagellate taxa, which include representatives from different taxonomic groupings but that share the common characteristic that they are able to swim and they are generally small. These include representatives from green algae (Class Chlorophyceae), dinoflagellates (Class Dinophyceae), chrysophytes (Class Chrysophyceae), and others.

4.2.1.2.1.3.2 Vegetation

Dominance of *P. arundinacea* in the LCRE has been previously documented (Sagar et al. 2013). However, in this study *C. lyngbyei* was present as a dominant species in 23 percent of the samples, while *P. arundinacea* was only present as a dominant in 17 percent (Table 13). This is primarily because four of the six study sites are located in the lower portion of the LCRE, below rkm 89, where salinity and the tidal dominated hydrology reduces the probability of *P. arundinacea* occurrence (Borde et al. 2012; Sagar et al. 2013). *C. lyngbyei* was only present at the four lower LCRE sites, frequently occurring as a single dominant species, particularly at the rkm 6 site, and was more frequently mixed with other species at the sites located at rkms 37 and 53. The site at rkm 72 had a contiguous patch of *C. lyngbyei* while the rest of the high marsh was a mix of *P. arundinacea* and other species. Sixty of the 65 samples with *P. arundinacea* came from the three sites located at rkms 72, 149, and 221. *P. arundinacea* was mixed with other species from the sites at rkms 37 and 53 and was absent from samples at the lowest site at rkm 6. *Sagittaria latifolia* occurred in the samples from the four sites. The SAV species *Zannichellia palustris* occurred only at the rkm 6 site, whereas the other two dominant SAV species were present in samples from the other three lower river sites. SAVs were nearly non-existent in the two up-river sites.

4.2.1.2.2 Secondary Production

4.2.1.2.2.1 Quantity

Zooplankton abundances (considered here to be the sum of rotifers, copepods, and cladocerans) were present in greatest abundance at Campbell Slough in Reach F relative to all other sites (p < 0.001). Far lower total abundances were observed at the Reach B (Secret River and Welch Island), Reach C (Whites Island), and Reach A (Ilwaco) sites compared to Campbell Slough (abundances at Franz Lake were between lower reach sites and Campbell Slough). It should be noted that the abundances do not reflect zooplankton biomass, since there is a large size difference between the small rotifers and the larger crustaceans (copepods and cladocerans).

4.2.1.2.2.2 Species composition

Zooplankton species composition varied more by season than by site, with the exception of the Ilwaco time series, which was generally dominated by copepods. Interestingly, a comparison of two nearby sites in Reach A at Ilwaco showed that rotifers were much more abundant in a stream-type site (Ilwaco site sampled approximately at low tide at the USGS monitoring site) than an open-water site at the Ilwaco marina (Figure 79). There are a large number of rotifer taxa, which include several genera that live in association with vegetation and soils. At the other sites, however, there was a higher proportional abundance of small zooplankton (mainly rotifers, Phylum Rotifera) during the spring and prior to and during the Columbia River freshet. Following the decline of the freshet, the zooplankton assemblage became dominated by pelagic crustaceans (Class Crustacea, including Order Copepoda and Order Cladocera).

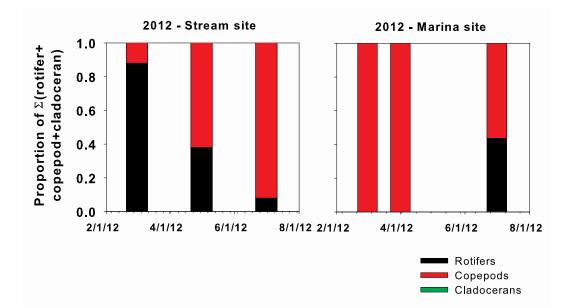


Figure 79. Proportional abundance of the dominant taxa at two sites at Ilwaco in 2012. *Left*: site sampled at low tide at the US Geological Survey continuous monitoring site and *Right*: site sampled at the Ilwaco marina. Rotifers were present at a proportionally higher abundance early in the year at the stream site, while they were proportionally more abundant later in the season at the marina site.

4.2.2 Trophic pathways

4.2.2.1 Stable Isotope Analysis

4.2.2.1.1 Summaries of stable isotope ratios

4.2.2.1.1.1 Stable isotope ratios of juvenile salmon tissues

The stable isotope analysis includes data collected 2010–2012. For this analysis, salmon muscle samples collected through 2012 were available from Campbell Slough and Whites Island. Salmon tissue samples were not available from Franz Lake Slough or Ilwaco because the very few salmon that were caught at those sites (n = 0 - 5) were used for analyses by NOAA Fisheries. Samples were collected during May, June, and July fish sampling trips conducted by NOAA Fisheries. The month of catch correlated both fish length and weight, so it is used as a proxy for size in this analysis. Unless otherwise specified, the variance measure reported here is standard deviation.

Among all salmon muscle samples (n=48), mean δ^{13} C of marked salmon muscle was -21.3 ± 2.1‰ and mean δ^{15} N was 12.3 ± 0.9‰ (n = 21; Table 15). For unmarked salmon muscle, mean δ^{13} C was -25.5 ± 2.4‰ and mean δ^{15} N was 11.5 ± 0.9‰ (n = 27). Dual isotope ratios of marked and unmarked salmon muscle differed significantly (R = 0.239, *p* = 0.001). Muscle from marked and unmarked salmon decreased in δ^{13} C and δ^{15} N ratios with later months of catch (i.e., increasing size/age). Dual stable isotope ratios of muscle samples from unmarked salmon collected from Campbell Slough and Whites Island differed significantly (R = 0.245, *p* = 0.007; Table 16). A site-to-site comparison of muscle samples from

marked salmon is not appropriate, because the dataset only includes one marked salmon from Whites Island.

	Number of samples	Mean δ ¹³ C	SD δ^{13} C	Mean δ ¹⁵ N	SD δ ¹⁵ N
mucus	4	-26.2	0.7	9.1	0.5
marked	3	-25.9	0.6	9.2	0.6
Jul	3	-25.9	0.6	9.2	0.6
unmarked	1	-27.0	N/A	9.1	N/A
Jun	1	-27.0	N/A	9.1	N/A
muscle	48	-23.7	3.1	11.9	1.0
marked	21	-21.3	2.1	12.3	0.9
May	14	-20.2	0.9	12.8	0.4
Jun	2	-21.6	0.7	12.2	1.3
Jul	5	-24.3	2.2	11.1	0.8
unmarked	27	-25.5	2.4	11.5	0.9
May	3	-22.4	2.1	12.8	0.6
Jun	18	-25.8	2.4	11.6	0.8
Jul	6	-26.2	1.4	10.6	0.4

Table 15. Mean and standard deviation of δ^{13} C and δ^{15} N values for juvenile salmon tissues analyzed by USGS 2010 – 2012, by hatchery marking and month [SD, standard deviation].

Table 16. Mean and standard deviation of δ^{13} C and δ^{15} N values from food web samples collected 2010 –
2012, grouped by site [SD, standard deviation].

	Number of samples	Mean δ ¹³ C	SD δ¹³C	Mean δ ¹⁵ N	SD δ ¹⁵ N
salmon muscle	48	-23.7	3.1	11.9	1.0
Campbell	37	-22.9	2.9	11.8	1.1
Whites	11	-26.2	2.4	12.1	0.7
salmon mucus	4	-26.2	0.7	9.1	0.5
Campbell	4	-26.2	0.7	9.1	0.5
hatchery food	3	-20.9	0.2	8.1	0.1
invertebrates	28	-25.5	4.7	6.9	1.5
Franz	4	-29.6	1.5	5.4	1.4
Campbell	10	-28.8	3.3	5.8	0.9
Whites	3	-26.7	2.9	7.7	1.6
Ilwaco	11	-20.6	1.3	8.2	0.7
periphyton	17	-25.8	2.8	4.7	1.3
Franz	4	-27.9	1.6	3.6	1.8

	Number of samples	Mean δ ¹³ C	SD δ^{13} C	Mean δ ¹⁵ N	SD δ ¹⁵ N
Campbell	3	-27.9	1.2	4.3	0.7
Whites	5	-26.7	1.0	4.7	0.9
Ilwaco	5	-22.0	0.8	5.8	0.8
РОМ	26	-30.1	2.3	4.4	1.7
Franz	6	-30.8	1.6	2.5	2.0
Campbell	8	-31.5	2.4	4.4	1.2
Whites	6	-30.3	1.1	4.7	0.6
Ilwaco	6	-27.2	1.2	6.0	0.7
vegetation	78	-26.1	3.8	5.6	1.5
Franz	3	-29.1	0.6	3.9	1.1
Campbell	24	-28.1	0.9	4.6	1.6
Whites	28	-26.5	3.1	5.4	1.0
Ilwaco	23	-23.0	4.7	6.9	0.8

4.2.2.1.1.2 Stable isotope ratios of invertebrate prey

Mean stable isotope ratios for chironomids were -29.4 ± 3.8 ‰ for δ^{13} C and 5.7 ± 1.0 ‰ for δ^{15} N. Mean ratios for amphipods were -22.6 ± 3.6 ‰ for δ^{13} C and 7.9 ± 0.9 ‰ for δ^{15} N. Dual isotope ratios of these prey types were significantly different (R = 0.68, p = 0.001). Values for δ^{13} C and δ^{15} N of invertebrates increased at further downstream sites (Table 16).

4.2.2.1.1.3 Stable isotope ratios of organic matter sources

Delta ¹³C and δ^{15} N signatures from our dataset are in line with typical values for major organic matter sources in a review of scientific literature by Finlay & Kendall (2007). Organic matter sources were grouped by various levels of detail for this exploratory analysis. Level 1 organic matter types are grouped as vegetation, periphyton, or POM—a proxy for phytoplankton. Level 2 organic matter types include freshwater vascular vegetation, saltmarsh vascular vegetation, aquatic macrophytes (submerged aquatic vegetation, SAV), freshwater periphyton, brackish periphyton, POM, and benthic macroalgae (Table 17). Across all sites, Level 1 organic matter types (vegetation, periphyton, and POM) differed significantly from each other (R = 0.279, p = 0.001), although pairwise tests indicated that periphyton and vegetation did not differ significantly from one another (R = 0.034, p = 0.311). Therefore, the SIAR model using the entire dataset may have difficulty discriminating the relative contributions of periphyton and vegetation.

When compared by Level 2 organic matter types, the signatures of the sources are significantly different from each other for only some pairs (Table 18). Among freshwater organic matter sources, freshwater vegetation and POM differed significantly (R = 0.353, p = 0.001). Saltmarsh vegetation and benthic macroalgae (both organic matter types unique to Ilwaco) had stable isotope ratios that differed significantly from those of freshwater periphyton, aquatic macrophytes, and POM.

	Number of samples	Mean δ13C	SD 813 C	Mean δ15N	SD δ15N
periphyton	17	-25.8	2.8	4.7	1.3
freshwater	12	-27.4	1.3	4.3	1.2
brackish	5	-22.0	0.8	5.8	0.8
РОМ	26	-30.1	2.3	4.4	1.7
vegetation	78	-26.1	3.8	5.6	1.5
freshwater emergent veg	47	-27.9	1.3	5.0	1.4
freshwater macrophyte	8	-24.0	4.5	4.6	1.0
saltmarsh vegetation	13	-25.9	3.4	7.0	0.8
brackish macroalgae	10	-19.1	2.9	6.9	0.7

Table 17. Mean and standard deviation of δ^{13} C and δ^{15} N values from organic matter samples collected 2010 – 2012, by Level 1 (bold) and Level 2 groupings [SD, standard deviation].

Table 18. Pairwise ANOSIM values by Level 2 organic matter type. ANOSIM R values are in the lower section; p values are in the upper section. Bold font indicates significant differences at p < 0.05. [fwveg, terrestrial or freshwater emergent vegetation; fwperi, freshwater periphyton; POM, particulate organic matter; smveg, saltmarsh vegetation; swperi, brackish periphyton].

	fwveg	fwperi	POM	macrophyte	macroalgae	smveg	swperi
fwveg		0.226	0.001	0.061	0.239	0.481	0.664
fwperi	0.055		0.249	0.091	0.001	0.001	0.097
РОМ	0.353	0.042		0.627	0.022	0.012	0.445
macrophyte	0.165	0.114	-0.396		0.002	0.001	0.599
macroalgae	0.059	0.590	0.226	0.445		0.599	0.599
smveg	-0.003	0.430	0.199	0.513	-0.056		0.011
swperi	-0.057	0.176	-0.008	-0.056	-0.056	0.312	

Stable isotope signatures of organic matter sources differed significantly by site (R = 0.274, p = 0.001), as did each individual Level 1 organic matter type (vegetation by site: R = 0.246, p = 0.001; POM by site: R = 0.229, p = 0.002; periphyton by site: R = 0.304, p = 0.007).

Within sites, Level 1 organic matter types differed significantly from one another at all sites except Franz Lake Slough [Franz (R = 0.1165, p = 0.188); Campbell Slough (R = 0.317, p = 0.003); Whites (R = 0.295, p = 0.008); Ilwaco (R = 0.309, p = 0.001]. The low number of samples from Franz Lake Slough likely affected this result.

4.2.2.1.2 SIAR models

4.2.2.1.2.1 SIAR models: juvenile salmon diets

Results from the SIAR model of marked salmon as consumers indicates that hatchery food is likely the most important food source for marked salmon, but that hatchery food loses importance over time during spring to early summer, when juvenile salmon are using these shallow water habitats. This was the case

when the model was run with USGS data only (muscle tissue) and when the USGS and NOAA datasets were combined (muscle tissue and whole bodies, respectively).

The proportions of chironomids and amphipods in the diets of marked salmon are difficult to determine using the SIAR model with the existing dataset. While determining their relative proportions in salmon diets was not a key question of this study, a clearer answer would provide context with which to interpret the results of the invertebrate diet models examining the contributions of organic matter sources into the food web.

For unmarked salmon, stable isotope data from whole bodies analyzed at the NOAA Northwest Fisheries Science Center lab were combined with data from muscle samples analyzed by USGS. An ANOSIM was used to compare the δ^{13} C and δ^{15} N of muscle samples and whole body samples collected from the same site and day. Stable isotope ratios from the two sets of samples did not differ significantly. Therefore, the two datasets were combined for this analysis. The SIAR model on unmarked salmon muscle and whole bodies estimated that the dietary proportion of chironomids in the diets of unmarked juvenile salmon increased over time (month of fish catch) during the spring.

Overall, salmon epidermal mucus had significantly lower δ^{13} C and δ^{15} N ratios than muscle (R = 0.562, p = 0.003). Moreover, mucus had lower δ^{13} C and δ^{15} N values compared to muscle samples collected the same month (comparisons were made between muscle and mucus collected from the same fish). When plotted in δ^{13} C vs. δ^{15} N isospace, the isotopic values of mucus plotted closer to environmental food sources than did muscle samples indicating that mucus is a better measure of diet (Figure 80).

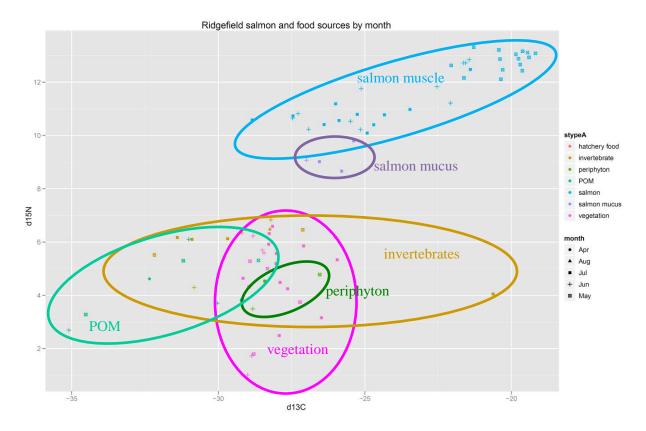


Figure 80. Delta plot of salmon tissues and food sources from Campbell Slough in Ridgefield National Wildlife Refuge collected 2010 - 2012, grouped by month.

4.2.2.1.2.1.1 SIAR models: invertebrate prey diets

The SIAR model evaluating chironomids as the consumers with potential organic matter sources estimated that particulate organic matter (POM; a proxy for phytoplankton) was likely the largest source of organic matter supporting chironomids collected from 2010 to 2012. This was the case for Level 1 organic matter sources (vegetation-periphyton-POM) and more refined groupings (Level 2 organic matter sources). POM most likely contributed to the largest proportion of chironomid diets within individual sites (Franz Lake Slough and Campbell Slough) and when data were combined for the two sites (Figure 81). Estimated contributions of periphyton, aquatic macrophytes, and terrestrial vegetation were less, and their contributions were less clear to separate from one another (Figure 82). However, periphyton and aquatic macrophytes (SAV) appear to have been more important contributors compared to terrestrial or emergent vegetation. When terrestrial or emergent vegetation sources were broken out by species, the likely contributors to chironomid diets were difficult to separate from one another, but the model consistently found wapato (Sagittaria latifolia) was not a contributor to chironomid diets.

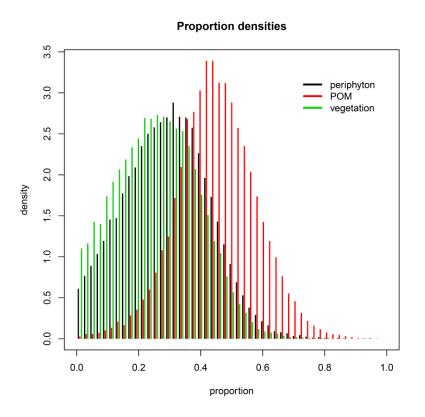


Figure 81. SIAR proportion density plot of chironomid dietary sources at Franz Lake Slough and Campbell Slough, grouped by Level 1 organic matter type.

Proportion densities

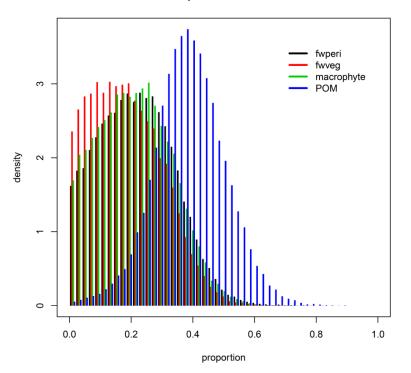


Figure 82. SIAR proportion density plot of chironomid dietary sources from Franz Lake Slough and Campbell Slough, grouped by relevant Level 2 organic matter types [fwperi, freshwater periphyton; fwveg, terrestrial or freshwater emergent vegetation; POM, particulate organic matter].

The SIAR model estimated that the largest contributor to diets of chironomids collected in May 2010-2012 was POM (Figure 83). For chironomids collected in June and July, the model estimated that chironomid diets were more mixed, with closer to equal proportions of POM, aquatic macrophytes, terrestrial vegetation, and periphyton.

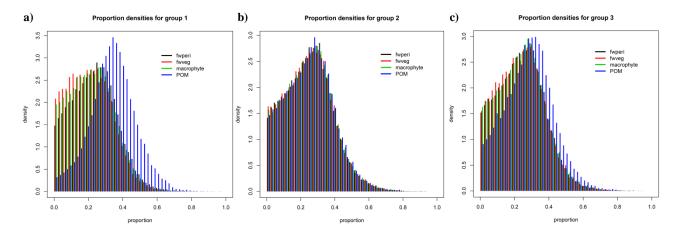


Figure 83. SIAR proportion density plots of chironomid dietary sources grouped by relevant by Level 2 organic matter type and month: a) May; b) June; c) July [fwperi, freshwater periphyton; fwveg, terrestrial or freshwater emergent vegetation; POM, particulate organic matter]

The SIAR model with *Corophium spp.* amphipods from Whites Island and Ilwaco as consumers indicated that vegetation was likely the most important organic matter source contributing to the *Corophium spp.* diet, but that periphyton was important to a lesser extent (Figure 84). The model indicated that POM was not a likely food source for *Corophium spp.*

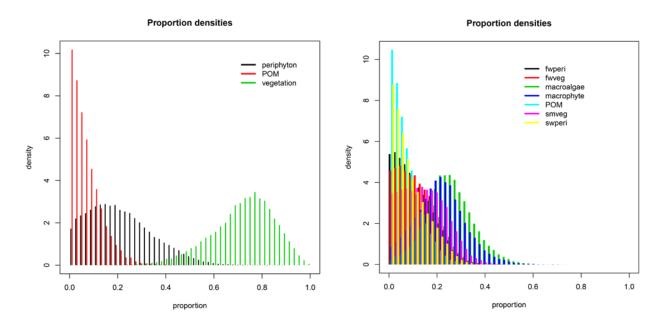


Figure 84. SIAR proportion density plots of *Corophium spp.* dietary sources at Whites Island and Ilwaco grouped by a) Level 1 organic matter sources; b) Level 2 organic matter sources [fwperi, freshwater periphyton; fwveg, terrestrial or freshwater emergent vegetation; POM, particulate organic matter; smveg, saltmarsh vegetation; swperi, brackish periphyton].

When the model was run using *Corophium spp*. (amphipods) and organic matter sources only from Ilwaco, the likely proportion of vegetation in the diet increased and the model became more certain (Figure 85). When examining Level 1 organic matter sources, the relative contributions of periphyton and

POM at Ilwaco could not be teased apart from one another, but their contributions were less than that of vegetation. When grouped by Level 2 organic matter sources, benthic macroalgae and saltmarsh vegetation were the most important contributors to *Corophium spp*. diets. Periphyton may have also contributed to a lesser extent, and POM was likely the smallest contributor. Therefore, organic matter sources from vegetation were the most important component in *Corophium spp*. diets at Ilwaco.

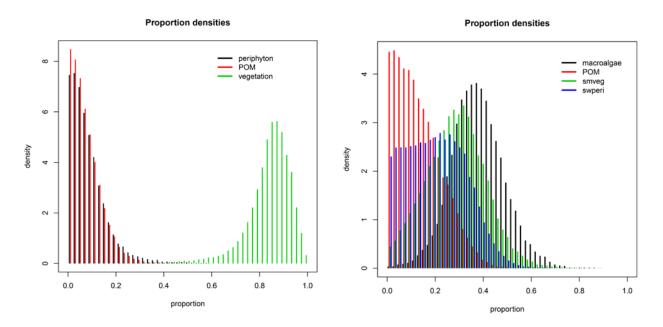
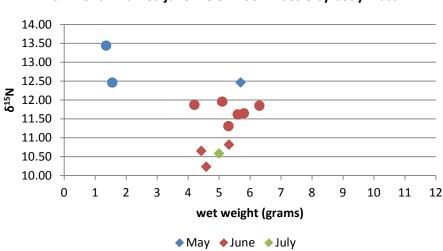


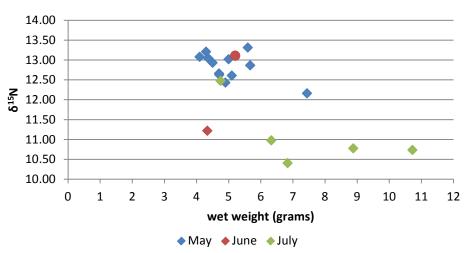
Figure 85. SIAR proportion density plots of *Corophium spp*. dietary sources from the Ilwaco site only grouped by a) Level 1 organic matter sources; b) Level 2 organic matter sources [POM, particulate organic matter; smveg, saltmarsh vegetation; swperi, brackish periphyton].

Unmarked juvenile Chinook salmon could be approaching equilibrium (leveling out of δ^{15} N that shows the switch from maternal to environmental food sources) at smaller body weights because they began eating environmental food sources earlier than marked fish, which fed on hatchery food early in life. The higher δ^{15} N values in muscle of unmarked individuals caught at Whites Island in Reach C (represented as circles in Figure 85 and Figure 86) compared to those caught at Campbell Slough in Reach F (represented as diamonds) during the same month of catch are likely due to the higher δ^{15} N values of food sources at the downstream site. Since fish were different sizes between the two sites in May, it is unknown whether all small fish at these sites would have begun to show δ^{15} N values from environmental food sources or if there is a spatial component to the data.



 δ^{15} N of unmarked juvenile Chinook muscle by body mass

Figure 86. Delta ¹⁵N of unmarked salmon muscle as a function of body mass. Circles represent salmon caught at Whites Island. Diamonds represent salmon caught at Campbell Slough.



 δ^{15} N of marked juvenile Chinook muscle by body mass

Figure 87. Delta ¹⁵N of marked salmon muscle as a function of body mass. Circles represent salmon caught at Whites Island. Diamonds represent salmon caught at Campbell Slough.

4.2.3 Interdisciplinary Relationships

4.2.3.1 Water Temperature Analysis

There was no difference between water temperature collected by PNNL and the USGS (two-sided p-value = 0.65 from a two-sample t-test, Figure 88) at reference sites in the same month and year. The mean

temperature is estimated to be 0.407° C higher in USGS temperature data than the PNNL data for the same locations and time (95% confidence interval from -1.41 to +2.23°C).

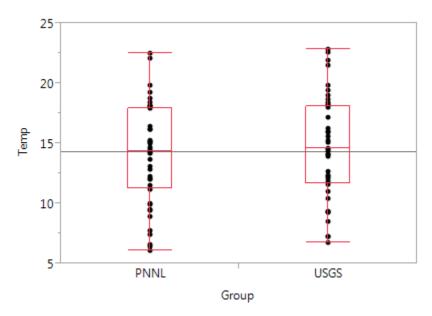


Figure 88. Box plot comparison of mean water temperature (°C) data collected by the USGS and PNNL over multiple months and years.

4.2.3.2 Multivariate Analysis

4.2.3.2.1 Abiotic factors and primary production

To explore potential environmental gradients which influence primary production, we constructed two matrices – a phytoplankton/periphyton matrix and an environmental matrix containing water quality parameters. The same sample units are represented in both matrices (Table 19).

Site	Month	Year	Site	Month	Year	Site	Month	Year
Campbell Slough	April	2013	Franz Lake	June	2011	Ilwaco	June	2013
Campbell Slough	May	2011	Franz Lake	June	2012	Whites Island	April	2013
Campbell Slough	May	2013	Franz Lake	June	2013	Whites Island	May	2011
Campbell Slough	June	2012	Ilwaco	April	2012	Whites Island	May	2012
Campbell Slough	June	2013	Ilwaco	April	2013	Whites Island	May	2013
Franz Lake	April	2012	Ilwaco	May	2011	Whites Island	June	2011
Franz Lake	April	2013	Ilwaco	May	2012	Whites Island	June	2012
Franz Lake	April	2011	Ilwaco	May	2013	Whites Island	June	2013
Franz Lake	May	2012	Ilwaco	June	2011			
Franz Lake	May	2013	Ilwaco	June	2012			

Table 19. Site, month, and year used to examine abiotic factors and primary production.

The periphyton/plankton matrix was constructed of 27 sample units (rows) and six measures of primary production (columns). Periphyton data were reported in units of periphyton chlorophyll a (mg/m²),

periphyton pheophytin a (mg/m²), and periphyton biomass (g/m²). Phytoplankton was reported in units of phytoplankton chlorophyll a (mg/m³), phytoplankton pheophytin a (mg/m³), and phytoplankton volatile solids (mg/L). Sites with multiple replicates were averaged by collection month.

Initial primary production analysis showed a data range of 4.1 orders of magnitude. Ilwaco in May 2012 was identified as a strong outlier at 3.8 standard deviations from the grand mean of distance between sample units and even after generalized log-transforming the dataset, Ilwaco in May 2012 remained a strong outlier at 3.9 standard deviations. Generalized log-transformation tends to preserve the original order of magnitude and results in values of zero when the initial value was zero (McCune and Mefford 2002); therefore this method was chosen for the analysis. A sample unit total that was 20 times greater than the average total of sample units also contributed to Ilwaco in May 2012 being an outlier and we removed the sample unit from the analysis due to this large difference. Campbell Slough in June 2013 was also identified as an outlier at 2.1 standard deviations. The dataset was relativized by primary production parameters to reduce the influence of high total values relative to parameters with lower total values. General relativization has been to shown to improve the relationship between the patterns in the original data and ordination axes (Peck 2010). Following relativization, Whites Island in May 2012 was also identified as a weak outlier due to a lower than average sample unit total (for all sample unit totals); although, due to weak influence of the outlier and the use of Sorenson distance measure, Whites Island in May 2012 was retained for analysis. Sorenson distance measure shifts the emphasis onto the responses that are actually present in the sample unit retaining sensitivity even in heterogeneous datasets without excessive sensitivity to outliers (Peck 2010).

The environmental matrix was constructed of 10 sample units (rows) and 15 habitat variables (columns). Water quality data consisted of grab samples collected during site visits. Water temperature, pH, and dissolved oxygen data were collected continuously and were averaged by site and month. The environmental matrix was evaluated for outliers. Conductance and water temperature were identified as outliers due to scale of those data compared to other measurements within the dataset. Conductance and water temperature were generalized log transformed to reduce the scale of the data. Following transformation, no outliers were detected. In addition to 12 water quality variables, month and year were added to the environmental matrix for a total of 14 variables (Table 20).

Month	Total Kjeldahl Nitrogen+ Nitrate+Nitrite (TKN+NO ₃ +NO ₂)
Year	Ortho-phosphate (Ortho-P)
Nitrate/Nitrite (NO ₃ /NO ₂)	Total Phosphate (T-Phos)
	Total Nitrate+Total Phospate
Nitrate (NO ₃)	(TN+TP)
Nitrite (NO ₂)	Temperature
Ammonia (NH ₃)	Conductance
Total Kjeldahl Nitrogen (TKN)	рН

Table 20. Variables included in analysis primary production.

Differences between groups when year (p = 0.354, A = 0.0045) or month (p = 0.527, A = -0.0097) were used as the grouping variable were not apparent. However, when sites were used as a grouping variable, phytoplankton and periphyton abundance significantly differed among sites (p = 0.0036, A = 0.1563).

A NMS ordination with a two-dimensional solution of plots in primary production space was used (Final Stress = 7.59, final stability ≤ 0.00001 , number of iterations = 43) and the solution rotated so conductance, ortho-phosphate, and ammonia were parallel with axis one. The first two axes explained 96.7% of the variation in the data. A moderate correlation related to conductance (r = 0.517), orthophosphate (r = 0.470), and ammonia (r = 0.450) was found with axis one (Figure 89).

Measures of periphyton, periphyton chlorophyll *a* (r = 0.953), periphyton pheophytin *a* (r = 0.957), and periphyton biomass (r = 0.861) had a strong positive correlation with axis one. Phytoplankton volatile solids were moderately correlated with axis two.

The Ilwaco site correlated with high levels of conductance, ortho-phosphate, and ammonia, and showed similarities in production of periphyton and plankton across months and years. Franz Lake, Campbell Slough, and Whites Island sites were found to be highly variable and showed little similarity in periphyton and plankton production across months and years.

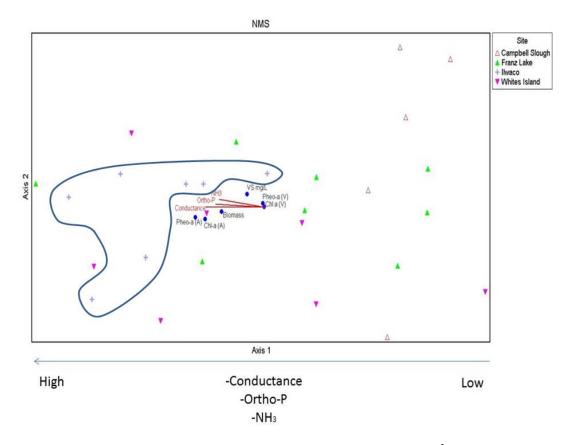


Figure 89. NMS ordination of sites in site space. Habitat variables with $r^2 > 0.20$ are displayed with a vector joint plot. Sites associated with conductance, ortho-phosphate (Ortho-P), and ammonia (NH₃) are circled. Chl-a (A)=Periphyton chlorophyll *a* (mg/m²); Pheo-a (A)=Periphyton pheophytin *a* (mg/m²); Biomass= Periphyton biomass (g/m²); Chl-a (V)=Phytoplankton Chlorophyll *a* (mg/m²); Pheo-a (V)=Phytoplankton pheophytin *a* (mg/m²); VS=Phytoplankton volatile solids (mg/L).

When the Ilwaco site was excluded from the analysis, no difference was found when grouped by month (p = 0.54, A = -0.011) or year (p = 0.29, A = 0.014). When grouped by site, a suggestive but inconclusive difference was found (p = 0.059, A = 0.073).

A NMS ordination with a two-dimensional solution of plots in primary production space was used (Final Stress = 7.90, final stability \leq 0.00001, number of iterations = 99). The solution was rotated so orthophosphate and ammonia were parallel with axis one. The first two axes explained 95.5% of the variation in the data. Ortho-phosphate and ammonia were found to be weakly correlated with axis one.

Measures of periphyton (A), periphyton chlorophyll a (r = -0.953), periphyton pheophytin a (r = -0.950), and periphyton biomass (r = -0.841) had a strong negative correlation with axis one. Phytoplankton volatile solids had a strong negative correlation with axis two (r = -0.825).

4.2.3.2.2 Vegetation and water quality gradients

We constructed two matrixes – a species matrix consisting of percent cover of vegetation and an environmental matrix containing water quality measurements. In order to identify abiotic environmental gradients associated with plant species, the same sample units are presented in both matrixes (Table 21).

Site	Month	Year	Site	Month	Year	Site	Month	Year
Campbell Slough	April	2012	Franz Lake	May	2012	Ilwaco	June	2012
Campbell Slough	April	2013	Franz Lake	May	2013	Ilwaco	June	2013
Campbell Slough	May	2011	Franz Lake	June	2011	Ilwaco	July	2012
Campbell Slough	May	2012	Franz Lake	June	2012	Whites Island	April	2012
Campbell Slough	May	2013	Franz Lake	June	2013	Whites Island	April	2013
Campbell Slough	June	2011	Franz Lake	July	2012	Whites Island	May	2011
Campbell Slough	June	2012	Ilwaco	April	2012	Whites Island	May	2012
Campbell Slough	June	2013	Ilwaco	April	2013	Whites Island	May	2013
Campbell Slough	July	2012	Ilwaco	May	2011	Whites Island	June	2011
Franz Lake	April	2012	Ilwaco	May	2012	Whites Island	June	2012
Franz Lake	April	2013	Ilwaco	May	2013	Whites Island	June	2013
Franz Lake	May	2011	Ilwaco	June	2011	Whites Island	July	2012

Table 21. Site, month, and year used to examine abiotic factors and vegetation.

The vegetation species matrix was constructed of 36 sample units and 110 species and vegetation data were reported in percent cover. Average percent cover at each site was calculated by averaging identified species present in survey quadrats. Any species with no occurrence were removed prior to analysis (Table 22). Deleting species that occur in less than 5% of the sample units reduces noise in the dataset without losing much information; furthermore, it often enhances the detection of relationships between community composition and environmental factors (McCune and Mefford 2002).

	Species		Species
BESY	Beckmannia syzigachne	LISC	Lilaea scilloides
CAAT	Carex athrostachya	LW	Live wood
CASP2	Carex spp.	LYPO	Lythrum portula
CEDE	Ceratophyllum demersum	MAVE	Marsilea vestita
COCO	Cotula coronopifolia	MEPU	Mentha pulegium
COMA	Collomia mazama	PLDI	Platanthera dilatata
CYST	Cyperus strigosus	PONO	Potamogeton nodosus
ELNU	Elodea nuttallii	PORI2	Potentilla rivalis
FOAN	Fontinalis antipyretica	POZO	Potamogeton zosteriformis
GNUL	Gnaphalium uliginosum	RINA	Ricciocarpos natans
HYAN	Hypericum anagalloides	SASP	Salix spp.
HYSC	Hypericum scouleri	SCTR	Schoenoplectus triqueter
IMSP	Impatiens capensis,Impatiens noli-tangere	SPEU	Sparganium eurycarpum
ISSP	Isothecium spp.	TRWO	Trifolium wormskioldii
JUEN	Juncus ensifolius	TYSP	Typha angustifolia
LAPA	Lathyrus palustris	VESP	Veronica spp.
XAST	Xanthium strumarium		

Table 22. Vegetation species removed from analysis.

Initial percent cover data had a range of 3.7 orders of magnitude. Canadian sanspurry (SPCA; *Spergularia canadensis*) was identified as an outlier at 2.1 standard deviations from the grand mean of distance between species units. After arcsine squareroot transforming the dataset to eliminate unequal variance and improve normality (Sokal and Rohlf 1995), Canadian sanspurry was 2.2 standard deviations from the grand mean. The dataset was then relativized by species to reduce the influence of species with high total abundance relative to species with lower total abundance. After data transformation and relativization, outliers were no longer present and Sorenson distance was applied.

The environmental matrix was constructed of 10 sample units (rows) and 15 habitat variables (columns). Conductance and water temperature were identified as outliers due to the scale of those data compared to other measurements within the dataset. Outliers were no longer detected and the scale of the data was reduced after generalized log transforming the conductance and water temperature data. In addition to the 12 water quality variables, month and year were added to the environmental matrix for a total of 14 variables (Table 23).

Month	Total Kjeldahl Nitrogen+ Nitrate+Nitrite (TKN+NO3+NO2)
Year	Ortho-phosphate (Ortho-P)
Nitrate/Nitrite (NO3/NO2)	Total Phosphate (T-Phos)
Nitrate (NO3)	Total Nitrate+Total Phosphate (TN+TP)
Nitrite (NO2)	Temperature
Ammonia (NH3)	Conductance
Total Kjeldahl Nitrogen (TKN)	pH

Table 23. Habitat Variables included with analysis of vegetation.

Vegetation significantly differed by year (p = 0.0004, A = 0.090) and site (p < 0.0001, A = 0.472). Month was not used as a grouping variable because vegetation cover is only collected once per year. Thus, vegetation cover was applied to multiple months for analysis, which means the vegetation values for each month within a given year are the same.

A NMS ordination with a two-dimensional solution of plots in species space was used (Final Stress = 7.07, final instability \leq 0.000001, number of iterations = 52) and the solution rotated so conductance, ortho-phosphate, total phosphate, and ammonia were parallel with axis one. The first two axes explained 63% of the variation in the data. We found a very strong correlation related to conductance (r = -0.913), a strong correlation related to ortho-phosphate, and a moderate correlation to ammonia (r = -0.544) and total phosphate (r = -0.557) (Figure 90).

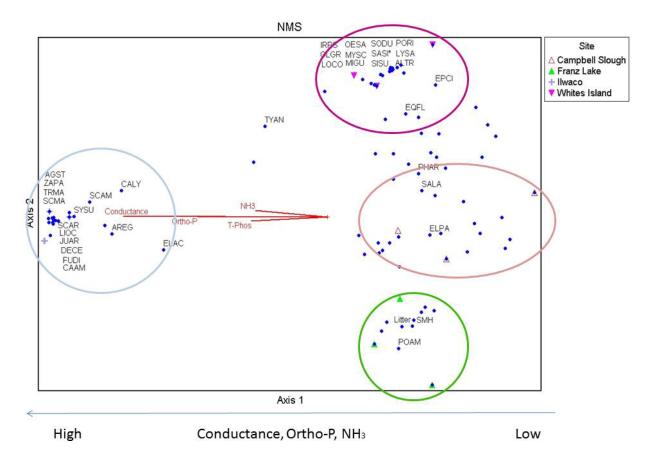


Figure 90. NMS ordination of sites in site space. Habitat variables with $r^2 > 0.20$ are displayed with a vector joint plot. Specific plant species assemblages were associated with specific sites are circled. T-Phos=total phosphate, Ortho-P=ortho-phosphate, and NH₃=ammonia.

The Ilwaco site was associated with native wetland obligate and facultative wetland plants (i.e., AGST (r = -0.962), AREG (r = -0.946), CAAM (r = -0.952), CALY (r = -0.958), DECE (r = -0.877), ELAC (r = -0.906), FUDI (r = -0.896), JUAR (r = -0.747), LIOC (r = -0.929), SCAM (r = -0.906), SCAR (r = -0.915), SCMA (r = -0.882), SYSU (r = -0.874), TRMA (r = -0.956), ZAPA (r = -0.805)). The Ilwaco site was also associated with higher conductance, ortho-phosphate, total phosphate, and ammonia. Campbell Slough was associated with native and invasive wetland obligate and facultative wetland plants (i.e., ELPA (r = 0.792), PHAR (r = 0.760), and SALA (r = 0.872)) and with lower conductance, orthophosphate, total phosphate, and ammonia (Figure 90). Whites Island was associated with native and invasive wetland obligate and facilitative plants (i.e., ALTR (r = 0.823), EPCI (r = 0.640), EQFL(r = 0.765), GLGR (r = 0.832), IRPS (r = 0.838), LOCO (r = 0.841), LYSA (r = 0.810), MIGU (r = 0.816), MYSC (r = 0.820), OESA (r = 0.835), PORI (r = 0.803), SASI* (r = 0.835), SISU (r = 0.757), SODU (r = 0.832), TYAN (r = 0.839)), with a higher number of invasive species compared to other sites. Franz Lake was only associated with one plant species (POAM) along with litter and unidentified small mixed herbs.

With the Ilwaco site excluded, significant differences were found between site (p < 0.0001, A = 0.380) and year (p < 0.0001, A = 0.167). A NMS ordination with a three-dimensional solution of plots in species space was used (Final Stress = 2.75, final instability ≤ 0.000001 , number of iterations = 61) and the

solution rotated so total kjeldahl nitrogen, total phosphate, pH, and nitrate/nitrite were parallel with axis one. The first three axes explained 86% of the variation in the data and we found a moderate correlation related to total kjeldahl nitrogen (r = -0.609), total phosphate (r = -0.536), pH (r = 0.507) and a weak correlation to nitrate/nitrite (r = 0.444; Figure 90).

The Campbell Slough site was associated with native and invasive obligate and wetland plants (i.e., LYNU (r = 0.807), PONA (r = 0.805)). Franz Lake was associated with native wetland and obligate plants (i.e., CASP (r = 0.846), ELA (r = 0.805), POAM (r = 0.833), SALU (r = 0.8700). Whites Island was associated with native and invasive obligate and facultative wetland vegetation (i.e., ALTR (r = 0.862), CALY (r = 0.844), EQFL (r = 0.923), GLGR (r = 0.885), IRPS (r = 0.871), LOCO (r = 0.918), LYSA (r = 0.814), MIGU (r = 0.871), MYSC (r = 0.892), OESA (r = 0.874), PORI (r = 0.883), SASI (r = 0.863), SODU (r = 0.849), TYAN (r = 0.857)) and was characterized by lower levels of total phosphate and higher pH.

4.2.3.2.3 Macroinvertebrates and water quality

We constructed two matrixes – a species matrix consisting of macroinvertebrate abundance and an environmental matrix containing water quality measurements. To compare macroinvertebrate species with water quality parameters, the same sample units must be represented in both matrices (Table 24).

Site	Month	Year
Campbell Slough	May	2011
Campbell Slough	May	2012
Campbell Slough	June	2012
Campbell Slough	May	2013
Campbell Slough	June	2013
Ilwaco	May	2011
Whites Island	May	2011
Whites Island	June	2011
Whites Island	June	2012
Whites Island	June	2013

Table 24. Site, month, and year used to examine macroinvertebrate abundance and water quality.

The species matrix was constructed of 10 sample units (rows) and 25 species (columns). Macroinvertebrate tow data were reported as the number of macroinvertebrates per meter towed and samples were collected in open water habitats and near-shore emergent vegetation habitats. Near-shore emergent vegetation habitats were used in the multivariate analysis since biomass can be 20 times greater in samples collected from near-shore emergent habitats (Sagar et al. 2013, 2012). Sites with multiple tows were averaged by month and year. Similar to vegetation species, macroinvertebrate species with no occurrence (i.e., Chilopoda, Decapoda, Diplopoda, Neuroptera, Opilines, Plecoptera, Psocoptera, Trubellara) were removed from the analysis in order to reduce noise in the dataset and to enhance the detection of relationships between community composition and environmental factors (McCune and Mefford 2002). Following the removal of species of no occurrence, 25 species remained in the analysis (Table 25).

Acari	Coleoptera	Hydrozoa	Ostracoda
Amphipoda	Collembola	Hymenoptera	Polychaeta
Araneae	Copepoda	Isopoda	Thysanoptera
Bivalvia	Diptera	Lepidoptera	Trichoptera
Branchiobdellida	Ephemeroptera	Nemata	
Cladocera	Gastropoda	Odonata	
Clitellata	Hemiptera	Oligochaeta	

Table 25. Macroinvertebrate species used in the analysis.

Initial species abundance data had a range of 3.6 orders of magnitude. The dataset was checked for outliers and Isopoda was identified an outlier at 2.4 standard deviations from the grand mean of distance between species units. To reduce the high degree of variation among species and large range of the data, the dataset was generalized log-transformed (McCune and Mefford 2002). Following the transformation, polychae became an outlier at 2.1 standard deviations from the grand mean of distance between sample units. Although the dataset was relativized by species to reduce the influence of species with high total abundance relative to species with lower total abundance, polychae was still identified as a weak outlier at 2.0 standard deviations from the grand mean. In addition, with transformation and relativization, beta diversity and variation of species abundance were reduced to improve sensitivity of ordination distance measures when evaluating relationships to environmental gradients (McCune and Mefford 2002). The factors of high variation of sample units, lack of linearity, and weak outlier in the dataset favor the use of Sorenson distance over Euclidian distance since Sorenson distance measure shifts the emphasis onto the responses that are actually present in the sample unit, retaining sensitivity even in heterogeneous datasets without excessive sensitivity to outliers (Peck 2010).

The environmental matrix was constructed of 10 sample units (rows) and 15 habitat variables (columns). Water quality data consisted of grab samples collected during site visits. Water temperature data, which were collected continuously, was averaged by site and month. The environmental matrix was evaluated for outliers. Conductance and water temperature were identified as outliers due to scale of those data compared to other measurements within the dataset and were generalized log transformed to reduce the scale of the data. Following transformation, outliers were no longer detected. In addition to 13 water quality variables, month and year were added to the environmental matrix to total 15 variables (Table 26).

Month	Total Kjeldahl Nitrogen+ Nitrate+Nitrite (TKN+NO3+NO2)
Year	Ortho-phosphate (Ortho-P)
Nitrate/Nitrite (NO3/NO2)	Total Phosphate (T-Phos)
Nitrate (NO3)	Total Nitrate+Total Phospate (TN+TP)
Nitrite (NO2)	Temperature
Ammonia (NH3)	Conductance
Total Kjeldahl Nitrogen (TKN)	рН

Table 26. Environmental variables used in analysis of macroinvertebrates.

No difference in macroinvertebrate abundance was found between months (p = 0.695, A = -0.016) or years (p = 0.290, A = 0.020). A NMS ordination with a three-dimensional solution of plots in species space was applied (Final Stress = 2.96, final instability \leq 0.000001, number of iterations = 39) and the solution was rotated so conductance, ortho-phosphate, and ammonia were parallel with axis one, pH with axis two, and year with axis three. The first three axes explained 86% of the variation in the data. A strong correlation related to conductance (r = 0.752), and a moderate correlation related to orthophosphate, (r = -0.516) and ammonia (r = 0.444) was found with axis one. A strong correlation related to pH (r = -0.747) was found with axis two and a moderate correlation related to year (r = -0.501) and orthophosphate (r = 0.555) was associated with axis three (Figure 91 and Figure 92).

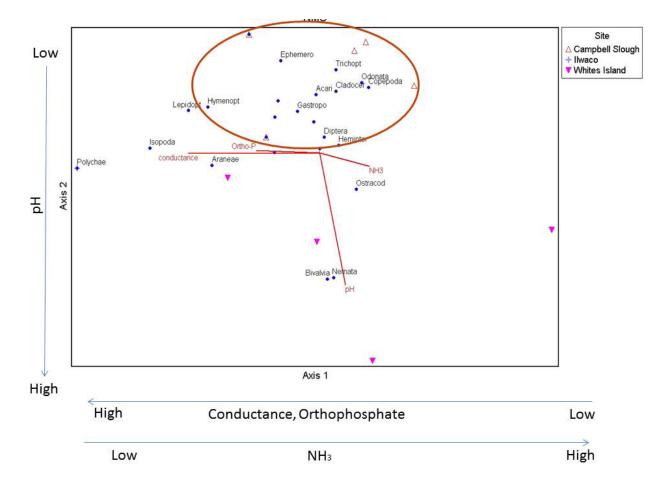


Figure 91. Axis one and two of a NMS ordination of sites in site space. Habitat variables with $r^2 > 0.20$ are displayed with a vector joint plot. Specific macroinvertebrates associated with Campbell Slough are circled.

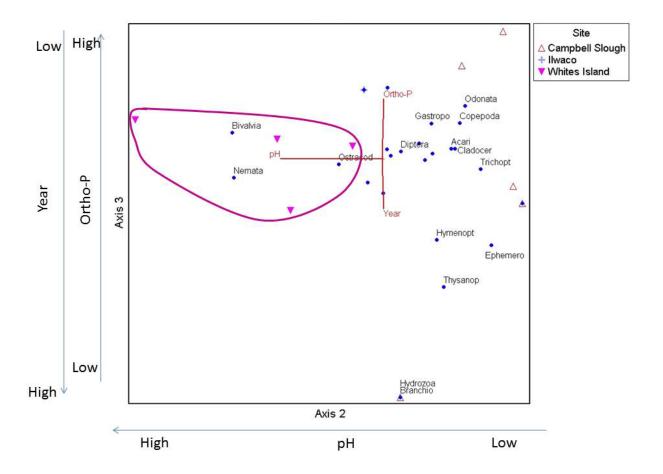


Figure 92. Axis two and three of a NMS ordination of sites in site space. Habitat variables with $r^2 > 0.20$ are displayed with a vector joint plot. Specific macroinvertebrates associated with Whites Island are circled.

Polychae (r = -0.683), Isopoda (r = -0.709), Lepidotera (r = -0.668), Hymenoptera (r = -0.582), Araneae (r = -0.840) were associated with higher levels of conductance and ortho-phosphate and lower levels of ammonia, while Hemipetera (r = 0.588) followed an opposite trend (Figure 91). Acari (r = 0.806), Cladocera (r = 0.719), Copepoda (r = 0.632), Diptera (r = 0.552), Ephemeroptera (r = 0.557), Gastropoda (r = 0.627), Odonata (r = 0.557), and Trichoptera (r = 0.688) were associated with lower pH levels, while Bivalvia (r = -0.714), Nemata (r = -0.747), and Ostrcods (r = -0.594) associated with higher pH levels (Figure 92). Branchiobdellida (r = -0.819), Ephemeroptera, (r = -0.515), Hydrozoa (r = -0.819), Hymenoptera (r = -0.536), and Thysanoptera (r = -0.873) were associated with later years and less Orthophosphate, while Gastopoda (r = 0.544) followed the opposite trend (Figure 92).

Campbell Slough was associated with the specific macroinvertebrate species Acari, Cladocera, Copepoda, Diptera, Ephemeroptera, Gastropoda, Odonata, and Trichoptera (Figure 91). Whites Island was associated with Bivalvia, Nemata, and Ostrocods (Figure 92). Ilwaco was removed from the analysis and sites were grouped by month and year. No difference was found when sites were grouped by month (p = 0.705, A = -0.021) or year (p = 0.335, A = 0.018), although a significant difference was found when site was used as a grouping variable (p = 0.005, A = 0.112).

A NMS ordination with a two-dimensional solution of plots in species space was applied (Final Stress = 7.25, final instability \leq 0.000001, number of iterations = 37) and the solution was rotated so ammonia was parallel with axis one and pH with axis two. The first two axes explained 86% of the variation in the data. A moderate correlation related to ammonia (r = -0.619) was found with axis one and a moderate correlation related to pH (r = 0.499) was found with axis two. Campbell Slough was strongly correlated with Acari (r = 0.789), Cladocera (r = 0.812), Diptera (r = 0.619), and Trichoptera (r = 0.744) and was characterized by lower pH. Whites Island was strongly correlated with Bivalvia (r = -0.784) and was characterized by higher pH.

4.2.3.2.4 Macroinvertebrates and vegetation

A macroinvertebrate abundance matrix and a vegetation matrix were constructed to explore potential correlations. To compare macroinvertebrate abundance and vegetation, the sample units represented in both matrices are provided in Table 27.

Site	Month	Year	Site	Month	Year	Site	Month	Year
Burke Island	May	2011	Secret River	February	2012	Welch Island	June	2013
Campbell Slough	May	2011	Secret River	April	2012	Welch Island	July	2013
Campbell Slough	May	2012	Secret River	June	2012	Whites Island	May	2011
Campbell Slough	June	2012	Secret River	May	2013	Whites Island	June	2011
Campbell Slough	May	2013	Secret River	June	2013	Whites Island	April	2012
Campbell Slough	June	2013	Washougal	April	2012	Whites Island	May	2012
Deer Island	May	2011	Washougal	May	2012	Whites Island	June	2012
Goat Island	May	2011	Welch Island	April	2012	Whites Island	May	2013
Ilwaco	May	2011	Welch Island	May	2012	Whites Island	June	2013
Lemon Island	March	2012	Welch Island	June	2012	Whites Island	July	2013
Lemon Island	April	2012	Welch Island	March	2013			
Lemon Island	May	2012	Welch Island	May	2013			

Table 27. Sites, months and years used in analysis of macroinvertebrate communities and vegetation.

The species matrix was constructed of 34 sample units (rows) and 23 species (column).

Macroinvertebrate tow data were reported in number of macroinvertebrates per meter towed. Samples were collected in both open water habitats and near-shore emergent vegetation habitats, although near-shore emergent vegetation habitats were included in the multivariate analysis since biomass can be 20 times greater in samples collected from near-shore emergent habitats (Sagar et al. 2013). Sites with multiple tows were averaged by month and year. Species of macroinvertebrates with no occurrence (Chilopda and Diplopda) and species with less than 5% of sample units (Branchiobdellida, Clitellata, Decapoda, Newuroptera, Opiliones, Plecoptera, Polychacta, Psocoptera) were removed from the dataset to reduce noise in the dataset without losing much information (McCune and Mefford 2002). Following the removal of species with rare or no occurrence, 23 sample units remained (Table 28).

Acari	Diptera	Nemata
Amphipoda	Ephemeroptera	Odonata
Araneae	Gastropoda	Oligochaeta
Bivalvia	Hemiptera	Ostracoda
Cladocera	Hydrozoa	Thysanoptera
Coleoptera	Hymenoptera	Trichoptera
Collembola	Isopoda	Turbellaria
Copepoda	Lepidoptera	

Table 28. Macroinvertebrates included in the analysis with vegetation.

Initial species abundance data had a range of 3.7 orders of magnitude and the dataset did not contain any outliers. Sorenson distance measure was chosen for this analysis due to high variation of sample units and lack of linearity.

The environmental matrix was constructed of 34 sample units and 114 habitat variables. Vegetation data consisted of percent cover averaged by site. No species outliers were found; however, the very large variation in species can have a strong influence on the outcome of the analysis (McCune and Medford 2002). Thus, species data were relativized to retain the variation in abundance across species units and improve the relationship between the patterns of the original data (Peck 2010).

Macroinvertebrate abundance was found to differ between years (p = 0.018, A = 0.030), but no difference was found between months (p = 0.254, A = 0.011). A NMS ordination with a two-dimensional solution of plots in species space was applied (Final Stress = 12.29, final stability ≤ 0.000001 , number of iterations = 38) and the solution was rotated so species were parallel with axis one and two. The first two axes explained 74.7% of the variation in the data. On axis one, a strong correlation was found with overhanging Pacific willow (SALU*, r = -0.702) and a moderate correlation related to bare ground (BG, r = -0.572), rice cutgrass (LEOR, r = 0.448), creeping jenny (LYNU, r = -0.492), open water (OW, r = -0.523), curly leaf pondweed (POCR, r = -0.533), floating-leaved pondweed (PONA, r = -0.464), and creeping buttercup (RARE, r = -0.495). On axis two, a moderate correlation related to creeping bentgrass (AGST, r = 0.484), (COAR, r = -0.499), tufted hairgrass (DECE, r = 0.478), fowl mannagrass (GLST, r = -0.499), common rush (JUEF, r = -0.499), mixed grass (MG, r = -0.508), western watermilfoil (MYHI, r = -0.499), Himalayan blackberry (RUAR, r = -0.499), and American bulrush (SCAM, r = 0.449; Figure 93).

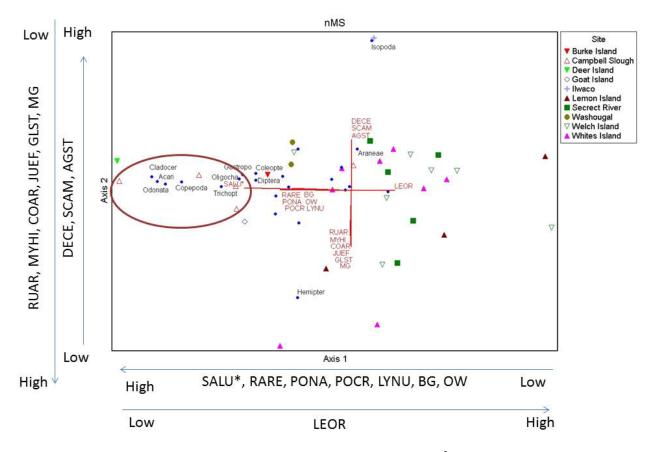


Figure 93. NMS ordination of sites in site space. Habitat variables with $r^2>0.20$ are displayed with a vector joint plot. Specific plant species assemblages associated with Campbell Slough are circled.

Copepoda (r = -0.741), Cladocera (r = -0.682), Diptera (r = -0.682), Oligocha (r = -0.685), Gastropoda (r = -0.514), Odonata (r = -0.534), Trichoptera (r = -0.598), Acari (r = -0.482), and Coleoptera (r = -0.464) were associated with higher percent cover of native and invasive plants (SALU*, RARE, PONA, BG, POCR, OW, LYNU) and lower percent cover of native LEOR. Hemiptera (r = -0.606) was associated with higher percent cover of native vegetation (RUAR, MYHI, COAR, JUEF, GLST, and MG). Araneae (r = 0.461) and Isopoda (r = 0.481) were associated with higher percent cover of both native and invasive vegetation (DECE, SCAM, and AGST).

After removing Ilwaco from the analysis, no significant difference was found when sites were grouped by month (p = 0.239, A = 0.013), but they differed by year (p = 0.017, A = 0.033). When grouped by site, a significant difference was also found (p = 0.0004, A = 0.100).

A NMS ordination with a two-dimensional solution of plots in species space was used (Final Stress = 12.15, final stability \leq 0.000001, number of iterations = 47) and the solution was rotated so species were parallel with axis one and two. The first two axes explained 75.4% of the variation in the data. Curly leaf pondweed (POCR, r = -0.641), common spikerush (ELPA, r = -0.559), creeping buttercup (RARE, r = -0.640), Pacific willow (SALU*, r = -0.666), Carex species (CASP, r = -0.561), creeping jenny (LYNU, r = -0.473), bare ground (BG, r = -0.493), and algae (r = -0.467) were moderately correlated with axis one. Fowl mannagrass (GLST, r = 0.578), soft rush (JUEF, r = -0.578), mixed grass (r = 0.585), western milfoil (MYHI, r = 0.578), Richard's pondweed (PORI, r = 0.495), Himalayan blackberry (RUAR, r =

0.578), morning glory (COAR, r = 0.578), water speedwell (VEAN, r = 0.526), moss (r = 0.511), and standing dead vegetation (SD, r = 0.508) were moderately correlated with axis two.

Campbell Slough was associated with Acari (r = -0.571), Cladocera (r = -0.649), Copepoda (r = -0.675), Odonata (r = -0.635), and Oligocha (r = -0.617) and was characterized by higher percent cover of native and invasive obligate and facultative plants (ELPA, SALU, CASP, POCR, RARE, LYNU). Lemmon Island, Secret River, Washougal, Welch Island, and Whites Island were not associated with specific macroinvertebrates.

4.2.3.2.5 Fish abundance and macroinvertebrate abundance

To explore potential gradients between fish abundance and macroinvertebrates, two matrices were constructed – a fish species matrix and a macroinvertebrate matrix. To compare fish species with macroinvertebrate species, the same sample units must be represented in both matrices (Table 29).

Table 29. Sites, months, and years included in analysis of salmonid abundance to a macroinvertebrate gradient.

Site	Month	Year	Site	Month	Year
Burke Island	May	2011	Washougal	April	2012
Campbell Slough	May	2011	Washougal	May	2012
Campbell Slough	May	2012	Welch Island	April	2012
Campbell Slough	June	2012	Welch Island	May	2012
Campbell Slough	May	2013	Welch Island	June	2012
Campbell Slough	June	2013	Welch Island	March	2013
Deer Island	May	2011	Welch Island	May	2013
Goat Island	May	2011	Welch Island	June	2013
Ilwaco	May	2011	Welch Island	July	2013
Lemon Island	March	2012	Whites Island	May	2011
Lemon Island	April	2012	Whites Island	June	2011
Lemon Island	May	2012	Whites Island	April	2012
Secret River	February	2012	Whites Island	May	2012
Secret River	April	2012	Whites Island	June	2012
Secret River	June	2012	Whites Island	May	2013
Secret River	May	2013	Whites Island	June	2013
Secret River	June	2013	Whites Island	July	2013

The species matrix was constructed from salmon density data reported in number of fish per 1000 m². Three types of fish sampling tows were used, beach seine-PSBS, modified-PSBS, modified block net; however, this does not preclude comparison between sampling sites and types (L. Johnson, personal communication, November 13^{th} 2013). At sites with multiple tows, number of fish was averaged by site, month, and year. Deleting species that occur in less than 5% of the sample units is a useful way of reducing noise in the dataset without losing much information; furthermore, it often enhances the detection of relationships between community composition and environmental factors (McCune and Mefford 2002). Thus, pink salmon and brown trout were removed from the dataset due to non-occurrence

and unmarked rainbow/steelhead trout were excluded due to less than 5% occurrence in sample units. Following the removal of rare species, nine fish species remained (Table 30).

Chinook marked	Coho unmarked
Chinook unmarked	Coho total
Chinook total	sockeye total unmarked
Chum unmarked	cutthroat trout unmarked total
Coho marked	

Table 30. Fish species retained for analysis.

Initial species density data had a range of 2.7 orders of magnitude and the dataset was checked for outliers in the sample units. Ilwaco in May 2011 and Secret River in June 2013 were found to be outliers at 2.6 and 2.0 standard deviations from the grand mean of distances between sample units, respectively, because they had far fewer fish per 1000 m² than other sample units. The dataset was generalized log-transformed to reduce the high degree of variation among species and large range of the data. Following the transformation Ilwaco in May 2011, Secret River in June 2013, and Washougal May 2012 were 2.7, 2.0, and 2.0 standard deviations from the grand mean of distance between sample units, respectively. The dataset was relativized by species to reduce the influence of species with high total abundance relative to species with lower total abundance. Reduction in variation of species improves sensitivity of ordination distance measures when evaluating relationships to environmental gradients (McCune and Mefford 2002). Sorenson distance measure was chosen for this analysis due to high variation of sample units and lack of linearity in the dataset.

The environmental matrix was constructed of 34 sample units (rows) and 25 macroinvertebrate variables (columns). Macroinvertebrate tow data were reported in number of macroinvertebrates per meter towed. Samples were collected in open water habitats and near-shore emergent vegetation habitats; however, near-shore emergent vegetation habitats were used in the multivariate analysis since biomass can be 20 times greater in samples collected from near-shore emergent habitats (Sagar et al. 2013). Sites with multiple tows were averaged by month and year. Species of macroinvertebrates with no occurrence (Chilopoda, Diplopoda, Megaloptera, Mysida, Orthoptera) and macroinvertebrate species which occurred in less than 5% of sample units (Branchiobdellida, Clitellata, Decapoda, fish, Neuroptera, Opiliones, Plecoptera, Polychaeta, Psocoptera) were removed. The environmental matrix was examined for species outliers. Due to its small abundance relative to other macroinvertebrate species, Turbellaria was identified as an outlier species and was removed from the dataset due to its rare occurrence. Following the removal of rare species, 22 macroinvertebrate species remained (Table 31). In addition to 22 macroinvertebrate habitat variables, month and year were added to the macroinvertebrate matrix.

Acari	Coleoptera	Gastropoda	Lepidoptera	Thysanoptera
Amphipoda	Collembola	Hemiptera	Nemata	Trichoptera
Araneae	Copepoda	Hydrozoa	Odonata	
Bivalvia	Diptera	Hymenoptera	Oligochaeta	
Cladocera	Ephemeroptera	Isopoda	Ostracoda	

Table 31. Macroinvertebrate species retained for analysis with fish abundance.

Fish abundance was found to be similar across years (p = 0.062, A = 0.032), but a significant difference was found between months (p = 0.007, A = 0.097). A NMS ordination with a two-dimensional solution of plots in species space was used (Final Stress = 13.15, final instability ≤ 0.000001 , number of iterations = 64) and the solution was rotated so Hymenoptera abundance was parallel with axis two. The first two axes explained 84.4% of the variation in the data. A moderate correlation related to Hymenoptera abundance (r = 0.593) was found with marked Chinook salmon (r = 0.473), unmarked sockeye salmon (r = 0.543) and unmarked chum salmon (r = -0.425; Figure 94).

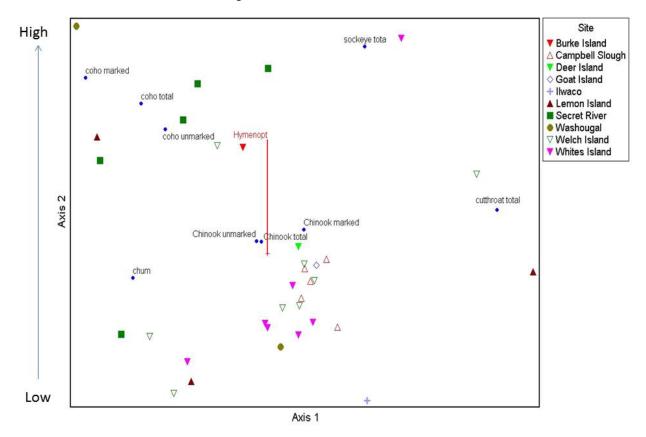


Figure 94. NMS ordination of sites in species space. Habitat variables with r^2 >0.20 are displayed with a vector joint plot.

The environmental characteristics of increasing Hymenoptera abundance is moderately correlated with marked Chinook salmon and unmarked sockeye salmon abundance. Unmarked chum salmon are moderately associated with lower abundances of Hymenoptera compared to other salmonid species.

Sites with a single occurrence of fish capture were removed from the dataset in order to look at group differences. A significant difference was found when site was used as a grouping variable (p = 0.01, A = 0.10). No significant difference was found when year was used as a grouping variable (p = 0.135, A = 0.02), but a significant difference was found when site were grouped by month (p = 0.015, A = 0.097).

4.2.3.2.6 Fish abundance and environmental/species abundance

To explore potential gradients between fish abundance and environmental measures and multiple species abundance, two matrices were constructed – a fish species matrix and environmental/species abundance matrix. To compare fish species with macroinvertebrate species, the same sample units must be represented in both matrices (Table 32).

Site	Month	Year	Site	Month	Year
Campbell Slough	May	2011	Washougal	April	2012
Campbell Slough	May	2012	Washougal	May	2012
Campbell Slough	June	2012	Welch Island	April	2012
Campbell Slough	May	2013	Welch Island	May	2012
Campbell Slough	June	2013	Welch Island	June	2012
Lemon Island	March	2012	Welch Island	March	2013
Lemon Island	April	2012	Welch Island	May	2013
Lemon Island	May	2012	Welch Island	June	2013
Secret River	May	2013	Welch Island	July	2013
Secret River	June	2013			

Table 32. Sites, months, and year included in analysis of salmonid abundance and environmental/species abundance gradient.

The species matrix was constructed from salmon density data reported in number of fish per 1000 m². Three types of fish sampling tows were used, beach seine-PSBS, modified-PSBS, modified block net; however, this does not preclude comparison between sampling sites and types (L. Johnson, personal communication, November 13th 2013). At sites with multiple tows, fish density was averaged by site, month, and year. Deleting species that occur in less than 5% of the sample units is useful way of reducing noise in the dataset without losing much information; furthermore, it often enhances the detection of relationships between community composition and environmental factors (McCune and Mefford 2002). Thus, pink salmon and brown trout were removed from the dataset due to non-occurrence and unmarked rainbow/steelhead trout were excluded due to less than 5% occurrence in sample units. Following the removal of rare species, nine fish species remained (Table 30).

The species matrix consisted of 19 sample units initial species density data had a range of 2.5 orders of magnitude. The dataset was checked for outliers in the sample units. No outliers were found, but due to the high coefficient of variation (CV% = 150) in columns, the dataset was generalized log transformed to reduce the high degree of variation among species and large range of the data. Following the transformation, both variation among species and range of the data was reduced and Sorenson distance measure was chosen for this analysis due to high variation of sample units and lack of linearity in the dataset.

The environmental matrix was constructed of 19 sample unites and 37 environmental/species variables. Environmental variables included average monthly water temperature, mainstem river kilometer, average flow at Bonneville Dam, average site elevation, and site SEV. Macroinvertebrate species and vegetation species cover were added to the second matrix. The top ten macroinvertebrate species weight for the analysis and vegetation species which occurred in more than 50% of sites were chosen for the analysis. In

addition to species density and abundance, species richness and Shannon's species diversity for macroinvertebrates, vegetation, and fish were calculated (Table 33). Species richness is the calculated number of species in a sample unit, while Shannon-Wiener diversity index characterizes species diversity in a sample unit.

Environmental Variables	Vegetation Variables
Temperature	Algae
River Kilometer	Bare Ground (BG)
Flow	Carex lyngbyei (CALY)
Avg Site Elevation	Detritus
Avg marsh elev SEV	drift wrack (DW)
Fish Variables	Eleocharis acicularis (ELAC)
Fish Richness	Elodea canadensis (ELCA)
Fish Diversity Index	Eleocharis palustris (ELPA)
Macroinvertebrate Variables	Juncus oxymeris (JUOX)
Amphipoda	Leersia oryzoides (LEOR)
Cladocera	Litter
Coleoptera	Small wood debris (LWD)*
Diptera	Phalaris arundinacea (PHAR)
Hemiptera	Polygonum persicaria (POPE)
Hymenoptera	Sagittaria latifolia (SALA)
Nemata	small mixed herbs (SMH)
Trichoptera	Symphyotrichum subspicatum (SYSU)
Macroinvertebrate Richness	Plant Richness
Macroinvertebrate Diversity Index	Plant Diversity Index

Table 33. Environmental, fish, macroinvertebrate, and vegetation variables included in the analysis with fish species.

*Small wood debris is characterized by wood found in vegetation sampling quadrats with a diameter <5cm and >25 cm.

No significant difference was found when site was grouped by year (p=0.828, A=-0.023) or when site was used as a grouping variable (p = 0.121, A = 0.066). When sites were grouped by month, a significant difference was found (p = 0.011, A = 0.130). A NMS ordination with a three-dimensional solution of plots in species space was used (Final Stress = 5.62, final instability \leq 0.000001, number of iterations = 48) and the solution was rotated so plant diversity and small woody debris were parallel with axis one (Figure 95). Average site elevation, PHAR, SALA, ELCA, fish species richness, fish diversity, and Hymenoptera were rotated to be parallel with axis two. The first three axes explained 96% of the variation in the data.

Unmarked Chinook salmon (r = -0.864), total Chinook salmon (r = -0.748), marked Coho salmon (r = -0.673), and total Coho salmon (r = -0.631) were moderately correlated with higher percent cover of small woody debris (r = -0.448) and lower plant diversity (r = 0.546). Marked Coho salmon (r = 0.659) were moderately correlated with higher percent cover of ELCA (r = 0.686), Hymenoptera abundance (r = (r = -1.448)) and lower plant diversity (r = 0.546).

0.552), fish richness (r = 0.681), and fish diversity (r = 0.576) and lower percent cover PHAR (r = -0.524) and SALA (r = -0.633) and lower average site elevation (r = -0.485). Marked Chinook salmon (r = 0.600) and Total Chinook salmon (r = 0.715) were moderately correlated with higher fish species richness (r = 0.493).

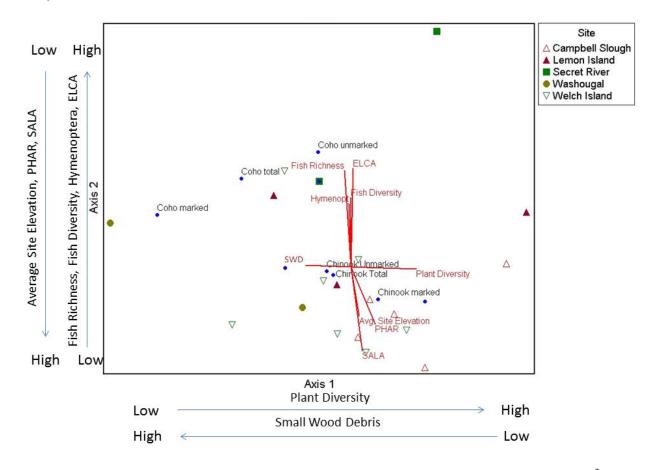


Figure 95. Axis one and two of a NMS ordination of sites in species space. Habitat variables with $r^2 > 0.20$ are displayed with a vector joint plot.

5 Discussion

5.1 Variability

5.1.1 Habitat Structure

5.1.1.1 Temporal Variability

Longer term datasets result in a more clear understanding of habitat changes over time. The temporal variability analysis from this study showed that the average site similarity (a comparison of an individual site's conditions to itself over time), based on vegetation cover, decreased significantly with an increasing number of years between observations. This is in part due to a set of longer-term records (nine years) from sites in the upper river (rkm 145 and 149) where the signal of seasonal and inter-annual hydrologic variability is much stronger (Jay et al. in review). This hydrologic variability has a direct effect on the vegetation cover in up-river locations as shown by a previously conducted trend analysis (Figure 96; Sagar et al. 2014), which indicates that in higher water years, the increase in inundation (as measured by the sum exceedance value [SEV]) results in decreased vegetation cover. Thus, the decrease in site similarity with time is primarily due to hydrologic variability between years at a site rather than a gradual vegetation community shift over time.

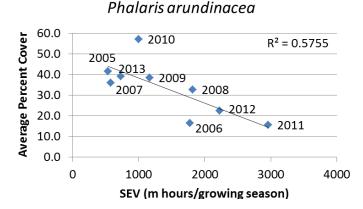


Figure 96. Regression of inundation (SEV) verses average percent cover of *P. arundinacea* at the Cunningham Lake site for the years 2005 to 2013.

At the lower river sites, the conditions over time are more stable, with subtle changes between years. Among these sites, the lowest similarity was observed at the low marsh site at Secret River. The reason for the difference between the cover measured in 2008 and that measured in 2012 and 2013 is not clear. The difference could be related to variation in winter flooding from the local watersheds as opposed to Columbia River hydrologic variability. The site is one of the lowest elevation sites we have surveyed in the estuary, located at the emergent vegetation elevation threshold, and perhaps slight variations in hydrology and sediment effect these sites more dramatically than higher elevation sites.

5.1.1.2 Spatial Variability

Spatial variability among tidal marsh sites in the LCRE was focused on vegetation cover. We did not have enough data for other habitat metrics measured as part of the EMP to adequately test the variability in the same way the vegetation cover data were analyzed.

Spatial variability among tidal marsh sites in the LCRE is dependent on location of the sites relative to the hydrologic gradient of the river and on location of the sites relative to each other. We found that site similarity based on vegetation cover decreases with increasing between-site distance. Similarity was generally highest within EM zones, enforcing the establishment of these zones (Jay et al. in review, modified from Sagar et al. 2012). Higher variability occurred in some EM zones indicating the need for additional sampling to determine if additional zones or stratification within current zones are necessary (e.g., tributaries vs. mainstem). Future habitat monitoring efforts could use EM zone rather than hydrogeomorphic reach to stratify vegetation sampling.

Tidal emergent marsh habitats provide rearing and refugia habitat (i.e., habitat capacity) for juvenile salmonids during seaward migration. Spatial and temporal variability in vegetation cover was identified across and within some EM zones, driven primarily by hydrological processes, particularly in the upper reaches. There is a need for understanding the range and source of natural variability in these relatively undisturbed habitats in order to direct and provide end points for restoration and action effectiveness monitoring efforts. Continued long term sampling could account for more years of fluctuating hydrological conditions and aid in determining the range of variability of existing vegetation characteristics in the lower river.

5.1.2 Fish

5.1.2.1 Temporal Variability

Power analyses indicate that we have conducted sufficient sampling to identify seasonal and spatial trends in fish community and salmon occurrence at the EMP sites. Salmon species diversity was highest in April and May, reflecting the presence of chum, Chinook and coho salmon at this time, but diversity was also surprisingly high in December, although data for this month were limited. We observed a succession of salmonid species throughout the system, with peaks in occurrence of chum salmon in the winter-spring (February to April), Chinook salmon in May-June (coinciding with the freshet), and coho salmon in the fall and winter (September-December). These observations are consistent with previous studies that documented the highest densities of chum (and highest proportion of catches where chum were present) in late winter and early spring during their typical season of outmigration (Myers 1980; Johnson et al. 1997; Roegner et al. 2008; Johnson et al. 2011). Unmarked Chinook salmon densities peaked during May-June, although they were present throughout the year, in contrast to marked Chinook salmon, which were present only between April and July (with a peak in May-June). The highest densities of marked coho salmon coincided with hatchery releases (Columbia River DART: http://www.cbr.washington.edu/dart/hatch.html), which peaked in May.

In general, the juvenile Chinook salmon genetic stock composition was similar to that reported by others (Bottom et al. 2008, Sather et al. 2009, Johnson et al. 2011). Very low proportions of Chinook salmon from outside the Lower Columbia River ESU were observed in the winter-spring (February and March), with increasing proportional representation over the sampling season. By the winter (November-

December), higher proportions of non-Lower Columbia ESU stocks (primarily Upper Willamette Spring Chinook salmon) were found, but data for these months are limited.

5.1.2.2 Spatial Variability

A spatial spread in peak densities and proportional representation of the different salmon species were observed in the EMP data. Chum and unmarked juvenile Chinook salmon were found at the highest densities in reaches downstream of the Portland metropolitan area (Chum salmon mainly in Reach A, Chinook salmon in Reaches B-E). Although we did not observe high densities of Chinook salmon in the emergent marsh at Ilwaco Slough, located in Reach A, Roegner et al. (2008) reported high densities of Chinook salmon at various other sites in Reach A, including Clatsop Spit, West Sand Island, and Point Adams, representing locations nearer to the river mainstem than Ilwaco Slough, which may account for the difference in Chinook salmon observations between the two studies.

This study found intriguing patterns in the upper reaches of the LCRE. In contrast to chum and unmarked Chinook salmon, marked Chinook salmon had the highest absolute and relative abundances in Reach F, closer to sites of their release, as well as the highest stock diversity. Non-native species (potential competitors to juvenile salmonids) as well as higher abundances of predator species were observed in Reaches F-H, similar to other studies (Roegner et al. 2008; Sanderson et al. 2009; Sather et al. 2009; Johnson et al. 2011). Salmon species diversity was greatest in Reach H, upstream of the Willamette-Columbia confluence, and coho salmon (marked and unmarked) had peak densities in Reaches G and H. The percentage of Chinook salmon belonging to stocks other than those that are part of the Lower Columbia ESU increased in upriver reaches, with highest proportions in Reaches G and H, consistent with other studies (Bottom et al. 2005; Sather et al. 2009; Roegner et al. 2010; Johnson et al. 2011; Sagar et al. 2013).

Marked fish tended to be larger, showing less variability in size over time, compared to unmarked Chinook salmon, similar to other work (Bottom et al. 2005, 2008; Roegner et al. 2008, 2010; Sather et al. 2009; Johnson et al. 2011; Sagar et al. 2013). Overall, condition factor for juvenile Chinook salmon was higher in marked compared to unmarked fish. Unmarked fish showed an increase in size and condition factor between February and July and condition factor declining in the winter months. Lipids did not vary with season in unmarked fish, although higher values were observed in Reach F (Campbell Slough) compared to other sites. In marked fish, lipid content did vary, with occasional high values presumably due to capturing of fish soon after hatchery release. High lipids in these fish tended to coincide with proximity to hatcheries (i.e., high values were observed in Reach H). Genetic stock contributed significantly to variability in lipid content, as well as fish size (length), with the largest fish being Willamette River spring Chinook salmon. However, the number of fish belonging to non-Lower Columbia ESU stocks was relatively small, making it difficult to comprehensively evaluate differences in growth, conditions and habitat use across stocks and to differentiate them from impacts of hatchery rearing. Additionally, marked fish and fish from various genetic stocks vary in their abundance and distribution both seasonally and by sampling site, resulting in gaps in spatial and temporal information.

While we have captured broad seasonal and spatial patterns from EMP data analyzed thus far, our ability to detect temporal trends in many parameters is limited. In particular, we have seen little evidence of increasing or decreasing trends in fish variables (e.g., size, condition, lipids, growth rates) at the multi-

year sampling sites. Although significant variability among years was observed at some sites for certain parameters, we have generally seen a fair degree of consistency in our observations from year to year. However, of the six trend sites, only two (i.e., Campbell Slough and Whites Island) have been sampled consistently throughout the sampling season for more than a few years. While Franz Lake has been a trend site since 2008, we have salmon occurrence data for May and June (the peak months of Chinook salmon density) in 2008 and 2009 only, as a result of the difficulties in sampling this site during high water years (e.g., 2011, 2012). We have similar problems at Campbell Slough during high water months in 2011 and 2012. An apparent declining trend in Chinook salmon density at Franz Lake is more likely due to our inability to sample this site during the spring and early summer months than to actual changes in habitat use by these fish. Another difficulty encountered with these data is that because not all sites were sampled over the same time period, the ability to simultaneously evaluate the effects of sampling year and sampling site is limited. This makes it difficult to identify temporal changes that might be influencing sites throughout the estuary.

Sampling throughout the lower river provides an important opportunity to detect spatial changes that may occur along the longitudinal estuarine tidal freshwater habitat gradient and whether differences in fish metrics correlate to differences in habitat capacity in the lower river. However, knowledge gaps still remain, including the extent of emergent wetland habitat use, physical condition, and growth of non-lower Columbia River ESU salmonid stocks as they outmigrate through the lower river. Continued long term sampling in the freshwater tidal habitats of the lower Columbia River will expand on past fisheries work conducted in the lower reaches (Bottom et al. 2005, 2008, 2011; Roegner et al. 2010, 2012), forming a more comprehensive annual dataset. Such data are essential for identifying limiting factors affecting juvenile salmonid survival, fitness, and habitat use, and for informing salmon recovery efforts in the lower Columbia River.

5.1.3 Invertebrate prey availability and Chinook salmon diets

While the abundance of salmon invertebrate prey varies by site, month and habitat (i.e., emergent vegetation vs. open water), habitat is the most important factor explaining variation in invertebrate densities and biomass. The densities and biomass of invertebrates were consistently higher in the emergent vegetation (EV) habitats compared to the open water (OW) habitats, not only for taxa more commonly associated with benthos such as dipterans and amphipods, but also among pelagic species such as copepods and cladocerans. There was little correlation between the abundance of prey from EV and OW habitats collected during the same sampling event for dipterans and amphipods (n = 60 pairs; r = 0.28 for all invertebrates, r = 0.29 for Diptera, r = 0.24 for amphipods), but somewhat higher correlations for taxa less associated with the benthos (cladocerans and copepods), suggesting there may be more movement of these taxa between habitat types.

Prey densities and prey biomass in the EV habitat showed seasonal trends, in that they increased throughout the spring/summer months, which is coincident with an increase in the cover of live vegetation (grass and other vegetation; $r^2 = 0.06$). However, the influence of percent cover on Diptera density was minor in comparison to the effects of month and site. Similar to the fish sampling, only two EMP sites (Campbell Slough and Whites Island) were sampled in similar months over a period sufficient to evaluate annual trends (i.e., four years). Based on these two sites, there was little evidence of annual variability on invertebrate abundance.

Diptera is by far the most represented insect order in the diets of juvenile Chinook salmon, both on the basis of abundance and of biomass, with amphipods being the second most abundant taxa consumed; However, since the data did not meet the assumptions of most parametric statistical analyses, it was not possible to identify the factors that explain differences in diets among sites, years and months sampled.

5.2 Food Web Synthesis

5.2.1 Productivity and Trophic Pathways

The role that food availability in the tidal freshwater and estuary play in salmon survival is an area of recent focus for the region (ISAB 2011; Naiman et al. 2012; Bellmore et al. 2013). Major gaps remain in our understanding of the factors that drive salmon prey populations and the food web they depend upon. The contribution of pelagic phytoplankton (mainly diatoms, Class Bacillariophyceae) to total primary production in the system has increased over the past several decades, due to the creation of impoundments associated with hydropower operations. This has increased water clarity and allowed greater light penetration through the water column as sediments become deposited in impoundments behind the dams, increasing primary production by phytoplankton in the river (Sullivan and Prahl 2001). At the same time, reductions in marsh habitats and reduced channelization and geomorphic complexity in the lower river has led to a reduction in the inputs of macrodetritus from vascular plants, which have traditionally fueled salmon food webs. At different times of year, the juvenile salmon diet consists of dipterans, crustaceans (cladocerans and copepods, both planktonic taxa), and other terrestrial and aquatic insects (Maier and Simenstad 2009). Some of these taxa would have traditionally fed on macrodetritus but now phytoplankton may play a larger role in their diet. Phytoplankton now plays a key role in river food webs since the macrodetrital sources of organic matter have been reduced. Such changes to the basis of the food web could result in conditions advantageous for pelagic feeding species (e.g., American shad, Alosa sapidissima) rather than epibenthic feeding species such as juvenile salmonids (Bottom et al. 2005). Monitoring the seasonality and species composition of phytoplankton and understanding their pathways in the salmon food web (for example through grazing by zooplankton and consumption as phytodetritus by macroinvertebrates) is a critical component of ecosystem monitoring in post-hydropower salmon food webs.

For salmon in particular, the role that phytoplankton play in fueling growth of macroinvertebrates (and zooplankton, which are also salmon prey) should be elucidated more clearly. For this, it is important to know if and how shallow water habitats concentrate organic matter to feed salmon prey. In other locations, benthic invertebrates have been shown to consume significant amounts of phytoplankton, along with woody debris and plant matter (Chessman 1986). There appears to be a difference among chironomid species as to which proportion of plankton versus plant matter is preferred. Therefore, an effective monitoring program requires a fundamental understanding of (1) links between environmental conditions and population dynamics of primary producers, (2) links between primary producers and salmon prey, either macroinvertebrates or pelagic zooplankton, and (3) the effect that different prey items have on salmon physiological condition and, potentially, diversity.

The study of phytoplankton and zooplankton populations is important for a number of reasons, which are outlined below:

- Phytoplankton control water quality by producing oxygen, altering pH (by taking up CO₂ through photosynthesis and modifying carbonate chemistry), and reducing oxygen upon decay (when respiration by bacteria increases)
- Phytoplankton themselves are food for salmon prey, particularly benthic species or those found in association with aquatic plants
- Phytoplankton are food for zooplankton, which are also salmon prey
- Zooplankton are important at certain times of year as prey items for salmonids
- Zooplankton can keep phytoplankton stocks low, reducing threat of enhanced respiration upon algal decay

Finally, it is important to understand all levels of the food web in order to 1) assess natural conditions at sites and across the ecosystem, 2) assess what juvenile salmon are eating at the site and ecosystem scale, and the conditions that limit or improve prey resources, 3) evaluate whether prey available to salmon are what they historically would have eaten, whether there has been a shift in prey available as a result of anthropogenic impacts and whether any shifts that have occurred are detrimental to salmon growth and survival, and 4) evaluate if there is sufficient prey available to juvenile salmon and other aquatic species using the site, or ecosystem throughout each year or if the site or ecosystem has reached its carrying capacity.

At the lowest level of the food web, algal species composition can affect site conditions, by reducing dissolved oxygen levels or releasing cyanotoxins, to make the site prohibitive or harmful to juvenile salmon or their prey base or by improving it, such as providing food for zooplankton or benthic macroinvertebrates. Salmon exhibit clear preferences in prey and in turn, prey are highly influenced by their own food resources and site conditions. A visual representation of food web dynamics in emergent wetlands in the LCRE are shown in Figure 97.

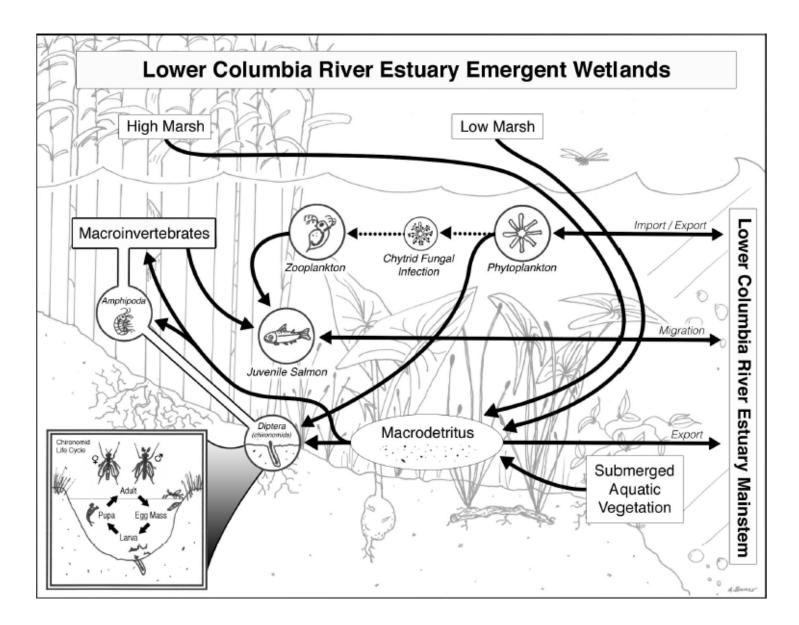


Figure 97. Conceptual model of food web interactions within Lower Columbia River estuary emergent wetlands.

Overall, isotope model results suggest that POM is likely a major contributor to the diets of Diptera (chironomids) midges in May, which are consumed by juvenile salmon throughout the lower river and estuary. Vegetation contributes to diets of chironomids in June and July and to the diets of amphipods, (*Corophium spp.*, consumed by salmon in the lower reaches, A-C) throughout the spring and summer.

The importance of POM in the diets of chironomids collected in the spring before the spring freshet and then a shift to a more mixed (terrestrial and fluvial-derived) diet later in the season during 2010 - 2012sampling is consistent with the findings of Maier and Simenstad (2009); although, we used a different model for the analysis, included samples from a larger spatial range (including upper reaches), had a larger sample size of macroinvertebrate prey, and used different types of tissue samples from salmon. The EMP showed the abundance of phytoplankton in the Columbia River was high in the spring before the freshet (Sagar et al. 2013), but decreased following the freshet. Further, the observed shift to a more strongly macrodetritus-based isotopic signature later in the season for chironomids may reflect a requirement for microbial processing of the detrital matter prior to consumption. In general, fluvial phytoplankton are generally thought to have a high nutritive value and are thus readily available to consumers (Lamberti and Moore 1984; Campeau et al. 1994) in contrast to vascular plant detritus which is thought to require processing ("pre-conditioning") by microbes (Moran et al. 1988) or fungi (Bärlocher and Kenrick 1975). However, it is not known whether chironomids opportunistically forage on phytoplankton when it is available in the spring (even though they would otherwise consume macrodetritus) or if chironomids are selecting for phytoplankton due to a limited availability of macrodetritus in the upper river.

In addition to EMP findings that showed organic matter sources contributing to chironomid diets at a single site varied by month, Maier and Simenstad (2009) also found variability in the dominant organic matter sources contributing to chironomid diets at different sites (in lower river reaches, A - C) during the same month. Our results were also consistent with their findings in that amphipods appear to be primarily supported by vascular plants, although benthic macroalgae and aquatic plants can also be important for some months and sites. Given that fish habitat use across the different sites sampled in the EMP varied substantially (particularly between marked and unmarked fish), it is important to better understand the factors that drive both spatial and temporal differences in organic matter production and consumption. The continuous data collected from the two mainstem sites at River Mile 53 and River Mile 122 clearly illustrate a suite of phytoplankton "blooms" of varying magnitude throughout the spring, summer, and autumn months. Preliminary analysis using results from the EMP suggest that river discharge is an important factor driving changes in the magnitude of the phytoplankton population.

Likewise, vegetation detritus production is tied to variation in river discharge. Detritus production varied between sites, with a lower amount of detritus production occurring in the fluvial dominated, upper river sites. This spatial trend is likely due to the effects of the spring freshet on the upper river sites, particularly the two relatively high water years that occurred in 2011 and 2012, which reduced the vegetation cover and biomass production in the upper river sites during those years (see Figure 96). It is not known whether the higher ratio of plankton to vascular plant detritus seen in the consumer (chironomid) diets in the upper river sites was due to the abundance of phytoplankton (and opportunistic feeding) or low vegetation detritus production in the upper river sites during these study years, in combination with less wetland area in this reach, or a combination of factors.

Phytoplankton populations were invariably dominated in the spring by diatoms (Class Bacillariophyceae). Diatoms are generally lipid-rich, fast growing organisms that fuel efficient growth of secondary producers (primary consumers). The primary species was *A. Formosa*, a very large, colony-forming diatom and is generally thought to escape consumption by zooplankton because of the large size of the colonies. However, we have documented – in part through the EMP – that this species is prone to heavy parasitism by aquatic fungi, which are broadly considered to be highly nutritious for large and small zooplankton species. Thus, parasitism of dominant primary producers could, in fact, increase the efficiency of consumption of carbon, transferring it to higher trophic levels. This process, termed the 'mycoloop', is not well understood and represents a potentially important and overlooked contributor to aquatic food webs.

During the spring bloom period in the river, the zooplankton community was composed mainly of small species, likely incapable of consuming large, colonial diatoms. Later in the season, larger crustacean species increased in abundance, particularly in areas with slower flushing such as Campbell Slough. In addition, fluctuations in the population size of fluvial primary producers could be related either to changes in river discharge (increased discharge events produced a dilution effect in the biomass) or changes to elevated rates of herbivory by zooplankton or fish. In the late spring/summer, events where a rapid demise of phytoplankton populations was observed were likely caused by increased grazing pressure (suggested by a decline in the concentration of chlorophyll *a* and primary production without a decrease in the net ecosystem metabolism observed, using dissolved oxygen data from the in situ sensors).

Data on plant production from marshes in the LCRE is limited. A comprehensive marsh primary production study was conducted in the lower portion of the estuary as part of the Columbia River Estuary Data Development Program (CREDDP; MacDonald 1984). The EMP study found similar quantities and patterns of marsh primary production to the previous study, with high marsh having greater plant biomass production than low marsh. In addition, data collected as part of the EMP on marsh plant biomass production provides data from the submerged aquatic vegetation (SAV) stratum, provides trend data over multiple years, and for the first time provides data from sites sampled in the upper reaches of the river. In addition, the EMP study is providing data on species level primary production and the resulting detritus potential.

In addition to historical changes in detritus production in the estuary due to diking of wetlands and other large-scale land conversion disturbances for urban, industrial, and agricultural development, changes have undoubtedly occurred due to a dramatic increase in the non-native invasive reed canarygrass (*P. arundinacea*). The effect of this species on the food web is uncertain, however results from this study indicate that the detrital contribution from reed canarygrass is potentially much less (average = 291 g/m^2) than native species such as Lyngby's sedge (*Carex spp.*, average = 1021 g/m^2). This difference is likely due in part to differences in the breakdown of the two species, with *P. arundinacea* having a greater amount of standing stock that remains between years. However, the detrimental effect of the prolonged inundation on *P. arundinacea* that occurred during the study years (see Figure 96) must also be taken into account. The *Carex spp*. that was sampled in this study only occurs in the lower river sites where flooding effects are not observed so prominently. Although the spatial pattern of lower detrital contribution in upper river sites was perhaps more pronounced in the high water years of this study, the high cover of *P. arundinacea* in the upper river areas could also be contributing to low detrital levels. Restoration of

wetlands in the upper reaches of the river, with a focus on native species establishment, could potentially increase the amount of macrodetritus production in this area.

5.2.1.1 Limitations and Sampling Recommendations

5.2.1.1.1 Stable Isotope Analysis

In some cases, it was difficult to determine the relative proportions of some organic matter sources in invertebrate diets using stable isotope analysis because the isotopic signatures of the organic matter sources were often too similar to one another. In addition, for unmarked salmon, we only had two potential food sources, chironomids and amphipods, because those invertebrates were selectively sampled for based on stomach content analyses from earlier years of this study. Therefore, the SIAR model estimated results based on the two food sources, although amphipods were an unlikely food source for juvenile salmon at upper reach sites. While the SIAR model indicated that chironomids become increasingly important in juvenile Chinook salmon diets during the spring and early summer, the higher δ^{13} C and δ^{15} N amphipods likely served in the model as a substitute for the influence of higher δ^{13} C and δ^{15} N marine-derived maternal signatures that were passed on to the egg. Therefore, future model efforts could be improved by the addition of other invertebrate taxa that were present (although to a lesser extent than chironomids and amphipods) in juvenile Chinook salmon stomachs.

It appears from our data that the muscle tissues of many of the salmon sampled had not approached a new equilibrium from reflecting maternal influence to reflecting utilized environmental food sources until the juveniles reached at least 5 – 6 grams (for unmarked salmon) or \geq 6 grams (for marked salmon) of total body weight (Figure 86 and Figure 87). The new equilibrium is determined based on a leveling out of the δ^{15} N. Unmarked juvenile Chinook salmon could be approaching equilibrium (leveling out of δ^{15} N that shows the switch from maternal to environmental food sources) at smaller body weights because they started eating environmental food sources earlier than marked fish, which fed on hatchery food early in life. Analysis of muscle from larger juveniles would be more useful in distinguishing environmental food sources.

Because δ^{15} N is an indicator of trophic position (it typically increases by ~3.4‰ per trophic level), the decrease in muscle δ^{15} N with later month of fish capture (and the continuation of that trend in mucus, which reflects more recent food sources) indicates that there is a shift from higher δ^{15} N food sources (i.e., foods that are from a higher trophic level) to lower δ^{15} N food sources. The higher δ^{15} N values of smaller fish caught earlier in the season (before June-July) likely reflect the high δ^{13} C and δ^{15} N marine-influenced maternal inputs. Bilby et al. (1996) reported high δ^{13} C and δ^{15} N signatures in eggs and muscle from spawned Coho salmon compared to subyearling Coho in western Washington. However, data reporting δ^{13} C and δ^{15} N values of eggs or spawning adult Chinook salmon could not be found in the literature. For marked salmon, the pattern likely also reflects a shift from high δ^{15} N hatchery foods, which are composed of fish meal and fish oils to lower δ^{15} N food sources found in the environment (invertebrates). There was much more variability in carbon and nitrogen isotope signatures in muscle from salmon caught in June and July compared to relatively tightly clustered values in May, probably reflecting the shift in diet from maternal to environmental food sources.

It may not be possible to collect muscle samples from larger salmon at these sites, since out-migrating juveniles typically move out of shallow water habitat sites by the time they are suitably sized. Therefore,

analyzing stable isotopes of carbon and nitrogen from epidermal mucus from all juvenile Chinook salmon collected throughout the sampling season could provide more useful information than analysis of muscle tissue, particularly for fish caught early in the season (prior to June).

5.2.1.1.2 Vegetation Primary Productivity

Sampling in the upper reaches of the river occurred in years that were strongly affected by high inundation levels. The results indicate that two high water years were followed by a "recovery" year that was similar in cover to previous years (Figure 96), however the plants were smaller and biomass levels were similar to those collected during the high water years. While this timing was fortuitous to evaluate the effects of high water, it provided limited data on the vegetation conditions during average or lower water conditions. Additionally, the two sites that were sampled in the upper reaches responded differently to the high water conditions, with the uppermost site experiencing a shift in cover and biomass production to a more aquatic species, while the other site exhibited a reduction in cover and biomass with no other species filling the niche. The inundation was likely not high enough at the latter site to allow a shift to occur over the time frame of the high water. Further limiting the results from the upper reaches, was the occurrence of cow grazing at one of the sites during the 2012 sampling year. Overall, more sampling at additional sites in the upper reaches is necessary to evaluate spatial patterns in this part of the river, to further evaluate the effects of reed canarygrass in the upper reaches, and to determine the macrodetritus potential from this reach in average or low water years.

5.2.2 Mainstem conditions

Several years of observations from RM-53 (Reach C) allow for some generalizations to be made concerning the biogeochemistry and water quality of the mainstem lower Columbia River. One important observation was that water temperatures above the recognized threshold for suitable salmon habitat (Bottom et al. 2011) occur each summer in the mainstem portion of the river. The collection of highresolution data allows for an exact tally of the number of days above 19°C. The data reveal that 2009 and 2013 had significantly more days exceeding this threshold than did 2010 or 2012. The sensor platform is located at > 20 m deep and the water column at this site is well mixed. Therefore, localized temperature gradients do not develop at this site and the sensor data are representative of the whole water column.

One important correlation observed every winter at RM-53 was an increase in river discharge and the concomitant increases in nitrate concentration, turbidity, and CDOM (colored dissolved organic matter) that persist for days to weeks. These episodic discharge events were typically caused by large storms from the Pacific Ocean that resulted in significant precipitation in the Columbia River watershed. Furthermore, these events were more closely associated with increased discharge in lower Columbia River tributaries compared to the flow of the mainstem measured at Bonneville Dam, as illustrated by the difference in discharge between RM-53 and Bonneville Dam. This pattern can be partly attributed to the runoff that directly results from precipitation in the lower elevation regions of the lower Columbia River, whereas precipitation at higher elevations (above Bonneville Dam) adds to the region's snowpack and does not immediately contribute to river discharge. In addition, regulation of discharge by Bonneville Dam (and dams upstream of Bonneville) acts to decouple winter precipitation from discharge. The Willamette River (the largest tributary in the lower Columbia River) has fewer dams and its watershed has many low elevation regions; therefore, this tributary is likely an important source of water and materials detected at RM-53 during these episodic events.

Nitrate flux at RM-53 was determined for the 2009-2010 water years to be approximately 47,000 tonnes nitrogen, occurring mostly during the winter and before the spring freshet. This value agrees with historical estimates by the USGS (see Aulenbach 2006) using a more coarse temporal resolution for estimating fluxes that indicate the Columbia River flux varied between 32,000 - 117,000 tonnes per year during 1975 - 2002. These values are similar to the Susquehanna River (29,000 - 106,000 tonnes yr⁻¹) and an order of magnitude smaller than the Mississippi River (495,000 - 1,820,000 tonnes yr⁻¹) for the same time period. Both the flux in the Susquehanna and the Mississippi rivers are associated with deleterious environmental conditions in their estuaries related to eutrophication; however, these same issues are less apparent in the Columbia River estuary and the coastal ocean. The reasons why the lower Columbia River does not show more signs of eutrophication are likely due to the physical processes that keep water residence times low and the seasonal timing of the nitrate flux, which occurs during winter when algae growth conditions are unfavorable. However, some signs of localized eutrophication are evident, for example in Campbell Slough (high cyanobacteria abundance) and Reach A (low dissolved oxygen concentrations).

Another important observation from the mainstem river measurements is that fluvial phytoplankton exhibit seasonal patterns consistent with the general temperate ecological pattern observed for freshwater and marine primary production. The temperate zone pattern typically includes a spring bloom in phytoplankton caused by increased daylight that relieves light limitation, followed by a bloom crash caused by nutrient exhaustion. The summer period is characterized by a lower biomass of algae that grow using recycled nutrients, and a fall bloom that results from additional nutrient inputs from late season storms. The Columbia River phytoplankton population exhibits many of these general characteristics, with a few exceptions. It does not appear that nutrients lead to a bloom crash during the spring; rather the spring freshet can restrict phytoplankton biomass, likely as a result of high turbidity and high turbulence. Also, "flushing" action that is typical in riverine dominated estuaries is apparent, whereby seasonal algal blooms are washed out to more marine-influenced waters. Summer blooms are associated with low nutrients, but it remains unclear what limits phytoplankton biomass. Since nitrate always remains above 5 μ M, it is likely another nutrient, such as phosphate, that limits growth rate. Results from this study demonstrate that this is the case, in the tidal freshwater portion of the lower Columbia River.

Net ecosystem metabolism measurements corroborate the chlorophyll *a* measurements and indicate that primary production is highest during spring and summer in the mainstem portion of the river. Rates of primary production compared to estimates of biomass suggest that the doubling time of algal biomass is on the order of days (data not shown). Since biomass does not continuously increase during the same periods of high positive primary production (especially in summer), there must be considerable grazing and transfer of primary production into the food web of the river and coastal zone. These observations of algal abundance and phytoplankton growth are in agreement with earlier studies that clearly show riverine phytoplankton are abundant (Sullivan et al. 2001, Small and Prahl 2004) and that the Columbia River is likely 'greener' than similar large rivers of the world. Indeed, some of the results of our mainstem monitoring in chlorophyll *a* levels demonstrate a potential issue with nutrient enrichment. For example, in Oregon water quality impairment is determined when chlorophyll *a* levels are greater than 15 μ g/L for three consecutive months. Our results from both RM-53 and RM-122 demonstrated this. This should be explored further, including the severity, frequency, timing and magnitude of phytoplankton blooms, and sources of pollutants causing increased blooms, if a consistent and sufficiently significant pattern is found.

The flux of particulate organic carbon (POC) associated with phytoplankton measured at RM-53 represents an important source of organic carbon to the saltwater region of the Columbia River estuary (Reach A). Unlike the nitrate flux, which occurs mainly in winter, the POC flux occurs in spring and summer when conditions in the coastal zone are more biologically active. The water column of Reach A is typically considered to be net heterotrophic – that is, due to the POC flux from the river there is more organic carbon respired in the ecosystem than is produced by photosynthesis (Small and Prahl 2004), leading to a net drawdown of water column dissolved oxygen. In addition, Reach A is also influenced by low oxygen oceanic waters moving into the estuary through tidal exchange (e.g., Roegner et al. 2011). These low oxygen water masses are predicted to worsen due to climate change and therefore low oxygen conditions in Reach A will persist. The added influence of POC from the Columbia River is thus an important variable in understanding the overall hypoxic conditions of Reach A.

An important addition to the EMP in 2013 was the installation of a mainstem observatory platform upstream of the Willamette-Columbia confluence at RM-122. The biogeochemical measurements in the mainstem Columbia River from this platform provide important data upstream of the Willamette River, and when compared with the RM-53 platform it can be used to better understand how conditions change throughout the entire lower river and estuary. For example, water temperatures between the two sites were found to be nearly the same at all times of year, including during the warmest summer periods. This observation implies that local heating and cooling has no influence on the mainstem water temperature, and that the Willamette and other tributaries do not change the water temperature of the Columbia River as measured at RM-53. Instead, water temperature follows seasonal climate conditions and is likely a result of upstream processes. The mainstem observations indicate that water temperatures above the recognized threshold for suitable salmon habitat (19°C; Bottom et al. 2011) occur each summer in the mainstem portion of the river. Temperatures above 19°C occurred for more days in 2013 than in the previous two years, a result that is correlated to lower discharge levels associated with the spring freshet as compared to 2011 and 2012. Given that the temperature between the two sites is similar at all times of the year and that there is no gradient in temperature with water depth, we conclude that the observed periods when temperature surpasses 19°C represent the *lowest* temperatures in the mainstem lower Columbia River for these time periods. Therefore, if salmonids are seeking cold water refuges during this time, they would need to be out of the mainstem Columbia and likely associated with localized regions of cold water input, such as tributaries, deltas or underground springs.

In contrast to temperature, other water quality indicators change significantly between upstream and downstream of the Willamette River. For example, fluxes and concentrations of CDOM, nitrate, and turbidity increase during winter storms at RM-53 but show little or no response at RM-122. This observation supports earlier evidence that winter storms cause increased precipitation and discharge to the lower Columbia River, but upstream of the Willamette River confluence the discharge is not significantly affected, either as a result of hydropower management or because precipitation falls as snow and therefore does not lead to increased runoff. The difference between fluxes at the two sites can be precisely quantified by summation of the daily nitrate flux calculation (i.e., from the data presented in Table 34). For the period 09/2012-09/2013 the nitrogen flux of nitrate at RM-53 was 64.6 thousand metric tons. The nitrogen flux (associated with nitrate) measured for the same time period at RM-122 was 34.8 thousand metric tons. Therefore, a 46% increase in nitrogen flux occurred downstream of RM-122. This finding has significant implications for predicting future water quality scenarios in the lower Columbia River and estuary, since tributaries are responsible for approximately half of the total nutrient input to the lower

river nutrient budget. Similar calculations were made for other important biogeochemical parameters (Table 34). The data show similar results for turbidity (61% increase) and a lesser influence of CDOM (26% increase). The smallest change was in the chlorophyll *a* flux (10% increase), which reflects the different biogeochemical factors influencing chlorophyll *a* concentrations compared to the other parameters (discussed below). We can attribute most of the increased flux in Table 34 to the episodic events that occurred during winter of 2013; therefore, these events have a significant contribution to the annual flux of material to the estuary and ocean. Similar findings have been reported for many of the smaller mountainous rivers in the Oregon coastal range (Wheatcroft et al. 2010) and suggest that year round monitoring is critical to understand movement of materials through rivers in the Pacific Northwest.

Table 34. Calculation of % increase in fluxes between RM-122 and RM-53 for the time period 09/2012-09/2013.

	RM-122	RM-53	% Increase
Nitrate	3.5E+04	6.5E+04	46
Turbidity	3.9E+11	9.9E+11	61
CDOM	2.0E+12	2.7E+12	26
Chlorophyll a	4.0E+07	4.4E+07	10

Following the freshet, there is a summer phytoplankton bloom that also persists throughout the river (as measured by the two observational platforms). Based on multiple years of data at RM-53 and supported by the 2013 data from RM-122, the summer biomass of phytoplankton is typically lower than the preceding spring bloom. Three likely reasons for this observation are, nutrient limitation of growth, topdown control of biomass by grazing or lower nutrient availability, and phytoplankton contributions to the mainstem Columbia from the Willamette River and upriver when water levels go down. It is important to distinguish between these mechanisms, since nutrient limitation implies that increased growth of phytoplankton may occur under scenarios where nutrient loading increases, whereas grazing control of biomass implies that phytoplankton growth rates are normal but because of grazing the biomass does not increase. This would lead to a large transfer of carbon to the food web relative to a nutrient limited food web. In fact, there is evidence for both mechanisms to operate in the Columbia River. As described in the 2012 EMP annual report (Sagar et al. 2013), Net Ecosystem Metabolism (NEM) can provide a direct measure of phytoplankton growth rates. This measure of growth is based on changes in dissolved oxygen concentration and thus does not depend on biomass measurements. A careful analysis of NEM was presented in the 2012 annual report for RM-53, and from those data it is evident that the summer period had much lower chlorophyll a concentrations than the spring bloom; however Gross Primary Production (GPP) was similar during the two time periods. This is strong support for grazing as an important factor in summer for controlling biomass concentrations, however direct measurement of grazing rates were not conducted. These measurements should be attempted in future work as a means to constrain the carbon flow from phytoplankton into the food web.

Tidal freshwater habitats in the lower Columbia River provide areas of refuge for juvenile salmon, salmon prey production, and phytoplankton deposition. The complex nature of the salmonid food web in the lower Columbia River requires careful, long term study to understand how seasonal and spatial factors influence habitat quality and prey dynamics. Through the EMP, we have observed the importance of phytoplankton in the diets of salmon prey and reduced plant detritus inputs in the upper reaches,

supporting the concept of a shift from a historic detritus based food web to a pelagic phytoplankton based system. Continued food web sampling, such as studying the relationship between phytoplankton community and zooplankton dynamics (e.g., grazing rates and competition with salmon for food sources) will contribute to understanding salmon food availability. Currently, EMP food web sampling efforts have focused on emergent marsh habitats and have not evaluated diet information for fish caught within the Columbia River mainstem or tributaries. Expanding food web sampling to include juvenile salmon caught within the mainstem and in tributaries would provide key information on salmon dietary sources and how tidal freshwater habitats benefit stocks and species that typically spend less time in the lower Columbia River than juvenile Chinook salmon (e.g., steelhead and sockeye salmon). In addition, continuously monitoring mainstem conditions will be useful for detecting declining conditions, such as increasing water temperatures and eutrophication, and identifying whether management actions are effective in moderating such conditions.

5.2.3 Multivariate Analysis

The small subset of overlapping data coupled with variability in the ecosystem is likely obscuring fine scale environmental associations; however, the nonparametric multivariate method used for this analysis detected strong associations between species and environmental factors. It is important to remember the multivariate analysis used in this analysis is essentially looking at specific food web elements filtered through selected abiotic and/or species abundance factors. The number of overlapping datasets limits the scope and applicability of results.

5.2.3.1 Abiotic Factors

The multivariate analysis approach for assessing food web components began with identifying possible impacts of abiotic conditions, specifically water quality, on biotic factors. This exploratory approach was an attempt to draw linkages between different levels of the food web; however, the results of the multivariate analysis did not illuminate new linkages. Certain results from the multivariate analysis were comparable to spatial trends found in other univariate analyses performed in this study, such as the similarity in spatial trends and site differences regarding periphyton production. Other results related to water quality and biological gradients were inconclusive and generally showed associations of co-occurrence in space and time rather than mechanisms indicating co-variation.

5.2.3.2 Biotic factors

Biotic food web components such as vegetation, macroinvertebrate abundance, and fish abundance were examined in association with other biotic elements to explore any influences on abundance or composition. Macroinvertebrate abundance was associated with both native and invasive vegetation species, but the mechanism driving the association is not clear. For example, since vegetation composition is only collected once per year, we are not able to examine if macroinvertebrate abundance and vegetative biomass. When the relationship between macroinvertebrate abundance and vegetation was examined, no difference was found between years, but a significant difference was evident between sites, mirroring results examining macroinvertebrate abundance at EMP sites (Section 4.1.3.1).

It is uncertain how macroinvertebrate abundance correlates with fish abundance in this analysis. For all applicable sites, fish abundance with respect to macroinvertebrate abundance differed between months, but did not differ between years. Marked Chinook salmon and unmarked sockeye salmon were found to have a positive association with Hymenoptera, which corresponds with observed patterns of high

selectivity for this taxon in Chinook salmon diets at some sites located in the upper reaches (Figure 50f). Other macroinvertebrate abundance gradients were not related to other salmonid species, probably as a result of the large heterogeneity in fish and macroinvertebrate species abundances (McCune and Mefford 2002).

Regarding fish abundance, it is unclear how vegetation or macroinvertebrates correlate with unmarked Chinook salmon. Total Chinook salmon abundance is associated with higher number of fish species and lower plant diversity. Marked and unmarked Chinook salmon were found at sites characterized by small wood debris (diameter greater than 5cm and less than 25 cm), overall lower plant diversity, and overall higher fish species richness. The plant diversity measure used in this analysis is influenced by two factors, the number of species and the distribution of species. Sites with lower plant diversity could either have fewer overall species at the site or have a large number of species at the site of which a few plant species are dominant. The sites used in this analysis have multiple species and lower plant species diversity is a result of undisturbed sites characterized by a few dominant native wetland plants. Fish species richness is the number of species of fish captured at a site. Chinook salmon appear to be caught at sites that have a higher number of salmonid species present. Higher species richness could indicate favorable conditions for salmonids in general, but this analysis was not able to elucidate the combination of factors which create these favorable juvenile salmonid conditions.

5.3 Policy Implications

Shallow water emergent wetlands have been shown in this study to provide not just habitat for salmon but cover and complexity for salmon prey. In addition, emergent wetlands provide an area of phytoplankton deposition that concentrates this resource close to salmon prey. Preliminary analyses indicate that wetland macrodetritus and fluvial phytoplankton may both be important energetic sources for invertebrates that are selected prey items for juvenile Chinook salmon, although their importance may vary across sites. Even at sites where phytoplankton appeared to be the most important direct food source for invertebrate prey, emergent vegetation provided important habitat for those invertebrates. Protection (land protection, controlling shoreline armoring, lower pesticide use) and restoration of these habitats therefore provides benefits for multiple aspects of the salmonid food web. Further study to better understand how wetland characteristics (native vs. non-native cover for example) interact with salmon prey would help to understand which functional aspects are important to restore for salmon.

Preliminary data indicate a higher contribution of macrodetritus to wetlands from a native wetland species, Lyngbye's sedge, compared with the contribution of the invasive non-native species reed canarygrass. Restoration of wetlands in the upper reaches of the river, with a focus on native species establishment, could potentially increase the amount of macrodetritus production in this area. Further work to elucidate differences in macroinvertebrate and macrodetritus availability and production between these two vegetation types in the LCRE is planned for 2014 in the EMP.

Wetland biomass productivity data from this study can be used to estimate macrodetritus contribution from LCRE restoration areas. These data would be very useful for making more specific estimates of macrodetritus production in restored areas, looking at the cumulative effects of restoration on macrodetritus production in the LCRE and providing benchmarks for biomass production at restorations sites.

Fish habitat occurrence data highlight potential effects of human activities, even in the relatively undisturbed habitats sampled as part of the EMP. A prevalence of non-native fish species, high summer water temperatures in both shallow water habitats and the river mainstem, and dominance of marked (hatchery) fish were all found in these relatively 'undisturbed' sites. Chemical contaminants, not covered in detail in this document but covered in the earlier synthesis, are also a concern. If these anthropogenic effects are found in the least disturbed sites in the LCRE, the impacts to salmon would be greater when considering the estuary as a whole, including more disturbed sites. The fish data also indicate differences among genetic stocks in factors such as size range, growth rate, and lipid content. These genetic differences in stocks should be taken into account when evaluating the influence of habitat condition on salmon performance. For example, to ensure adequate habitat opportunity, habitat restoration designs should aim to achieve hydrologic and inundation patterns that correspond to migration timing of the stocks and life stages intended to benefit most from the restoration action.

Hydrology in the LCRE, particularly the spring freshet, has a strong influence on both wetland vegetation cover and abundance, and species composition of pelagic phytoplankton (prey of salmon prey). The regulation of the magnitude and duration of flood events would directly impact these two aspects of the salmonid food web. For example, in this study, spring freshet flows in the upper river reaches (farther away from salt water influence) have been shown to reduce vegetation cover (important habitat for salmon prey) and to reduce plankton standing stocks. The prevalence of phytoplankton as an important food source in salmon preferred prey (dipterans) also diminishes post-freshet as contributions from vegetation detritus increase. While very high flows tend to reduce the availability of organic matter to fuel growth of salmon prey, it is likely that during periods of low flow in the summer months, spill at Bonneville Dam provides a source of organic matter to downstream sites in the form of phytoplankton biomass which has accumulated behind the dam. Reduced suspended sediment (i.e., increased water clarity), extended water residence time, and decreased flower velocity in the reservoirs have created conditions conducive to phytoplankton growth. Spill patterns result in summer peaks in plankton biomass associated with elevated river discharge. We predict that monitoring conditions in the impoundment behind the dam would provide a much better understanding of how river discharge patterns influence the amount of phytoplankton-based organic matter available to salmon prey downstream. This could be accomplished by installing continuous monitoring sensors (similar to the sensor platform currently installed at RM 122) behind the dam and retrieving periodic samples from the impoundment for analysis of planktonic assemblages. The results provide a host of implications for river management and reinforce the importance of gaining a better understanding of the temporal and spatial variability of food web resources

Mainstem chlorophyll *a* levels assessed through the EMP suggest a potential issue with nutrient enrichment (eutrophication) in the lower Columbia River. Through the EMP and CMOP, we should continue to evaluate patterns of chlorophyll *a* concentrations and identify potential nutrient sources that are contributing to increased bloom events. Additionally, some evidence of cyanobacteria species have been noted at two trend sites in Reach F and Reach H, as well as in the mainstem river near Beaver Army Terminal (RM 53), further supporting the concern of nutrient enrichment.

6 Recommendations

6.1.1 Fish

<u>Continue to collect trends data to document long-term changes in salmon habitat occurrence, health and condition, and fish community composition.</u> To date we have only limited data for temporal assessment, with datasets of five years or more in only two of the eight hydrogeomorphic reaches of the Lower Columbia River and Estuary.

<u>Improve our understanding of interactions between salmon and non-native fish species and piscivorous predators.</u> Our findings show high percentages of non-native fish in catches, especially in reaches F-H, as well as some fish species that are known predators for juvenile salmon. These species are generally present in high numbers during the summer months when juvenile salmon densities are low, so the non-native species may not interact with out-migrant juveniles directly, but their impacts on the salmon prey base and other indirect effects are not known. Moreover, nearshore habitats may serve as nursery areas for species that may later act as predators or have other negative ecological interactions with salmon. Climate change may exacerbate this situation, as warmer temperatures are often favorable for many non-native fish species.

Improve understanding of the relationship between habitat conditions, prey availability, and salmon health and function. As noted in the discussions of the salmon food web and the multidisciplinary analysis, our data suggest important relationships between emergent vegetation cover and prey abundance, which in turn may influence both habitat occurrence and performance of juvenile salmon. However, limited data and gaps in data collection make a comprehensive analysis difficult at this point. More information on habitat with higher levels of disturbance might also improve our ability to understand these relationships, as this would provide a clearer gradient from very poor to relatively good conditions. Chemical contaminants, while not discussed in detail in this synthesis, are another factor that must be considered, including impacts of herbicides and pesticides that affect vegetation cover and insect prey.

Improve our understanding of the influence of genetic stock on salmon size, growth rates, and condition. Our data suggest differences in size distribution, condition and growth rates among Chinook salmon from various genetic stocks, differences that must be taken into account when investigating the influences of habitat quality on salmon. Additional data on size ranges and growth rates for Chinook salmon stocks, especially interior Columbia stocks for which information is limited, will help with this analysis.

<u>Continue monitoring of salmon habitat occurrence in late fall and winter.</u> Recently collected data on juvenile salmon habitat occurrence in late fall and winter suggest seasonal shifts in stocks present, with spring Chinook salmon showing greater abundance during this period. However, our data from this season are still very limited and more information is needed to confirm these patterns.

6.1.2 Food Web

<u>Pinpoint linkages between primary production and salmonid prey populations</u>. Data from 2011-2013 suggest that fluvial phytoplankton populations track river discharge patterns and reflect connections between shallow water habitats and the mainstem Columbia River. However, it is uncertain how changes in the availability (amount and species composition) of phytoplankton affect macroinvertebrate population growth, size, and composition. Two approaches to disentangle this relationship are suggested:

(1) longer monitoring period to determine correlations between fluvial phytoplankton abundances and macroinverebrate prey populations; (2) targeted studies to examine gut contents of preferred macroinvertebrate prey of salmonids (chironomids and amphipods). The latter could be carried out using microscopy and DNA sequence analysis. A third approach is to use stable isotope signatures of different types of organic matter; however, the stable isotope mixing model would benefit strongly from an independent form of validation, which the second approach provides.

Determine relationship between flow regime and deposition profiles of fluvial phytoplankton species. It has been suggested that the loss of tidal channels and wetland environments of high complexity has contributed to the loss of suitable habitat for juvenile salmon. Part of the story might involve the quantity of particulate organic matter that is deposited within salmonid habitats. While some of the deposited material comes from macrodetritus, some probably comes from the sinking and deposition of phytoplankton. Therefore, it is likely that rapid flows found in deeper (e.g., mainstem) habitats lead to the loss of fluvial phytoplankton from the system, while shallow water, higher-complexity habitats with vegetation and channels, might provide an environment more suitable for deposition of phytoplankton, which could contribute to increased macroinverebrate productivity. A modeling approach (i.e., a numerical circulation model) could be used to test this hypothesis using the data collected from the EMP. Since it is not known whether macroinvertebrate prey populations would increase with additional supply of fluvial phytoplankton, it is difficult to recommend specific restoration strategies; however, if a relationship is found, then increasing the acreage of depositional areas would be one strategy to try.

Determine competition for particulate organic matter between invertebrate species that are preferred vs. non-preferred for juvenile salmon. Work from the EMP has clearly demonstrated juvenile Chinook salmon preference for dipteran insects (and amphipods and hymenoptera) over other taxa, including cladocerans and copepods. It is quite possible that high levels of the latter could compete with preferred prey items for food (i.e., fluvial phytoplankton). If this piece of information was known (i.e., competitive interactions between invertebrate taxa), then recommendations regarding ideal flushing rates, for example, could be put forward.

Determine whether a threat from cyanotoxin exposure to fish and other wildlife exists with the lower reaches of the Columbia River. Phytoplankton species counts revealed that between 2011 and 2013, high abundances of potentially toxic cyanobacteria were present, especially during the summer months at Campbell Slough and to some degree at Franz Lake Slough. There is some evidence to suggest that in other systems, fish accumulate these types of toxins within various tissue types. The threat from exposure to cyanobacterial toxins by wildlife (for example, the endangered Columbian white-tailed deer and by fish species using shallow water habitats) is unknown. It would be helpful to measure levels of one or more cyanotoxins (e.g., microcystin, saxitoxin, anatoxin) during periods when cyanobacterial biomass is elevated.

<u>Reduce uncertainty in macrodetrital production in the upper estuary.</u> Through the EMP and other studies, we have an improved understanding of spatial and temporal trends associated with the production and contribution of macrodetritus in the *lower* estuary (downstream of rkm 89). In this zone, we have begun to quantify the inter-annual trends associated with varying water years and with different vegetation communities; however we have not been able to quantify these trends in the riverine dominated portion of the river (upstream of rkm 89). The data we have from the upper 146 km have been collected during two

high water years and a recovery year, and only from two sites resulting in very little knowledge of the production and export patterns and trends in this large reach. Preliminary results indicate that macrodetrital production in the upper estuary could be much more limited than in the lower estuary.

Apply wetland biomass productivity data to land cover data to estimate macrodetritus contribution for the <u>LCRE</u>. The recent land cover analysis for the lower river (Sanborn Map Company and LCEP 2011) could be used to estimate the quantity of macrodetritus production in the LCRE using the data we have collected through this and other studies. In the upper reaches, we need more data to be able to do this kind of analysis; however the existing data for the lower river could be used to make these calculations. This analysis would give us a better understanding of the areas of the river that may be deficient in macrodetritus production and those that would be good targets for restoration of these indirect benefits. This would also be a first step in developing a carrying capacity model for the estuary.

Apply wetland biomass productivity data to restoration sites to estimate macrodetritus contribution from LCRE restoration areas. Previous studies have done some preliminary quantification of biomass production in restoration sites the lower river (downstream of rkm 89; Diefenderfer et al. 2013), using restoration area estimates and some of the early data collected through the EMP and other studies. In more recent years for the EMP, we have collected additional and more detailed data on wetland plant production. These data would be very useful for advancing the previous analysis and making more specific estimates of macrodetritus production in restored areas.

<u>Create a ratio-based estimator for macrodetritus production</u>. With more focused and additional data collection, we may be able to develop a relationship between cover estimates and biomass production and macrodetritus production. If this relationship could be developed, then estimates of biomass production could be made based on the lower-cost and easier-to-measure metric of cover rather than biomass collection and processing. These data would then be more easily measured at restoration sites to be able to determine the potential contribution of macrodetritus from restored areas.

6.1.3 Mainstem abiotic conditions

<u>Understanding the links between abiotic conditions (i.e., water quality) and the sources of carbon</u> for higher trophic levels is central to any ecosystem assessment or monitoring program. By accurately characterizing nutrients, energy flow, and water quality conditions such as dissolved oxygen concentrations, it is possible to better assess overall ecosystem function for targeted species such as Chinook salmon. Nitrate can cause water quality issues if it is used by plants and algae as a nutrient source, leading to eutrophication (Cloern 2001). Increased DOC and turbidity from runoff are associated with a range of changes to ecosystems including reduced algae growth, underwater vision for predators, and increased sedimentation (Cloern 2001).

<u>More information about the temporal variability of the primary producers in the mainstem</u> portion of the river (i.e., phytoplankton) and salmon prey is critical to inform future food web studies, including those associated with reed canarygrass and other emergent habitats where particulate material from the river likely accumulates on the benthos and is consumed by zooplankton and macroinvertebrates that colonize those habitats. The mechanisms and quantification of the phytoplankton – prey – salmon food chain are very poorly understood for the system. This food chain may also be the mechanism through which contaminants are transferred from water to fish, and should be an active and complementary area of research.

6.1.4 Vegetation and Hydrology

<u>Develop a predictive model of the effects of sea level rise and climate-derived hydrologic change</u>. The data we have collected in the EMP and other studies have allowed us to quantify the effects of hydrologic variability on wetland plant communities. These data could now be used to develop a predictive model to estimate changes that will occur with sea level rise, climate change, and/or Treaty related hydrologic changes.

To fully understand the effects of hydrologic change (e.g., increased inundation) on LCRE wetlands, we need a better understanding of sediment processes. The expected rates of sediment accretion or erosion rates will dictate the rates at which wetlands will be able to "keep up with" hydrologic change. In order to understand this, additional and more accurate sediment accretion measurements are needed. Sediment evaluation tables (SETs) and lead210 cores would complement the data collection we have completed to date and allow more accurate estimates of how LCRE wetlands will be affected by sea level rise and other future hydrologic changes.

<u>Evaluate Carbon Sequestration Potential in Tidal Wetlands (Blue Carbon)</u>. Carbon sequestration in wetlands is an area of growing interest globally and is now also getting attention from the coastal wetland restoration community. Currently, working groups in OR and WA are convening to figure out the specifics of methods for establishing a system for carbon mitigation. A recent report (Crooks et al. 2014) from the Snohomish estuary in Puget Sound has outlined a method for determining the carbon sequestration potential in tidal wetlands. In the LCRE, we have begun to evaluate some of the same metrics they recommend, such as total organic carbon in sediments and sediment accretion. Additional and more accurate data would allow the same calculations to be made in the LCRE.

6.1.5 Multivariate Analysis

<u>Increase sampling sites</u>. In the multivariate analysis, site was repeatedly identified to differ significantly more often than year or month for most elements of the food web, which indicates a need to monitor as many sites as possible to build a coherent picture of baseline conditions.

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8 Appendices

Appendix 1

Ecosystem Monitoring Program Sampling Effort

Table A1-1. Summary of sampling effort by site and year(s) for sites between 2005 and 2013, trend sites in bold font. *Lord-Walker Island 2 was sampled by the EMP in conjunction with the Reference Site Study; thus, only vegetation and habitat data were collected at Lord-Walker 2.

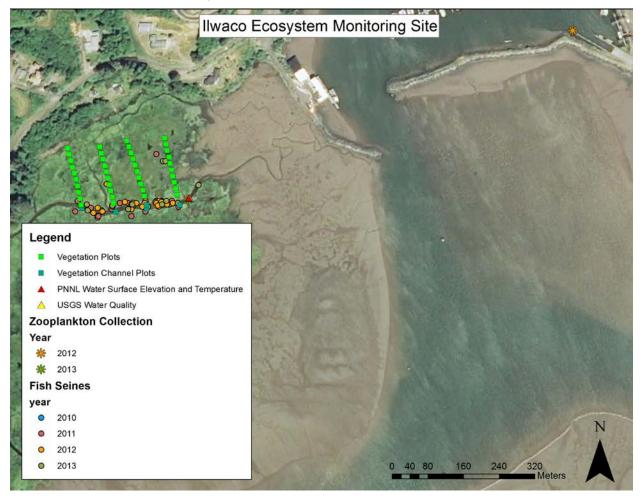
Reach	Type of Site	Site	Vegetation & Habitat	Fish &Prey	Abiotic Conditions	Food Web (in Reach B no USGS sampling)	Mainstem abiotic conditions
А	Trend	Ilwaco	2011-2013	2011-2013	2011-2013	2011-2013	
В	Trend	Secret River	2012, 2013	2012, 2013		2012, 2013	
	Trend	Welch Island	2012, 2013	2012, 2013		2012, 2013	
	Status	Ryan Island	2009	2009			
	Status	Lord-Walker Island 1	2009	2009			
С	Status	Lord-Walker Island 2*	2009				
	Trend	Whites Island	2009-2013	2009-2013	2009, 2011- 2013	2011-2013	
	Status	Jackson Island	2010	2010			
	Status	Wallace Island	2010	2010			

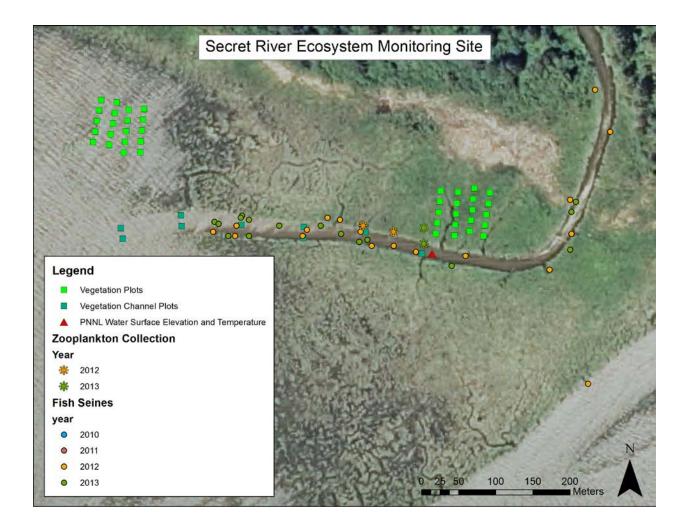
Reach	Type of Site	Site	Vegetation & Habitat	Fish &Prey	Abiotic Conditions	Food Web (in Reach B no USGS sampling)	Mainstem abiotic conditions
	Status	Bradwood Landing	No access	2010			
	Status	Cottonwood Island small slough	2005				
D	Status	Cottonwood Island large slough	2005				
	Status	Dibble Slough	2005		2005		
	Status	Sandy Island 1, 2	2007	2007			
Е	Status	Lewis River Mouth	2007				
	Status	Martin Island	2007				
	Status	Sauvie Cove	2005				
	Status	Hogan Ranch	2005				
F	Status	Goat Island	2011	2011			
	Status	Deer Island	2011	2011			
	Status	Burke Island	2011	2011			
	Trend	Cunningham Lake	2005-2013	2007-2009			
	Trend	Campbell Slough	2005-2013	2007-2013	2008-2013	2010-2013	

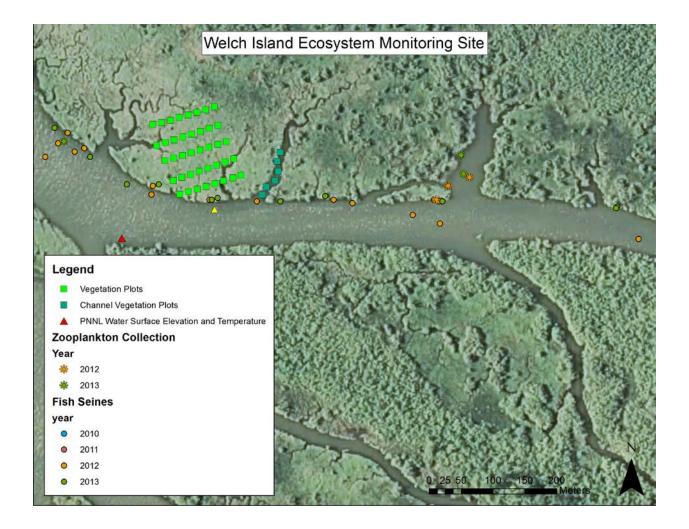
Reach	Type of Site	Site	Vegetation & Habitat	Fish &Prey	Abiotic Conditions	Food Web (in Reach B no USGS sampling)	Mainstem abiotic conditions
	Status	Water Resources Center	2006				
	Status	McGuire Island	2006				
	Status	Old Channel Sandy River	2006			2006	
G	Status	Chattam Island	2006				
	Status	Government/Lemon Island	2012	2012			
	Status	Reed Island	2012	2012			
	Status	Washougal Wetland	2012	2012			
	Trend	RM122					2012, 2013
Н	Trend	Franz Lake (slough)	2008-2009, 2011- 2013	2008-2009, 2011-2013	2011-2013	2011-2013	
	Status	Sand Island	2008	2008	2008		
	Status	Hardy Slough	2008	2008			

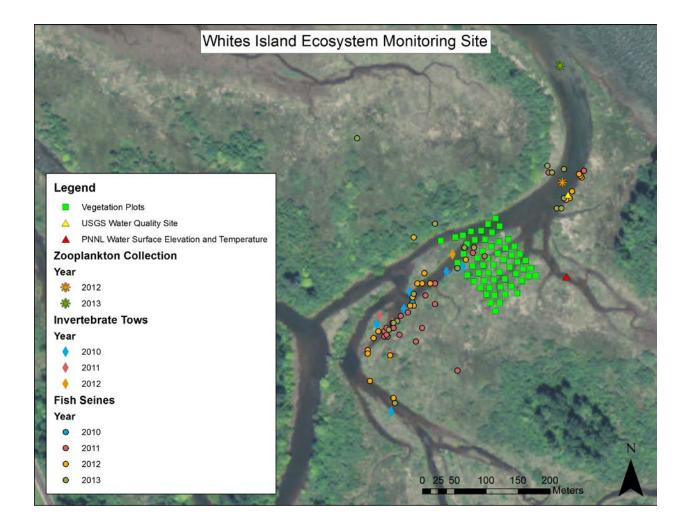
Appendix 2

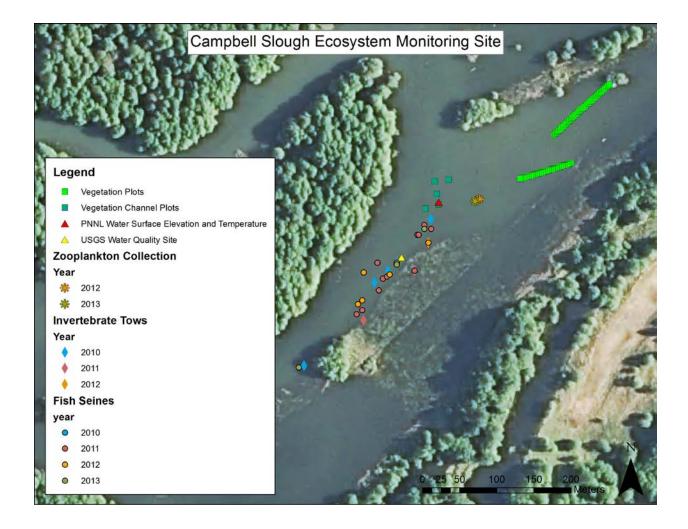
Figure A2-1. Maps of overlap in Ecosystem Monitoring Program sampling for the six trend sites in the lower Columbia River and Estuary.

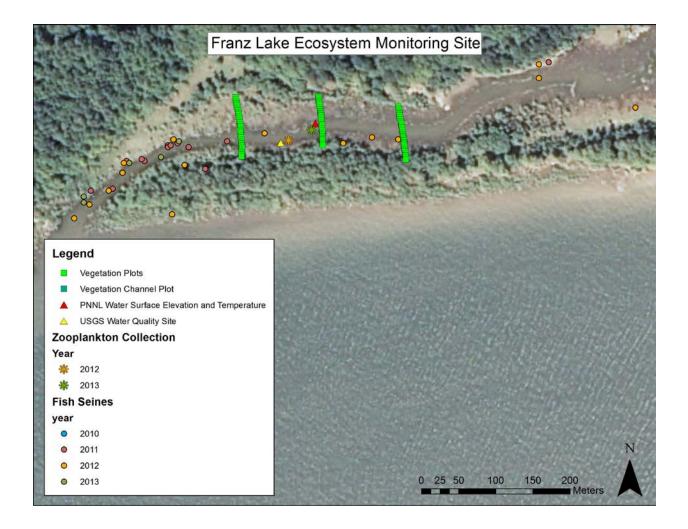












Appendix 3

Fish and Fish Prey Methods

	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
A-Ilwaco Slough	3	4	8	11	9	8	8	6	6	6	6	75
B-Secret River	1	6	3	5	6	6	6	6	1	3	0	43
B-Welch Island	3	2	6	2	3	3	6	6	3	3	3	40
C-Ryan Island	0	0	3	2	4	3	3	0	0	0	0	15
C-Bradwood	0	0	2	1	1	1	1	0	0	0	0	7
Slough C-Jackson Island	0	0	2	1	2	2	2	0	0	0	0	9
C-Whites Island	3	5	11	11	13	14	15	9	6	5	5	97
C-Wallace Island	0	0	2	1	1	2	2	0	0	0	0	8
C-Lord/Walker	0	0	2	1	2	3	3	0	0	0	0	11
Island												
E-Burke Island	0	0	0	1	0	2	2	3	3	3	3	17
E-Goat Island	0	0	0	1	0	1	2	3	3	3	3	16
E-Deer Island	0	0	0	1	0	1	3	3	3	3	3	17
E-Sandy Island	0	0	9	6	3	0	0	0	0	0	0	18
F-Campbell	0	0	6	22	21	20	18	9	3	0	0	99
Slough												
G-Lemon Island	0	2	2	1	1	1	3	1	3	3	2	19
G-Reed Island	3	0	0	0	0	2	0	0	0	0	2	7
G-Washougal	2	2	1	1	1	1	2	2	0	3	1	16
H-Sand Island	0	0	3	3	0	3	2	0	0	0	0	11
H-Franz Lake	3	4	6	5	0	10	12	8	6	6	6	66
H-Pierce Island	0	0	2	3	1	1	0	0	0	0	0	7
H-Hardy Slough	0	0	3	3	2	3	3	0	0	0	0	14

Table A3-1. Seasonal fish collection showing number of sampling sets by month at each EMP sites.
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Table A3-2. Seasonal	sampling by year	at trend sites.
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	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
A-Ilwaco Slough												
2011	0	0	2	5	3	2	3	3	3	3	3	27
2012	3	3	3	3	3	3	2	0	3	3	3	29
2013	0	1	3	3	3	3	3	3	0	0	0	19
B-Secret River												
2012	1	3	2	3	3	3	3	3	1	3	0	9
2013	0	3	2	2	3	3	3	3	0	0	0	19
B-Welch Island												
2012	3	1	3	1	1	1	3	3	3	3	3	25
2013	0	1	3	1	2	2	3	3	0	0	0	15
C-Whites Island												
2009	0	0	3	2	5	3	3	0	0	0	0	16
2010	1	0	2	2	2	3	3	0	0	0	0	17
2011	0	0	0	5	2	2	3	3	3	3	3	24
2012	2	2	3	1	1	3	3	3	3	2	2	25
2013	0	3	3	1	3	3	3	3	0	0	0	18
F-Campbell												
Slough												
2007	0	0	0	12	12	3	0	0	0	0	0	18
2008	0	0	3	3	3	3	3	0	0	0	0	12
2009	0	0	0	2	4	3	3	0	0	0	0	16

2010	0	0	3	1	1	3	3	0	0	0	0	11
2011	0	0	0	1	0	2	3	3	3	0	0	12
2012	0	0	0	2	1	3	3	3	0	0	0	12
2013	0	0	0	1	3	3	3	3	0	0	0	13
H-Franz Lake												
2008	0	0	0	3	3	0	3	3	0	0	0	12
2009	0	0	3	2	0	6	0	0	0	0	0	11
2011	0	0	0	0	0	1	3	3	3	3	3	16
2012	3	1	0	0	0	0	3	2	3	3	3	18
2013	0	3	0	0	0	0	3	3	0	0	0	9

Table A3-3. The number of invertebrate tow samples collected at each site per sampling event between 2008 and 2013.

	2	2008		200)9	· · · ·	201	.0		2	2011			2	2012				201	13		
	April	Мау	June	Мау	June	April	Мау	June	July	April	May	June	February	March	April	Мау	June	March	Мау	June	July	total tow samples
Ilwaco	0	0	0	0	0	0	0	0	0	2	8	4	0	0	0	0	0	0	0	0	0	14
Secret River	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4	1	6	0	4	4	0	23
Welch Island	0	0	0	0	0	0	0	0	0	0	0	0	0	2	5	4	4	4	4	4	4	31
Ryan Island	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Bradwood Slough	0	0	0	0	0	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	16
Jackson Island	0	0	0	0	0	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	12
Whites Island	0	0	0	4	0	4	4	4	4	0	10	4	0	2	6	4	4	0	4	3	6	63
Wallace Island	0	0	0	0	0	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	16
Lord/Walker Island	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Burke Island	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4
Goat Island	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4
Deer Island	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4
Campbell Slough	3	6	0	5	4	4	4	4	2	0	4	0	0	0	0	4	4	0	4	4	0	52
Lemon Island	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	4	2	0	0	0	0	13
Washougal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	4	0	0	0	0	10
Sand Island	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Franz Lake	6	6	0	4	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	18
Hardy Slough	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
total tow samples	15	12	4	20	4	20	20	20	14	2	36	8	4	7	21	21	24	4	16	15	10	297

	2	008		200)9		2	010			2	2011			2	2012				201	3		
	April	May	June	Мау	June	April	May	June	yuly	August	Мау	June	yuly	February	March	April	May	June	March	May	June	July	total diet samples
Secret River	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	15	0	14	0	12	1	2	59
Welch Island	0	0	0	0	0	0	0	0	0	0	0	0	0	16	14	14	30	15	9	30	23	25	176
Ryan Island	0	0	0	9	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19
Bradwood Slough	0	0	0	0	0	10	17	9	10	8	0	0	0	0	0	0	0	0	0	0	0	0	54
Jackson Island	0	0	0	0	0	19	15	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42
Whites Island	0	0	0	10	0	16	14	18	19	13	10	25	2	0	13	10	11	15	0	15	13	0	204
Wallace Island	0	0	0	0	0	6	14	11	11	0	0	0	0	0	0	0	0	0	0	0	0	0	42
Lord/Walker Island	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Burke Island	0	0	0	0	0	0	0	0	0	0	10	0	2	0	0	0	0	0	0	0	0	0	12
Goat Island	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	13
Deer Island	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	10
Campbell Slough	6	19	0	10	9	12	24	18	15	0	22	0	0	0	0	0	18	15	0	34	9	1	212
Lemon Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	7	15	15	0	0	0	0	50
Washougal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	18	36	0	0	0	0	69
Sand Island	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
Franz Lake	15	7	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30
Pierce Island	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Hardy Slough	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
total diet samples	43	26	13	43	19	63	84	64	55	21	65	25	4	31	40	61	92	110	9	91	46	28	1033

Table A3-4. The number of juvenile Chinook diet samples collected at each site per sampling event between 2008 and 2013.

Appendix 4

Table A4-1. Stepwise regression and power analysis results for effects of site, reach, and month on fish	
community variables.	

Factor	Predictor	Least significant number	% variation explained	P value
Species diversity	Site	75	37	< 0.0001
(n=597)	Reach	36	37	< 0.0001
	Month	235	7	< 0.0001
Species richness	Site	72	35	< 0.0001
(n=597)	Reach	41	35	< 0.0001
	Month	98	13	< 0.0001
% non-natives	Site	83	35	< 0.0001
(n=597)	Reach	37	35	< 0.0001
	Month	149	11	< 0.0001
% predatory fish	Site	243	12	< 0.0001
(n=597)	Reach	144	12	< 0.0001
	Month	321	7	< 0.0001

Factor	Site	Total current	Least	p-value
		sets	significant	
			number	
Species diversity	Campbell Slough	99	75	0.0012
	Franz Lake	66	96	0.1614
	Ilwaco Slough	75	97	0.0979
	Secret River	43	537	0.5813
	Welch Island	40	1067	0.6629
	Whites Island	97	148	0.1811
Species richness	Campbell Slough	99	41	< 0.0001
2	Franz Lake	66	43	0.0057
	Ilwaco Slough	75	199	0.3329
	Secret River	43	2092	0.7800
	Welch Island	49	798	0.6629
	Whites Island	97	67	0.0086
% non-natives	Campbell Slough	98	113	0.0894
	Franz Lake	65	144	0.3647
	Ilwaco Slough	75	106	0.1198
	Secret River	43	862	0.6710
	Welch Island	39	206	0.3966
	Whites Island	91	171	0.2797
% predatory fish	Campbell Slough	98	379	0.7692
	Franz Lake	65	22	< 0.0001
	Ilwaco Slough	75	ND ¹	ND^1
	Secret River	43	113	0.2395
	Welch Island	39	85	0.1850
	Whites Island	91	315	0.5991

Table A4-2. Analysis of variance and power analysis results for temporal trends in fish community variables at the EMP trend sites.

¹Could not be determined; all values were 0.

Table A4-3. Power analysis results for EMP salmon composition variables by site, reach, month, and season.

Factor	Predictor	LSN	%	p-value
			variation	
			explained	
% unmarked	Site	90	29	< 0.0001
Chinook in catches				
(n=223)	Reach	50	23	< 0.0001
	Month	105	13	< 0.0001
% marked	Site	128	22	< 0.0001
Chinook in catches				
(n=223)	Reach	69	17	< 0.0001
	Month	100	8	< 0.0001
% unmarked coho	Site	118	12	0.0012
in catches				
(n=223)	Reach	99	15	0.0052
	Month	39	37	< 0.0001

% marked coho in catches	Site	79	32	< 0.0001
(n=223)	Reach	59	27	< 0.0001
	Month	39	20-24*	< 0.0001
% chum in salmon catch (includes 2007 data)	Site	80	31	< 0.0001
(n=266)	Reach	39	23	< 0.0001
	Month	94	12	0.0001
Salmon species diversity (includes 2007 data)	Site	101	27	< 0.0001
(n=266)	Reach	79	15	< 0.0001
	Month	170	9	0.0006 < p < 0.0008*

*Value varies depending on whether site or reach is used in the regression model

Table A4-4. Stepwise regression and power analysis results for variability in salmon density by site, reach, and month. LSN – Least significant number of observations needed to determine significant differences for that predictor and factor. Note that LSN was determined for each predictor separately, while variation explained indicates results in stepwise regression incorporating site or reach and month.

Factor	Predictor	LSN	% variation explained	p-value
Density marked Chinook	Site	633	6	0.0132
(n=567)	Reach	704	3	0.0099
	Month	308	6	0.00012
Density unmarked Chinook	Site	160	15	< 0.0001
(n=567)	Reach	316	5	0.0001
	Month	100	16	< 0.0001
Density chum	Site	1298	2	0.9041
(n=567)	Reach	588	2	0.0590
	Month	600	3	0.0517
Density unmarked coho	Site	313	9.3	< 0.0001
(n=567)	Reach	682	2	0.1058
	Month	2005	0.7	0.8733
Density marked coho	Site	260	10	< 0.0001
(n=567)	Reach	335	4	0.0009
	Month	220	8	< 0.0001

Table A4-5. Analysis of variance and power analysis results for temporal trends in salmon density at the EMP trend sites. LSN = Least significant number of observations needed to determine significant differences for that predictor and factor. ND = not determined because there was no variance for that parameter.

Factor	Site	Total sets	LSN	p-value
Density unmarked coho	Campbell Slough	72	ND ¹	ND ¹
	Franz Lake	66	210	
	Ilwaco Slough	75	288	
	Secret River	43	264	
	Welch Island	40	261	
	Whites Island	97	42	
Density marked coho	Campbell Slough	72	ND ¹	ND ¹
	Franz Lake	66	73	
	Ilwaco Slough	75	ND^1	ND^1
	Secret River	43	212	
	Welch Island	40	261	
	Whites Island	97	ND^1	ND^1
Density chum	Campbell Slough	72	173	
	Franz Lake	66	81	
	Ilwaco Slough	75	380	
	Secret River	43	21196	
	Welch Island	40	825	
	Whites Island	97	124	
Density marked Chinook	Campbell Slough	72	209	0.580
	Franz Lake	66	70	0.0636
	Ilwaco Slough	75	256	0.4167
	Secret River	43	10177	0.8992
	Welch Island	40	103	0.2550
	Whites Island	97	120	0.1043
Density unmarked Chinook	Campbell Slough	72	180	0.4280
	Franz Lake	66	150	0.3814
	Ilwaco Slough	75	99	0.1023
	Secret River	43	40	0.5235
	Welch Island	40	21205	0.9326
	Whites Island	97	184	0.2855

Table A4-6. Power analysis results for temporal trends in salmon catch composition variables at EMP trend sites.

Factor	Site	Total sets	LSN	p-value
% unmarked Chinook	Campbell Slough	29	51	0.2710
	Franz Lake	17	15	0.0275
	Ilwaco Slough	13	69	0.5703
	Secret River	18	182	0.5435
	Welch Island	21	1044	0.7838
	Whites Island	43	76	0.2491
% marked Chinook	Campbell Slough	29	50	0.2553
	Franz Lake	17	20	0.0994
	Ilwaco Slough	13	36	0.3541
	Secret River	18	401	0.5235
	Welch island	21	818	0.7566
	Whites Island	43	184	0.2855
% unmarked coho	Campbell Slough	29	ND ¹	ND ¹
	Franz Lake	17	9	< 0.0001
	Ilwaco Slough	13	145	0.7653
	Secret River	18	43	0.2071
	Welch island	21	136	0.4469
	Whites Island	43	48	0.0749
% marked coho	Campbell Slough	29	ND^1	ND^1
	Franz Lake	17	42	0.4229
	Ilwaco Slough	13	ND ¹	ND ¹
	Secret River	18	90	0.3872
	Welch island	21	136	0.4469
	Whites Island	43	ND^1	ND ¹
% chum ²	Campbell Slough	47	101	0.4368
	Franz Lake	17	48	0.5703
	Ilwaco Slough	13	66	0.5580
	Secret River	18	40	0.1944
	Welch Island	21	419	0.6725
	Whites Island	43	109	0.4370
Salmon diversity ²	Campbell Slough	47	123	0.5520
· · ·	Franz Lake	21	21	0.0475
	Ilwaco Slough	13	ND ¹	ND ¹
	Secret River	19	3270	0.8830
	Welch Island	22	336	0.6203
	Whites Island	43	69	0.2000

¹not determined because all values were 0. ²2007 data included for Campbell Slough

Table A4-7. Power analysis results for EMP salmon composition variables by site, reach month and month. For this analysis, n refers to the number of sampling events by month and year, not beach seine sets. LSN = Least significant number of observations needed to determine significant differences for that predictor and factor.

Factor	Predictor	LSN	% variation	p-value
			explained	
% non-LCR stocks	Site	61	42	0.0011
(n=91)	Reach	65	18	0.0079
	Month	136	7-9	0.4888-0.4970
Genetic stock	Site	74	34	0.0088
diversity				
(n=91)	Reach	86	14	0.0399
	Month	173	7	$0.5221 - 0.5654^{1}$

¹p-value and/or variance explained varied depending on whether site or reach was used in the stepwise regression.

Table A4-8. Analysis of variance and power analysis results for temporal trends in Chinook salmon stock composition variables at the EMP trend sites. For this analysis, n refers to the number of sampling events by month and year, not beach seine sets. LSN = Least significant number of observations needed to determine significant differences for that predictor and factor.

Factor	Site	Total sampling events	LSN	p-value
% non-LCR stocks (includes 2007)	Campbell Slough	16	19	0.1143
	Franz Lake	3	20	0.5653
	Ilwaco Slough	2	ND	ND
	Secret River	5	ND	ND
	Welch Island	8	ND	ND
	Whites Island	19	40	0.2983
Chinook stock diversity (includes 2007)	Campbell Slough	16	19	0.1135
	Franz Lake	3	10	0.4296
	Ilwaco Slough	2	ND	ND
	Secret River	5	ND	ND
	Welch Island	8	ND	ND
	Whites Island	19	81	0.6790

Table A4-9. Power analysis results for EMP fish monitoring variables related to Chinook salmon size	
distribution, condition and health.	

Factor	Predictor	Least significant number	% variance explained	p-value
Chinook length (n=1196; includes only fish where stock is known)	Site	89	10	< 0.0001
	Reach	48	7	< 0.0001
	Month	46	24	< 0.0001
	Marked vs. unmarked	10	35	< 0.0001
	Stock	78	2	< 0.0001
Chinook condition factor (n=1196; includes only fish where stock is known)	Site	235	6	< 0.0001
	Reach	150	3	< 0.0001
	Month	49	28	< 0.0001
	Stock	609	1	$0.0602 - 0.0843^{1}$
	Marked vs. unmarked	8978	1	0.0001
Chinook % lipid	Site	93	22	< 0.0001
(n=221)	Reach	46	18	< 0.0001
	Month	70	5	0.0324, 0.0198
	Stock	169	5	0.0133. 0.0273
	Marked vs. unmarked	7584	0	0.9438. 0.9396

Table A4-10. Power analysis results for temporal trends in salmon catch composition variables at EMP trend sites. For this analysis, n refers to the number of sampling events by month and year, not beach seine sets. LSN = Least significant number of observations needed to determine significant differences for that predictor and factor.

Factor	Site	Total samples	LSN	p-value
Chinook length (unmarked fish only)	Campbell Slough	156	165	0.0636
•	Franz Lake	27	37	0.1281
	Ilwaco Slough	6	4	0.0089
	Secret River	123	29	0.0001
	Welch Island	266	27	0.0001
	Whites Island	348	50	0.0001
Chinook condition factor (unmarked fish only)	Campbell Slough	156	19	0.0002
	Franz Lake	27	53	0.2630
	Ilwaco Slough	6	267	0.7827
	Secret River	122	22	0.0001
	Welch Island	266	113	0.0026
	Whites Island	346	101	0.0001
Chinook lipid content (%)	Campbell Slough	16	27	
	Franz Lake	3	50	
	Ilwaco Slough	2	ND	
	Secret River	5	ND	
	Welch Island	8	ND	
	Whites Island	19	70	

Appendix 5

Table A5-1. Descriptive statistics (mean, standard deviation, and coefficient of variation) for percentage of non-native fish in catches by reach and month. N = the number of years that reach was sampled for that month. Includes data from 2007-2013.

	Reach A	Reach B	Reach C	Reach E	Reach F	Reach G	Reach H
February	26.1	0.19	0 ± 0	NS	NS	5.48	2.22
-	-	-	-			-	-
	n=1	n=1	n=2			n=1	n=1
March	0	0	0 ± 0	NS	NS	7.69	50.00 ± 70.71
	-	-	-			-	141%
	n=2	n=2	n=2			n=1	n=2
April	0.13 ± 0.11	0.09 ± 0.13	0.65 ± 0.77	NS	13.03 ± 12.16	2.64	10.05 ± 9.50
	87%	141%	117%		93%	-	95%
	n=3	n=2	n=4		n=2	n=1	n=2
May	0.07 ± 0.12	1.79 ± 1.83	0.42 ± 0.85	8.2 ± 6.9	6.78 ± 10.16	0.28	0.60 ± 0.85
-	173%	102%	200%	84%	150%	-	141%
	n=3	n=2	n=5	n=2	n=7	n=1	n=2
June	0.02 ± 0.03	1.17 ± 0.68	0.62 ± 1.20	15.88	32.31 ± 23.61	0.61	0.90
	173%	58%	194%	-	73%	-	-
	n=3	n=2	n=5	n=1	n=5	n=1	n=1
July	0.17 ± 0.26	1.41 ± 0.70	1.13 ± 0.67	44.37 ± 20.72	38.60 ± 25.52	2.44	10.54 ± 8.70
	155%	50%	60%	47%	66%	-	83%
	n=3	n=2	n=5	n=2	n=7	n=1	n=3
August	0.03 ± 0.06	1.47 ± 0.22	2.38 ± 1.73	32.53	74.8 ± 21.3	8.95	31.87 ± 23.75
	173%	15%	73%	-	28%	-	75%
	n=3	n=2	n=5	n-1	n=6	n=1	n=4
September	0.06 ± 0.08	0.58 ± 0.48	0.43 ± 0.46	22.33	97.37 ± 2.00	64.84	25.08 ± 17.37
	141%	82%	106%	-	2%	-	69%
	n=2	n=2	n=3	n-1	n=3	n-1	n=3
October	0.18 ± 0.25	0.89	0.16 ± 0.22	3.13	7.36	27.54	39.65 ± 46.81
	141%	-	141%	-	-	-	118%
	n=2	n=1	n=2	N=1	n=1	n=1	n=2
November	0.07 ± 0.10	0.33	4.17 ± 5.89	2.17	NS	8.29	1.06 ± 0.44
	141%	-	141%	-		-	42%
	n=2	n=1	n=2	n=1		n=1	n=2
December	0.35 ± 0.61	0	0 ± 0	0.16	NS	33.33	34.94 ± 30.91
	173%	-	-	-		-	88%
	n=3	n=1	n=2	n-1		n=1	n=3

	Reach A	Reach B	Reach C	Reach E	Reach F	Reach G	Reach H
February	0	0	0 ± 0	NS	NS	0	0
	-	-	-			-	-
	n=1	n=1	n=2			n=1	n=1
March	0	0	0 ± 0	NS	NS	0	0 ± 0
	-	-	-			-	-
	n=2	n=2	n=2			n=1	n=2
April	0	0	0 ± 0	NS	0 ± 0	0	0.11 ± 0.15
	-	-	-		-	-	141%
	n=3	n=2	n=4		n=2	n=1	n=2
May	0	0	0 ± 0	0 ± 0	0.08 ± 0.21	0.56	1.76 ± 1.37
	-	-	-	-	256%	-	78%
	n=3	n=2	n=5	n=2	n=7	n=1	n=2
June	0	0.09 ± 0.12	0.03 ± 0.07	0.97	1.28 ± 1.52	0.20	0.00
	-	144%	224%	-	119%	-	-
	n=3	n=2	n=5	n=1	n=5	n=1	n=1
July	0	0.06 ± 0.09	0.01 ± 0.01	0.66 ± 0.94	2.55 ± 3.92	0.09	12.61 ± 20.68
	-	141%	150%	141%	154%	-	164%
	n=3	n=2	n=5	n=2	n=7	n=1	n=3
August	0	0.01 ± 0.01	0.23 ± 0.51	0.06	1.88 ± 1.85	3.88	3.46 ± 4.69
	-	141%	224%	-	98%	-	136%
	n=3	n=2	n=5	n-1	n=6	n=1	n=4
September	0	0 ± 0	0.20 ± 0.35	0.27	7.72 ± 3.52	0.00	1.59 ± 1.03
	-	-	173%	-	46%	-	65%
	n=2	n=2	n=3	n-1	n=3	n-1	n=3
October	0	0	0 ± 0	0.00	1.03	0.00	0.43 ± 0.61
	-	-	-	-	-	-	141%
	n=2	n=1	n=2	n=1	n=1	n=1	n=2
November	0	0	0 ± 0	0.00	NS	0.00	0.18 ± 0.05
	-	-	-	-		-	31%
	n=2	n=1	n=2	n=1		n=1	n=2
December	0	0	0 ± 0	0.00	NS	0.00	3.11 ± 5.38
	-	-	-	-		-	173%
	n=3	n=1	n=2	n-1		n=1	n=3

Table A5-2. Descriptive statistics (mean, standard deviation, and coefficient of variation) for percentage of fish that are potential salmon predators in catches by reach and month. N = the number of years that reach was sampled for that month. Includes data from 2007-2013.

Table A5-3. Descriptive statistics (mean, standard deviation, and coefficient of variation) for density of unmarked Chinook salmon (fish per 1,000 m²). N = the number of years that reach was sampled for that month. Data from 2007 are not included as marked status of some fish was uncertain and sampling that year was not designed to measure density.

	Reach A	Reach B	Reach C	Reach E	Reach F	Reach G	Reach H
February	2.5	48.9	1.6 ± 2.2	NS	NS	1.2	000
	-	-	141%			-	-
	n=1	n=1	n=2			n=1	n=1
March	0.0	20.3 ± 28.7	6.11 ± 8.7	NS	NS	12.4	0 ± 0
	-	141%	141%			-	CV = -
	n=2	n=2	n=2			n=1	n=2
April	0.4 ± 0.8	15.7 ± 22.2	18.9 ± 16.0	NS	13.0 ± 2.8	16.4	23.5 ± 15.2
_	173%	141%	85%		21%	-	65%
	n=3	n=2	n=4		n=2	n=1	n=2
May	$0.6 \pm$	16.9 ± 23.8	66.7 ± 71.4	144.4	13.8 ± 16.7	170.8	13.3 ± 14.3
-	0.57	141%	107%	CV = -	121%	-	107%
	94%	n=2	n=5	n=1	n=6	n=1	n=2
	n=3						
June	0.4 ± 0.7	38.3 ± 54.2	46.2 ± 30.5	NS	13.4 ± 15.8	91.7	40.3
	173%	CV =144%	66%		118%	-	-
	n=3	n=2	n=5		n=4	n=1	n=1
July	0	7.6 ± 10.8	4.2 ± 4.7	1.5	2.1 ± 3.8	13.6	2.1 ± 3.5
	-	CV = 141%	110%	CV = -	182%	-	173%
	n=3	n=2	n=5	n=1	n=6	n=1	n=3
August	0	0.23 ± 0.32	0.7 ± 1.5	0.5	0.0 ± 0.0	0.00	0.0 ± 0.0
	-	CV = 141%	CV =	-	-	-	-
	n=3	n=2	n=5	n-1	n=6	n=1	n=4
September	0	0 ± 0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.00	0.0 ± 0.0
	-	-	-	CV = -	-	-	-
	n=2	n=2	n=3	n-1	n=3	n-1	n=3
October	0	0	0 ± 0	0.0	0.0	0.00	0.0 ± 0.0
	-	-	-	CV = -	-	-	-
	n=2	n=1	n=2	N=1	n=1	n=1	n=2
November	0	0	0 ± 0	0.0	NS	2.30	0.0 ± 0.0
	-	-	-	CV = -		-	-
	n=2	n=1	n=2	N=1		n=1	n=2
December	0	0	0 ± 0	0.0	NS	19.72	2.2 ± 3.1
	-	-	-	CV = -		-	141%
	n=3	n=1	n=2	n-1		n=1	n=2

Table A5-4. Descriptive statistics (mean, standard deviation, and coefficient of variation) for density of unmarked coho salmon (fish per 1,000 m²). N = the number of years that reach was sampled for that month. Data from 2007 are not included as marked status of some fish was uncertain and sampling that year was not designed to measure density.

	Reach A	Reach B	Reach C	Reach E	Reach F	Reach G	Reach H
February	0.00	0.0	0.0 ± 0.0	NS	NS	0.6	0.00
_	-	-	-			-	-
	n=1	n=1	n=2			n=1	n=1
March	0.7 ± 1.0	0.0 ± 0.0	0.00 ± 0.0	NS	NS	0.00	0 ± 0
	141%	-	-			-	-
	n=2	n=2	n=2			n=1	n=2
April	0.0 ± 0.0	0.8 ± 1.1	0.0 ± 0.0	NS	0.00 ± 0.00	0.00	5.6 ± 6.3
	-	141%	85%		-	-	112%
	n=3	n=2	n=4		n=2	n=1	n=2
May	0.0 ± 0.0	0.9 ± 1.3	4.3 ± 9.7	0.93	0.00 ± 0.00	15.86	5.7 ± 5.7
	-	141%	224%	-	-	-	100%
	n=3	n=2	n=5	n=1	n=6	n=1	n=2
June	0.0 ± 0.0	1.8 ± 2.5	0.0 ± 0.0	NS	0.00 ± 0.00	0.00	5.9
	-	144%	-		-	-	-
	n=3	n=2	n=5		n=4	n=1	n=1
July	0.0 ± 0.0	0.9 ± 1.3	0.9 ± 1.9	0.00	0.00 ± 0.00	0.00	0.15 ± 0.25
	-	141%	224%	-	-	-	173%
	n=3	n=2	n=5	n=1	n=6	n=1	n=3
August	0.0 ± 0.0	0.23 ± 0.32	0.6 ± 1.1	0.00	0.0 ± 0.0	11.2	$18.6{\pm}37.2$
	-	141%	194%	-	-	-	200%
	n=3	n=2	n=5	n-1	n=6	n=1	n=4
September	0.0 ± 0.0	0 ± 0	0.0 ± 0.0	0.00	0.0 ± 0.0	0.00	0.0 ± 0.0
	-	-	-	-	-	-	-
	n=2	n=2	n=3	N=1	n=3	n-1	n=3
October	0.0 ± 0.0	0	0 ± 0	0.00	0.0	0.00	7.8 ± 6.2
	-	-	-	-	-	-	79%
	n=2	n=1	n=2	N=1	n=1	n=1	n=2
November	0.0 ± 0.0	0	0 ± 0	0.00	NS	0.58	1.1 ± 1.6
		-	-	-		-	141%
	n=2	n=1	n=2	N=1		n=1	n=2
December	0.0 ± 0.0	0	0 ± 0	0.00	NS	2.41	11.9 ± 14.6
	-	-	-	-		-	123%
	n=3	n=1	n=2	n=1		n=1	n=2

Table A5-5. Descriptive statistics (mean, standard deviation, and coefficient of variation) for density of chum salmon (fish per 1,000 m²). N = the number of years that reach was sampled for that month. Data from 2007 are not included as marked status of some fish was uncertain and sampling that year was not designed to measure density.

	Reach A	Reach B	Reach C	Reach E	Reach F	Reach G	Reach H
February	0.0	2.2	0.00 ± 0.00	NS	NS	0.00	0.00
_	-	-	-			-	-
	n=1	n=1	n=2			n=1	n=1
March	0.7 ± 1.0	0.0 ± 0.0	1.0 ± 1.4	NS	NS	0.59	0 ± 0
	141%	-	141%			-	-
	n=2	n=2	n=2			n=1	n=2
April	148.1 ± 258.2	1.5 ± 2.2	3.7 ± 5.3	NS	1.9 ± 1.1	0.00	6.6 ± 7.7
_	171%	141%	146%		58%	-	116%
	n=3	n=2	n=4		n=2	n=1	n=2
May	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 4.1	0.0	0.00 ± 0.00	1.22	0.10 ± 0.15
	-	-	CV =182%	-	-	-	141%
	n=3	n=2	n=5	n=1	n=6	n=1	n=2
June	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	NS	0.00 ± 0.00	0.00	0.00
	-	-	224%		-	-	-
	n=3	n=2	n=5		n=4	n=1	n=1
July	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.00 ± 0.00	0.00	0.00 ± 0.00
	-	-	-	-	-	-	-
	n=3	n=2	n=5	n=1	n=6	n=1	n=3
August	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.00	0.0 ± 0.0	0.00	0.00 ± 0.00
	-	-	-	-	-	-	-
	n=3	n=2	n=5	n=1	n=6	n=1	n=4
September	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.00	0.0 ± 0.0	0.00	0.00 ± 0.00
	-	-	-	-	-	-	-
	n=2	n=2	n=3	n=1	n=3	n-1	n=3
October	0.0 ± 0.0	0	0.0 ± 0.0	0.0	0.0	0.00	0.00 ± 0.00
	-	-	-	-	-	-	-
	n=2	n=1	n=2	n=1	n=1	n=1	n=2
November	0.0 ± 0.0	0	0.0 ± 0.0	0.0	NS	0.00	0.00 ± 0.00
	-	-	-	-		-	-
	n=2	n=1	n=2	n=1		n=1	n=2
December	0.0 ± 0.0	0	0.0 ± 0.0	0.0	NS	0.00	0.00 ± 0.00
	-	-	-	-		-	-
	n=3	n=1	n=2	n-1		n=1	n=2

Table A5-6. Descriptive statistics (mean, standard deviation, and coefficient of variation) for Chinook salmon genetic stock diversity. N = the number of years that Chinook salmon were collected and analyze for genetic stock for that month. Includes data from 2007 to 2012. NS = not sampled. ND = no data (not Chinook salmon caught and sampled for genetics).

	Reach A	Reach B	Reach C	Reach E	Reach F	Reach G	Reach H
February	ND	0.675	ND	NS	NS		ND
		-					
		n=1					
March	ND	0540	0.271	NS	NS	0.6971	ND
		-	-			-	
		n=1	n=1			n=1	
April	ND	0.414	0.584 ± 0.367	NS	0.597 ± 0.844	0.914	0.950
		-	63%		141%	-	
		n=1	n=2		n=2	n=1	n=1
May	0.00	0.748	0.698 ± 349	0.9369	0.612 ± 0.559	0.1.27	0.318 ± 0.450
	-	-	50%	-	91%	-	141%
	n=1	n=1	n=4	n=1	n=5	n=1	n=2
June	0.00	0.892	0.636 ± 0.043	0.419 ± 0.592	0.980 ± 0.279	0.628	1.04
	-	-	7%	141%	28%	-	-
	n=1	n=1	n=4	(n=2)	n=4	n=1	n=1
July	ND	0.404	0.304 ± 0.373	0.693	0.777 ± 0.198	0.937	ND
		-	123%	-	25%	-	
		n=1	n=4	n=1	n=2	n=1	
August	ND	0.00	0.849	ND	ND		ND
		-	-			ND	
		n=1	n=1				
September	ND	ND	ND	ND	ND	ND	ND
October	ND	0	ND	ND	ND	ND	ND
		-					
		n=1					
November	ND	ND	ND	ND	NS	0.562	ND
						-	
						n=1	
December	ND	ND	ND	ND	NS	0.00	ND
						-	
						n=1	

Table A5-7. Descriptive statistics (mean, standard deviation, and coefficient of variation) for the percentage of unmarked Chinook salmon that belong to non-Lower Columbia River stocks. N = the number of years that Chinook salmon were collected and analyze for genetic stock for that month. Includes data from 2007 to 2012. NS = not sampled. ND = no data (not Chinook salmon caught and sampled for genetics).

	Reach A	Reach B	Reach C	Reach E	Reach F	Reach G	Reach H
February	ND	3.3	ND	NS	NS		ND
		-					
		n=1					
March	ND	0	0.0	NS	NS	7.7	ND
		-	-			-	
		n=1	n=1			n=1	
April	ND	11.5	3.8 ± 5.4	NS	10.0 ± 14.1	20.0	40
		-	142%		141%	-	-
		n=1	n=2		n=2	n=1	n=1
May	0.0	3.3	10.8 ± 7.9	44.4	18.8 ± 20.2	36.7	16.7 ± 23.6
	-	-	73%	-	107%	-	141%
	n=1	n=1	n=4	n=1	n=5	n=1	n=2
June	0.0	25.4	20.7 ± 11.5	13.8 ± 19.5	43.9 ± 37.7	93.3	100
	-	-	56%	141%	86%	-	-
	n=1	n=1	n=4	n=2	n=4	n=1	n=1
July	ND	0.0	32.5 ± 45.6	50.0	56.1 ± 15.0	76.9	ND
		-	140%	-	27%	-	
		n=1	n=4	n=1	n=2	n=1	
August	ND	0.0	22.2	ND	ND		ND
_		-	-			ND	
		n=1	n=1				
September	ND	ND	ND	ND	ND	ND	ND
October	ND	0.0	ND	ND	ND	ND	ND
		-					
		n=1					
November	ND	ND	ND	ND	NS	75	ND
						-	
						n=1	
December	ND	ND	ND	ND	NS	50	ND
						-	
						n=1	

Appendix 6

Abiotic conditions

Continuous, in situ measurements of temperature (°C), specific conductance (μ S cm⁻¹ at 25°C), pH, and dissolved oxygen (mg L⁻¹) were determined at four fixed sites in shallow water habitats: Ilwaco, Whites Island, Campbell Slough, and Franz Lake Slough during 2011-2013. Data from 2011 and 2012 will be discussed here since the 2013 data were not fully verified at the time of writing.

The temperature time series at each site clearly showed increases over the spring-summer season (Figure 98). The highest variability in temperature was observed at the shallowest sites (Ilwaco and Campbell Slough), where the regression showed greater scatter (lower R^2) compared to the other two sites (Whites Island and Franz Lake Slough).

Water temperatures fluctuate widely at Ilwaco due to the strong ocean influence at this site, particularly during the summer months. The weekly maximum temperature standard of 17.5°C was exceeded at times in April and May and consistently after late May 2012. However, because of the influence of cool, marine waters, the daily mean temperature at Ilwaco was $<17.5^{\circ}$ C until early July, except for a brief period in mid-June. At Whites Island (Reach C), the water temperature did not exceed the weekly maximum temperature standard (17.5°C) until early July, at which point even the daily minima exceeded the 17.5°C threshold. Similarly, at Campbell Slough in Reach F and at Franz Lake Slough in Reach H, water temperature exceeded the weekly threshold of 17.5°C by the first or second week of July. However, shorter-term temperature peaks earlier in the season at Campbell Slough resulted in weekly temperatures in excess of the threshold in late May, early and late June, and nearly all of July. The y-intercepts produced by the regression analyses suggest that the average spring temperatures differed significantly, with both Franz Lake Slough and Whites Island having early spring temperatures of ~7.8°C. In contrast, early spring temperatures extrapolated from the regression at Ilwaco and Campbell Slough were 10.6 and 9.2°C, respectively. While these differences are small relative to the seasonal fluctuation in temperature at each of the sites, the differences most likely correspond with habitat depth and cover, which ultimately influences the resilience of the habitats to temperature extremes. A question that is generated by these data: is the starting temperature related to the number of days the habitat temperature is close to or exceeding temperature thresholds?

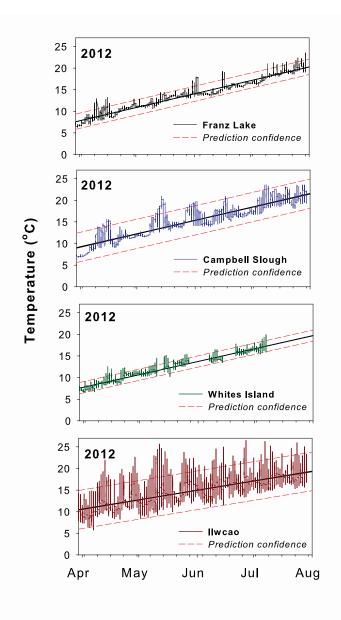


Figure 98. Date-temperature relationships at four fixed EMP sites (Franz Lake, Campbell Slough, Whites Island, and Ilwaco) during 2012.