DIET PREFERENCES OF JUVENILE STEELHEAD

(*ONCORHYNCHUS MYKISS*):

A COMPARISON BETWEEN THREE HOOD CANAL RIVERS

by

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ABSTRACT

Diet preferences of juvenile steelhead (*Oncorhynchus mykiss*):
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Hatchery programs have been established in Washington State for decades to supplement declining wild steelhead (*Oncorhynchus mykiss*) populations. However, these hatchery programs have been implemented with a limited understanding of how the introduction of large numbers of hatchery raised steelhead in Puget Sound river systems impact wild steelhead populations. This information can inform current steelhead supplementation programs throughout the Pacific Northwest by providing the composition and quantity of important prey items and will allow for a better understanding of juvenile steelhead needs. This study examined differences between wild and hatchery steelhead in three Hood Canal rivers of various sizes. Results showed that Ephemeroptera aquatic larvae are the dominant items in the drift and the dominant prey items in wild and steelhead juvenile diets. At the river scale, wild and hatchery steelhead diets were found to differ significantly in the middle sized river ($A=0.1617$, $p<0.001$). At the reach scale, wild and hatchery diets were found to differ significantly in the lower reaches of the medium ($A=0.0370$, $p<0.001$) and large sized rivers ($A=0.1230$, $p<.001$), but not in the small river. This could possibly be due to the lack of resources in the small river, where the abundance of items in the drift was lowest, and as such, fish had less selection, thereby eliminating differences between wild and hatchery diet. This is consistent with the observation that wild and hatchery diets were found to have a wider variety of prey items in the smallest size river. Furthermore, wild and hatchery fish rejected fewer items

relative to the larger rivers, with wild fish consistently consuming all items available relative to drift. Overall, wild fish consumed a greater diversity of prey relative to hatchery fish. These results show that wild and hatchery steelhead have been found to consume different items in some rivers and reaches and that river size seems to influence the number of items available. These diet differences could possibly be due to rearing environment differences or competition between wild and hatchery steelhead.

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Introduction

Puget Sound steelhead (*Oncorhynchus mykiss*) play important cultural, economic and ecological roles in Washington State. Declines in Puget Sound steelhead populations in recent decades led to the development of hatchery programs in the 1960's and 1970's designed to bring back historic steelhead populations (WDFW 2014a). The Puget Sound wild steelhead population has declined 97% since the 1800's. In 1895, steelhead populations ranged from 330,000-820,000 fish, while the current population is at an average of 22,000 fish (WFC (b)). These hatchery programs have been implemented with limited understanding of how the introduction of large numbers of hatchery raised steelhead in Puget Sound river systems impacts wild steelhead populations. Furthermore, despite these hatchery programs, steelhead populations have continued to decline. It is imperative that the interactions between wild and hatchery steelhead are better understood so it can more clearly be determined if current hatchery practices are helping or harming wild steelhead populations.

Through better understanding of wild and hatchery steelhead feeding behaviors, hatchery programs can be developed in order to ensure that hatchery fish are not negatively impacting wild populations. Diet analysis provides the composition and quantity of important prey items, allowing for a better understanding of juvenile steelhead needs (Wright 2010). An understanding of juvenile steelhead diets while in the freshwater ecosystem can inform current steelhead supplementation plans throughout the Pacific Northwest, where large numbers of steelhead populations also continue to decline. Measures can be taken to allow for hatchery programs to more closely mimic the conditions experienced by wild juveniles through a better understanding of the feeding habits and the factors that influence these feeding behaviors.

A difference in diet and prey preference could have several implications for hatchery practices and management. A difference in diet and preference may reflect hatchery practices that are rearing fish that have diets not found in wild fish. This could indicate that hatchery fish are not consuming the appropriate prey and are possibly not receiving the nutrients needed to allow for their survival to maturity. However, diet differences may also indicate competition between wild and hatchery steelhead. If larger and more aggressive hatchery fish (Abbot 1985; Hill 2006; Keeley & McPhail 1998 & McMichael et al. 1999) are consuming the preferred items, wild fish may be forced to specialize their diets, consuming the less desirable items that don't require confrontations with hatchery fish. Conversely, if the wild and hatchery juvenile populations are found to have similar diets it may indicate that hatchery supplementation programs are rearing fish that exhibit the same natural feeding patterns of wild fish.

Differences in diet could also have implications in regards to trophic dynamics. Steelhead juveniles feed almost exclusively on macroinvertebrates found in the drift. If hatchery fish are consuming macroinvertebrates that are not often food items for wild fish, the food chain could be drastically altered. This is due to the role of macroinvertebrates in stream ecosystems. Macroinvertebrates influence nutrient cycling, primary production and decomposition (Wallace & Weber 1996). If the balance of the ecosystem is geared toward what wild steelhead consume, introducing hatchery steelhead that consume different macroinvertebrate species may impact these essential ecosystem functions.

Understanding juvenile steelhead diets can also influence habitat restoration planning. Food webs are not often considered when planning habitat restoration projects. By taking into consideration steelhead feeding habitats and including numerous habitat types that allow for multiple feeding options, there is an increased chance that one of these habitats will become favorable to steelhead when environmental and ecological conditions change (Bellmore et al. 2013). This habitat restoration strategy will better enable future steelhead populations to thrive in the face of continued anthropogenic impacts including global climate change. Through the understanding of juvenile steelhead diets and feeding behaviors, restoration teams can provide an environment that will provide juvenile steelhead with the resources necessary to ensure proper growth and survival of these populations. Combining this with the identification of wild and hatchery steelhead diet preferences can provide the optimal combination of hatchery rearing and habitat restoration practices that will allow for the most beneficial conditions to restore wild steelhead population levels in Washington and throughout the Pacific Northwest.

In order to determine what the actual interactions are between wild and hatchery steelhead I will be focusing specifically on juvenile wild and hatchery steelhead in three Hood Canal rivers: the Dewatto River, Duckabush River and South Fork Skokomish River. I will be attempting to answer the following research question: Do wild and hatchery steelhead juveniles exhibit different diet preferences and do these preferences vary within rivers and between rivers? The middle, upper and lower reaches of these rivers is also of great importance to my study because it provides a more complete picture of each river system and how habitat and prey dynamics may impact juvenile steelhead diet and preference. These are three of six rivers included in a larger study, the Hood

Canal Steelhead Project (HCSP), in which the effects of hatchery steelhead supplementation are being examined. The three supplemented rivers included in this study contain hatchery fish that were collected from these rivers, after spawning occurred naturally, making them genetically wild. These eggs were then raised in a conservation hatchery setting where food and water temperatures were closely regulated.

Following this introduction, a comprehensive literature review will provide an in-depth look into steelhead life history, causes for steelhead population decline, the establishment of Washington State fish hatcheries and the known impacts of hatchery steelhead on wild populations. The second portion of the literature review will focus more specifically on juvenile steelhead diets and feeding behaviors. It will also provide a detailed look into past and present research conducted in the area of juvenile steelhead diet analysis. Next contains the findings of this study, presented in the form of a manuscript. The manuscript contains study background, methods used and data analysis conducted, as well as a discussion of the results and findings of the research questions. The final chapter focuses on the key findings of this research and the implications of these findings on steelhead management plans in Washington State and the Pacific Northwest.

CHAPTER II: Literature Review

Puget Sound steelhead populations have declined 97% since the 1800's resulting in the development of hatchery programs in Washington State in an attempt to boost wild steelhead populations. However, steelhead populations have continued to decline despite these programs (WFC (b)). These hatchery programs have been implemented with a limited understanding of the interactions between wild and hatchery steelhead and how hatchery steelhead impact wild populations. It is imperative that these interactions are better understood to determine if current hatchery programs are truly helping to boost wild populations.

Understanding juvenile steelhead diets while in freshwater systems is an important element to consider when developing hatchery supplementation plans with the goal of improving wild steelhead populations. Steps can be taken to allow hatchery programs to more closely align with the conditions experienced by steelhead juveniles in the wild. For this to be possible, the feeding habits of wild and hatchery steelhead must be understood. This literature review will provide the background information needed to understand the complexities of wild and hatchery steelhead interactions and more specifically, what factors have been found to influence wild and hatchery steelhead feeding preferences and diet.

Steelhead Life History

Steelhead are anadromous salmonids. Adults return from the ocean to lay their eggs in freshwater rivers and streams where the eggs hatch and spend 1-2 years rearing in freshwater. Smolts out-migrate to the saltwater environment where they spend 1-3 years before returning to freshwater to spawn (Sheppard 1972; Quinn 2005). Native steelhead populations extend from the Bering Sea to southern California. Steelhead have been introduced to freshwater lakes and streams in the United States, Canada, Europe, South America, Africa and Asia (Gilbert & Williams 2002; Krueger & May 1991). Within the United States steelhead have been introduced to the Appalachian Mountains (Krueger & May 1991) and in the late 1800's steelhead were introduced to the Great Lakes (Seelbach 1993).

Embryos

The length of time it takes for steelhead egg development is temperature dependent. On average the eggs need 50 days of 10°C water. For every degree below 10°C, the eggs will need an additional day of incubation and for every degree above 10°C the eggs need one less day of incubation (Sheppard 1972). Water temperatures vary extensively between rivers resulting in a wide range of steelhead incubation periods. Eggs that are laid in cooler streams will require much longer incubation periods than eggs in warmer water temperatures. For example, steelhead eggs laid in a stream with an average temperature of 5°C, will take 68 days to hatch, while in contrast, eggs in a stream with 11°C water will only require 28 days of incubation. This acceleration of hatching in warmer waters is due to the fact that higher temperatures increase metabolic rate and so increase the rate of development. Salmonid embryos, including steelhead, survive best in water temperatures between 5-11°C. Any temperatures below 2°C or above 14°C are lethal (Quinn 2005). Eggs are typically incubating from March to May, depending on when spawning occurred (Sheppard 1972).

Dissolved oxygen (DO) is the second most important factor in determining the time required between fertilization and hatching and can actually have a slowing effect in steelhead development in warm water temperatures. This is due to the fact that DO concentrations decrease with warmer temperatures because of the water's limited capacity to hold oxygen at higher temperatures. However, metabolic rate of embryo development increases with these warmer temperatures requiring a higher exchange of oxygen that is not readily available, resulting in delayed hatching. A decrease in DO concentrations at 10°C can delay the hatching of steelhead by 35-40 days, depending on the decrease in DO concentration (Quinn 2005). Therefore, water temperature is the main factor in determining the length of time required between egg fertilization and hatching, but DO levels can also add extra time to embryo development when water temperatures are high.

Alevins and Fry

After hatching, steelhead briefly enter the alevin stage, which occurs while still within the gravel of the redd. Alevins still have a yolk sac attached to their bodies to provide nutrients until they become large enough to capture sufficient amounts of food. Immediately after hatching alevins bury themselves deeper into the gravel, then gradually move up through the stream's substrate. Fry emerge from the gravel completely once the yolk sac has been absorbed, emerging as fry. The length of the alevin life stage is related to the same water temperature and DO factors that influence embryonic development (Quinn 2005).

Once the steelhead fry emerge they feed on microscopic organisms floating by in the current. As juvenile steelhead grow larger in size, they move to deeper parts of the stream to establish feeding territories where there are larger rocks and riffles. Steelhead juveniles will stay in freshwater for an average of one to four years before they begin smoltification (Busby et al. 1996).

<u>Smolts</u>

During smoltification juvenile steelhead undergo physiological changes that prepare them for their entrance into salty ocean waters. Steelhead smolts can out migrate to the ocean any time during the year but the majority migrates from April to June, with the peak occurring in mid-April. However, some steelhead never migrate to the ocean and become resident steelhead, also referred to as rainbow trout (Sheppard 1972).

<u>Marine Adults</u>

Once steelhead smolts reach the ocean, they begin a rapid growth process, reaching 5-30 lbs after two to three years (Sheppard 1972), though they can continue to grow larger once they return to the ocean after spawning. This rapid growth is due to the large amount of food available in the marine environment (Sheppard 1972). While in the ocean, steelhead feed on zooplankton, krill, squid, amphipods and schooling fish such as herring and sand lance (Sheppard 1972 & Quinn 2005). The distance that steelhead will travel while in the ocean varies greatly by population. Steelhead in the Pacific Northwest have been found to travel west to Kamchatka Peninsula in Russia. However, not all steelhead will travel that great a distance with some southern Oregon and northern

California populations only spending one summer at sea (Busby et al. 1996 & Quinn 2005).

Spawning Adults

There are two steelhead runs, winter run and summer run. The winter run steelhead begin to enter their natal stream in October and November, with the highest numbers occurring in January through March. Winter steelhead spawning occurs from late March to early May (Sheppard 1972). The summer steelhead, often referred to as "stream maturing" (Quinn 2005), begin to enter their native streams in late spring and summer, with highest densities generally reached in the late summer months of August through September. Summer run steelhead enter the rivers as sexually immature adults and remain in freshwater until they reach sexual maturity and spawn the following spring (Sheppard 1972).

Steelhead will spawn in main river channels and smaller side streams (Sheppard 1972). Females dig redds in stream bottoms, where eggs are deposited and simultaneously fertilized by males (Quinn 2005), with about 95% of the deposited eggs becoming fertilized (Sheppard 1972). Once the eggs have been fertilized, the females will cover the redd with gravel to protect the eggs until the eggs hatch. Female steelhead may have multiple redds in a single spawning season with each successive redd containing fewer and fewer eggs. The average female steelhead (~ 700m in length) will lay around 5,000 eggs. Steelhead are iteroparous, which means they can survive spawning and repeat the migration to the ocean and return to freshwater to spawn more than once (Quinn 2005). Steelhead have been documented to make the journey from the ocean to spawn in freshwater as many as ten times (Anderson 2014), meaning that depending on when smolting occurs, steelhead may live to be 15 years old.

Steelhead Population Decline

Throughout the coastal and interior waters of the western United States, steelhead populations have been in decline in recent decades. Currently there are eleven steelhead populations protected under the Endangered Species Act (ESA) including: the Columbia, Snake and Willamette rivers, Central California Valley, the California coast, and the Puget Sound in Washington State (NOAA 2014). On May 11, 2007, Puget Sound steelhead was listed as a threatened species. (Dept. of Commerce 2007), meaning that this distinct steelhead population is “likely to become endangered in the foreseeable future throughout all significant portions of its range” (Dept. of Commerce 2007). Population growth rates continue to decline 3-10% every year putting Puget Sound steelhead at a high risk of going extinct in the next 100 years (NOAA 2011). This population decline is due to overfishing, habitat loss in both freshwater and estuary environments, development of hydropower, poor ocean conditions and hatchery practices (NOAA 2011).

Overfishing has contributed to the decline in Puget Sound steelhead and has greatly impacted the summer run populations. Since the mid-1900’s commercial fishing of steelhead has been limited, but sport fishing of steelhead has grown in popularity (Sheppard 1972). Management of the steelhead sport fishing industry may have contributed to the drastic decline, and even elimination, of summer run steelhead. After the introduction of hatchery steelhead, open fishing for steelhead shifted to earlier in the season to accommodate for the returning hatchery fish, which were genetically selected for early return in order to reduce inbreeding between wild and hatchery populations. This focus on the early run hatchery fish also allowed for the simultaneous catching of wild summer run steelhead due to the fact that previous state law allowed the capture of wild steelhead. However, current Washington State law prohibits keeping wild steelhead, year round. Only catch and release fishing of wild steelhead is permitted (DFW306243, 2012). As a result of these early regulations, the summer run populations have declined greatly and resulted in the shifting of steelhead life histories to favor later winter runs (McMillan 2006).

Habitat loss has also been a major factor in steelhead population decline. There are many factors that contribute to the loss of steelhead habitat including increased fine sediment loads due to land use practices, changes in stream temperatures and light levels and decreased levels of large woody debris **(**Collins 1976, Hicks et al. 1991 & Suttle et al. 2004**).**

Human caused activities, such as road building, forestry practices, livestock grazing and mining practices (Collins 1976 & Hicks et al. 1991**)** have increased fine sediment (silt and clay particles that are <0.0625mm (Woo et al. 1986)) storage throughout the steelhead's range (Suttle et al. 2004) resulting in the loss of suitable habitat. An investigation of juvenile steelhead in a California stream concluded that as fine-sediment loads increased, the growth of juveniles decreased. This reduced growth is attributed to the impact of large amounts of fine sediment on the macroinvertebrate populations which juvenile steelhead primarily rely on for food. An increase in fine sediment amounts is found to shift these macroinvertebrate communities to be dominated by burrowing taxa that can better escape the impacts of fine sediments. However, steelhead juveniles only consume organisms that are available in the drift, or main water column, making these burrowing macroinvertebrates an unusable food source. This lack of food availability results in decreased growth of steelhead juveniles (Suttle et al. 2004). Human impacts that cause an increase in fine sediment loads, even at low concentrations (Suttle et al. 2004) ultimately create habitat conditions that are unsuitable for steelhead populations, resulting in the loss of habitat available to these populations.

The removal of riparian vegetation along streams and rivers changes the light and temperature of these waterways, which in turn can impact primary and secondary production in the stream, as well as timing of the emergence and survival of juvenile salmonids, including steelhead (Hicks et al. 1991). Increases in water temperatures beyond those preferable for steelhead can inhibit adults from returning upstream to spawn, increase risk of disease outbreaks and alter the metabolism of fish, reducing their efficiency in converting food into energy. An increase in light and temperature can lead to an increase in primary and secondary production which may allow for an increase in food for steelhead juveniles. However, this increase in food production is offset by the detrimental impacts associated with increased stream temperatures (Hicks et al. 1991).

An alteration in stream temperatures can also negatively impact steelhead in winter months. A lack of riparian vegetation along streams in the winter reduces insulation potential and can result in the formation of ice and even a “freeze up” in higher elevation areas. Once air temperatures warm and the ice breaks up, it can scour stream bottoms, disrupting the recently created redds (Hicks et al. 1991).

Through logging practices, as well as other land-use changes, the removal of riparian vegetation and surrounding trees leads to a decrease in the amount of available large woody debris (LWD). Large woody debris includes entire trees that fall into or near a stream, as well as large branches, tree crowns and root balls that enter stream channels (Sickle & Gregory 1990). LWD has also been removed from streams for navigation and to reduce property damage during floods. Historically, LWD was removed from rivers because it was initially believed to inhibit fish migration (Hicks et al. 1990). However, LWD is now recognized to play an important role in steelhead and salmonid fish habitat by creating dynamic areas of water movement, with stretches of faster moving water and deep pools where steelhead can rest and hide from predators (Roni & Quinn, 2001).

Of all the anthropogenic challenges that threaten steelhead habitat, dams have had the greatest impact (Collins 1976). Dams create barriers for adult steelhead returning to spawn and for out-migrating juveniles. Dams also disrupt river flows by creating large reservoirs in areas of the river in which water used to flow freely, as well as cause increases in water temperature. Predator and prey dynamics are also altered, food availability is disrupted and disease rates increase with the presence of dams (Collins 1976).

Dams greatly impact juvenile steelhead, and all other salmonid populations. As the water passes over the spillway, or through the turbines, the juvenile fish flow with it. Juveniles that pass through the turbines may be injured or killed by the movement of the turbine blades themselves or by the high water velocity, turbulence and larger pressure changes associated with the large volumes of water passing through the turbines (Collins

1976 & Petrosky & Schaller 2010). Fish that become injured or disoriented after their passage over the spillway or through the turbines are highly susceptible to predation from predators waiting below the dams (Collins 1976 & Petrosky & Schaller 2010). In the 1980's and 1990's, turbine screens were installed in order to avoid this dam related mortality. These screens directed out migrating juveniles to bypass systems or to collection systems in which juveniles were collected and then transported in tanker trucks around the dams (Collins 1976). However, these practices still can have detrimental impacts on juvenile steelhead including high levels of stress and exposure to pathogens while in holding areas and transport vehicles (Petrosky & Schaller 2010).

In addition to the direct dangers juvenile steelhead face during their seaward migration, dams also negatively impact habitat and environmental conditions. For example, a study conducted on a dammed portion of the Columbia River found that juvenile fish were delayed anywhere from three days to a month before out migrating to estuaries, making them more susceptible to predation and disease (Collins 1976). Due to the increased surface area associated with the impounded waters that dams create, an increase in water temperatures also occurs, which can reach lethal levels during the summer months. Furthermore, thermal stratification occurs in these large water bodies, in which warmer waters are located in the top of the water column, while cooler waters sink to the bottom. This stratification is more detrimental to juvenile steelhead rather than adults. Due to their small size and inability to swim into deeper waters, juveniles are only able to occupy these warmer, top areas of the water column, also making them susceptible to disease and death (Collins 1976).

Changes in river habitat are not the only challenges that steelhead face. Poor ocean conditions are also believed to be a contributing factor in the decline if steelhead populations. Lower steelhead survival rates have been found to be associated with warmer ocean waters and reduced spring upwelling (Petrosky & Schaller 2010). Hatchery practices are believed to also be contributing to steelhead population declines and will be discussed further below.

Washington State Fish Hatcheries

The first hatchery was established in Washington State in 1895 on the Kalama River in order to mitigate for large areas of altered habitat. Since then, Washington State has established a large network of 146 hatcheries that are focused on salmonids and trout, including steelhead. Eighty-three of these hatcheries are operated by the state, fifty-one are tribal hatcheries and twelve hatcheries are federally managed (WDFW 2014a). Hatcheries are now a large part of the state's economy, providing salmonids and trout for commercial and recreation fisheries. Currently 88% of steelhead caught in commercial and recreational fisheries are of hatchery origin (WDFW 2014a). After the listing of many salmon and steelhead populations under the Endangered Species Act in 1997 and 1998, Washington State hatcheries began supplementation programs in order to boost wild fish population numbers (WDFW 2014a). The most recent available records available through the Washington Department of Fish and Wildlife state that in 2013, 5.3 million hatchery steelhead were released in Washington State, with 1.2 million of released into Puget Sound (WDFW 2014a). Release of hatchery steelhead and salmonids is still on-going, with the release of millions of fish a year (WDFW 2014a).

However, these hatchery practices have been implemented with little understanding of the impacts of hatchery fish on wild populations (Arkai et al. 2007). A growing number of scientists and conservation organizations are concerned that these large releases of hatchery steelhead can have significant impacts on wild steelhead populations including: negative genetic interactions (Arkai et al. 2007; Kostow 2009 & Mackey et al. 2001), declining steelhead survivability (Chicolte 2003 & Smith & Li 1983) and significant size differences between hatchery and wild steelhead juveniles (Abbot 1985; Berejikian et al. 1996; Hill 2006; Keeley &McPhail 1998; Kostow 2009 & McMichael 1999).

Many organizations have become concerned about the impacts of hatchery fish on wild steelhead populations, including The Wild Fish Conservancy (WFC). WFC is a non-profit conservation organization established in Duvall, WA in 1989 which states: "through science, education and advocacy, WFC promotes technically and socially responsible habitat, hatchery and harvest management to better sustain the region's wild-fish heritage." In March of 2014, the WFC filed a lawsuit against the Washington State Department of Fish and Wildlife (WDFW). The WFC claimed that WDFW was violating the Endangered Species Act by imperiling wild steelhead, salmon and bull trout recovery (WDFW 2014b) by operating hatchery programs without hatchery genetic management plans (HGMP) approved by the National Oceanic and Atmospheric Administration (NOAA). NOAA approval is required before hatchery programs can be implemented (WFC 2014).

In April 2014, WDFW and WFC reached an agreement. The only hatchery released steelhead in Puget Sound will be 180,000 fish into the Skykomish River in

Snohomish County in 2014 and 2015 to support the steelhead recreation fishery (WDFW 2014b; WFC 2014 & Yuasa 2014). The remaining steelhead will be released for sport fishing in Washington lakes that have no connection to Puget Sound (Yuasa 2014 & WDFW 2014b). The agreement also stated that WDFW will not release winter run steelhead into Puget Sound rivers until the National Marine Fisheries Service has reviewed and approved each state hatchery's HGMP (WDFW 2014b). In addition, a 12 year research study will be conducted on the Skagit River, in which no winter run hatchery steelhead will be released. This study will allow for the evaluation and possible establishment of hatchery programs in the Skagit Watershed using wild hatchery stock (WDFW 2014b). This lawsuit is a clear indication that people are concerned about hatchery steelhead affecting wild runs, making research focused on these interactions essential.

Impacts of Hatchery Reared Steelhead on Wild Fish

Current research into hatchery and wild steelhead interactions has focused mainly on the genetic implications of cross-breeding between these two populations (Arkai et al. 2007; Kostow 2009 & Mackey et al. 2001). Previous research has also focused on studying the size difference between wild and hatchery smolts at the time of hatchery release and the dominance and aggression associated with these size differences (Abbot 1985; Berejikian et al. 1996; Hill 2006; Keeley &McPhail 1998; Kostow 2009 & McMichael 1999). Another emerging need for steelhead research is a closer examination of steelhead juvenile diets and the differences between wild and hatchery prey items and feeding behaviors. These areas of steelhead research focused on wild and hatchery interactions can allow for improved hatchery practices and can inform restoration and conservation efforts to reduce negative wild and hatchery steelhead smolt relationships and to aid in the removal of Puget Sound steelhead from the ESA's Threatened Species List.

Genetics

Research focused on steelhead genetics is important because genetic overlap between wild and hatchery populations is a major concern. It is believed that hatchery fish can reduce the fitness and survivability of wild fish when these populations interact during spawning. Steelhead raised in a traditional hatchery setting, meaning hatchery fish that are breed with other hatchery raised fish, have showed lower fitness than wild fish (Arkai et al. 2007). Although there is no clear definition of fitness, as used here, it is referring to the ability of a species or population to survive and reproduce in the environment in which it inhabits (Orr 2009). There are three possible explanations for this observed fitness decline in hatchery fish. The first is the accumulation of deleterious mutations. Research has shown that in hatcheries, the survival rate of hatchery fish from the egg to smolt stage is 85-95%, while the survival rate of wild steelhead during the same time frame has a survival rate of only 1-5% out in the wild (Arkai et al. 2008). However, in the wild, those fish that had genetic abnormalities would have never survived to adulthood. Therefore, when hatchery fish are released into the wild environment, those genetic abnormalities that may have been beneficial or had no effect while in captivity are not conducive to the environment experienced outside the hatchery (Akai et al. 2008 & Solberg et al. 2013). This can lead to a decrease in the ability of hatchery fish to survive and reproduce.

The second possible contributor to the decrease in the fitness of hatchery fish is inbreeding depression. This refers to the reduction in fitness related to mating between relatives. Some hatchery programs may only have small breeding populations to work with, causing inbreeding to occur. Inbreeding has been shown to decrease offspring survival rates by 10-30% (Araki et al. 2008). However, there is an ongoing debate on these survival rate decreases in regards to the exact genetic causes of fitness decline (Araki et al. 2008).

The third possible reason for decreased fitness in hatchery steelhead is domestication selection, in which traits that may be beneficial to steelhead in the hatchery environment may be detrimental to hatchery populations once they enter the natural environment. Selection for high growth rates are favored in conventional hatchery settings (Weber & Fausch 2003) because many hatchery programs raise steelhead to undergo smoltification at age-1, while the majority of wild steelhead will not advance to the smolt stage until at least age-2, and often not until age-3 or 4 (Hill et al. 2006 & Kostow 2009). Thus, hatchery fish are larger at an earlier age. Smolt size has been linked with improved survival to adulthood therefore hatchery programs produce fish to undergo early smoltification in order to increase the likelihood of fish surviving to adulthood and to reduce the cost associated of having to raise juveniles in a hatchery setting for an additional 2 or 3 years (McMichael et al. 1999). Although having larger smolts at an earlier age may be appropriate for the purposes of hatchery production it can have negative impacts on fish once they are released from the hatchery. Larger fish have higher metabolic rates and so require more food than smaller fish. If hatchery fish are released into environments with limited food availability or where wild steelhead and other fish species are present, they may be unable to meet their metabolic needs, resulting in decreased survival (Smith & Li 1983).

Hatchery fish are believed to have lower fitness possibly due to these genetic effects, making them less likely to survive and reproduce when they are released from the hatchery environment into the natural environment. Researchers have found indications that the genetic differences between wild and hatchery fish can have a negative impact on wild steelhead. A study of Oregon steelhead populations found that when hatchery fish made up 50% of the spawning population productivity declined (Chicolte 2003). Productivity refers here to the relationship between spawning adults and their ability to successfully reproduce offspring that will themselves return to spawn as adults. Researchers found that when the steelhead population was made up of equal numbers of wild and hatchery fish that 63% fewer recruits were produced per spawning adult (Chicolte 2003). This decrease in productivity indicates that hatchery and wild interactions can lead to a decrease in reproductive success.

Hatchery practices have been developed to limit the genetic interactions between wild and hatchery steelhead populations, but are not always 100% successful. It is common hatchery practice to breed hatchery fish so that they spawn months before the wild fish in order to reduce inbreeding of these two populations (Mackey et al. 2001). Hatchery fish usually return to spawn three months before wild fish. However, some population overlap has been observed where hatchery fish return late and mix with wild populations or wild fish return early and mix with hatchery populations (Mackey et al. 2001). With overlap occurring between wild and hatchery fish it is possible that these hatchery fish may be uncovering and exposing wild steelhead redds and reducing the chances that the wild steelhead can be successful in their reproduction (Kostow 2009). Although it was found that spatial interactions occurred between the two populations, wild fish tended to spawn further up stream than hatchery fish, perhaps limiting the amount of breeding between wild and hatchery steelhead populations (Mackey et al. 2001).

Resource Competition and Size Differences

Negative ecological effects have been found to be the greatest when wild and hatchery steelhead share a similar environment for an extended period of time, such as during freshwater development (Kostow 2009). "Hatchery adults and their juvenile offspring were [found to] be using resources that could have been supporting wild populations (Kostow 2009)." As is a usual hatchery practice, large amounts of steelhead hatchery smolts are released all at one time, which leads to increased food competition between wild and hatchery fish. Also a common hatchery practice is to release one year steelhead smolts, while wild steelhead generally smolt around age-3 or 4. Growth rates are accelerated in order to produce smolts that will be big enough to survive to adulthood in the most cost effective timeframe (McMichael et al. 1999). By releasing hatchery smolts so early, there are often higher numbers of residual steelhead (those fish that did not out-migrate right away but remain in freshwater) in stream systems, adding to the reduction of resources available to wild smolts (Kostow 2009).

Territory size tends to increase with steelhead size (Keeley & McPhail 1998) and larger fish tend to be more dominant (Abbot 1985). More dominant fish compete with smaller, more subordinate fish can consume food at higher rates. This is due to the fact that the subordinate fish have been found to reduce their own feeding rates when a dominant fish is present, thus allowing the dominate fish to have greater access to the food that is available (Abbot 1985).

Larger, dominant fish are also found to be more aggressive. These aggressive fish have more access to the central water column where prey is readily available (Keeley & McPhail 1998). Fish that are dominant can maintain their status, become larger and continue to consume more prey (Berejikian 1996) than wild, often smaller steelhead smolts. Hatchery fish have been found to be dominant to wild fish in most interactions, except when wild smolts were larger than hatchery smolts (McMichael et al. 1999). This competition between hatchery and wild steelhead limits resource availability and due to the aggressive behavior of hatchery fish, wild fish are displaced from preferred feeding areas. When wild fish are displaced from their original position because of avoidance of hatchery fish, the wild fish usually stop feeding altogether (McMichael 1999). This negative interaction found between wild and hatchery fish can harm wild steelhead populations by limiting the amount of food that these juveniles can consume. A lack of food can negatively impact steelhead growth, fitness and survivability.

Juvenile Steelhead Diets and Feeding Behaviors

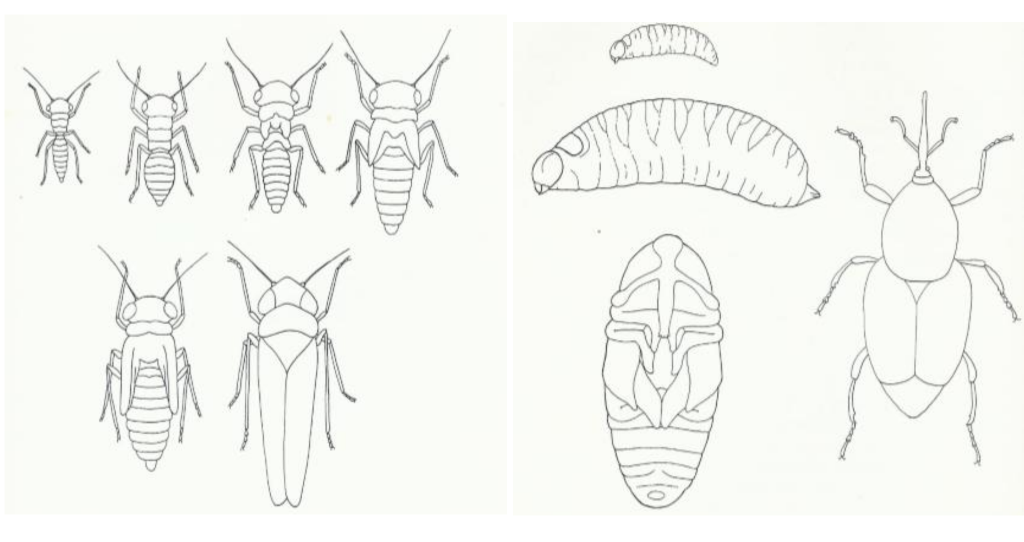
Juvenile Steelhead Diets

Juvenile steelhead diets mainly consist of terrestrial and aquatic macroinvertebrates (Mistak et al. 2003; Rundio & Lindley 2008 & Simpson et al. 2009) and smaller fish, often sub-yearling salmonids (Simpson et al. 2009). Because steelhead diets consist largely of aquatic and terrestrial macroinvertebrates, in all life stages, a brief description of the macroinvertebrate life cycle is necessary before going into the further specifics of what types of macroinvertebrates juveniles consume.

<u>Macroinvertebrate Life Cycle</u>

Depending on the species, macroinvertebrates undergo one of two metamorphic life cycles, complete metamorphosis and incomplete, or gradual, metamorphosis (Figure 1) (Lanham 1964). Stoneflies (*Plecoptera*) and grasshoppers are examples of macroinvertebrates that undergo incomplete metamorphosis (Lehmkuhl 1979 & Reece et al. 2011) During incomplete metamorphosis eggs develop into nymphs, which then develop into adults (Reece et al. 2011). Nymphs resemble adults, sometimes sharing the same feeding behaviors and habitats, but are smaller and do not have the ability to fly or reproduce. During this stage nymphs undergo multiple molts in which the wings become more developed with each molt (Lanham 1964 & Reece et al. 2011). After the molt, the macroinvertebrate emerges as a full sized, winged, sexually mature adult (Reece et al. 2011).

Figure 1 Diagrams of incomplete and complete metamorphosis (Lanham 1964).



Incomplete Metamorphosis   Complete Metamorphosis

Riffle beetles (*Coleroptera*) and butterflies are examples of macroinvertebrates that undergo complete metamorphosis (Lehmkuhl 1979). This cycle begins with the egg stage and progresses through the larva and pupae stages before becoming adults (Lehmkuhl 1979 & Reece et al. 2011). Unlike incomplete metamorphosis, the young look nothing like the adults and serve very different functions (Lanham 1964). The main function of young during the larval stage is to feed and grow as quickly and "economically" as possible (Lanham 1964). The majority of the actual metamorphosis occurs during the pupae stage in which the wings and legs develop. At this stage, the pupae stops feeding and does not move. All the development is occurring internally, where the larval tissues are replaced by adult tissue. Pupae are often contained within a protective shell such as a puparium, or a cocoon or chrysalis in the case of moths and butterflies (Lanham 1964).

Prey Consumption

As steelhead juveniles age their diet preferences change. A study focused on recently emerged steelhead fry concluded that the two main taxa that fry feed on are Chironomids (Johnson et al. 2013), also known as midges (Pacharsky et al. 1990) and baetids (Johnson et al. 2013), commonly referred to as mayflies (Lehmkuhl 1974), making up 20-42% and 14-34% of fry diets respectively (Johnson et al. 2013). Terrestrial macroinvertebrates made up 7-18% of steelhead fry diets (Johnson et al. 2013).

One year old juveniles in freshwater mostly consume insect larvae and pupae, adult insects and amphipods (crustaceans). At age two, steelhead eat the same number of insects, fewer amphipods and begin to eat small fish larvae. When steelhead juveniles are three years old they eat more fish larvae, consume more mollusks and consume fewer insects. And at age four, generally the age when steelhead begin to migrate out to sea, they feed mainly on small fish larvae and adult fish (Juncos et al. 2011).

<u>Feeding Behavior</u>

Juvenile steelhead hold their feeding position by swimming against the current and moving out of their position to capture prey drifting in the water column (Keeley & McPhail 1998). When steelhead feeding behaviors and diets were examined in main river channels and stream side channels, fish consumed almost all the prey available when in the main channels. However, the steelhead were found to utilize the food sources available in both the main and side channels, even though there were higher levels of macroinvertebrates in the main channels than the side channels. The fact that steelhead consumed macroinvertebrates in both the main and side channels, regardless of insect levels indicates that steelhead are flexible and will consume whatever prey is available (Bellmore et al. 2013).

"Feeding rate is a critical factor for survival during stream-rearing and subsequent life history stages (McCarthy et al. 2009)." Steelhead metabolism is affected by water temperature and fish body weight. The rate of metabolism determines the level of energy left for growth (Elliot 1993). Therefore, steelhead juveniles feed on drift in both winter and summer months because this method utilizes the least amount of energy. By feeding on what is already available in the drift steelhead are not required to expend energy actively looking for food resources (McCarthy et al. 2009 & Wright 2010).

Wild vs. Hatchery Steelhead Smolt Diets and Feeding Behavior

Wild and hatchery steelhead diets have been closely examined in the Pacific Northwest and in the Great Lake regions of the United States. Wild steelhead feeding during the day has been found to be dependent on prey availability in the stream (Elliot 1973). Wild steelhead diets contain a wide range of aquatic and terrestrial insects (Rundio & Lindley 2008). When hatchery steelhead and wild steelhead are compared, hatchery residual fish show more surface oriented feeding than wild steelhead and consume more Hemiptera (true bugs) and Archnida (eight-legged jointed invertebrates) than wild fish. This likely due to the fact that hatchery fish are used to feeding on the surface in hatcheries (Simpson et al. 2009). Wild smolts tend to use deeper water and larger substrate than hatchery fish (Hill et al. 2006). This could cause a difference in diets between the two populations.

In regards to the amount of prey consumed by wild and hatchery steelhead smolts, there has been no firm conclusion. Some research has found that there was no difference in the number of invertebrates consumed by wild and hatchery fish (Goby et al. 2007), while other research has shown that out-migrating wild steelhead smolts consumed more prey than hatchery smolts (Simpson et al. 2009). Evidence supporting the finding that wild smolts consumed more prey than hatchery juveniles points to the decline in the condition of hatchery fish after release. This decline is believed to be linked to the inability of hatchery fish to recognize available food, less time spent foraging and lower feeding efficiency (Simpson et al. 2009 & Weber & Fausch 2003) than wild steelhead (Simpson et al. 2009). Stomach contents of both wild and hatchery fish consisted of similar taxa, including sub-yearling salmonids, Diptera (true flies), Tricoptera (caddisflies) and Ephemeroptera (mayflies) (Simpson et al. 2009).

Methods Used in Steelhead Diet Analysis

Resident Drift Collection and Analysis

There are numerous methods that have been used to collect and analyze the prey that are available for steelhead smolt consumption. The most common method in which to collect the invertebrates that are drifting through the water column is to stretch drift nets across rivers and streams to collect anything floating downstream. Because steelhead stay in the water column, facing upstream, this is an appropriate method in which to collect prey items. These drift nets are often set along different reaches of the stream, such as upper, lower and middle reaches. The nets are left in the river for one to twenty-four hours. (Elliot et al. 1973; Johnson 2007; Johnson et al. 2013; Keeley & McPhail 1998 & McCarthy et al. 2009). In order to determine the prey availability on the bottom of the river Surber samples are collected (Bellmore et al. 2013; Elliot 1973; Johnson 2007; Johnson et al. 2013 & Rundio et al. 2008), while terrestrial samples are either included in the drift net analysis or pans are set out on the water's surface to collect prey that falls into the stream (Rundio et al. 2008).

Once the drift samples are collected, they are placed in a preserving liquid until the samples can be transported to a lab and analyzed (Keeley & McPhail 1998). Many of the invertebrate samples are dried (Johnson 2007 & McCarthy et al. 2009) and then identified to taxon, order and family (Johnson 2007; Keeley & McPhail 1998 &McCarthy et al. 2009). These are then often divided by functional groups (McCarthy et al. 2009).

The most common way to determine prey abundance is to weigh the dried samples and calculate prey abundance (Elliot 1973 & Johnson 2007). Another method that has been used is to measure the length of the macroinvertebrates (Keeley & McPhail 1998).

Stomach Content Collection and Analysis

Two common methods are used to determine the prey items consumed by steelhead. The first method is the use of gastric lavage, which forces water into the smolt's mouth and into the stomach, causing them to expel their stomach contents (Rundio et al. 2008 & McCarthy et al. 2009). The advantages in using gastric lavage include high rates of prey item removal and high rates of fish survival. Research focused specifically on steelhead found that 90% of stomach contents were able to be removed from fish stomachs with gastric lavage. A comparison between hatchery and wild coho showed that gastric lavage did not have a significant impact on the condition and survivability of hatchery or wild fish 30 days after stomach flushing (Meehan & Miller 1978). Disadvantages to the gastric lavage technique include the inadequate flushing of larger and more ridged prey items and the decreased success in removing stomach contents from larger fish. Larger fish have greater stomach muscle mass resulting in more difficulty in dislodging prey from their stomachs (Meehan & Miller 1978).

The other commonly used method to collect stomach contents is to euthanize the smolts and surgically remove their stomachs entirely (Kiffney et al. 2014). It is not always possible to use this method with ESA listed species such as steelhead due to permitting and laws regarding "take". Once the stomach contents have been removed , the contents are dried and identified using the same methods used with the drift, benthic and terrestrial samples.

Mass of Stomach Contents

Total amounts of prey in fish stomachs can be calculated using the total wet mass of stomach contents and expressed as a percentage of body mass. A liner regression analysis can then be conducted to determine mean percent of stomach content mass across river reaches and months of data collection. This information is useful because it allows for the detection of differences between the different river habitats and conditions. If relationships are found where steelhead in some rivers have a larger proportion of their body weight as prey than steelhead in other rivers, then further investigations can be made into the habitat conditions of those rivers. Water temperature, canopy cover, water velocity and water depth can then be correlated with fish stomach contents.

Another important calculation used is the calculation of the mean number of prey items found in each stomach and determining the percent composition of those prey items in relation to the stomach contents for each specific fish sampled. In order to compare the stomach contents between species, or in the case of this study between hatchery and wild steelhead, the prey taxa that make up 5% or more of the diet composition in each reach and in each month can be included in the data analysis (Mistak et al. 2003).

Electivity Indices

In order to determine if juvenile steelhead are consuming prey items base on preference of certain prey species electivity indices can be used. “Electivity indices measure the utilization of food types in relation to their abundance or availability in the environment (Lechowicz, 1982).” Foods that make up a larger proportion of the diet than is available can be considered as preferred. If a food item makes up a smaller proportion than the food available, that food item can be considered as being avoided. If the proportions are equal between food items found in the diet and that are available, than that food item is considered to be eaten at random (Lechowicz, 1982).

Lechowicz (1982) compared three commonly used electivity indices, Ivlev’s, Jacobs’s and Vanderploeg and Skavia’s . The author found that Ivlev’s and Jacob’s electivity index can only be used if looking at two prey types, making it unsuitable for most diet analysis as most species consume numerous types of food. Due to the fact that steelhead juvenile diets will contain more than one prey species Vanderploeg and Skavia’s electivity index is the most appropriate.

The Vanderploeg and Scavia electivity index is determined using the following equation:

$$E_i = \frac{[W_i - (1/n)]}{[W_i + (1/n)]} \text{ where } W_i = \frac{r_i/p_i}{\Sigma_i r_i/p_i}$$

r_i= proportion of taxon i in the diet
p_i= proportion of taxon in i environment
n= number of kinds of food items

The electivity index ranges from -1 to +1, with a negative number indicating avoidance of a prey item and a positive number indicating a preference for a specific macroinvertebrate prey species (Vanderploeg & Scavia, 1979). These preferences can be calculated and compared for each month of collection, each river reach and each river as a whole and can be compared between wild and hatchery steelhead populations.

There has been comprehensive research in regards to steelhead life histories and some understanding of the genetic impacts and food resource competition between wild and hatchery steelhead. There is a basic understanding of steelhead diets but knowledge in the area of wild and hatchery steelhead diets is still not well known. And the research that has been conducted, does not generally focus on wild and hatchery feeding interactions and diets in Puget Sound. This makes investigating the differences between wild and hatchery steelhead within Washington State and Puget Sound even more imperative. The diet interactions of wild and hatchery steelhead are investigated in the study that follows, focusing specifically on the Hood Canal region of Puget Sound.

CHAPTER II: Manuscript

Introduction

Steelhead (*Oncorhynchus mykiss*) populations have declined significantly since the 1800's due to overfishing and habitat loss (WFC (b)). Due to this continuous population decline, with Puget Sound steelhead listed as threatened under the endangered species act in 2007 (Dept. of Commerce 2007), hatchery programs have been implemented in Washington State in recent decades in an effort to increase wild steelhead populations (WDFW 2014a). However, there is a limited understanding of the impacts that the release of hatchery raised steelhead into Puget Sound rivers have on the wild population. It is of critical concern to determine if these hatchery steelhead are helping or harming wild steelhead populations.

Although there has been research in the areas of genetic interactions (Arkai et al. 2007; Kostow 2009 & Mackey et al. 2001) and food resource competition between wild and hatchery steelhead (Abbot 1985; Berejikian et al. 1996, Hill 2006; Kelley &McPhail 1998; Kostow 2009 & McMichael 1999), there is still a lack of understanding in how wild and hatchery steelhead diets differ in regards to actual types of food items consumed. Understanding differences in juvenile diets can provide baseline information for hatchery supplementation focused on improving wild steelhead populations. Measures can be taken to allow hatchery programs to more closely resemble the conditions experienced by wild juveniles in order to ensure better survival of hatchery released steelhead once they reach freshwater.

Understanding steelhead diets can also inform habitat restoration managers and allow for the implementation of restoration practices that will benefit juvenile steelhead the most. By comprehending what prey items juvenile steelhead are consuming, efforts can be made to incorporate habitat elements that will foster an environment where these prey species can thrive. This will ensure the proper growth and survival rates necessary to boost wild steelhead populations. Combining beneficial habitat restoration with the understanding of wild and hatchery steelhead diets and interactions will allow for the optimal combination necessary to increase wild steelhead populations.

This study focuses specifically on the diets of hatchery and wild steelhead in three rivers located on the Hood Canal in Washington State: the Dewatto River, the Duckabush River and the South Fork Skokomish River. These three rivers were specifically examined due to their inclusion in an ongoing study, the Hood Canal Steelhead project, in which the impacts of hatchery steelhead on wild steelhead are being studied. Of the six rivers included in the HCSP the Dewatto, Duckabush and South Fork Skokomish Rivers are the experimental rivers in which hatchery steelhead are released. In addition, these three rivers are of varying sizes and allow for diet comparisons between small, medium and large rivers. This provides a bigger picture of the factors that may impact the diets of juvenile steelhead. Downstream and upstream diet differences were examined in order to identify possible differences in diet assemblages due to the differing habitats that are present in the upper, middle and lower reaches in each of these rivers. Macroinvertebrate species may differ depending on which reach of the river they occupy. This in turn may have an impact on the items on which juvenile steelhead feed.

Drift in each of the rivers was examined in order to determine the types of items that are available for steelhead consumption. Preference and avoidance of item types found in juvenile steelhead diets were also examined. Comparisons were made between wild and hatchery steelhead juveniles at the river scale, with all reaches being combined. Comparisons were also made between wild and hatchery steelhead in the lower reaches of each river.

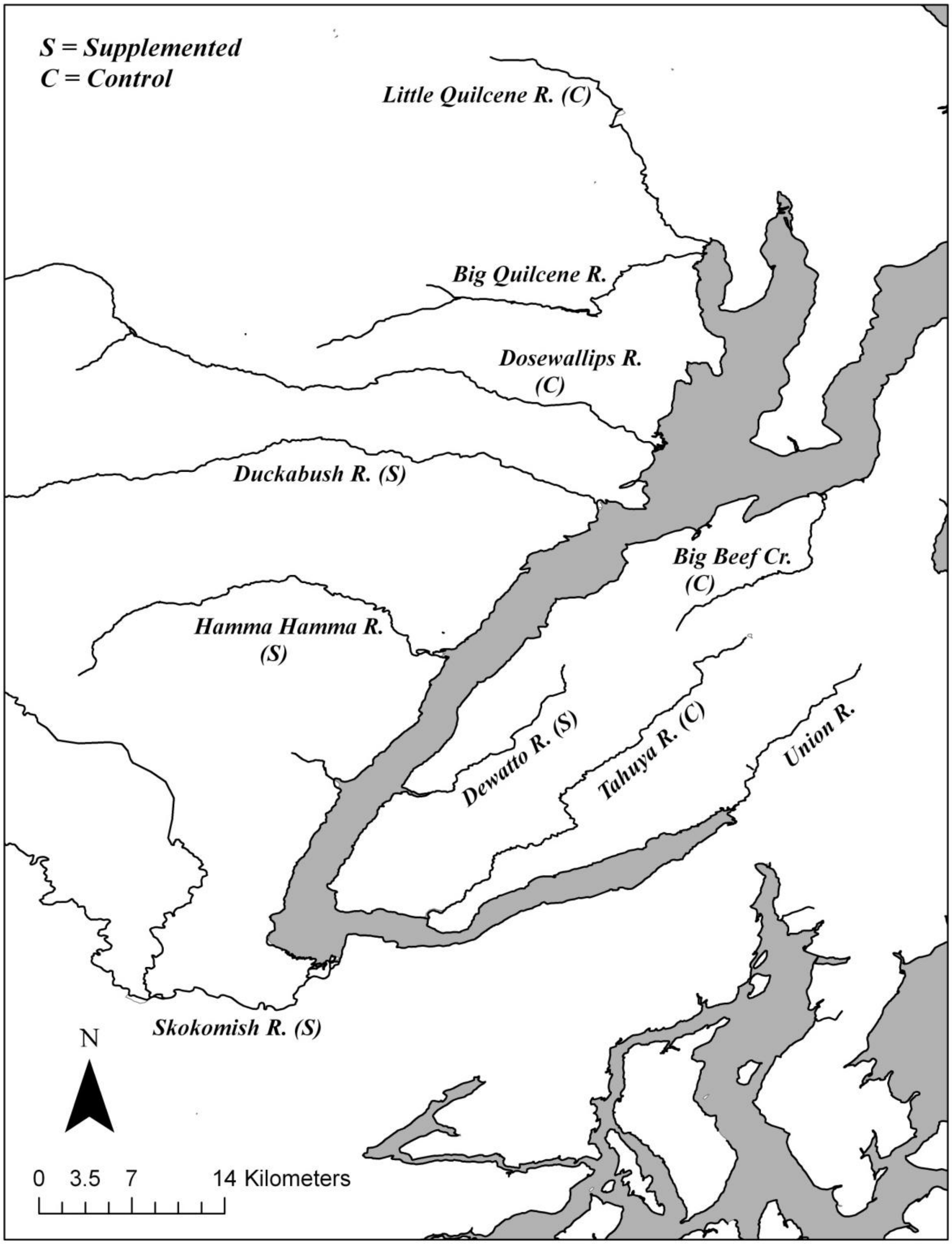
The findings of this study can be used to implement hatchery practices and habitat restoration measures that can more effectively improve wild steelhead population numbers, as well as ensure that the implementation of hatchery programs across Washington State are not having negative impacts on wild populations.

Methods

Hood Canal Steelhead Project

As previously discussed, this steelhead juvenile diet study is part of a larger research study, the Hood Canal Steelhead Project (HCSP). The HCSP is an on-going sixteen year study that began in 2006 (NOAA 2015). The HCSP aims to augment steelhead populations by supplementing Hood Canal Rivers with juvenile and adult steelhead over a fixed period of time. There are three supplemented rivers (Dewatto, Duckabush and South Fork Skokomish) and three control rivers where no supplementation has occurred (Tahuya, Little Quilcene and Big Beef Creek), although they are not studied here (Figure 2). This project is an expansion of a 10 year pilot study conducted on the Hamma Hamma River (Berejikian et al. 2008). The HCSP is a large

Figure 2. A map showing the locations of the supplemented (S) and control (C) streams in Hood Canal. The Hamma Hamma River supplementation was terminated in 2007 (Berejikian et al. study plan draft).



partnership with eleven different, federal, state, tribal and non-profit agencies (LLTK 2010).

Supplementation efforts began with the collection of eyed-eggs (the stage at which the eyes are visible within the eggs) from naturally-occurring steelhead redds on the supplemented rivers. This differs from traditional hatchery programs where eggs are collected and fertilized by hand. This method allows for natural spawning and egg fertilization, creating more genetic diversity than found in traditional hatchery programs where fertilization is human controlled. The number of eggs collected in each river will depend on the number of steelhead redds typically found in each of the three rivers. Once collected, these eggs are then brought to the U.S. Fish and Wildlife Service's Quilcene National Fish Hatchery (Dewatto and Duckabush eggs) and Washington State Department of Fish and Wildlife (WDFW) McKernan Hatchery (South Fork Skokomish eggs) where they are incubated and tested for pathogens (Berejikian et al study plan draft).

Once the eggs have hatched, the fry are transported to two rearing facilities, the Long Live the King's Lilliwaup Hatchery (Duckabush and Dewatto) and the WDFW McKernan Hatchery (South Fork Skokomish). Most of these fry will be reared to age-2 smolts and then released into their natal streams. This differs from traditional hatchery programs in which hatchery smolts are released at age-1. In this study smolts were released at age-2 in order to produce fish with "a more natural age at smolitification" (Berejikian et al. 2013). Some juvenile steelhead will be reared to age-4 adults and released into their natal streams for natural spawning (Berejikian et al. study plan draft). The desired number of embryos collected and the number of smolts and adults to be released into each of the supplemented rivers are as follows: Duckabush River: 8,620 embryos, 6,667 smolts and 229 adults ; Dewatto River: 9,566 embryos, 7,400 smolts and 253 adults; South Fork Skokomish River: 44,216 embryos, 34,507 smolts and 400 adults (Berejikian et al. study plan draft). The number of embryos, smolts and adult targets for each river are related to river size, with the lowest numbers in the small Dewatto River and the largest numbers in the large South Fork Skokomish River.

The HCSP uses the Before-After-Control-Impact study design to test if supplementation impacts the productivity, life-history or genetic characteristics of the wild steelhead populations. Four years prior to the introduction of hatchery steelhead baseline information was collected on both the supplemented and control rivers (2006-2010). Supplementation will last for seven years (2011-2018). Post supplementation monitoring will be conducted for four years following the last supplementation (2019-2022) on supplemented and control rivers. Pre and post monitoring includes redd surveys to estimate the number of steelhead spawners, smolt trapping to gather information on out-migrating juveniles to estimate steelhead productivity, life history monitoring to track the numbers of anadromous and resident steelhead (rainbow trout), and acoustic monitoring to gather further information on the number of out-migrating juveniles. (USDA 2011 a).

From March to July, steelhead redd surveys are conducted on the supplemental rivers and eggs fertilized from wild spawning adults are collected. Once these eggs are collected they are raised in similar conditions and feeding rations and growth rates mimic those of wild steelhead (USDA 2011a). The last egg collections were conducted in May 2014.

The HCSP will lead to an increased understanding of the efficacy of conservation hatcheries to restore wild steelhead populations, potential recovery of threatened steelhead populations and improved ecosystem functioning (USDA 2011a). The design and implementation of the HCSP allows for smaller sub-studies to be conducted to investigate other possible differences or similarities between wild and hatchery steelhead outside the realm of genetics. The study of juvenile steelhead diets is one such study.

Study Area

The Dewatto, Duckabush and South Fork Skokomish rivers are the focus of this steelhead juvenile diet study because these are the three rivers within the HCSP that are undergoing supplementation with hatchery fish. Each of these rivers varies significantly in elevation, hydrology, water source and canopy cover. The characteristics of Hood Canal and these rivers (Table 1) are described below.

Table 1. The physical attributes of the Dewatto, Duckabush and South Fork Skokomish Rivers.

Physical Attribute	**Dewatto**	**Duckabush**	**S.F. Skokomish**
River length (km)	14	39.4	44.2
Annual flow rate (m^2s^{-1})	2.01	11.8	21
Depth (m) at invert. collection sites	0.18 (2010) 0.14 (2011)	0.38 (2010) 0.43 (2011)	0.25 (2010) 0.29 (2011)
Annual Temperature (°C)	9.5	6.8	8.1

Hood Canal is an 80 km glacial-carved fjord and is the western most waterway in the Puget Sound Basin, located within Jefferson, Kitsap and Mason counties of western Washington State (Berejikian et al. 2013 & HCCC). The watershed is an interactive system that depends on the continued cycling of clean water and nutrients to maintain its "biological character" (HCCC).

The Dewatto River is a first order stream (WADOE 1998) and has an elevation of 134 m at its headwaters and is 14.0 km in length, however steelhead generally occupy only the lowest 4.8 km of the river (Berejikian et al. 2013). The Dewatto watershed is 59.6 km^2 (Mason County 2011). The river is located in the southwestern area of the Kitsap Peninsula, draining into Hood Canal at the tidal marsh and mud flats (PNPTC) of Dewatto Bay. The stream is rain fed with a mean annual flow of 2.01 m^3s^{-1} (Berejikian et al. 2013), with the highest flows occurring in January and the lowest flows occurring in September (Collings et al. 1968). During data collection the average water depth taken at invertebrate collection sites on the Dewatto River was 0.18 m in 2010 and 0.14 m in 2011 (Doctor 2014). The mean annual water temperature is 9.5°C (Berejikian et al. 2013). Land cover on the Dewatto includes 56% floodplain and riparian zone, 39% forest and 4% wetland (Mason County 2011).

Land use practices along the Dewatto River mainly consist of logging and timber production and development of residential areas and parks. Forestry makes up 98% of the land use, with residential and vacant areas making up the other 2%. Land ownership along the river is 100% private. The Port of Dewatto manages and operates a park area near Dewatto Bay, while the Manke Timber Company and Pope Resources own and manage the forest lands (Mason County 2011).

The Duckabush River is a third order stream (WADOE 2011) and has an elevation of 1724 m at its headwaters and is 39.4 km in length (Berejikian et al. 2013). The river begins in Olympic National Park on the Olympic Peninsula and enters the northwestern side of Hood Canal. The Duckabush watershed is 202 km^2 (USFS, 1998). The Duckabush has a transitional hydrologic regime, meaning the flow is influenced by both rainfall and snowmelt and has a mean annual flow of 11.8 m^3s^{-1} (Berejikian et al. 2013). During data collection the average water depth taken at invertebrate collection sites was 0.38 m in 2010 and 0.43 m in 2011 (Doctor 2014). The river has a mean annul water temperature of 6.8 °C (Berejikian et al. 2013). The riparian zone is composed of 66% mixed forest, 25% deciduous trees and shrubs, 5% conifers and 4 % grasses (Correa 2003).

Of the land in the Duckabush River watershed 89% is contained within the Olympic National Forest and Olympic National Park, while the remaining land use consists of forest land held by private owners, residential properties and parks (Masello 2013). A fourth of the riparian zone, located below river mile 3 consists of urban and commercial development, rural residences, roads and dikes (Correa 2003).

The South Fork Skokomish River has an elevation of 1646 m at its headwaters and is 44.2 km in length (Berejikian et al. 2013). The river begins in the Olympic National Forest on the Olympic Peninsula and enters the southern Hood Canal after being joined with the North Fork Skokomish River. The South Fork Skokomish watershed drains 268 km^2 (USDA, 2011 (b) (c)). The South Fork Skokomish also has a transitional rainfall and snowmelt hydrologic regime, with a mean annual flow of 21 m^3s^{-1} (Berejikian et al. 2013). During data collection the river's water depth taken at invertebrate collection sites was an average of 0.25 m in 2010 and 0.29 m in 2011 (Doctor 2014). The river has a mean annual water temperature of 8.1°C (Berejikian et al. 2013). A 1997 analysis of the upper reaches of the South Fork Skokomish found that there were areas of mature old growth forest as well large clear cut areas (USDA 2011 (b)).

The upper portion of the South Fork Skokomish River is managed by the National Forest Service for commercial and pre-commercial tree thinning, as well as for recreation use. Recreation uses within the national forest include camping, hiking, horseback riding, mountain biking, berry picking and hunting. Local tribes also utilize the lands near the South Fork Skokomish for berry picking, hunting and harvesting of plant materials for tribal practices (USDA 2011 (b)).

Macroinvertebrate Drift Collection

Macroinvertebrate drift collection was conducted in the upper and lower reaches of the Dewatto River and the upper, middle and lower reaches of the Duckabush and South Fork Skokomish rivers. Sampling locations in each of these reaches were in riffle habitats. Riffle habitats are defined as shallow river sections where water flows over course sediment to create mild to moderate water turbulence. The current is less than 0.5 m deep and has a flow greater than 0.3 m/s (Woo et al. 1986).

The drift data was collected in August of 2010 and August and September of 2011. Sampling occurred during the late summer months when water flows are lowest and is the most limiting time for juvenile steelhead food resources in these rivers. Collecting stomach and drift samples during the low summer flow period provides a more representative snapshot of juvenile steelhead feeding behaviors and diet preferences.

Drift nets were placed along a transect that ran the width of the riffle. The nets were lowered ~5cm above the stream bottom to insure that all drift in the water column and on the surface were collected, but nothing was sampled from the benthos. The drift nets were attached and held in place with rebar installed into the stream bottom. The three replicate drift samples were conducted at the same time of day in each river and reach ranging from 9am to 2pm. A flow meter was used to determine water volume and velocity through the net. Photos and GPS coordinates were collected at each drift location. Three replicates of drift sampling were conducted at each river reach location for two-hour increments in 2010 and one-hour increments during 2011 data collection. Once the nets had soaked, the entire contents of the net were emptied into a sieve. Large substrate was removed and finer particles and organic matter were further separated from the macroinvertebrates. Samples were then placed in a whirlpak with a 95% ethanol solution.

The drift samples were shipped to AquaticBio labs in Portland, Oregon. Insects and non-insects were included. The lengths of all macroinvertebrates were measured to 0.5 mm if the organism was less than 5 mm and to the nearest 1mm if larger than 5 mm. Insect life stages were identified to larvae, pupae and adult. Origins were identified as aquatic or terrestrial. Nematocera were identified to family and determined to be terrestrial or aquatic. Brachycera adults were all assumed to be terrestrial in origin. Chrionomidae were identified to family. Aquatic larvae and pupae were identified to PNW standard taxonomic effect.

<u>Steelhead Juvenile Stomach Sampling</u>

Juvenile steelhead sampling was conducted as broadly as possible throughout the upper and lower reaches of the Dewatto River and the upper, middle and lower reaches of the Duckabush River and South Fork Skokomish River where drift samples were collected in August 2010 and August and September 2011. Upper and lower reaches of each fish collection period were marked by GPS. Only steelhead larger than 90 mm were collected using barbless hook-and-line sampling. An attempt was made to sample thirty wild and thirty hatchery fish during each sampling period within each reach.

Once captured, fish were anesthetized using MS-222. Fork length, weight, rearing history (wild or hatchery), DNA, scales and diet samples were collected in the field using the Hood Canal Steelhead Project summer parr and diet sampling protocol methods established by Berejikian (2010 & 2011). Fish were held in a recovery bucket until the sampling in each reach was completed. Diet samples were collected by gastric lavage, using a squirt bottle to flush stomach contents into a funnel that flowed into a separate sieve. This allowed water to pass through while solid material was maintained. Stomach contents were placed into whirlpaks containing 95% ethanol solution. Stomach contents were sent to AquaticBio for analysis, identified and classified in the same manner as the drift macroinvertebrate samples described in the previous section.

Site Characteristics

Habitat sampling occurred in five random sampling sites downstream of the riffles in which the drift samples were collected. The start point for the five samples was randomly determined. At each of the five locations water velocity, water depth, substrate type, temperature and specific conductivity, turbidity, wetted width, gradient, bank full width and canopy cover were collected but will not be reported for the purposes of this study.

Data Analysis

All drift items and stomach content items were classified by order, origin (aquatic or terrestrial) and life stage (adult, larvae, pupae) when possible. This classification system was used in all data analyses discussed below.

Drift Analysis

Abundance percentages of drift items were calculated for each river at the river scale, meaning that drift was combined across the upper and lower reaches in the Dewatto River and across the upper, middle and lower reaches of the Duckabush River and South Fork Skokomish River. A percentage was calculated for each specific drift item. Abundance percentages were calculated using the following equation:

$$\%\ \text{drift abundance} = \left(\frac{\#\ \text{of the specific drift item}}{\text{total number of drift items in the river}}\right) * 100$$

Pie charts were created for Dewatto, Duckabush and South Fork Skokomish drift to demonstrate the abundance of each of the specific items in the drift of each river.

Stomach Content Analysis

Percent Abundance

In order to determine the abundance of each specific item found in wild and hatchery juvenile steelhead stomach contents, percent abundance calculations were made using the following equation:

$$\%\ \text{diet abundance} = \left(\frac{\#\ \text{of the specific item in the stomach contents}}{\text{total number of items in the steelhead's stomach}}\right) * 100$$

Numerous percent abundance calculations were made to determine the percent abundance of all items found in juvenile steelhead stomach contents in each of these three rivers. Additional percent abundance calculations were made to examine diet differences between wild and hatchery steelhead juveniles. Further percent abundance calculations were done at the reach scale in the lower reach of the South Fork Skokomish and Duckabush River following a significant result during NMS/MRPP analysis. Only the lower reaches were examined due to low numbers of hatchery fish captured in the upper and middle reaches.

Nonmetric Dimensional Scaling & Multi-response Permutation Procedure

Nonmetric multidimensional scaling (NMS) and multi-response permutation procedures (MRPP) were conducted on each of the Dewatto, Duckabush and South Fork Skokomish Rivers to analyze diet overlap between wild and hatchery steelhead in each river, as well as to analyze diet overlap in the upper, middle and lower reaches of each of the three rivers (Clarke 1993 & Tagliaferro et al. 2015). Across the three rivers a combined total of 523 fish were included in the NMS and MRPP analysis, consisting of 454 wild and 69 hatchery steelhead juveniles and a total of 41 different item types were included (Table 2).

Table 2. The number of hatchery and wild fish included in the NMS/MRPP analysis of the Dewatto, Duckabush and South Fork Skokomish River. Types of prey items indicates the number of distinct prey categories included in the analysis.

River	Wild Fish	Hatchery Fish	Total Fish	Types of prey items
Dewatto	149	35	184	25
Duckabush	133	22	155	29
South Fork Skokomish	172	12	184	27
Total	**454**	**69**	**523**	**41 (different items)**

The NMS and MRPP analysis were conducted using PC-ORD 6.0. These calculations allow for the analysis of diet overlap between wild and hatchery fish and between river reach. Data were relativized to prevent very abundant prey items from outweighing less abundant prey items during analysis.

For NMS analysis three 2-D plots were created for each individual river using the Sorenson’s distance measure. The similarity between items found in stomach contents of wild and hatchery steelhead were plotted for each river (all reaches were pooled).

Similarities between the prey items of each reach in each individual river were assessed separately. In addition, similarities between wild and hatchery steelhead diets in the lower reaches of all three rivers were assessed.

Using the Sorenson's distance measure an MRPP analysis was conducted for each of the Dewatto, Duckabush and South Fork Skokomish Rivers. Three MRPP analyses were conducted for each river, one calculation to determine prey item similarities between wild and hatchery steelhead juveniles and another calculation to determine diet overlap among river reaches within each river. An additional MRPP was conducted for the Dewatto, Duckabush and South Fork Skokomish River to determine prey similarities between wild and hatchery steelhead in the lower river reaches. Only the lower river reaches were examined due to the fact that those were the only reaches that contained enough hatchery and wild steelhead for a comparison.

The resulting A-statistic of an MRPP represents the effect size, in this case showing the amount of similarity between wild and hatchery steelhead diets. The larger the A value, the more difference there is within the two groups. The significance of difference is determined by the p value and denotes there is more difference than that which would be expected by chance (McCune & Grace, 2002). An A value of 1 indicates that steelhead diets are identical and hatchery diets are identical, while a value of 0 indicates that the differences within dietary groups is not more than expected by chance. A value less than 0 indicates that there is more difference within groups (i.e. hatchery or wild) than expected by chance (McCune & Mefford 2011).

Electivity Index

Vanderplog and Scavia Electivity Indices (Ei) were calculated for the Dewatto, Duckabush and South Fork Skokomish Rivers to determine if certain items were preferred by wild and hatchery origin juvenile steelhead. Due to an inability to determine exactly where these items were consumed, all river reaches for each river were combined. Items that were found in stomach contents but not the drift could not be included in this analysis and were eliminated, as well as items that were found in the drift but not in the stomach contents. These items were excluded because the item needs to be in both the drift and diet in order to conduct the Ei calculations. A total of 538 juvenile steelhead were used in this analysis, 452 wild and 86 hatchery origin fish (Table 3). Due to different drift compositions the number of prey items included varied by river (Appendix Table 1).

Table 3 The number of hatchery and wild fish included in the Ei analysis of the Dewatto, Duckabush and South Fork Skokomish River. Types of items indicates the number of distinct categories included in the analysis.

River	Wild Fish	Hatchery Fish	Total	Orders
Dewatto	149	35	184	32
Duckabush	133	22	155	32
South Fork Skokomish	170	29	199	39
Total	**452**	**86**	**538**	

Electivity was determined using the following equation:

$$E_i = \frac{[W_i-(1/n)]}{[W_i+(1/n)]} \text{ where } \mathrm{W_i} = \frac{\mathrm{r_i}/\mathrm{p_i}}{\Sigma_\mathrm{i}\,\mathrm{r_i}/\mathrm{p_i}}$$

r_i= proportion of prey item i in the diet
p_i= proportion of prey item i in the environment
n= number of possible kinds of food items in each river

The electivity index ranges from -1 to +1 where a value below 0 indicates negative electivity (discrimination) for a specific item type and a value above 0 indicates positive electivity (preference) for a specific item type (Vanderploeg & Scavia, 1979). Item categorization for Ei analysis was identical to that used in the NMS/MRPP analysis. Categories included orders, insect or non-insect, aquatic or terrestrial origin and life stage (adult, pupae, larvae)

The electivity values for each diet item were graphed to show the relationship between wild and hatchery steelhead diet electivity (Mistak et al. 2003 & Tagliaferro et al. 2015). However, due to a greater proportion of wild steelhead included in this study, not all items could be compared between wild and hatchery fish. Furthermore, not all stomach content items were found across all three rivers preventing the comparison of all items between rivers.

Results

Drift Composition

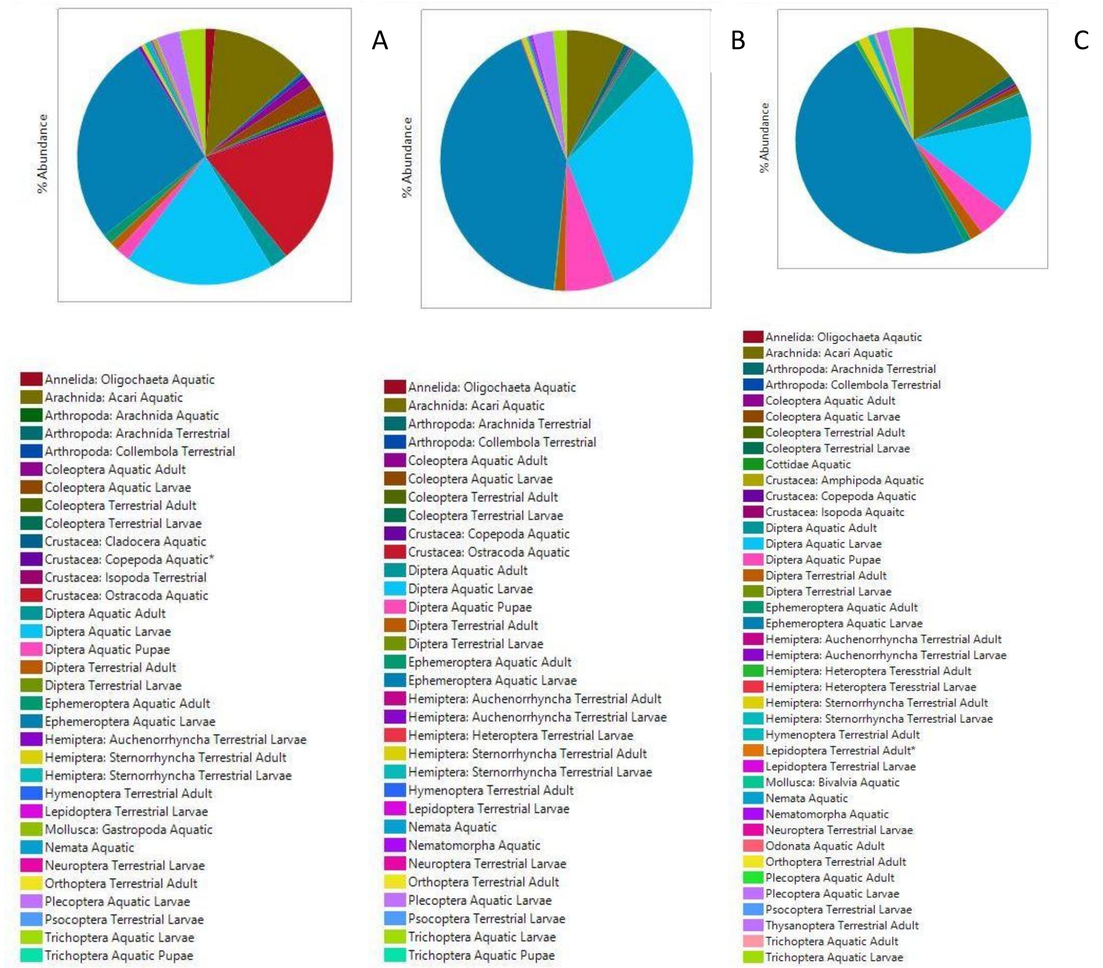
The most abundant item found in the drift of all three rivers was aquatic Ephemeroptera larvae composing 27% of the total drift collected in the Dewatto River, 42% of total drift in the Duckabush and 49% of the total drift in the South Fork Skokomish River (Figure 3, Appendix Table 1).

In the Dewatto River aquatic Crustacea: Ostracoda and aquatic Diptera larvae were also a significant proportion of the total drift, each comprising 19% of the total drift. Aquatic Diptera larvae were also large contributors to the drift in the Duckabush and

Skokomish Rivers comprising 31% and 14% respectively. In addition aquatic Arachnadia: Acari comprised 15% of the total drift in the South Fork Skokomish River (Figure 3).

The Dewatto River had 33 different drift item types, three of which were not found in the other two rivers and include aquatic Arthropoda: Arachnadia, aquatic Crustacea: Cladocera and aquatic Mollusca: Gastrapoda. The Duckabush River had 32 different drift item types and a similar composition of that found in the Dewatto with the exception of two additional Hemipotera types, terrestrial Hemipotera: Heteroptera larvae and terrestrial Hemipotera: Auchenorrhyncha adults. The South Fork Skokomish River was the most variable with 40 different drift item types. The South Fork Skokomish drift had one more Hemioptera item than the Duckabush, terrestrial Hemipoter: Heteroptera adults, as well as seven additional item types not found in the other two rivers. These include Cottidae, aquatic Crustacea: Amphipoda, terrestrial Lepidoptera adults, aquatic Mollusca: Bivalvia, aquatic Odonata adults, aquatic Plecoptera adults and terrestrial Thysanoptera adults (Appendix Table 3).

Figure 3 Percent abundance of drift items collected in the Dewatto River (A), Duckabush River (B) and South Fork Skokomish River (C).



<u>Diet Composition</u>

The number of hatchery and wild juvenile steelhead diets examined differed between the Dewatto, Duckabush and South Fork Skokomish rivers due to different population sizes in these three river. The Dewatto River had the fewest fish, with only 837. The Duckabush had the most fish captured with 2,022 and the South Fork Skokomish had the second highest number of fish captured, 1,405. The number of fish collected differed by river reach, with the majority collected in the lower reaches of all three rivers.

Variability in types of items consumed differed between the Dewatto River, Duckabush River and South Fork Skokomish River. The steelhead juvenile diets in the Dewatto River showed a greater variety in the types of items consumed with 45 item types, while the Duckabush had less variety with 38 types and the South Fork Skokomish had the least variety, with only 31 (Table 4).

Table 4. The number of wild and hatchery fish included in diet analysis on the Dewatto River, Duckabush River and South Fork Skokomish River and the number of different diet item types found in stomach contents. Fish are broken down by reach, upper (U), middle (M) and lower (L) and the drift totals are combined for all river reaches. (Note: No data was collected in the middle reach of the Dewatto River).

	Dewatto	**Duckabush**	**Skokomish**
Wild			
U	360	546	604
M	n/a	720	420
L	333	612	345
Hatchery			
U	13	0	0
M	n/a	20	0
L	131	124	36
Total # of juvenile steelhead	837	2022	1405
Total # of diet item types	45	38	31

Overall Juvenile Steelhead Diet

When hatchery and wild juvenile steelhead stomach contents were combined at the river scale, the most abundant item in all three rivers was aquatic Ephemeroptera larvae comprising 34% of the total diet in the Dewatto River, 54% in the Duckabush River and 59% in the South Fork Skokomish River (Figure 4). The second most abundant item found in stomach contents was aquatic Diptera larvae comprising 33% of total stomach contents in the Dewatto, 23% in the Duckabush and 13 % in the South Fork Skokomish Rivers (Figure 4, see Appendix Table 2).

Hatchery Juvenile Steelhead Diets

When hatchery juvenile steelhead were analyzed separately, aquatic Ephemeroptera larvae were found to be the most abundant item in hatchery fish diets in the Dewatto and Duckabush rivers, comprising 37% of stomach contents in the Dewatto and 48% in the Duckabush. Analyses of hatchery fish in the South Fork Skokomish River showed that 61% of hatchery steelhead diets were comprised of aquatic Ephemeroptera adults. Aquatic Diptera larvae were the second most abundant item found in hatchery stomach contents comprising 36% in the Dewatto, 23% in the Duckabush and 10% in the South Fork Skokomish (Figure 5).

Wild Juvenile Steelhead Diets

When wild steelhead diets were analyzed, aquatic Ephemeroptera larvae were the most abundant item in stomach contents, consisting of 37% of wild diets in the Dewatto River, 48% in the Duckabush River and 60% in the South Fork Skokomish River. Aquatic Diptera larvae were found to be the second most abundant item found in wild steelhead stomach contents in all three rivers, comprising 36% in the Dewatto River, 24% in the Duckabush River and 13% in the South Fork Skokomish River (Figure 6).

Figure 4 Percent abundance of items collected from Dewatto River (A) Duckabush River (B) and South Fork Skokomish River (C) wild and hatchery juvenile steelhead stomach contents.

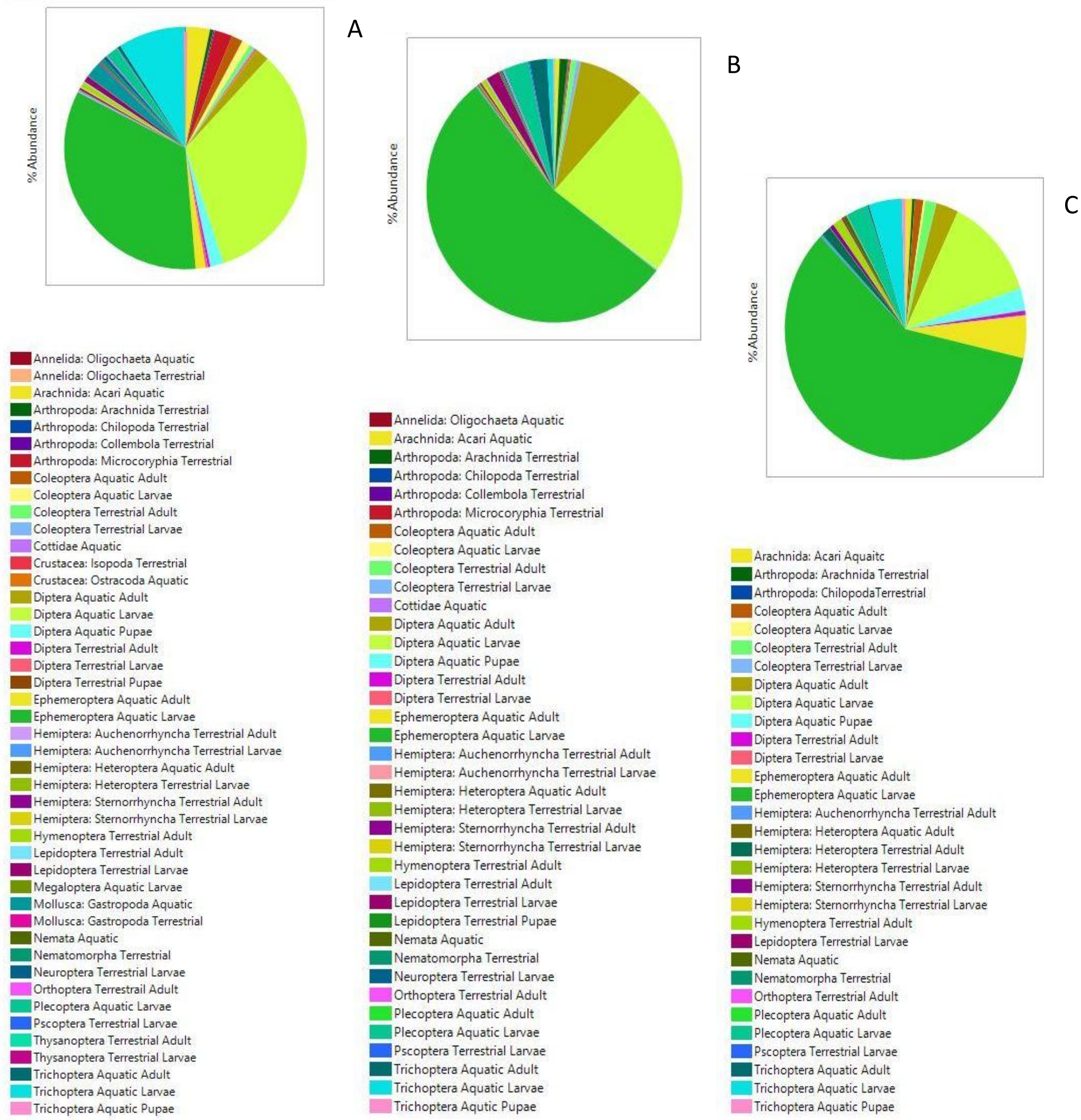


Figure 5 Percent abundance of items collected from Dewatto River (A) Duckabush River (B) and South Fork Skokomish River (C) hatchery juvenile steelhead stomach contents.

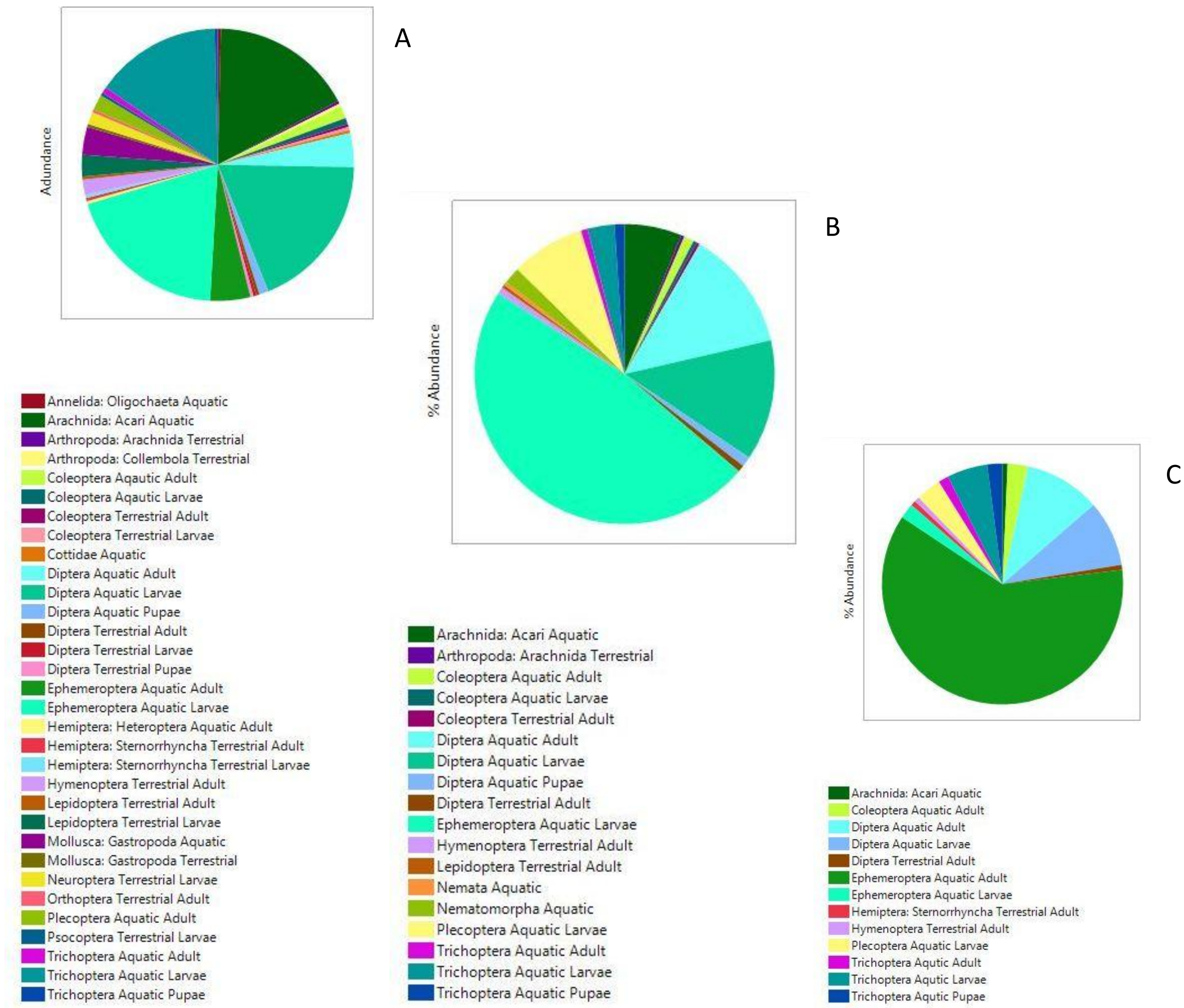
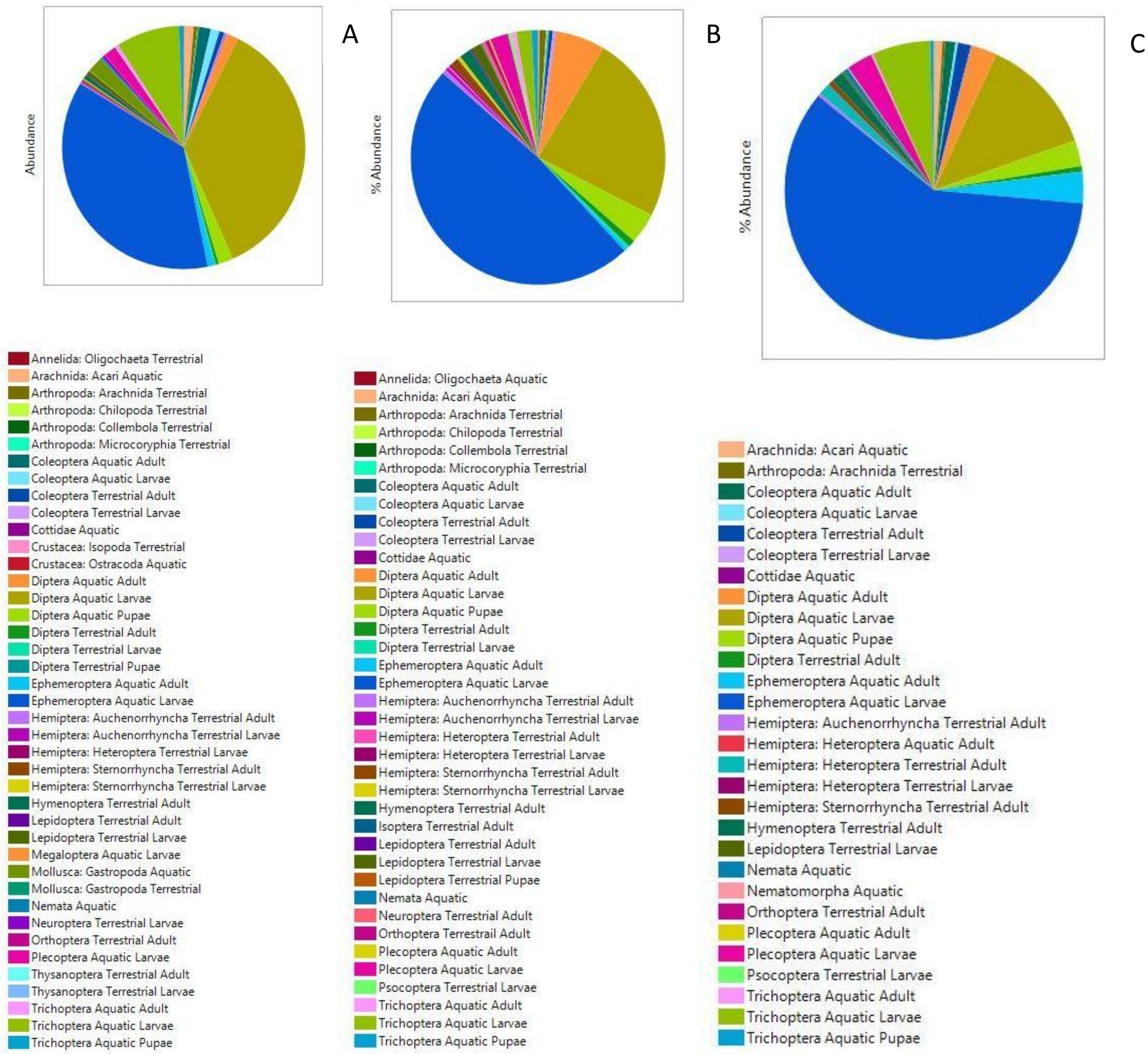


Figure 6 Percent abundance of items collected from Dewatto River (A) Duckabush River (B) and South Fork Skokomish River (C) wild juvenile steelhead stomach contents.



Diet Overlap

River Scale

NMS and MRPP analysis showed that in the Duckabush River wild steelhead and hatchery steelhead diets differed more than would be expected by chance (A=0.1617, $p<0.001$), indicating that wild fish and hatchery fish are not consuming the same items. However in the Dewatto and South Fork Skokomish Rivers wild steelhead and hatchery steelhead diets did not differ more than expected by chance ((A=0.0005680, p=0.427,

$A=0.001850$, $p=0.09612$, respectively)) indicating that wild fish and hatchery fish are consuming similar items (Figure 7).

Reach Scale

NMS/MRPP analysis showed that in the Duckabush and South Fork Skokomish Rivers juvenile steelhead diets (wild and hatchery pooled) differed more between river reaches than expected by chance (($A=0.0378$, $p<0.001$, $A=0.1238$, $p<0.001$, respectively) indicating that juvenile steelhead are not consuming the same items in the upper, middle and lower reaches of these two rivers. Analysis of the Dewatto River showed that juvenile steelhead diets did not differ between the upper and lower reaches of the river ($A=0.001211$, $p= 0.1603$) (Figure 8).

To further investigate these significant A-value findings, an additional NMS/MRPP was conducted for the reaches of the Duckabush and South Fork Skokomish for wild and hatchery fish. However, due to low hatchery numbers in the upper and middle reaches of both rivers, only the lower reaches were analyzed. Results for the lower Duckabush reach showed that wild steelhead and hatchery steelhead diets differed more than expected by chance ($A=0.02348$, $p<0.001$), indicating that wild steelhead and hatchery steelhead are not consuming the same items (Figure 9). Analysis of the lower South Fork Skokomish reach showed that wild steelhead and hatchery steelhead diets also differed more than expected by chance ($A=0.009190$, $p=0<0.05$), indicating that wild fish and hatchery fish are not consuming similar items (Figure 9).

Percent abundance calculations for wild and hatchery fish in these two lower reaches showed that wild steelhead juveniles in the Duckabush River consumed a greater number of items than the hatchery steelhead, consuming 34 and 14 item types, respectively. Aquatic Ephemeroptera larvae were the most abundant in both diets, comprising 41% of wild steelhead diets and 42% of hatchery steelhead diets. The second most abundant item in wild and hatchery diets in the Duckabush and South Fork Skokomish were aquatic Diptera larvae, comprising 24% and 16% of their diet, respectively. (Figure 10).

In the South Fork Skokomish River there was also a difference in the number of prey types found in steelhead juvenile diets, with wild fish consuming 21 item types and hatchery fish consuming 14 item types. Wild and hatchery steelhead diets consisted of vastly different compositions of items. Wild juvenile diets consisted mainly of aquatic larval stages of Ephemeroptera and Diptera, 29% and 24% respectively, while hatchery fish diets consisted mainly of aquatic adult stages of Ephemeroptera and Diptera, 61% and 10% respectively. (Figure 11).

In the Dewatto River juvenile steelhead (wild and hatchery) diets did not differ more than expected by chance between river reaches (A=0.001211, p=0.1603) indicating that juvenile steelhead are consuming the same items in the upper and lower reaches of the river (Figure 8).

Figure 7 Nonmetric multidimensional scaling (2D) showing the degree of diet overlap between wild and hatchery origin steelhead juveniles in the Dewatto River (A), Duckabush River (B) and South Fork Skokomish River (C) using the Bray-Curtis similarity index. The proximity of the symbols indicates degree of similarity. Solid black triangles indicate hatchery steelhead, hollow triangles represent wild steelhead.

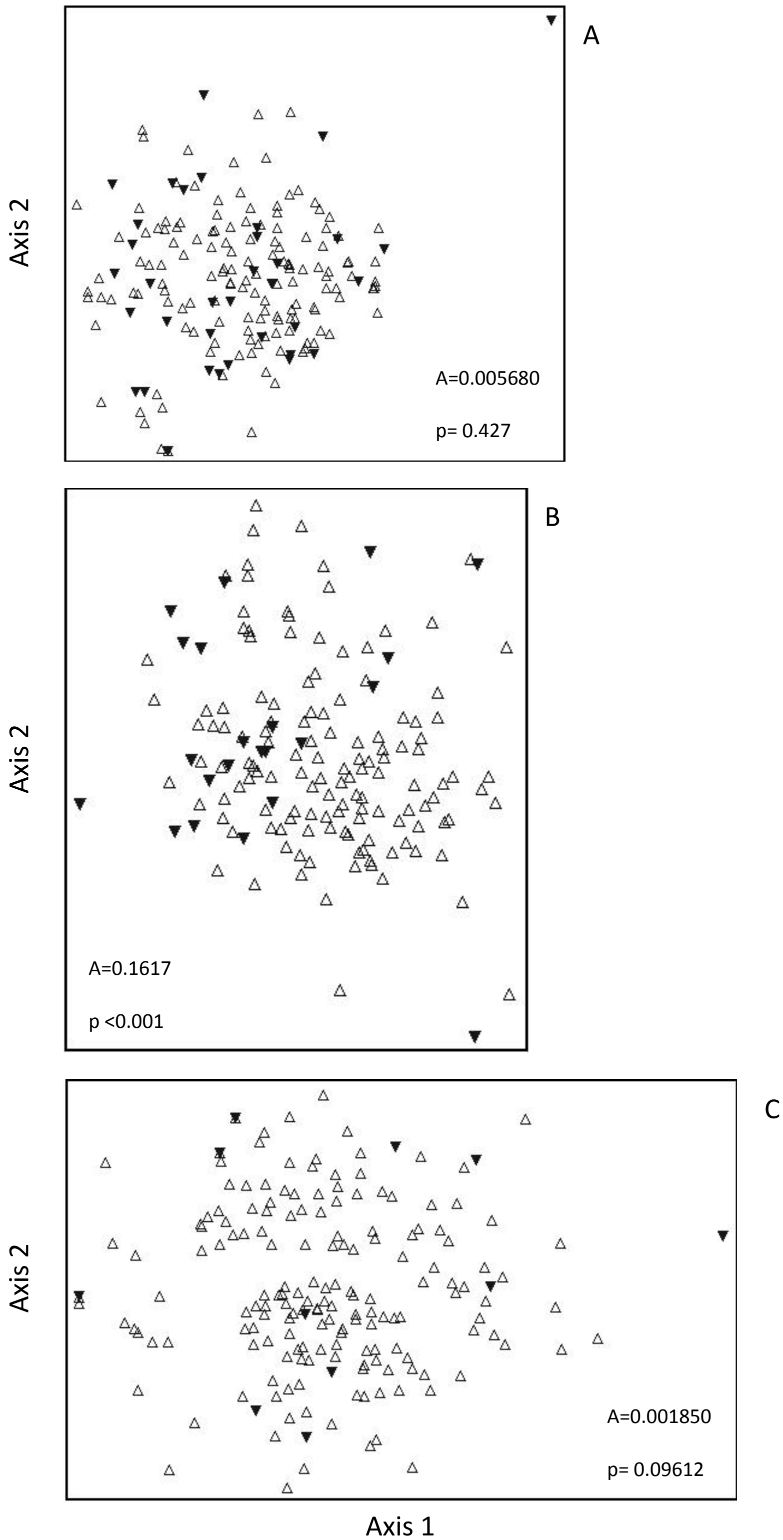


Figure 8 Nonmetric multidimensional scaling (2D) showing the degree of diet overlap between river reaches in the Dewatto River (A), Duckabush River (B) and South Fork Skokomish River (C) using the Bray-Curtis similarity index. The proximity of the symbols indicates degree of similarity. Hollow circles indicate the upper reaches, hollow squares indicate the middle reaches, solid circles indicates lower reaches. (Note that the Dewatto River only contains an upper and lower reach. No data was collected in the middle reach of the Dewatto River).

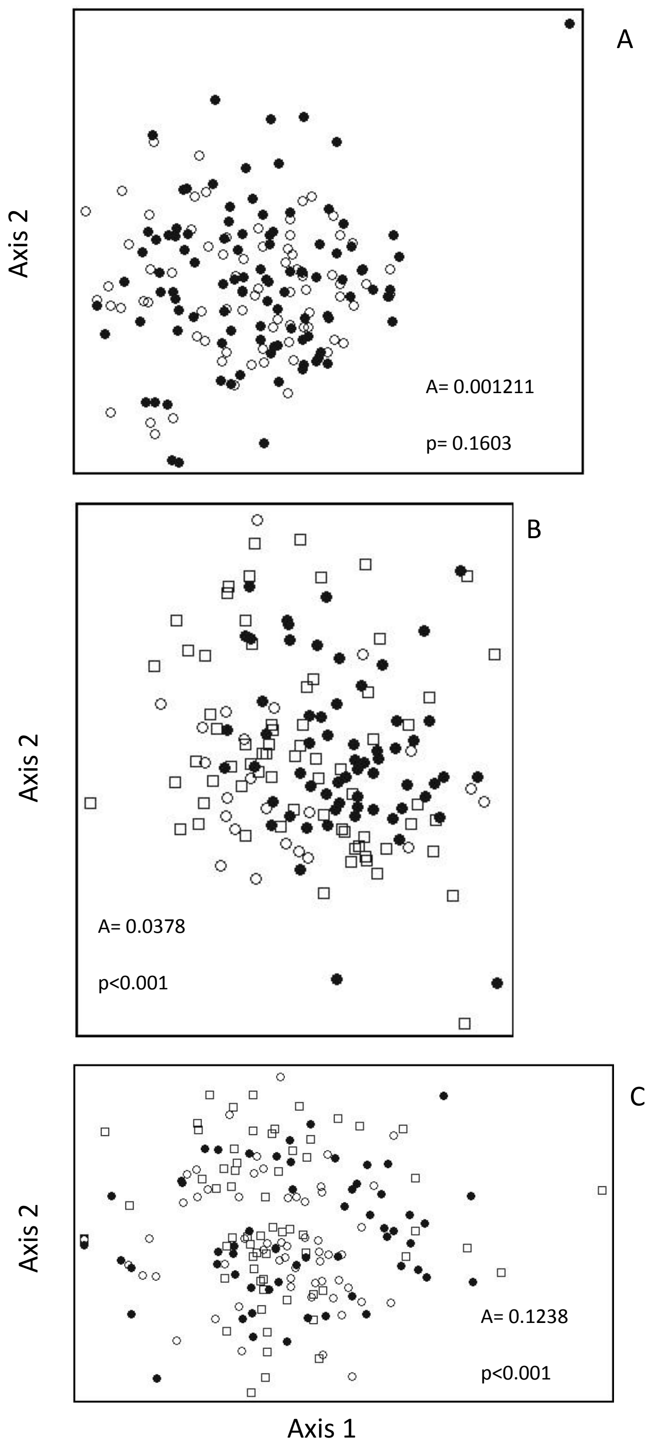


Figure 9 Nonmetric multidimensional scaling (2D) showing the degree of diet overlap between wild and hatchery steelhead juveniles in the lower reaches of the Dewatto River (A), Duckabush River (B) and South Fork Skokomish River (C) using the Bray-Curtis similarity index. The proximity of the symbols indicates degree of similarity. Solid triangle represent hatchery steelhead, hollow triangles represent wild steelhead.

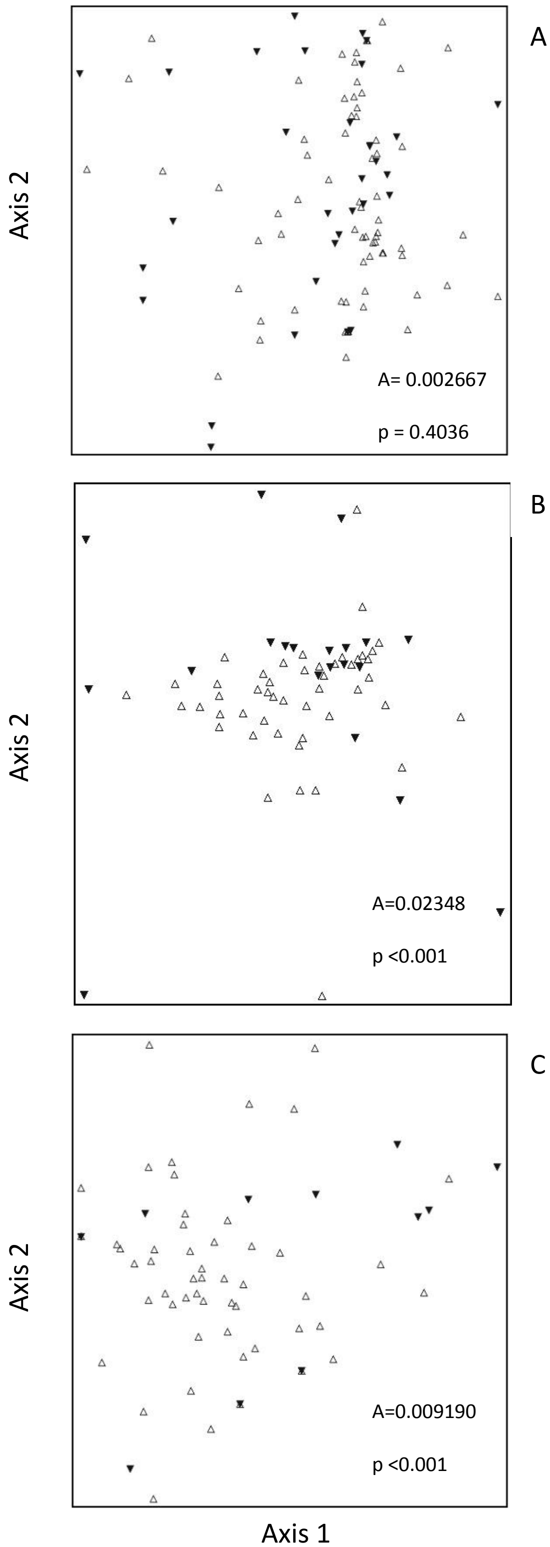


Figure 10 Percent abundance of items collected from lower Duckabush River wild (A) and hatchery (B) juvenile steelhead stomach contents.

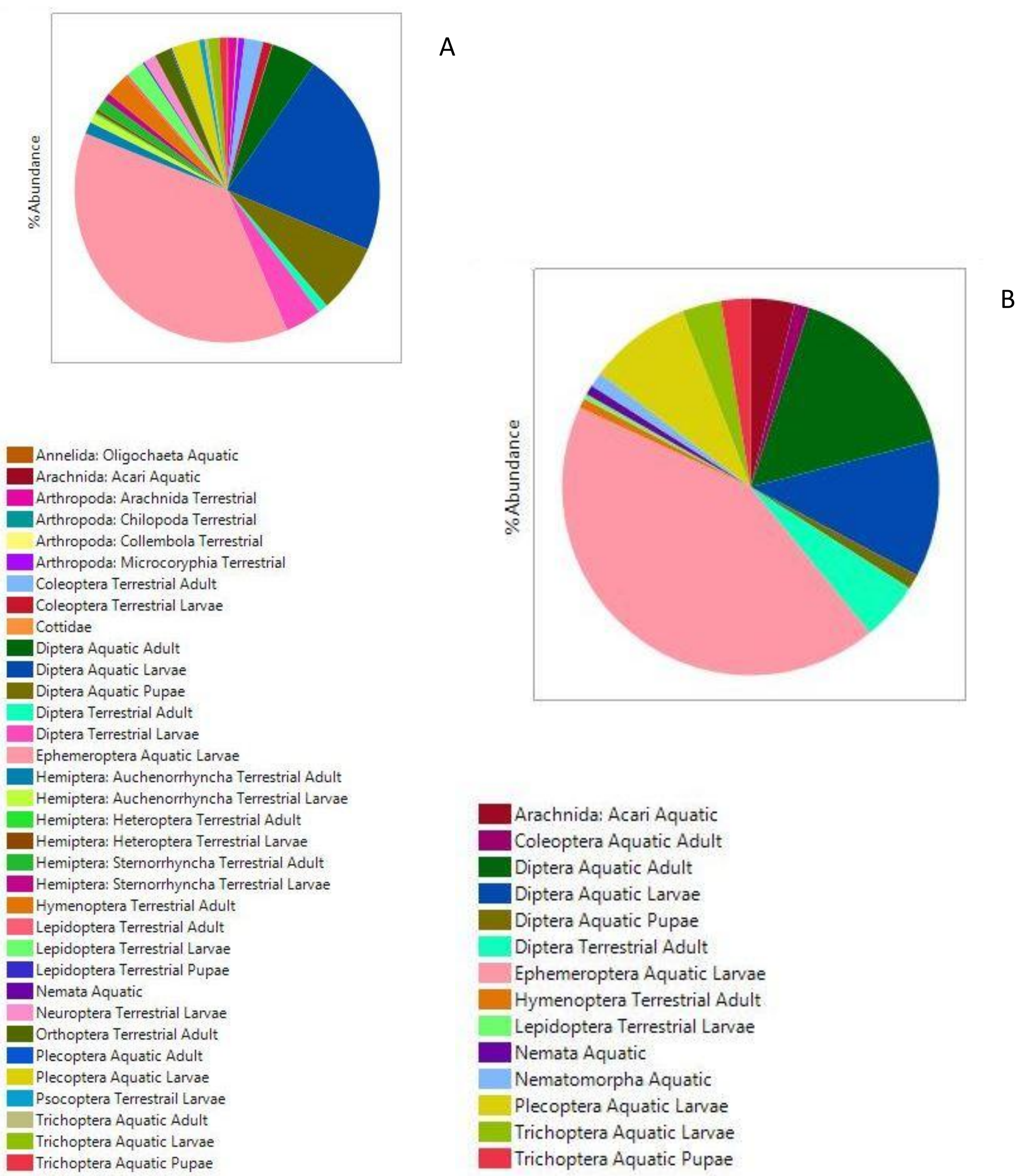
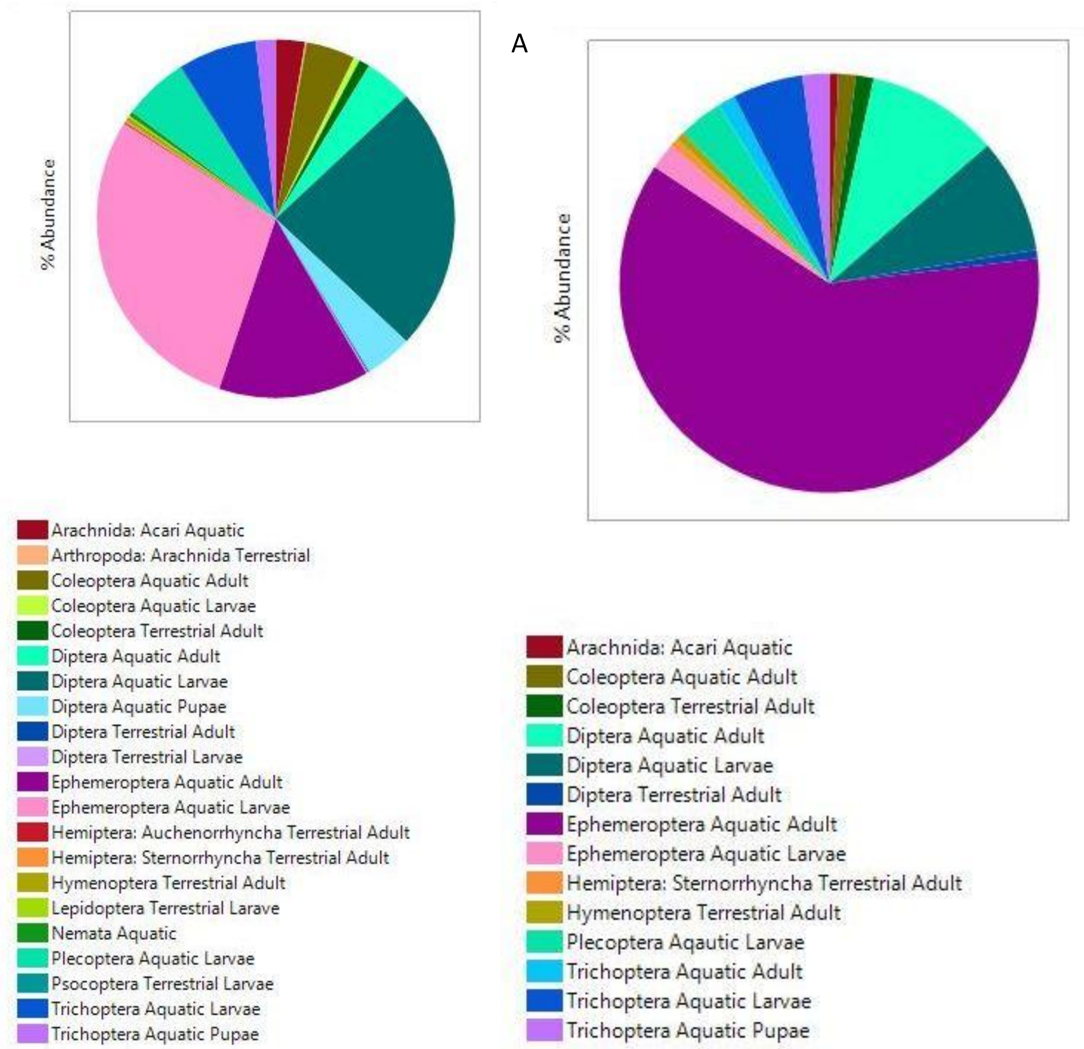


Figure 11 Percent abundance of items collected from lower South Fork Skokomish River wild (A) and hatchery (B) juvenile steelhead stomach contents.



Electivity Index

Vanderplog and Scavia Electivity Indices (Ei) were calculated for the Dewatto, Duckabush and South Fork Skokomish Rivers to determine if certain items were preferred by wild and hatchery origin juvenile steelhead. In this analysis a total of 454 wild and 69 hatchery steelhead juveniles were included (see Table 2 in methods). Due to the fact that there were significantly more wild juvenile steelhead included in this study than hatchery steelhead it is difficult to conclusively state diet preference differences between wild and hatchery juvenile steelhead.

Hatchery Juvenile Steelhead Electivity

Nonetheless, through this analysis a few patterns did develop across all three rivers. There was a distinct difference between the preferences of hatchery fish in the Dewatto, Duckabush and South Fork Skokomish Rivers. In the Dewatto River, hatchery fish preferred 100% of the available items (Figure 12), while in the Duckabush (Figure 13) and South Fork Skokomish River (Figure 14) hatchery fish showed more variation in preference of item types. For example, of the 29 available items in the Duckabush River included in this analysis, hatchery steelhead preferred 6 of the items indicating a preference for only 20% of the items (Figure 13). Similarly of the 27 available items in the South Fork Skokomish River, hatchery steelhead preferred 3 of the items indicating a preference for only 11% of the items (Figure 14).

In regards to preferential items, no patterns emerged between the hatchery steelhead across all three rivers. There was not one item that was preferred or not preferred consistently across the Dewatto, Duckabush and South Fork Skokomish Rivers Figure 12-14). However, this may be due to the fact that there are significantly fewer hatchery steelhead included in this analysis.

Wild Juvenile Steelhead Electivity

There was a distinct difference between preferences of wild steelhead in the three rivers. In the Dewatto River, wild fish preferred 68% of the available items (Figure 12), while in the Duckabush River (Figure 13) and South Fork Skokomish River (Figure 14) wild steelhead showed more variation in preference of items. Of the 29 available items in the Duckabush River wild steelhead preferred 6 of the available items indicating a preference for only 10% of the items (Figure 13). Of the 27 available items in the South Fork Skokomish River wild steelhead only preferred 8 prey items indicating a preference for 30% of the items (Figure 14). A few patterns did emerge in the preference of items by wild steelhead across all three rivers. Wild steelhead did not prefer aquatic Ephemeroptera larvae, aquatic Diptera (pupae, larvae or adult) or aquatic Coleoptera adults. However, wild steelhead in the Dewatto, Duckabush and South Fork Skokomish River did show a preference for aquatic Ephemeroptera adults (Figure 12-14).

Figure 12 Vanderplog and Scavia Electivity Index of item selectivity for wild and hatchery steelhead in the Dewatto River. Includes all items found in both the drift and steelhead stomach contents. Grey bars represent wild steelhead, black bars represent hatchery steelhead.

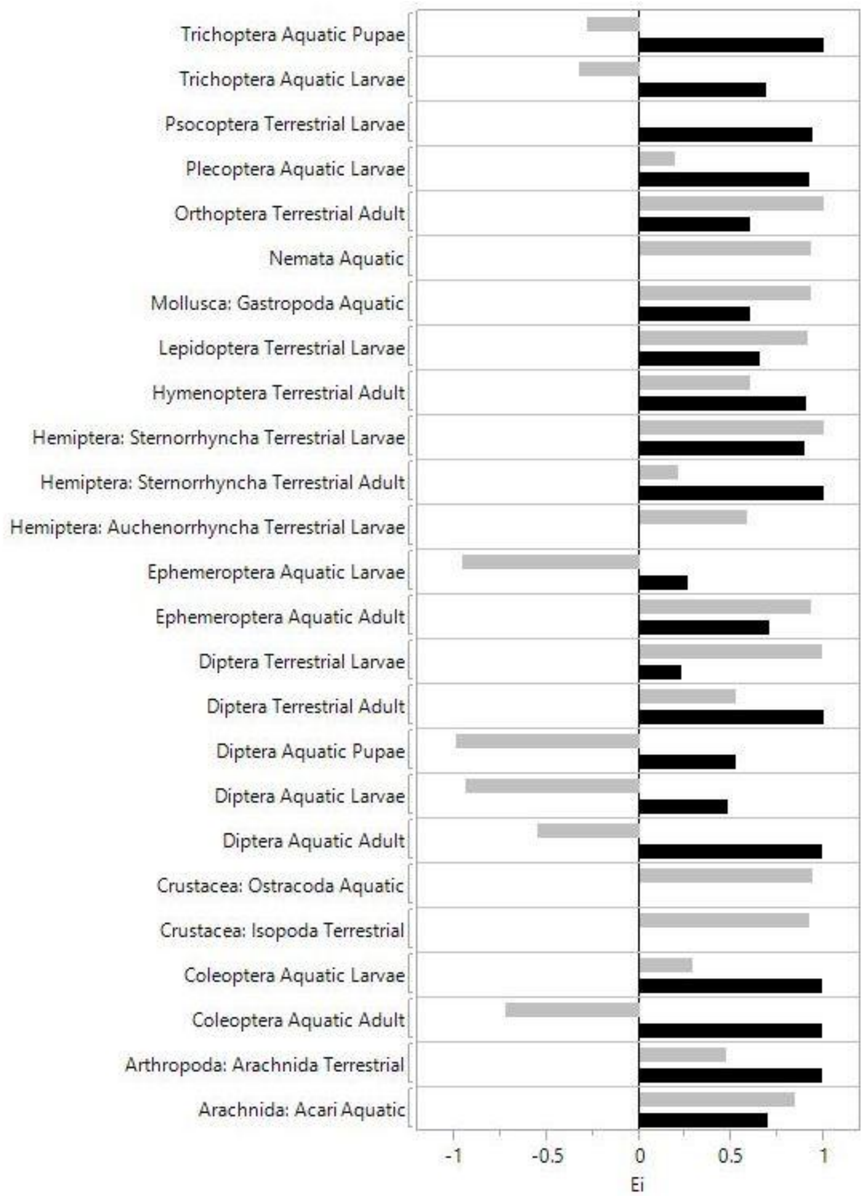


Figure 13 Vanderplog and Scavia Electivity Index of item selectivity for wild and hatchery steelhead in the Duckabush River. Includes all items found in both the drift and steelhead stomach contents. Grey bars represent wild steelhead, black bars represent hatchery steelhead.

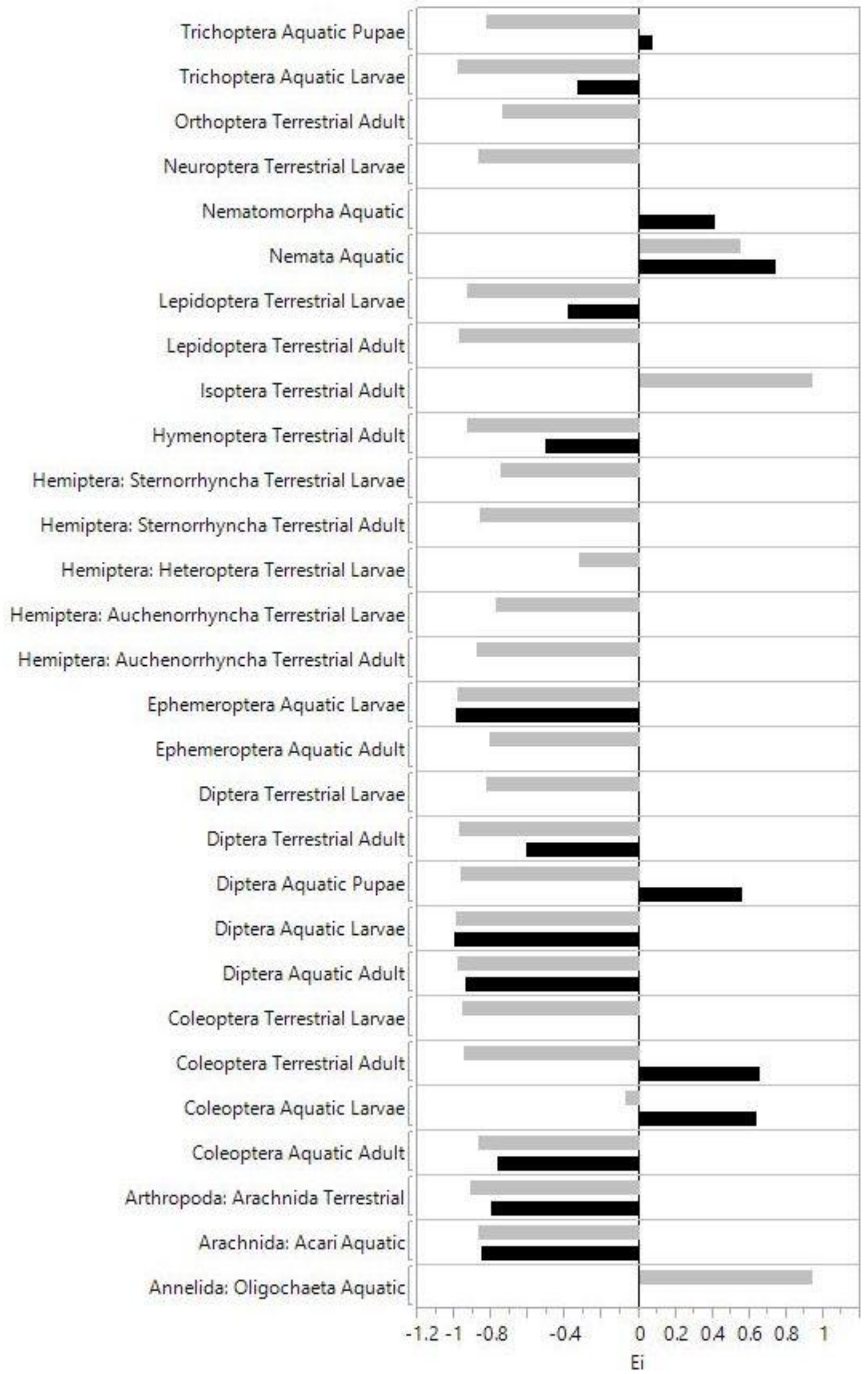
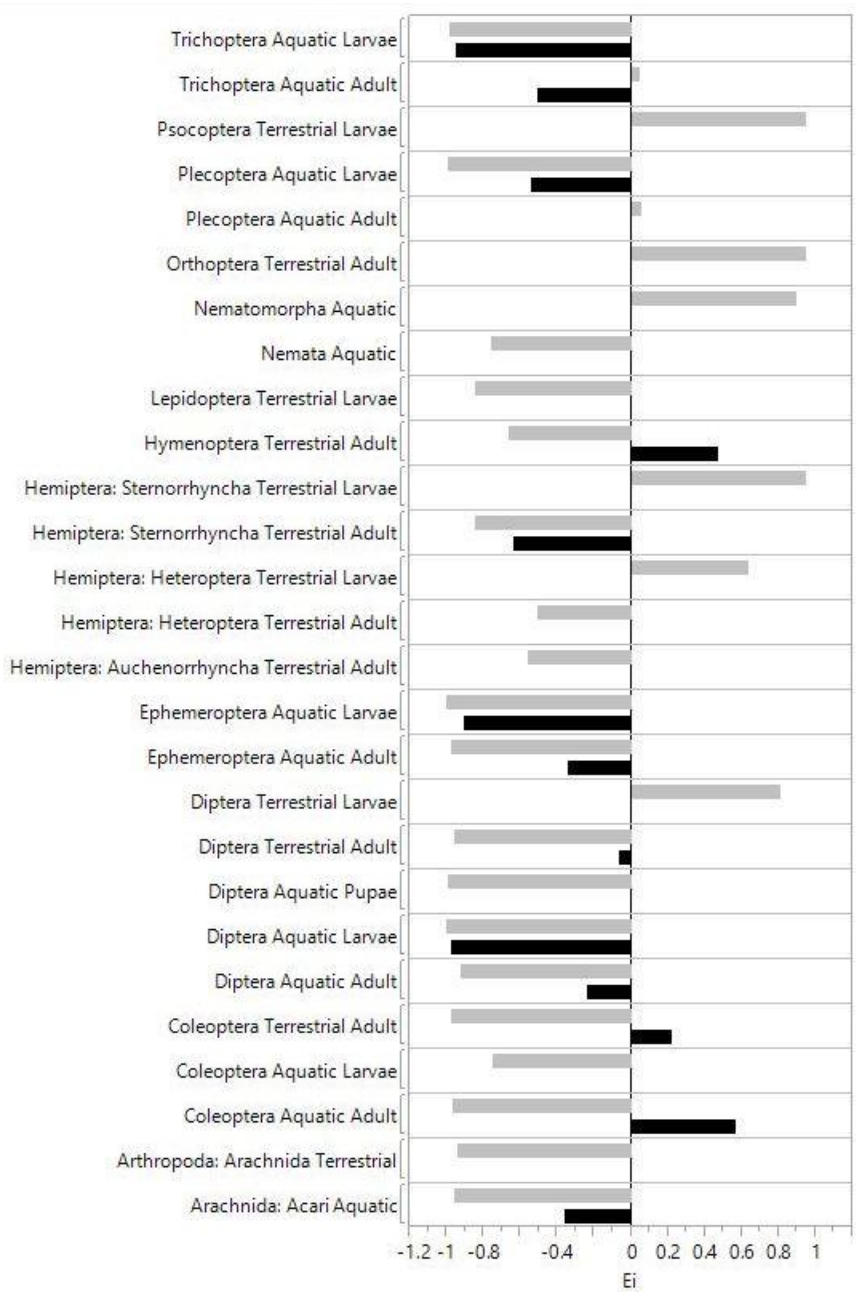


Figure 14 Vanderplog and Scavia Electivity Index of item selectivity for wild and hatchery steelhead in the South Fork Skokomish River. Includes all items found in both the drift and steelhead stomach contents. Grey bars represent wild steelhead, black bars represent hatchery steelhead.



Discussion

River Scale

Drift

There was greater variety of drift items in the South Fork Skokomish River compared to the Duckabush River and Dewatto River. This difference could possibly be due to a difference in river size. The South Fork Skokomish River is the largest of the three rivers, with a length of 44.2 km and encompassing the largest watershed area of 268 km^2. Because of its larger size, it is possible that the South Fork Skokomish River is receiving a larger input of drift items from lower order streams, further up in the watershed. As these smaller streams merge together and form the larger South Fork Skokomish River, the drift items from those streams are transported into the larger river (Wipfli & Gregovich 2002). These drift characteristics play an important role, as drift items are the main food source for juvenile steelhead and can greatly influence the diet composition of juvenile steelhead in these three rivers.

Diet Composition

The diet composition of the wild and hatchery steelhead juveniles in the Dewatto, Duckabush and South Fork Skokomish is consistent with results found in other studies focused on juvenile steelhead diets, with the most abundant items consisting of Ephemeroptera aquatic larvae and Diptera aquatic larvae (Godby et al. 2007; Johnson 2007 & Mistak et al 2003). This indicates that the juvenile steelhead in the these three rivers are consuming items that are characteristic of juvenile steelhead diets worldwide. However, there was a noticeable difference in juvenile steelhead diet composition across all three rivers. The juveniles in the Dewatto River consumed a greater variety of item types (72 types) than in the Duckabush River (56 types) and the South Fork Skokomish River (42 types). It is not entirely clear why the juvenile steelhead in the Dewatto River are selecting a greater variety of prey items than on the Duckabush and South Fork Skokomish Rivers. However, the size of the Dewatto River, as well as water depth, water velocity and water temperature, in comparison to the other two rivers, could explain this difference in juvenile fish diets.

The Dewatto River is significantly smaller, with a length of 14 km, while the Duckabush and South Fork Skokomish Rivers have a length of 39.4 and 44.2 km, respectively. In addition, during the months of data collection, the Dewatto River had the lowest stream depth and slowest water velocity when compared to the Duckabush River and South Fork Skokomish River. These differences in stream characteristics could influence the feeding behaviors of hatchery and wild fish in the Dewatto River. Due to the possible lower abundance of drift items available, because of low flow conditions, the steelhead juveniles have access to fewer resources and so are forced to eat all available item types. These may include items that the steelhead in the Duckabush River and South Fork Skokomish River do not often eat because these rivers are not as impacted by low flow conditions and so have more items available. This allows the steelhead in these rivers to be more selective about what items they eat.

In each of the three rivers, there were more item types found in juvenile steelhead stomach contents than were found in the drift. The largest discrepancy was in the Dewatto River where only 33 item types were found in the drift, while 72 item types were found in juvenile steelhead stomach contents. A similar pattern was found in the other two rivers. The Duckabush River had 32 item types found in the drift and 56 item types found in steelhead stomach contents. In the South Fork Skokomish River there were 40 item types found in the drift and 42 item types found in steelhead stomach contents. The most likely cause for this discrepancy is that terrestrial insects that entered the river were eaten right away by the juvenile steelhead, thus not making it into the drift. This likely explains why there was a larger difference between the number of drift and stomach content item types in the Dewatto River. Because the Dewatto River is much smaller than the other two rivers, it has a narrower stream channel with more overhanging vegetation, allowing for more terrestrial inputs into the stream. If juvenile steelhead are eating terrestrial inputs as soon as these items enter the river and the Dewatto has larger terrestrial inputs, due to its small size, then it would be expected that the steelhead in this river would have a larger variety of item types in their stomach contents than is found in the drift.

In all three rivers there was a noticeable difference in diet composition between wild and hatchery steelhead juveniles. Wild fish consumed a greater variety of item types than hatchery fish. In the Dewatto River, wild fish consumed 41 item types, while hatchery fish only consumed 32 types. This pattern holds true for the other two rivers, with Duckabush wild fish consuming 38 types and hatchery steelhead consuming 18. In the South Fork Skokomish wild fish consumed 29 types and hatchery fish consumed 13 types. It should be noted that there were far more wild steelhead analyzed in this study than hatchery fish, so some of these patterns may be related to the size difference between these two sample groups and may not fully reflect diet differences.

When looking at differences across all three rivers using the MRPP, the only significant difference at the river scale was found in the Duckabush River. There are a number of possibilities for these significant findings. One explanation may be that the Duckabush had the highest abundance of fish collected. This larger sample size may have allowed for the detection of a diet difference. In addition, in the Duckabush River, like all the rivers in this study, the majority of fish were collected in the lower reach. The lower reach of the Duckabush was also found to be significant between wild and hatchery diets, making it likely that the higher steelhead abundance in the lower reach is driving the pattern for the whole river.

Another possible explanation may be a difference in the rearing habitats experienced by wild and hatchery raised fish. Wild fish feed on live prey right away once they emerge as fry, but hatchery fish are pellet fed and don't experience live food until they are released into the wild (Simpson et al. 2009 & Weber & Fausch 2003). It has been suggested that hatchery fish juveniles may be unable to recognize available food sources and have been found to spend less time foraging and have lower feeding efficiency than their wild counterparts (Elliot 1973). This earlier experience with natural prey consumption may explain why wild steelhead are consuming such a wider array of prey items compared to the hatchery reared fish. This may also explain why wild fish consumed more larvae than adults in all three rivers, compared to hatchery fish who consumed more adult life stages. Larvae are more likely to be found in the drift than adult life stages of macroinvertebrates, who are located at or on the water surface. Because hatchery fish are accustomed to surface feeding, they go after the items located on top of the water, rather than items down in the water column, where it is likely more young life stages are available.

It is also possible that wild fish are eating a wider variety of items than hatchery fish due to competition. Hatchery fish are often larger and more aggressive than wild fish (Keeley & McPhail 1998). Studies focused specifically on competitive interactions between wild and hatchery steelhead have found that hatchery fish limit wild fish food resource availability due to the more aggressive behavior of hatchery fish (McMichael 1999). In the case of the wild and hatchery interactions in the Dewatto, Duckabush and South Fork Skokomish rivers, wild fish may be forced to consume different, perhaps less desirable, items because hatchery fish are holding the optimum feeding positions and therefore consuming the optimum, less available, prey. Although size data was collected it was not analyzed in this specific study. Further research is necessary in order to clearly determine if competition is occurring between wild and hatchery steelhead juvenile in these three Hood Canal Rivers.

Reach Scale

A significant difference was found between wild and hatchery diets on the lower reaches of the Duckabush River and South Fork Skokomish River. These differences may be due to the fact that the number of hatchery and wild fish captured in the three reaches of these rivers were very different. In the Duckabush River there were no hatchery fish captured in the upper reach and most hatchery fish were captured in the lower reach. Similarly, on the South Fork Skokomish River hatchery fish were only captured in the lower reach. The high abundance of hatchery fish in these lower reaches may have provided a large enough sample size to show a trend, although the number of hatchery fish captured in all three rivers was still much lower than wild fish.

Another possible factor that may have led to the significant findings in the lower reaches of these two rivers may be that the lower reaches have a larger input of items from upstream. Similar to the idea when discussing drift (Wipfli & Gregovich 2002) when those items from the upper reach are combined with the middle and lower reach, a wider variety of items are present (Wipfli & Gregovich 2002). .

Electivity

Despite the high abundance of aquatic Ephmeroptera and aquatic Diptera larvae in juvenile steelhead diets and the drift of all three rivers, these items both received negative electivity index values, with the exception of hatchery fish in the Dewatto River (which showed positive electivity for these two prey items). These negative electivity values indicate that despite high abundance in the drift, as well as high abundance in diets, steelhead juveniles selected against Ephemeroptera and Diptera aquatic larvae.

These findings are similar to that reported by Mistak et al. (2003) in a study focusing on juvenile steelhead diets in the Pine River. The authors found a similar situation in which items that were highly abundant in the drift and highly abundant in steelhead stomach contents were being selected against. They concluded that the negative electivity values were not necessarily indicating avoidance of these items but that the items were underutilized. A similar conclusion could be reached in the case of the Dewatto, Duckabush and South Fork Skokomish diet and drift composition. Although high abundance of Diptera and Ephemeroptera aquatic larvae exists in the drift and diet, in relation to all the other items consumed these two items are not being consumed in equal proportion to their availability. However, more research is necessary in this area to more clearly understand this discrepancy between high drift and diet abundance but low electivity values of these two items.

Due to the large discrepancy in the number of wild and hatchery fish included in this study it is hard to distinguish specific patterns in regard to electivity index (Ei) values. However the distinctive pattern between the Ei values of items consumed by hatchery steelhead in the Dewatto River was noticeable. Hatchery steelhead on this river have only positive Ei values, indicating that these hatchery fish selected for every single available item, while in the Duckabush and South Fork Skokomish River there was more variability in the preference and avoidance of the same items. Wild fish in the Dewatto also showed more preference than wild steelhead in the other two rivers.

There are a number of factors that may have contributed to the positive electivity values for both hatchery and wild steelhead in the Dewatto River. One factor may be the habitat availability in the Dewatto. Because data was collected during late summer, when stream flows are the lowest, there may be less habitat available to the fish in this smaller system, forcing hatchery and wild fish to be confined to a smaller area and to compete for the same limited food resources. This competition for food sources could be occurring in two different ways. Hatchery fish could be outcompeting wild fish (McMichael 1999), causing the wild steelhead to consume the less desirable (more discriminated against) items. On the other hand, it could be that wild fish are consuming the more desirable (less discriminated against) items, forcing hatchery fish to show a preference for all items.

Further Research

In order to delve deeper into some of the results found in this study there are three main areas in which more research is required. The most important finding that needs further investigation is the relationship between age and size differences between the wild and hatchery steelhead sampled in these rivers. By comparing the size and age differences between wild and hatchery fish, the idea that competition may be occurring between the two can be further substantiated.

Further investigation into the differing habitat characteristics along the entire lengths of the Dewatto, Duckabush and South Fork Skokomish rivers is also necessary. Looking at canopy cover, water temperature, turbidity, conductivity, substrate type and gradient (all of which were gathered during sample collection) may provide more insight into the drift and diet differences found in these three rivers. Using this information, it may be possible to determine exactly why the larger South Fork Skokomish River had a higher variety of drift items. These habitat characteristics could also be used to determine the diet differences observed between these three rivers.

Finally, as mentioned previously, more investigation is needed in order to explain the negative Ei values for the diet and drift items that were found to be the most abundant in all three rivers. Gaining a deeper understanding of this discrepancy will provide a clearer understanding of hatchery and wild juvenile steelhead diet preferences as well as investigate the usefulness of the Ei itself in diet analysis.

CHAPTER IV: Conclusion

There were two key findings in this study that were the most noteworthy. The first key finding of importance was the large variety of items in juvenile steelhead diets in the Dewatto River, while the Duckabush and South Fork Skokomish juvenile steelhead diets showed less variability. This finding is important because it may reveal a significant relationship between stream size and drift composition and how these two factors influence feeding behaviors of both hatchery and wild steelhead. Further research in this specific area will allow for a clearer understanding of the different diet requirements of steelhead in different sized rivers. Finding a link between stream size and diet may help inform management practices and allow for the greater success of steelhead juveniles in these lower order streams.

If steelhead juveniles are indeed relying on a larger proportion of the available drift items in small order streams, measures can be taken to ensure that these stream habitats are able to support these macroinvertebrate populations in the future. Climate change and other anthropogenic changes, such as alteration in land use practices, can negatively impact these ecosystems. In Washington State, climate change models are predicting increased precipitation levels in the winter, with more falling as rain rather than snow, and hotter and drier summers (Leung et al. 2004). This reduction in precipitation and large increase in temperatures could have great impacts on these smaller streams, creating even more drastic low flow conditions in the late summer months. Growing populations in the Puget Sound area as well will likely result in land use change, possibly resulting in reduced stream flows (Konrad & Booth 2002).

By anticipating these changes and incorporating them into current and future management plans, measures can be taken to maintain and/or create habitats in which low flow adapted macroinvertebrates can thrive and continue to be a food source for juvenile steelhead. These could include ensuring that there are enough pool areas available for juvenile steelhead and their food sources during low flow conditions, as well as ensuring that the hyphoreic zone is functioning properly and allowing for groundwater intrusion to keep stream sediments moist (Boulton et al. 1998). Many of the food sources that steelhead are eating in the Dewatto River have life history stages that are already adapted to flow conditions (Bunn & Arlington 2002). These macroinvertebrate species rely heavily on pools and burrowing into moist sediments for refuge (Williams & Hayes 1977). By creating areas where these macroinvertebrate and other food sources can survive, it will ensure that juvenile steelhead have access to necessary food sources.

The second finding of key importance was the significant diet differences between hatchery and wild steelhead juveniles in the Duckabush River, both at the river and reach scale. Depending on what further research reveals about the relationship between wild and hatchery steelhead diets in the Dewatto, Duckabush and South Fork Skokomish rivers, changes can be implemented to current hatchery program practices.

If the diet differences between wild and hatchery fish are due to rearing environments, measures can be taken to ensure that hatchery raised steelhead are exposed to live food items while still in the hatchery setting, as opposed to the current use of pellets, delivered at the water surface. If the management goal is ultimately to boost steelhead populations in Puget Sound, then steps need to be taken to ensure that once released, these hatchery fish have the best chance of survival. Providing live prey items while in the hatchery may be a way in which to ensure that these hatchery fish are adapted to the conditions experienced by wild steelhead, thus improving rates of survival.

Previous research in this area has shown that the exposure of hatchery raised salmonids to live prey while still in a hatchery setting are better able to forage for novel live prey items (Brown et al. 2003 & Maynard et al. 1994). However, this only occurred in settings where there was the addition of live prey as well as the addition of habitat complexity (i.e. tanks with rocks, wood and temperature variations). A similar approach could be used in the hatchery management plans in Washington State and could prove to be extremely beneficial in improving the post-release survival of hatchery steelhead (Brown et al. 2003 & Maynard et al. 1994), especially if Hood Canal rivers undergo hydrologic changes in the future. Hatchery fish that are able to identify novel prey items will be able to adapt more easily to changes in macroinvertebrate assemblages, making these fish more resilient. Ultimately hatchery fish with previous exposure to live food items will likely fare better and may be able to improve Puget Sound steelhead population numbers.

By looking deeper into the causes for the significant diet differences between wild and hatchery steelhead on the Duckabush Rivers, such as size and age differences between wild and hatchery fish and habitat characteristics that may be influencing drift and fish diet, a clearer understanding will be reached. It is imperative that fisheries managers and restoration ecologists work together to produce hatchery fish that are better adapted to natural environments and to create environments that promote healthy macroinvertebrate populations. Hatchery managers need to create programs in which hatchery fish are exposed to live prey items before release into streams to ensure that they will be able to feed efficiently and effectively in these wild environments. This will ultimately ensure the fitness of these animals in the wild. Restoration ecologists need to incorporate habitat structures and elements, such as pools and a healthy hyphoreic zone, to ensure that the food sources that juvenile steelhead rely on remain in these freshwater ecosystems.

The implementation of these management strategies is imperative and should begin as soon as possible. In addition, the ecological interactions between wild and hatchery steelhead, and salmonids in general, need to be more clearly understood. Despite the implementation of hatchery programs throughout Washington State in recent decades, steelhead populations have continued to decline, indicating that current management practices are not effective. A change in hatchery management plans that puts hatchery and wild steelhead on equal footing may be the only way for steelhead populations to bounce back.

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Appendices

Table 1 The type and abundance of prey items found in wild and hatchery steelhead juvenile diets in the Dewatto, Duckabush and South Fork Skokomish River. Insect prey items were classified by order and life stage. Non-insect prey items were classified by taxon. * indicates prey items found in stomach contents but not found in drift and were therefore excluded from the Electivity Index (Ei) calculations.

Insect Order	Life Stage	Life Stage	Dewatto Abundance	Duckabush Abundance	Skokomish Abundance
Coleoptera	Aquatic	Adult	33	8	54
Coleoptera	Aquatic	Larvae	24	2	13
Coleoptera	Terrestrial	Adult	14	28	69
Coleoptera	Terrestrial	Larvae	4	23	3
Diptera	Aquatic	Adult	40	369	144
Diptera	Aquatic	Larvae	732	1036	616
Diptera	Aquatic	Pupae	40	6	133
Diptera	Terrestrial	Adult	7	1	27
Diptera	Terrestrial	Larvae	6	2	2
Diptera	Terrestrial	Pupae	1*	0	0
Ephemeroptera	Aquatic	Adult	29	1	253
Ephemeroptera	Aquatic	Larvae	752	2389	2838
Hemiptera: Auchenorrhyncha	Terrestrial	Adult	3*	7*	14
Hemiptera: Auchenorrhyncha	Terrestrial	Larvae	3	2	0
Hemiptera: Heteroptera	Aquatic	Adult	1*	9*	1*
Hemiptera: Heteroptera	Terrestrial	Adult	0	0	62
Hemiptera: Heteroptera	Terrestrial	Larvae	1*	7	2
Hemiptera: Sternorrhyncha	Terrestrial	Adult	6	10	28
Hemiptera: Sternorrhyncha	Terrestrial	Larvae	4	8	1
Hymenoptera	Terrestrial	Adult	15	23	61
Lepidoptera*	Terrestrial	Adult	3*	6	0
Lepidoptera	Terrestrial	Larvae	18	75	10
Lepidoptera	Terrestrial	Pupae	0	2*	0
Megaloptera	Aquatic	Larvae	1	0	0
Neuroptera	Terrestrial	Larvae	11	8	0
Orthoptera	Terrestrial	Adult	3	7	1
Plecoptera	Aquatic	Adult	0	2*	3*
Plecoptera	Aquatic	Larvae	35	139	139
Pscoptera	Terrestrial	Larvae	1	7	1

Thysanoptera	Terrestrial	Adult	1	0	0
Thysanoptera	Terrestrial	Larvae	1*	0	0
Trichoptera	Aquatic	Adult	10*	99*	11
Trichoptera	Aquatic	Larvae	199	34	212
Trichoptera	Aquatic	Pupae	1	6	24*
Non-Insect Taxon			**Dewatto Abundance**	**Duckabush Abundance**	**Skokomish Abundance**
Annelida: Oligochaeta	Aquatic		1	1	0
Annelida: Oligochaeta	Terrestrial		1*	0	0
Arachnida: Acari	Aquatic		70	27	42
Arthropoda: Arachnida	Terrestrial		10	45	17
Arthropoda: Chilopoda	Terrestrial		1*	3	1*
Arthropoda: Collembola	Terrestrial		3	2	0
Arthropoda: Microcoryphia	Terrestrial		53*	5	0
Cottidae	Aquatic		2*	2*	0
Crustacea: Isopoda	Terrestrial		3	0	0
Crustacea: Ostracoda	Aquatic		3	0	0
Mollusca: Gastropoda	Aquatic		49	0	0
Mollusca: Gastropoda	Terrestrial		4*	0	0
Nemata	Aquatic		3	8	25
Nematomorpha	Terrestrial		11	5	2
Total Number of Prey Items			2132	4293	4780

Table 2. The type and abundance of prey items found in wild and hatchery steelhead juvenile diets in the lower reach of the Duckabush River.

Insect Order	**Hatchery**	**Wild**
Coleoptera Aquatic Adult	3	0
Coleoptera Terrestrial Adult	0	12
Coleoptera Terrestrial Larvae	0	14
Cottidae	0	1
Diptera Aquatic Adult	38	80
Diptera Aquatic Larvae	28	441
Diptera Aquatic Pupae	3	114
Diptera Terrestrial Adult	12	20
Diptera Terrestrial Larvae	0	10
Ephemeroptera Aquatic Larvae	102	748
Hemiptera: Auchenorrhyncha Terrestrial Adult	0	20
Hemiptera: Auchenorrhyncha Terrestrial Larvae	0	20
Hemiptera: Heteroptera Terrestrial Adult	0	4
Hemiptera: Heteroptera Terrestrial Larvae	0	6
Hemiptera: Sternorrhyncha Terrestrial Adult	0	39
Hemiptera: Sternorrhyncha Terrestrial Larvae	0	17
Hymenoptera Terrestrial Adult	2	35
Lepidoptera Terrestrial Adult	0	3
Lepidoptera Terrestrial Larvae	1	54
Lepidoptera Terrestrial Pupae	21	1
Neuroptera Terrestrial Larvae	0	12
Orthoptera Terrestrial Adult	0	21
Plecoptera Aquatic Adult	0	3
Plecoptera Aquatic Larvae	8	53
Psocoptera Terrestrail Larvae	0	12
Trichoptera Aquatic Adult	0	5
Trichoptera Aquatic Larvae	6	20
Trichoptera Aquatic Pupae	0	21
Non-Insects	**Hatchery**	**Wild**
Annelida: Oligochaeta Aquatic	9	1
Arachnida: Acari Aquatic	0	3
Arthropoda: Arachnida Terrestrial	0	16
Arthropoda: Chilopoda Terrestrial	0	1
Arthropoda: Collembola Terrestrial	0	2
Arthropoda: Microcoryphia Terrestrial	2	2
Nemata Aquatic	3	2
Nematomorpha Aquatic	0	0
Total Number of Prey Items	238	1813

Table 3. Items found in the Dewatto River, Duckabush River and South Fork Skokomish River. Those in bold indicate the drift items that were only found in one of the three rivers.

Dewatto River	**Duckabush River**	**South Fork Skokomish River**
Annelida: Oligochaeta Aquatic	Annelida: Oligochaeta Aquatic	Annelida: Oligochaeta Aquatic
Arachnida: Acari Aquatic	Arachnida: Acari Aquatic	Arachnida: Acari Aquatic
Arthropoda: Arachnida Aquatic	Arthropoda: Arachnida Terrestrial	Arthropoda: Arachnida Terrestrial
Arthropoda: Arachnida Terrestrial	Arthropoda: Collembola Terrestrial	Arthropoda: Collembola Terrestrial
Arthropoda: Collembola Terrestrial	Coleoptera Aquatic Adult	Coleoptera Aquatic Adult
Coleoptera Aquatic Adult	Coleoptera Aquatic Larvae	Coleoptera Aquatic Larvae
Coleoptera Aquatic Larvae	Coleoptera Terrestrial Adult	Coleoptera Terrestrial Adult
Coleoptera Terrestrial Adult	Coleoptera Terrestrial Larvae	Coleoptera Terrestrial Larvae
Coleoptera Terrestrial Larvae	Crustacea: Copepoda Aquatic	**Cottidae Aquatic**
Crustacea: Cladocera Aquatic	Crustacea: Ostracoda Aquatic	**Crustacea: Amphipoda Aquatic**
Crustacea: Copepoda Aquatic	Diptera Aquatic Adult	Crustacea: Copepoda Aquatic
Crustacea: Isopoda Terrestrial	Diptera Aquatic Larvae	Crustacea: Isopoda Aquaitc
Crustacea: Ostracoda Aquatic	Diptera Aquatic Pupae	Diptera Aquatic Adult
Diptera Aquatic Adult	Diptera Terrestrial Adult	Diptera Aquatic Larvae
Diptera Aquatic Larvae	Diptera Terrestrial Larvae	Diptera Aquatic Pupae
Diptera Aquatic Pupae	Ephemeroptera Aquatic Adult	Diptera Terrestrial Adult
Diptera Terrestrial Adult	Ephemeroptera Aquatic Larvae	Diptera Terrestrial Larvae
Diptera Terrestrial Larvae	Hemiptera: Auchenorrhyncha Terrestrial Adult	Ephemeroptera Aquatic Adult
Ephemeroptera Aquatic Adult	Hemiptera: Auchenorrhyncha Terrestrial Larvae	Ephemeroptera Aquatic Larvae
Ephemeroptera Aquatic Larvae	Hemiptera: Heteroptera Terrestrial Larvae	Hemiptera: Auchenorrhyncha Terrestrial Adult
Hemiptera: Auchenorrhyncha Terrestrial Larvae	Hemiptera: Sternorrhyncha Terrestrial Adult	Hemiptera: Auchenorrhyncha Terrestrial Larvae
Hemiptera: Sternorrhyncha Terrestrial Adult	Hemiptera: Sternorrhyncha Terrestrial Larvae	**Hemiptera: Heteroptera Terrestrial Adult**
Hemiptera: Sternorrhyncha Terrestrial Larvae	Hymenoptera Terrestrial Adult	Hemiptera: Heteroptera Terrestrial Larvae
Hymenoptera Terrestrial Adult	Lepidoptera Terrestrial Larvae	Hemiptera: Sternorrhyncha Terrestrial Adult
Lepidoptera Terrestrial Larvae	Nemata Aquatic	Hemiptera: Sternorrhyncha Terrestrial Larvae
Mollusca: Gastropoda Aquatic	Nematomorpha Aquatic	Hymenoptera Terrestrial Adult
Nemata Aquatic	Neuroptera Terrestrial Larvae	**Lepidoptera Terrestrial Adult**
Neuroptera Terrestrial Larvae	Orthoptera Terrestrial Adult	Lepidoptera Terrestrial Larvae
Orthoptera Terrestrial Adult	Plecoptera Aquatic Larvae	**Mollusca: Bivalvia Aquatic**
Plecoptera Aquatic Larvae	Psocoptera Terrestrial Larvae	Nemata Aquatic
Psocoptera Terrestrial Larvae	Trichoptera Aquatic Larvae	Nematomorpha Aquatic
Trichoptera Aquatic Larvae	Trichoptera Aquatic Pupae	Neuroptera Terrestrial Larvae
Trichoptera Aquatic Pupae		**Odonata Aquatic Adult**
		Orthoptera Terrestrial Adult
		Plecoptera Aquatic Adult
		Plecoptera Aquatic Larvae
		Psocoptera Terrestrial Larvae

		Thysanoptera Terrestrial Adult
		Trichoptera Aquatic Adult
		Trichoptera Aquatic Larvae
33 Total Item Types	32 Total Item Types	40 Total Item Types