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# DIETARY CHARACTERISTICS OF JUVENILE TROUT AND CHAR IN SEASONALLY INUNDATED STREAM SEGMENTS IN ROSS LAKE, WASHINGTON

By Emily Derenne

Accepted in Partial Completion

Of the Requirements for the Degree

Master of Science

Kathleen L. Kitto, Dean of the Graduate School

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MASTER'S THESIS

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Emily Derenne November 10, 2014

# DIETARY CHARACTERISTICS OF JUVENILE TROUT AND CHAR IN SEASONALLY INUNDATED STREAM SEGMENTS IN ROSS LAKE, WASHINGTON

A Thesis Presented to The Faculty of Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By Emily Derenne November 2014

#### **Abstract**

I investigated available prey items and the diet characteristics of juvenile fishes in three seasonally inundated tributaries to Ross Lake, Washington from March through June, 2013. Native fishes include Rainbow Trout (Oncorhynchus mykiss), Bull Trout (Salvelinus confluentus), and Dolly Varden (Salvelinus malma). Cutthroat Trout (Oncorhynchus clarkii), Brook Trout (Salvelinus fontinalis) and Redside Shiner (Richardsonious balteatus) comprise the introduced fishes in the lake. Both Cutthroat Trout and Redside Shiner are native to Washington, but not Ross Lake. Juvenile Bull Trout, Rainbow Trout, and Brook Trout are known to feed on items along the bottom of lakes or streams, such as larval and adult insects as well as items floating or drifting in the water column. Diet composition can be altered by the benthic macroinvertebrate community, season, and habitat type as well as anthropogenic interferences such as dams.

During each sampling event the stream was electrofished, benthic macroinvertebrate samples were collected, all captured fish over 50 mm were lavaged, and during the initial visit to each site, a habitat assessment occurred. Three fifty-meter reaches were selected for each stream to have representative sites at low, medium, high, and full pool elevations. Rapid habitat assessment was completed following USFS Stream Inventory Handbook for Region 6 on each of the streams during the first site visit, benthic macroinvertebrate sampling followed a modified version of the Environmental Protection Agency's Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, electrofishing followed American Fisheries Society and National Marine Fisheries Service Guidelines, and diet evaluation was completed using non-lethal gastric lavage of stomach contents mm following the modified protocols of Giles (1980), Strange and Kennedy (1981), Hartleb and Moring (1995).

My study suggests adequate food, in the form of benthic macroinvertebrates, is present based on the presence of few fish with empty stomachs in the system. The benthic macroinvertebrates found in the tributaries to Ross

Lake reflect those commonly found in Pacific Northwest Streams. A total of 3,645 individuals in 31 families were collected. Ephemeroptera was the most abundant and frequently occurring insect order across all samples, but not uniformly the most abundant at all sites, dates, or months. Abundance of families by date differed, but not site or reach.

Diets varied by sites, months, and most pool elevations, but not species. Sixty-five of the seventy-three fish collected had at least one diet item in their stomach (89% off all fish). Including those taxa that were identifiable only to terrestrial origin or class Insecta and those unrecognizable even at the class level, there were sixteen categories for analysis, seven of which were considered major and included in all analyses. Using Index of Relative Importance, I determined Diptera was the most important prey item overall, followed by Ephemeroptera. Stomach fullness, calculated by Instantaneous Ration, was correlated to the number of prey found in individual fish stomachs. As expected, stomach fullness followed benthos abundance trends.

Many studies are completed on adult feeding strategies, especially in comparisons between species or environments, but research on juvenile diets is less available, and to the best of my knowledge research on prey availability and selectivity on seasonally inundated streams is non-existent. Further research on Ross Lake juvenile trout diet, the most important prey taxa, and the benthic community they rely on will result in a better understanding of fish stock dynamics and Ross Lake ecology and perhaps influence management of the fish stocks and lake levels in the future.

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#### Introduction

Juvenile trout and char rely on streams during their early life history. Juveniles in the Pacific Northwest have adapted to the flow, temperature, and channel complexity in the streams found there. Trout and char have different habitat requirements and preferences, which allows for a variety of species to inhabit the same stream but different microhabitats based on substrate, depth, flow, temperature, and gradient (Quinn, 2005). Recently emerged trout and char fry school along streambanks while larger fry become territorial and require adequate space and forage. Specifically, juveniles of Bull Trout (Salvelinus confluentus), Rainbow Trout (Oncorhynchus mykiss), and Brook Trout (Salvelinus fontinalis) feed on aquatic prey found in streams, typically in the form of larval or adult insects, floating or drifting in the water column as well as prey found along the bottom (Wydoski and Whitney, 2003; Quinn, 2005; White and Harvey, 2007). Prey composition can be affected by the benthic macroinvertebrate community, season of the year, and habitat conditions (Hilderbrand and Kershner, 2004; Quinn, 2005). In addition to affecting prey composition, alterations to available habitat, canopy, flow, sediment rates, and primary productivity can adversely affect juvenile populations by over-crowding, altering temperature outside the suitable range, and reducing available forage (Anderson, 1971; Davis and Hughes, 1971; Korn and Smith, 1971; Quinn, 2005).

Reservoirs found on rivers often create habitat that is very different than both a natural lake and the previously existing stream habitat (Baxter, 1977). Water-level management of a reservoir can cause seasonally periodic inundation and exposure of former streams; new barriers may be created as well as access enabled to previously inaccessible habitat, primary productivity, the benthic community, and the littoral region altered, and fish and other vertebrates adversely affected (Isom, 1971; Korn and Smith, 1971; Taylor, 1971; Baxter, 1977; Scrimgeour et al, 2008; Northcote, 2010).

The purpose of this study is to characterize diet amongst the juvenile fish species during exposure of periodically inundated streams found in a reservoir with seasonally changing water levels. I hypothesized that the seasonally inundated streams would lack benthic macroinvertebrates and would therefore be unable to support fish life. If I was able to determine that fish were able to rear in the streams, I theorized that diet and benthos composition would differ with changing pool elevations, among stream reaches, and among sites. I projected that the upper stream reaches, those that had been exposed longer, would have a different diet composition than the lower reaches that spend more time lacustrine than riverine. Additionally, the tributaries to Ross Lake vary in size, location, and fish stocking history so I hypothesized a difference in benthos and diet characteristics would be seen among them. I tested these hypotheses by sampling the benthic invertebrate community, characterizing available habitat, and analyzing diets of juvenile trout and char in three tributaries to examine the relationship of habitat, fish, and food and to document changes in the existing prey community and diet characteristics by resident juvenile fishes.

#### **Study Site**

Ross Lake, a 37-km long reservoir created by the installation of Ross Dam on the upper section of the Skagit River, is surrounded by steep, rocky shorelines and is within the North Cascades National Park Complex (NOCA) (Figure 1). Ross Lake extends into Canada during summer months, but the majority of the lake is located within the United States. The North Cascade mountains rise from the Skagit River canyon to almost 1,525 meters at a nearly vertical pitch. Ross Dam, located at the southern end of the lake, is 165 meters high, and was constructed in two phases beginning in 1937 with final completion in 1949 (UWCFR, 1971; Johnston, 1989; NWDA, 2003). The installation of the dam inundated approximately 4,727 hectares of riparian habitat over 46 square kilometers and decreased primary productivity in the aquatic habitat to a point lower than naturally existed (Figure 2; Johnston, 1989; Brondi, 2006). Water levels are managed seasonally to provide hydropower, recreation, flood control, and fish habitat. About 20% of Seattle's total usage comes from the three Skagit River

dams, of which Ross Dam is the largest (SCL, 2014). The lake is deepest near the dam and varies in depth from 450 meters during the winter, when the lake is drawn down to provide storage for spring runoff and flood control, to 488 meters in the summer to allow recreation and power generation (Figure 3; Johnston, 1989). The lake is oligotrophic and monomictic; very little aquatic vegetation is found along the shorelines, dissolved oxygen is near saturation from surface to bottom, and during the summer the lake is thermally stratified (Johnston, 1989; Looff, 1995; Brondi, 2006; USGS, 2013).

Ross Lake exhibits several features that create a unique environment for fish. The water level regime of the lake is opposite that of natural lakes in the Pacific Northwest; the water level is high in the summer, when little precipitation occurs, and low during winter, when natural lakes are at their highest level from rainfall (Brondi, 2006). Ross Lake exists nearly entirely within the North Cascades National Park Complex and has only one unimproved access road via British Columbia. It is therefore largely unaffected by anthropogenic sources of pollution and disturbance, but it is still subject to human disturbances. Hydroelectric production is a major concern because operations are outside the jurisdiction of the National Park yet may have adverse impacts on the native trout. For example, although Seattle City Light manages the water levels to avoid spilling water over the top of the dam, occasional spilling does occur and has resulted in fish loss from Ross Lake. A spill in 1972 lasting 60 days resulted in the loss of an estimated 16,000 fish over the dam, with a mortality of 99.64% induced by the fall (Woodin, 1974; Johnston, 1989).

The reservoir is located within the Ross Lake National Recreation Area, created simultaneously with the North Cascades National Park in 1968, and together making up the North Cascades National Park Complex (UWCFR, 1971; Figure 1). This designation reduced activities in the watershed that were allowed when it was national forest land, particularly logging and mining (Luxenberg, 1986; Louter, 1998). Alterations to the reservoir have also been prevented. A request in 1970 to raise the dam and increase the water level 121 feet at full pool from 1602' AMSL to 1725' AMSL was denied, but resulted in a plethora of scientific and photographic evidence of

conditions at the time (Figure 2, SCL, 1970; UWCFR, 1971; Woodin, 1974; Johnston, 1989). The Ross High Treaty was signed in 1984, which in part provides for an international commission to facilitate research, education, protection and protection of the watershed.

The lake is fed by a number of tributaries that channel snow melt directly and indirectly into the reservoir. The Skagit River upstream of the dam is the largest tributary to the Lake, delivering an annual mean flow of 32 m<sup>3</sup>/s and draining 100,751 hectares in Canada (Johnston, 1989; Murray and Gaboury, 2005; Welch, 2012). Other prominent but smaller tributaries to the Lake include Hozomeen Creek, Silver Creek, Little Beaver Creek, Lightning Creek, Dry Creek, Big Beaver Creek, Skymo Creek, No Name Creek, Roland Creek, and Ruby Creek. All of these tributaries occur on the U.S. side of the border and combined drain approximately 160,579 hectares (Johnston, 1989). Aside the direct effects from the dam, the tributaries remain in a natural state, protected from anthropogenic impacts such as overuse, logging, and mining.

Among the recreational opportunities on the lake and tributaries, fishing is one of the most popular. Regulations open the fishery on July 1<sup>st</sup> allowing harvest of Rainbow Trout and Brook Trout, but requiring all Native Char to be immediately released.

#### **Macroinvertebrate Community**

Aquatic macroinvertebrates are extremely important in the food webs of the aquatic environment because they are responsible for converting plant material and detritus into energy useable by higher trophic-level organisms and thus are a main food source for fish (Waters, 1969; Brusven and Trihey, 1978; McCafferty, 1998; Pavluk et el, 2000). Terrestrial invertebrates, in addition to aquatic forms, are often found in the water column and are believed to be required to support fish communities (Laudon et al, 2005). Juvenile fishes will eat aquatic and terrestrial organisms at all stages of life but most often insects in the pupae, larvae, or nymph forms

(Wydoski and Whiney, 2003; Laudon et al, 2005). The most commonly found macroinvertebrates are in the form of benthos, which reside in, on, or near the bottom of the water body.

Macroinvertebrates are those organisms that are captured with a 200-µm mesh sieve, and those that may provide food for fish are the focus of this study (Thorp and Rogers, 2001). Aquatic insects (Phylum Arthropoda, Class Insecta) are the most commonly found macroinvertebrates. Of the ten taxonomic orders of insects that contain an aquatic life stage, five orders (Ephemeroptera, Plecoptera, Trichoptera, Megaloptera, and Odonata) have entirely aquatic larva while the remaining five orders (Diptera, Coleoptera, Lepidoptera, Hemiptera, and Neuroptera) are mainly terrestrial but have an aquatic stage in most families (Lehmkuhl, 1979; Hilsenhoff, 1991; McCafferty, 1998). Insects at all stages of life may be eaten by fish. After hatching from an egg, aquatic insects undergo metamorphosis as either incomplete, such as Plecoptera and Ephemeroptera, or complete, which includes a pupa stage, such as Trichoptera and Diptera (Lehmkuhl, 1979). Ephemeroptera, Plecoptera, Trichoptera, Lepidoptera, and Diptera, have larval forms that are exclusively submergent, meaning within or underwater, but no submergent adult representation (Hilsenhoff, 1991; Thorp and Rogers, 2001; McCafferty, 1998).

In addition to insects, other macroinvertebrates of various sizes that are eaten by fish include segmented worms, crustaceans, flatworms, mollusks, and spiders and mites. These taxa are often found in habitats that are lentic, dominated by fine sediments, or degraded. Freshwater Annelida (excludes Polychaeta, "segmented worms") are mostly composed of species in classes Oligochaeta and Hirudinea and are among the largest potential food items for juvenile fish in streams. Both water-dwelling terrestrial and aquatic Oligochaeta can be found in fish diets and terrestrial earthworms are often found in stream sediments (Northcote et al, 2007).

Amphipods ("crustaceans [scuds]", Phylum Arthropoda, Class Malacostraca) can encompass a substantial amount of the biomass in lakes and streams, may be extremely abundant having been documented to exceed 10,000 per m², and may be found in fish stomachs in high number (Pennak, 1978; Covich and Thorp, 1991).

Terrestrial mollusks (Phylum Mollusca, Class Gastropoda) are more common as prey for fish, but aquatic species are found in high number in Ross Lake, especially near Hozomeen Creek. Flatworms (Phylum Platyhelminthes) are smaller in size than the above, and are infrequently found in fish diets (Northcote et al, 2007). Water mites (Phylum Arthropoda, Class Arachnida) are among the smallest organisms and are the most commonly found aquatic arachnid although semi-aquatic spiders can also be found. Although they are consumed by juveniles, they are in low abundance and provide small energy gains so are not commonly found (Smith and Cook, 1991; Thorp and Rogers, 2001; Northcote et al, 2007).

The operations of Ross Dam provide unique challenges to the benthic community. Dams alter the flow of organic matter and sediment both above and below the structure, shifting the benthic structure to match the new environment (Baxter, 1977; Pavluk et al, 2000; Vallania and Del Carmen Corigliano, 2007). When dams are initially installed on rivers, it is expected that the lotic organisms will be replaced by lentic organisms more adapted to the lake-type habitat created behind the dam. Additionally, increased sedimentation, changes in temperature and oxygen availability due to the decrease of horizontal and vertical circulation, and draw-downs may trap, strand, suffocate, or drown organisms. Physical and chemical gradients unsuitable to the existing community may also alter the benthic composition (Baxter, 1977; Welch, 2012). Baxter (1977) found that initially following the installation of natural and man-made dams Ephemeroptera, Plecoptera, and Trichoptera decreased in abundance while chironomids (order Diptera) greatly increased. Brusven and Trihey (1978) found that rapid de-watering resulted in large scale stranding of benthos with the affects seen in the higher trophic levels. This is important in this study because the management of Ross Lake causes great annual variability in pool elevation and may be expected to have impacts on the benthic community in some habitats that would result from the annual cycle of transformation from a lotic to a lentic environment.

#### **Fish Community**

The operations of Ross Dam to control water levels may be problematic for resident fish as food availability and physical habitat in streams are decreased as the reservoir fills, providing potentially inadequate habitat for rearing and possibly forcing some juveniles to enter the lake before they would naturally do so. All of the native fishes, composed of Bull Trout, Dolly Varden (*Salvelinus malma*), and Rainbow Trout, and two of the three introduced species, Brook Trout and Cutthroat Trout (*Oncorhynchus clarkii*) utilize stream habitat for spawning and rearing of juveniles.

Among the various life history patterns that Bull Trout demonstrate, in Ross Lake they are adfluvial, spawning and rearing in streams before migrating to the lake for their adult life (Wydoski and Whitney, 2003; USFWS, 2004; Quinn, 2005). Juvenile Bull Trout diets consist mainly of aquatic insects and scuds, terrestrial insects, and fish, primarily sculpin, when available (Wydoski and Whitney, 2003). Ross Lake Bull Trout spawn in Big Beaver, Ruby, Lightning, Silver, and Roland Creeks in the fall, when the reservoir is still full (Downen, 2004; Welch, 2012). Because Bull Trout tend to be more sensitive to stream flow patterns and elevated temperatures than other trout species and also require complex in-stream channel features including cover, large woody debris, and pools, they are a sensitive indicator of conditions in the stream environment (Wydoski and Whitney, 2003; Quinn, 2005). Bull Trout were listed as Threatened in the coterminous 48 states under the Federal Endangered Species Act in 1999 (USFWS, 1999). Ross Lake Bull Trout have been shown to be genetically distinct from other Skagit River Bull Trout downstream of the dam; genetic analysis is ongoing to determine their origin (A. Rawhouser, North Cascades National Park, personal communication). Historically, upstream movement by the lower Skagit River Bull Trout population was likely naturally constrained by waterfalls in what is now the Diablo Lake reservoir below Ross Lake, thus they have remained geographically isolated and genetically unique (A. Rawhouser, North Cascades National Park, personal communication). The Ross Lake Bull Trout population has been increasing in number and size of individual fish (Welch, 2012; Anthony et al, in draft).

Dolly Varden closely resemble Bull Trout, but are difficult to physically distinguish and require genetic analysis to be certain (Quinn, 2005). Like Bull Trout, Dolly Varden spawn and rear in streams and over-winter and feed in lakes. They are opportunistic feeders consuming all stages of aquatic insects and other macroinvertebrates including leeches and snails (Wydoski and Whitney, 2003). Ross Lake is the only location in North America known to hold co-existing populations of Bull Trout and Dolly Varden in lacustrine habitat (E. Connor, Seattle City Light, personal communication). Although Bull Trout and Dolly Varden hybridize in the Ross Lake watershed, both species "maintain themselves as distinct and separate entities" (McPhail and Taylor, 1995; Smith and Naish, 2010; Anthony et al, in draft). McPhail and Taylor (1995) concluded that natural selection against hybrids and general differences in life histories must aid in keeping these two species from becoming a single gene pool. Rainbow Trout are native to the Ross Lake area with the population occasionally supplemented with hatcheryreared offspring from adults collected from the lake (A. Rawhouser, North Cascades National Park, personal communication). Rainbow Trout diet can change seasonally, but they are opportunistic and utilize the entire water column for forage. Past diet studies have found aquatic insects, amphipods, and aquatic worms among other items in the stomach contents of collected fish (Wydoski and Whitney, 2003). Similar to Bull Trout and Dolly Varden, many Rainbow Trout display an adfluvial life history in Ross Lake, spawning in the tributaries in May and June, and eventually migrating to the lake, although some remain residents in the tributaries (Woodin, 1974; Welch, 2012). Research in the 1970s found two populations within the Lake complex; a stream resident population that remained in the stream year round and a migratory population that occupied the Lake and returned to a specific tributary or stream mouth to spawn (Woodin, 1974). The timing of spawning by adfluvial fish is affected by lake levels as there are many barriers to access when the lake levels are low (Johnston, 1989; Welch, 2012). The growth rate for juvenile Rainbow Trout depends on water chemistry and food availability. A 1974 study found that juvenile Rainbow Trout grew more slowly when rearing in the tributaries to Ross Lake compared to those who moved into the lake, which is true of most stream-dwelling trout compared to their

migrating counterparts. However this is likely balanced by reduced predation for tributary rearing fish (Woodin, 1974; Wydoski and Whitney, 2003; Quinn, 2005). Rainbow Trout from the lake were stocked in Big Beaver Creek, which was isolated from the then Skagit River by a waterfall, in 1919 and continue to be planted at various sites to present (Johnston, 1989; CENR, 2014; A. Rawhouser, North Cascades National Park, personal communication). The population is larger than Bull Trout, Dolly Varden, and Brook Trout, but recent evidence indicates that the population in the lake is declining in number (Anthony et al, *in draft*). Potential causes are competition by juveniles for food with Redside Shiners, and predation on all life stages by adult Bull Trout (Johnston, 1989; Looff, 1995; Welch, 2012). Unlike Bull Trout and Dolly Varden, which cannot be harvested due to their Threatened status, Rainbow Trout are a sought-after food fish in the lake.

In the early 1900s, Brook Trout from Pennsylvania were stocked throughout the sub-alpine lakes found around Ross Lake and are now thriving in Hozomeen and Big Beaver Creeks. Brook Trout occur in smaller proportions than Rainbow Trout, Bull Trout, and Dolly Varden in lake samples, but have been increasing in number in recent samples (Johnston, 1989; Downen, 2004; Anthony et al, *in draft*). Juvenile Brook Trout are in streams in early spring following spawning in the late fall when the water temperature decreases (Wydoski and Whitney, 2003). Brook Trout are known to have a negative effect on Bull Trout by spawning earlier, displacing them via hybridization and sterile offspring, or predation as juveniles and Cutthroat Trout populations by forcing them upstream to less suitable habitat and competing for forage (Griffith, 1988; Leary et al, 1993; USFWS, 1999, Gunkel et al, 2002; Wydoski and Whitney, 2003; Rieman et al, 2006). Juveniles feed extensively on aquatic insects while rearing in streams and on zooplankton when in lakes (Wydoski and Whitney, 2003). Brook Trout populations appear to be increasing rapidly within the lake (Welch, 2012).

Much less is known about Ross Lake Cutthroat Trout than other species but they are believed to have been stocked in the early 20<sup>th</sup> century by anglers, County governments, and the US Forest Service (Downen, 2004).

The first recorded planting occurred in Big Beaver Creek in 1916 and included 47,000 Cutthroat Trout (Johnston,

1989; Welch, 2012). There have been at least 170,000 cutthroat stocked in the Ross Lake area since that original planting in 1916 (Johnston, 1989). Cutthroat Trout are native to Washington State, but not Ross Lake. Cutthroat Trout show adfluvial or resident life history patterns; both groups spawn in tributaries and adfluvial fish migrate to the lake. Juveniles remain nearly stationary in their feeding location in a stream eating aquatic and terrestrial insects and other invertebrates for up to four years before migrating to the Lake. They will consume prey from any location in the water column. Cutthroat Trout have a small, self-sustaining population in the lake and are not expected to increase in number (Anthony et al, *in draft*). Bull Trout and Cutthroat Trout are believed to have evolved together, allowing for limited competition for food and space (Griffith, 1988). Studies on cohabitation with Rainbow Trout or Dolly Varden have shown they will naturally segregate themselves by forage and habitat preferences and have been successfully doing so since the last glacial epoch (Griffith, 1988; Wydoski and Whitney, 2003).

Redside Shiner (*Richardsonius balteatus*), believed to be introduced in Ross Lake around 2000 but first noted in abundance in 2004, is a minnow (family Cyprinidae) that occurs in the region but was not previously found in the Upper Skagit watershed. Snorkel surveys of Redside Shiner completed within the lake have shown densities of hundreds per cubic meter in some places (Welch, 2012). In contrast to most lake populations of Redside Shiner which tend to school around the shore during cooler months, and head to the deep water during summer, the Ross Lake population appears to migrate to very deep water in the winter, returning to the nearshore habitat around May as temperatures increase (Wydoski and Whitney, 2003; Welch, 2012). Redside Shiners mature around 2 years of age and live to around 5 years. Redside Shiner were not a focus of my study because they prefer slower velocities and warmer temperatures than trout and char and are consequently not found in streams (Wydoski and Whitney, 2003; Welch, 2012).

#### Methods

#### **Site Location and Description**

I based site selection on accessibility, discharge, and available habitat. The streams needed to be perennial, wadeable, and provide appropriate habitat for fish and macroinvertebrates. Four sites were found to meet these criteria: Roland Creek, Dry Creek, Silver Creek, and Hozomeen Creek (Figure 4). Because of its location on the northwest side of the watershed and associated lack of accessibility by boat or foot at low pool elevations, I removed Silver Creek from the study and was unable to find a suitable replacement site. I proceeded using only Roland, Dry, and Hozomeen Creeks. Roland Creek is the shortest and most southern site (Figure 5). The headwaters of Roland Creek are at 2657.8 meters of elevation. There are four tributaries that feed into Roland Creek. The whole watershed is approximately 518.00 hectares with 54.1% in forested canopy and 88.6% over 30% slope (USGS, 2012). Roland Creek is 4.18 kilometers long and is generally steep with rapids and waterfalls (Johnston, 1989). Dry Creek, the next largest, is located between Dry Creek campground to the north and Tenmile Island Campground to the south (Figure 6). The Dry Creek watershed, above full pool, is approximately 1,072.25 hectares with 1719 meters of relief from the headwaters to Ross Lake. The watershed is approximately 71.5% forested and has a similar gradient as Roland Creek with 88.7% of the watershed over 30% slope (USGS, 2012). Dry Creek is 6.10 kilometers long, has two forks, and is generally steep with rapids, falls, and step pools (Johnston, 1989). Hozomeen Creek, the most northern site, flows adjacent to the Hozomeen Creek Campground and is 7.0 kilometers long (Johnston 1989; Figure 7). The watershed is 1,914.00 hectares and contains two lakes: Hozomeen Lake and Ridley Lake. There is 1950.7 meters of elevation change within the watershed and 75.5% has a slope greater than 30% (USGS, 2012). Silver Creek, the only site located on the west of Ross Lake and just south of Hozomeen Creek, has the largest watershed at 4,252.76 hectares and flows from Silver Lake (Figure 8). The mean slope of Silver Creek is 67.2% and over 94% of the watershed has greater than 30% slope (USGS, 2012). The creek is 9.98 kilometers long and has rapids and falls throughout (Johnston, 1989).

During the first sampling event it became clear that Hozomeen Creek would have to be accessed via the logging road from Hope, BC.

#### **Sampling Periods**

By using NPS's Snow Telemetry (Snotel) stations, which monitors snow-pack, and the expertise of their staff, I was able to determine low pool would occur in late March, which is when stream lengths would be at the maximum. Sampling for my project began March 29 and continued through June 21, 2013. Soon after this last sampling event, NPS staff alerted me to Rainbow Trout spawning and asked that further sampling events be cancelled to avoid potential negative impacts to adults. The reservoir reached full pool over a month later, on July 25<sup>th</sup>, at 1601.98.

Each site was sampled three times at varying water levels ranging from a low of 1513.6 feet AMSL on March 29<sup>th</sup> to a high of 1588.90 feet AMSL on June 21<sup>st</sup> (USGS, 2013;

Table 1 and Figure 9). At the lowest pool elevation relative to full pool, an additional 416.0 meters of Roland Creek was available, 448.7 meters at Dry Creek, and 1972.7 meters at Hozomeen Creek (Figure 10). The reservoir began filling the first week of April and was at 1520.0 feet AMSL when I completed my first round of sampling at Hozomeen Creek on April 11, 2013.

#### **Sample Collection and Field Techniques**

During each sampling event fish and macroinvertebrate samples were collected, and the stomach contents of fish were non-lethally removed. During the initial visit to each site, a habitat assessment occurred. Three fifty-meter reaches were selected within each stream to have representative sites at low, medium, high, and full pool elevations. The downstream reach was as close to the confluence with the Skagit River or Ross Lake as possible. The most upstream end of the upstream reservoir reach was approximately 20 meters downstream from the full-pool shoreline. The remaining reach was located in the middle of these two reaches. The second sampling event at Roland and Dry Creeks required the lower reach to shift upstream as the reservoir had filled beyond the reach. For the final sampling event at all sites, both of the lower reaches were underwater. The most upstream reach was shortened, and an additional reach upstream of the full-pool level was added to compare reaches above full pool with those inundated during the summer months below full pool.

#### **Habitat Assessment**

Rapid habitat assessment was completed following U.S. Forest Service Stream Inventory Handbook for Region 6 on each of the streams during the first site visit (USFS, 2012). Locations of pools, riffles, large wood, sediment type, and other notable habitat structures were recorded in an effort to quantify quality of habitat for juvenile fish utilizing the system. I used <u>The Aquatic Habitat Assessment: Common Methods</u> (1999) and data collected from the Rapid Assessment to compare habitat conditions among the three sites.

#### **Bank Stability**

Bank stability was not quantified but was visually assessed below and above full-pool. Noting the stability of the streambank is important because it quantifies the erodibility, complexity, and available fish habitat, found in the form of undercut banks, along the creek. Stable banks have low erosion while unstable banks are highly erodible. Heterogeneous plant communities provide roots of various depths which aid in reducing erosion (Stevenson and Mills, 1999).

#### Substrate

By measuring substrate, it is possible to determine the channel roughness and associated complexity of the reach. Substrate can also be used to explain local influences on habitat quality based on land disturbances such as logging or mass wasting in the system (Bain, 1999). Dominant substrate and the second-most common substrate are used to describe variability within the system and compare to other systems. Bottom substrate was classified as a percentage of total reach and separated into the five following size classes:

<0.08 inches

>160 inches

,	
GR – Gravel	0.08 – 2.5 inches
CO – Cobble	2.5 – 10 inches
BO – Boulder	10.0 – 160 inches

SA – Sand, silt and clay

BR – Bedrock

#### Large Woody Debris

Large woody debris was counted at each site to quantify amount of cover and channel complexity for refuge from physical conditions such as high flow and sunlight or from predation. Additionally, many macroinvertebrates attach to large woody debris or decompose it, increasing the prey diversity and abundance

of the site (Bain, 1999). Large woody debris was counted and classified into the following size classes using USFWS Region 6 protocols:

Size Class	Diameter	At length
Small	>12 inches	25 feet from large end
Medium	>24 inches	50 feet from large end
Large	>36 inches	50 feet from large end
Rootwad	No definable trunk	

Wood was only counted if a portion was below the bankfull channel and met the size class requirements or had a length at least twice the bankfull width.

#### Canopy Coverage and Riparian Vegetation

Canopy coverage over the stream and riparian vegetation are important habitat factors for a variety of reasons including shade to reduce solar radiation and the associated increase in stream temperature, as a source for nutrient and organic matter and large woody debris, habitat for organisms that will eventually become fish food, and reduced scour during high flow events (Bain, 1999). Canopy coverage and riparian vegetation were subjectively noted in the lower reaches of the three streams because they were absent. The reach located above full pool was examined to determine general make-up of trees and shrubs and approximate amount of overhead coverage associated with intact vegetation.

#### **Benthic Macroinvertebrate Community Composition**

Benthic macroinvertebrates were collected from each reach to confirm their presence and ensuing potential food base for fish. Sampling of benthic macroinvertebrates followed a modified version of the Environmental Protection Agency's Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish (Barbour et al, 1999). Using a D-frame kick net with 500-μm

mesh I sampled three 2-ft² sections in each 50-m reach, in riffled, gravelly habitat. This resulted in a total of 6-ft² per reach and 18-ft² per stream per sampling event. This net size was selected to target organisms most useful in bioassessment metrics. A trowel was used to disturb sediment and substratum upstream of the kick net and to remove clinging invertebrates and suspend those in the sediment. The area was disturbed for approximately one minute after which the material captured in the kick-net was transferred to a bucket. The three individual samples were composited into a single sample representative of the reach. This sample was preliminarily processed in the field by removing large stones and organic material by hand sorting and straining through a 125-µm mesh sieve. Two samples at Dry Creek were sorted in the field while all remaining samples were strained in a 125-µm sieve, preserved in 70% formalin or 95% ethyl alcohol in Nasco WhirlPacs®, and transferred to the Fish Ecology Lab at Huxley College for identification and counting. The two field-sorted samples were not used in reach-level analysis due to the potential bias caused by loss of small organisms. All of Dry Creek (3/29/2013) was removed from site-level and month-level analyses to eliminate potential bias caused by comparing one reach to the other samples with three.

#### **Fish Collection**

Juvenile fish were collected from each reach to identify the species, size distributions, and relative abundances of fishes inhabiting each stream. I sampled each reach using a backpack electrofisher following American Fisheries Society and National Marine Fisheries Service Guidelines (NMFS, 2000; Reynolds and Kolz, 2012). All sampling at Roland Creek and Dry Creek and the June 21, 2013 sampling at Hozomeen Creek used an Appalachian Aquatics AA-24 backpack electrofisher while the other two samples in Hozomeen Creek used a Smith Root LR-20B electrofisher. A team of three walked the reach from the downstream end working upstream. The output of the electro-fisher was adjusted to ensure stunning of small fish. The voltage was adjusted to produce less than 8 amperes on the AA-24 and 3 to 6 amperes on the LR-20B. Captured fish from each reach recovered in a container of stream water until stomach contents were removed by gastric lavage and

they were released. Because Bull Trout is a federally protected species I took extra care to avoid loss of life by monitoring my backpack electrofisher settings and ensuring adequate recovery time before fish were released. Electrofishing effectiveness and capture success of stunned fish appeared to vary depending on stream size and discharge. In particular, my success rate at Hozomeen seemed adversely affected by the larger width, discharge, depth, and the occurrence of isolated pockets with low-conductivity groundwater upwellings. However, all of

my collections appeared to be representative of the kinds of fish that were present.

#### **Diet Collection**

Diets were evaluated based on analysis of stomach contents. Stomach contents were collected by using non-lethal gastric lavage on fish between 50 mm and 152 mm following protocols modified from Giles (1980), Strange and Kennedy (1981), and Hartleb and Moring (1995) (E. Morgan, University of Washington Wetland Ecosystem Team, personal communication). Fish smaller than 50 mm were not lavaged because they have been reported to have poor survival following lavage and those greater than 152 mm were precluded from being anesthetized because Tricaine Methanesulfonate (MS 222) has a 21-day withdrawal time and this size of fish could potentially be caught and eaten by an angler (Ross and Ross, 2008; AVMA, 2013).

All fish within the appropriate size range were placed in a container of stream water containing a moderate amount (~100 mg/L) of the anesthetic MS 222 for 30-60 seconds to make handling easier and reduce stressing of the fish (Ross and Ross, 2008). Anesthesia and euthanasia, when required, were reached following guidelines from the 2013 American Veterinary Medical Association and Ross and Ross (2008). During my sampling, measurement of total length (mm), weight (g), and identification of species were completed for each of the captured fish before release. Dolly Varden and Bull Trout were recorded as Native Char because they are indistinguishable in the field.

Stream water used for lavaging was strained through a 125-µm mesh sieve to reduce the possibility of accidently collecting suspended material that could later be confused as diet items. Based on the recommendations of the University of Washington's Wetland Ecosystem Team, a modified garden pump sprayer for larger fish, as described in Hartleb and Moring (1995), or a syringe of either 60 cc or 25 cc for smaller fish, depending on gape, was utilized to lavage. The lavage devices used brass tips for insertion into the stomach. I created the brass tips by cutting a hypodermic needle and securing a curved brass sleeve on the tip in an effort to mimic the natural path into the stomach and reduce injury. I made three sizes of tips in order to have the most appropriate size for gape. To collect stomach samples, fish were held in the hand with the head pointed down and towards the sieve while the brass tip was carefully inserted into the mouth to the depth of the stomach. The fish was slowly moved back and forth along the tip while water was gently squirted into its gut. Typically, stomach contents flowed out of the mouth and were caught in the 125-µm mesh sieve. When stomach contents appeared to be exhausted, the mouth was checked to ensure no items were lodged. If not, the fish was transferred to a container of stream water for recovery (approximately 3-5 minutes) and eventual release. All Brook Trout were euthanized following the recommendation of USPS staff on site. In one case a Native Char was fatally injured during capture and was euthanized using MS 222. Stomach contents were placed into Nasco WhirlPacs® and preserved with either 70% isopropyl alcohol or 90% ethyl alcohol. Samples were transported back to Western Washington University's Fish Ecology lab for later analysis.

#### **Laboratory Techniques**

All benthic macroinvertebrate and fish stomach samples that were collected in the field were analyzed in Huxley College of the Environment's Fish Ecology Lab between June and December, 2013. Prey items were identified to the lowest possible taxon; benthic macroinvertebrate samples were distinguished to family and diet samples generally to order or class due to digestion and mastication making higher level identification difficult. Items that I was able to identify as class Insecta but not to the order level were recorded as

Unidentified. Items that I was unable to identify even to class level were recorded as Unknown. Order, or lowest identifiable taxa if lower than order, was used in all diet analyses but lower taxa were utilized in discussion.

#### Benthic Macroinvertebrate Samples

Benthic samples were sorted to separate individuals from other material such as organic matter and sediment (Carter and Resh, 2001). I identified all individual specimens using an Olympus SZ51 dissecting scope; no subsampling occurred. I examined all samples twice to ensure that I removed all individuals collected in the field. All insects, which were the dominant benthic taxa, were identified to the Order or Family level using a variety of dichotomous keys (Pennak, 1978; Lehmkuhl, 1979; Voshell, 2002; Thorp and Rogers, 2011; Adams and Vaughan, 2003; Clapp, 2006; Edwards, 2008; Merritt et al, 2008). Other specimens collected during kick-net sampling were identified to lowest possible taxonomic level, generally class or subclass. Terrestrial organisms were recorded as such, snails and clams were identified to class, and leeches and worms were identified to subclass. Although family is ideally the highest taxonomic level used in analysis, this study was focused on fish diet and food availability so this level of taxonomic distinction was appropriate because diet items could not be consistently identified below order (Lenat and Resh, 2001; Marshall et al., 2006). Family-level identifications have been found to be suitable for detecting differences in these situations.

I sent examples of each family that I identified to Bob Wisseman at Aquatic Biology Associates Inc. for quality assurance purposes. Results indicated that I needed to re-evaluate my identifications within Trichoptera and Plecoptera. With assistance from my committee and the correctly identified samples from Bob Wisseman, I reexamined each individual Trichoptera and Plecoptera and corrected my identification if indicated. Members of other taxa were correctly identified the first time.

#### **Diet Samples**

The diet of each fish was identified to the lowest possible taxon, weighed, and counted. I estimated individual count of prey items by the number of heads as this body part is the least digestible so it persists in the stomach the longest. The diet of each fish had been preserved in either 95% ethanol or 70% isopropyl alcohol in the field and then transferred to 95% ethanol at the lab. The sensitivity of the balance along with the high rate of evaporation of ethanol required diet items to be soaked in tap water for 15 minutes and then blotted on tissue paper to remove excess water before weighing to the nearest 0.00001 g (Garvey and Chipps, 2012; Hyslop, 1980; Windell and Bowen, 1978). Without this step the scale would not equilibrate. Any diet items that did not register at 0.00001 g were recorded as 0.000009 g and used in analysis. The total wet mass and wet mass for each category of stomach contents for each fish were measured using a Mettler Toledo AB135-S analytical balance.

#### **Data Analysis**

Multivariate analyses were used to compare within and among group variation as determined by relative abundance and presence/absence of different taxa but also test the contribution of specific taxa or individuals to further explain differences. I used non-metric multidimensional scaling (MDS) and PRIMER v6.1 software to determine general similarity between points (Clarke and Gorely, 2006). The MDS uses Bray-Curtis to construct a plot and associated stress value of rank-order distances between samples. A stress value of less than 0.1 suggests representation of actual conditions. A stress greater than 0.3 suggests points are not related and could be arbitrarily placed.

For those results that had a less than 0.25 stress value, I utilized analysis of similarities (ANOSIM) to examine difference between abundance of available prey items, as identified from benthic macroinvertebrate sampling and captured prey items. ANOSIM is an available function in PRIMER v6.1 (Clark and Gorely, 2006) and is useful in determining whether a significant difference between two groups exists, analogous to a parametric t-test, but

more useful with unbalanced data such as mine. If ANOSIM resulted in a significant model (p≤0.05), I used the similarity percentage test (SIMPER) to breakdown the contribution of each species to the similarity, or dissimilarity. Both ANOSIM and SIMPER are multivariate analyses and are based on the Bray-Curtis measure of similarity which compares each sample in group 1 to each sample in group 2.

#### Benthic Macroinvertebrates Community Analysis

Mean density of macroinvertebrates was calculated based on number of individuals collected in a 2 ft<sup>2</sup> area per reach (8 ft<sup>2</sup> per stream). This was calculated at the order and family levels. I compared relative abundance of different taxa among sites, months, reaches, and pool elevations with ANOSIM and SIMPER. Bray-Curtis clustering was used to explore relationships among sites, pool elevations, and reaches.

#### **Diet Analysis and Description**

I quantified the diet of each fish to compare among sites, months, species of fish, and pool elevations. Following standard techniques for analyzing fish diets, I calculated percent by weight (%G, Equation 1) and percent by number (%N, Equation 2) for each diet item. I also used frequency of occurrence (%O, Equation 3) to characterize diet (Garvey and Chipps, 2012). Mean percent by weight was used to evaluate the importance of a given prey item at various pool elevations, locations, and within species of fish. Mean percent by number was calculated solely for inclusion in the index of relative importance (IRI, Equation 4). Frequency of occurrence was utilized to determine if frequency of a given prey item, or overall breadth of diet changed by location, fish species, or pool elevation. Frequency of occurrence on its own cannot explain importance to overall diet because regardless of how often a given taxa occurs in stomachs, it may represent a minimal percentage of the actual diet by number or weight as compared to other taxa (Garvey and Chipps, 2012). Index of relative importance, a metric which includes %O, %N, and %V, was used to evaluate the relationship of prey taxa while addressing the sensitivity of sampling error found in frequency of occurrence and prey size bias created in %O and %N. Index of relative importance provides a more accurate and balanced description of prey importance

(Pianka et al., 1971; Fraley and Shepard, 1989; Liao et al, 2001). Because it has been suggested the IRI be reported on a percent basis for each prey taxa so that comparisons among various food taxa are less difficult, I included this factor in my analyses (Equation 5; Liao et al, 2001; Merz, 2001; Ahlbeck et al, 2012). Percent IRI was calculated for various factors including site, month, species of fish, and pool elevation; those taxa that had an average % IRI greater than 5% for a given factor were considered "major taxa" and included in further analysis.

Equation 1: Mean percent by weight 
$$(\%G_i) = \frac{1}{p} \sum_{j=1}^p \left( \frac{W_{ij}}{\sum_{i=1}^Q W_{ij}} \right) \times 100$$

Equation 2: Mean percent by number (%
$$N_i$$
) =  $\frac{1}{p}\sum_{j=1}^p \left(\frac{N_{ij}}{\sum_{i=1}^Q N_{ij}}\right) \times 100$ 

Equation 3: Frequency of occurrence (%
$$O_i$$
) =  $\frac{J_i}{P} \times 100$ 

Equation 4: Index of relative importance 
$$(IRI_i) = (\%N_i + \%G_i)\%O_i$$

Equation 5: Percent Index of relative importance (%
$$IRI_i$$
) =  $\frac{100IRI_i}{\sum_{i=1}^n IRI_i}$ 

Where Q is the number of prey categories found, p is the total number of fish with a diet sample,  $W_{ij}$  and  $N_{ij}$  are the weight and the number of prey item j in i fish respectively, and  $J_i$  is the number of fish with prey i.

I also used a cluster analysis to analyze diet composition. The Bray-Curtis index of similarity assesses degrees of similarity among prey taxa. Diet composition analysis did not include individual fish with empty stomachs or incidental non-nutritional items such as cases associates with Trichoptera or debris.

Instantaneous Ration, a relative measure of feeding intensity used to compare stomach fullness among different sized fish, was calculated by taking the total wet weight of an individual's stomach contents divided by the total

weight of that fish. This allows for diet overlap as well as relative fullness and stomach content quantity comparisons (Olegario, 2006; Spilseth, 2008; Cordell et al, 2012). A one-way analysis of variance (ANOVA), with type III sum of squares to account for the unbalanced data, was completed on pool elevation, site, month, reservoir reaches, reservoir reaches compared to full-pool reaches, and species of fish to detect differences. To compare stream with month I used a two-way ANOVA with both factors fixed. Spearman's correlation coefficient is non-parametric and does not require the assumption of normality allowing it to be used to examine the relationship between the number of prey found in a fish stomach, the number of prey in the environment, and the fullness of the stomach.

Multi-dimensional scaling (MDS) used prey composition to compare relationships among a variety of factors.

This technique uses Bray-Curtis similarities to relate diet composition among factors such as site, date, and pool elevation. To assess the differences by factor ANOSIM and SIMPER were applied to the Bray-Curtis similarity matrix. Abundances were compared between the following factors: month, site, reach, fish species, pool elevation, reservoir/creek, and northern/southern relative location. Two-way analyses were completed for month and site. All analyses were performed using the PRIMER v6.1 statistical package (Clarke and Gorely, 2006).

#### **Results**

A total of sixty-five fish had stomach contents; thirty-six Rainbow Trout, eighteen Native Char, ten Brook Trout, and one Cutthroat Trout were collected.

#### Habitat

Riparian vegetation, canopy coverage, and bank stability were similar among the three sites while substrate, large woody debris, and overall length of habitat varied. Hozomeen Creek, the longest of the three creeks, was 1,807 meters in length at lowest pool. We began sampling 300 meters upstream of the confluence because the water was too deep and the flow too large to effectively sample at the confluence. Hozomeen Creek had a mean width of 9.56 meters and a mean depth of 0.65 meters. Dry Creek, the next longest at 610 meters in length, had a mean width of 4.79 meters and mean depth of 0.26 meters. Roland Creek, the shortest of the three, was 578 meters at low pool and had a mean width of 1.67 meters and mean depth of 0.20 meters.

In general the sediment size was much larger at Dry Creek than Hozomeen or Roland Creeks. Twenty-four percent of reaches at Dry Creek had boulders as the dominant sediment type and four percent as the subdominant type while no reaches in Hozomeen or Roland Creeks contained boulders (

Table 2). Roland Creek was mainly gravel and cobbles. Hozomeen Creek was predominantly sand and silt with only 2% of the dominant sediment in a class larger than gravel and often no subdominant size class was present.

Wood with a diameter of at least 12 inches at a length of 25 feet from the large end was assessed in all three creeks. Wood outside of the bankfull channel and the length and diameter requirements were not included. Hozomeen Creek, due to its large size and higher flow, was more difficult to assess than Roland or Dry Creeks. Wood at Hozomeen Creek was counted the first 1,198 meters downstream and resulted in 44 pieces of small wood (diameter between 12" and 24") and seven pieces of medium wood (diameter between 24" and 36"). In total, fourteen pieces of small wood and seven rootwads were counted at Dry Creek. No measurable wood was found within the bankfull channel of Roland Creek. Rootwads were present throughout the banks of all three creek channels and could become part of the active channel from year to year depending on channel migration, sediment loads in the creek, and the rate of lowering or raising reservoir.

There was no woody vegetation downstream of the full-pool level at any of the three creeks resulting in no assessable canopy coverage or riparian vegetation. A grassy mat grew at Hozomeen Creek during May and June; Roland and Dry Creeks remained bare. Although no systematic riparian assessment occurred upstream of full pool, the area consisted of generally intact, native riparian vegetation dominated by coniferous trees with an understory of ferns and native shrubs.

The banks at the lower reaches of all three creeks were unstable, meaning easily erodible, in comparison to the upstream (above full-pool) reaches. Bank stability can be affected by plant roots and hardened or rocky banks, which increase the erosion resistance of the bank (Stevenson and Mills, 1999). The lack of woody vegetation found below full-pool at the three creeks likely contributed to the instability found there.

## **Benthic Macroinvertebrates**

A total of 3,645 individuals in 31 families were collected and identified from the useable samples. Specimens included pupae, nymph, larvae, and adult forms of aquatic and terrestrial insect species.

Ephemeroptera was the most abundant and frequently occurring insect order across all samples, but not uniformly the most abundant (Figure 11). The overall mean density of Ephemeroptera was 188.13 individuals per sample, and they comprised 41.3% of the 3,645 total individuals (Table 3). Ephemeroptera was the most abundant order at Roland Creek and Dry Creek during all three sample periods, but only during the pool elevation of 1520' AMSL at Hozomeen Creek. Interestingly, although the geographic location of sample reaches at 1520' AMSL and 1561.3' AMSL were the same and the three dominant taxa across all events remained unchanged, the percent composition of macroinvertebrates changed. During the April sample, Ephemeroptera comprised 59% of the total sample, Oligochaeta 27%, and Diptera only 1%. The May sample shifted to Oligochaeta becoming the most dominant (35%), followed by Diptera (33%), and then Ephemeroptera (18%). By June, Diptera had become the overwhelmingly dominant taxa at 68%, Ephemeroptera dropped to 14%, and Oligochaeta to 8%. Oligochaeta was the most abundant macroinvertebrate in the lowest Dry Creek site on the second sampling (45% of total), the lowest reach during the first sample at Roland Creek (36%) and both samples in the second reach in Hozomeen Creek (1520' AMSL: 56%; 1561.3' AMSL: 85%). I did not identify Oligochaeta beyond the subclass level but I believe a majority of these worms were terrestrial, coming from the streambank as the channel migrated and expanded.

At the family level, Chironomidae (Order Diptera) was the most frequently occurring across all samples, contributing 25% of the collected individuals (Figure 12). Heptageniidae and Baetidae (Order Ephemeroptera) were tied as the second most commonly occurring family across all samples, and often were the most abundanct during specific sampling events (Table 3). Heptageniidae at Roland Creek in particular was the most abundant. These flat-head mayflies comprised 47% of the 888 total macroinvertebrates collected there. They

increased from 24% of the sample in March to 47% in May and finally peaked during the June sample when they were responsible for 66% of the total 229 specimens sampled. The most abundant family at Hozomeen Creek varied depending on the sampling month. Baetidae (Order Ephemeroptera; 39%) was dominant during the April sampling event and Chironomidae during the June sampling event (68%). This included taxa that I identified to the lowest possible level, even if that level was higher than family. In May the class of Oligochaeta was most common (35%); the most frequently occurring family in May was Chironomidae (Order Diptera; 32%). Baetidae was the most abundant family at Dry Creek across all sample periods comprising 22% of the total of the 907 collected individuals. Baetidae was the most abundant family in March at 25% of the 408 collected individuals. In May Chironomidae was the most abundant family at 31%. Abundance shifted to Heptageniidae in June with 26% of the collected individuals. Terrestrial organisms were the least common across all samples with a density of 2.13 individuals/sample. Non-insects were generally identified to class and were included in the Order level analysis.

Abundance of families by date differed (ANOSIM, p=0.006, Global R: 0.311, Table 4). Dry Creek was not included in the March samples because they were field sorted rather than sorted in the lab, likely insufficiently representing the actual abundance of benthos found at the time. Pair-wise comparisons show the 3/29/2013 (1513.6' AMSL) and the 5/5/2013 (1521.3' AMSL) samples to be different (ANOSIM, p=0.036, R: 0.477) because Baetidae, Heptageniidae, and Chironomidae were in greater abundance during the 5/5/2013 sample than the 3/29/2013 sample (SIMPER, mean dissimilarity: 80.8; Table 5). The 5/5/2013 sampling and the 6/2/2013 (1572.3' AMSL) sampling at Dry and Roland Creeks differed (ANOSIM, p=0.032, R: 0.438) due to increased abundance of Baetidae, Chironomidae, and Oligochaeta during the 5/5/2013 sampling (SIMPER, mean dissimilarity: 68.0; Table 5). The 5/19/2013 (1561.3' AMSL) sample at Hozomeen Creek was different from the 6/2/2013 sampling at Dry and Roland (ANOSIM, p=0.029, R: 0.685) due to the increased abundance of

Oligochaeta and Chironomidae during the 5/19/2013 event and the increased abundance of Heptageniidae on 6/2/2013 (SIMPER, mean dissimilarity: 76.3; Table 5).

Family abundance differed by sample month (ANOSIM, p=0.01, Global R: 0.248). Pair-wise comparisons show March and March and May (p=0.024, R: 0.53) and May and June (p=0.048, R: 0.222) to be the only months different from one another. one another. The difference between March and May is further explained by the increased abundance of all families except families except for Taeniopterygidae and unidentified insects during the May sample however the ecological importance of importance of this comparison is limited because the March sample was made up solely of Roland Creek while the May and the May and June contained all three sites (SIMPER, average dissimilarity = 81.72;

sites (SIMPER, average dissimilarity = 71.33;	
	20
	29

Table 6). Greater abundance of Chironomidae and Heptageniidae in June accounted for 47% of the difference between the

Table 6). May had a greater abundance of Oligochaeta and Baetidae contributing 27% of the difference between the sites. Chironomidae, Heptageniidae, Oligochaeta, and Baetidae account for over 75% of the difference between the two months.

Ross Lake sites did not differ by benthos at the family level (ANOSIM, p=0.052, Global R: 0.144; Figure 13), however as anticipated, each of the creek samples generally clustered within one another, demonstrating that a change in location can cause alterations, albeit sometimes minimal, on the composition and abundance of available prey. Dry and Roland Creeks appear more similar to one another than to Hozomeen Creek when clustering by presence/absence at the family level (Figure 14). This is further explained using SIMPER (Table 7). Roland and Hozomeen Creeks were the most dissimilar with a mean dissimilarity of 76.63. This was mainly explained by the abundance of Heptageniidae at Roland Creek and the Chironomidae and Oligochaeta at Hozomeen Creek. Roland and Dry Creeks had a mean dissimilarity of 72.17 again due to the increased abundance of Heptageniidae at Roland Creek and Chironomidae at Dry Creeks. This compared to a dissimilarity of 71.16 between Dry and Hozomeen Creeks. Chironomidae, Oligochaeta, and Baetidae were all much more abundant at Hozomeen Creek than Dry Creeks.

The reach, independent of the stream, did not affect the abundance of benthos (ANOSIM, Global R: 0.154, p=0.084). The ecological importance of this comparison is limited because the reaches were sampled at different frequencies; therefore no further analysis was conducted. The lowest reach (Reach 1) was underwater during the second sampling at Roland and Dry Creeks so only one sample was obtained. During the second visit, a newly established low reach (Reach 1.5) was sampled at those creeks, allowing for only one sample at that location as well. The lower reach (Reach 1) at Hozomeen Creek and the middle reach (Reach 2) at all three sites were able to be sampled during the first two visits but were underwater during the last sampling event. The upper reach (Reach 3) located below full pool was sampled at all three visits at each site. The above pool reach (Reach 4) was sampled only on the last visit to each site. Using a Bray-Curtis cluster it was possible to determine

that reaches did not cluster together and the low reach at the second sample was not closely related to reach 1 or 2 at either site (Figure 15).

Hozomeen Creek, located in the northern part of Ross Lake, had a different composition of benthos than Dry and Roland Creeks, located in the southern part of Ross Lake (ANOSIM, p=0.031, R: 0.199). Hozomeen Creek had a greater composition of Chironomidae, Baetidae, and Oligochaeta while Dry and Roland Creeks had more Heptageniidae (SIMPER, mean dissimilarity: 71.61). Hozomeen Creek is a much larger system than either Dry or Roland Creeks, with more exposed creek bottom for a longer period of time and much smaller sediments. This likely contributes greatly to the different composition found there.

Although the streams by pool elevation are not different from one another (ANOSIM, Creek within pool elevation; Global R: 0.51, p= 0.05) their examination is interesting (Figure 16). When the pool elevation was the lowest, Roland Creek clustered most closely to the highest pool elevation at Dry Creek. As expected, the two Hozomeen Creek samples at the low and middle pool elevations clustered closely together. The middle Dry Creek sample clustered with the three Hozomeen Creek samples, rather than the other Dry Creek sample or Roland Creek samples.

## <u>Diet</u>

Sixty-five of the seventy-three fish collected had something in their stomach (89% off all fish). Juvenile salmonids ranged from 41 mm to 238 mm in total length, weighed between 0.50 g and 124.30 g and consumed prey from a variety of taxonomic groups. These included insects, snails, clams, leeches, amphipods, and various worms, segmented and not. A total of fourteen Orders were identified. Many of the organisms were too masticated or digested to identify beyond Order, but it was possible to identify some Chironomidae (Order Diptera), Simullidae (Sub-class Collembola), Sminthuridae (Order Symphypleona), Blephariceridae (Order Diptera), and a variety of families within Trichoptera, Ephemeroptera, and Plecoptera. Including those taxa that

were identifiable only to terrestrial origin or class Insecta and those unrecognizable even at the class level, there were sixteen categories for analysis.

Diptera and Ephemeroptera were the most commonly collected diet items. Diptera was the most frequently occurring diet item and the most important numerically at the order level across all samples. Ephemeroptera was the most abundant by percent by weight across all samples and Diptera was the second. Unidentified insects, those diet items unidentifiable even to the class level, were the second most abundant diet item found. This highlights the level of mastication and digestion found in many of the samples. Amphipods were abundant in the lower two reaches of all sites, but absent from the upper reach in early spring in both the benthic samples and diet. Amphipods were absent from both diet and benthic samples in late spring when the lower reaches were inundated. Plecoptera in the diet were found in low numbers at Hozomeen Creek, with none collected during the April sample there, compared to Roland and Dry Creeks.

Although it was not possible to identify all prey taxa to family, I did find a variety of identifiable taxa in stomachs that were not sampled in the kick-nets. For example, two Blephariceridae were found fully intact in a single fish stomach, and numerous Sminthuridae were found in fish stomachs from all sites. Blephariceridae are strong clingers and are unlikely to be dislodged easily during kick-net sampling. Sminthuridae are very small organisms that are terrestrially dominated and unlikely to be found in benthos. Items categorized as incidental included cases of Trichoptera and other debris. These items were weighed but where not used in analysis except for graphical representation or frequency of occurrence.

Importance of prey species to diet differed between sites (% IRI; ANOSIM, p=0.001; R: 0.327), dates (%IRI; ANOSIM, p=0.001; R: 0.357), and months (% IRI; ANOSIM, p=0.005; R: 0.136) but above-pool reaches compared to below-pool reaches did not differ (% IRI; ANOSIM, p=0.478, R: -0.001).

A summary of results from percent by number, percent by weight, and frequency of occurrence follow. Percent index of relative importance was used to test differences between metrics.

# Percent by Number

Percent of diet by number was calculated by order for each of the sixty-five fish for all prey items, excluding Trichoptera cases and other incidental items (Table 8). Numerically, Diptera was the most common food item across all sample dates composing 31.38% of the total number of food items found in all stomachs. Ephemeroptera was the second most abundant food item by percent by number composing 25.66% of the total food items. This is in contrast to the benthic abundance where Ephemeroptera were most abundant and Diptera second.

Percent by number was calculated for use in determining index of relative importance and comparing instantaneous ration. Percent by number is not believed to accurately reflect the significance of each food item yet in all but four individuals the most abundant food item as calculated by percent by number was also the most abundant food item as calculated by percent by weight (Table 8). Incidental items were always recorded as zero because it was unrealistic to determine how the incidental items were collected and to accurately count them; one Trichoptera case could equate to numerous particles making it appear that incidentals were the most abundant stomach item by percent by number, when in reality they were incidentally collected or a by-product of feeding.

# Percent by Weight

At the order level, Ephemeroptera was the most abundant food item by percent by weight of all samples, composing 25.2% of the total weight of all stomach contents (Table 8). Diptera was the second most important food item gravimetrically composing 21.6%. This reflects the findings of the benthic abundance sampling but differs from percent by number findings. Percent of diet by weight was calculated for the order (or

origin in the case of terrestrial insects) of each of the sixty-five fish for all prey items, excluding whole

Trichoptera cases and incidental items because it was not possible to differentiate between gravel arbitrarily

collected and that from a masticated casing. I do acknowledge that using wet weight after prey items have been

preserved and comparing it to "fresh" weight of the fish can result in errors, however Hyslop (1980) argues if the

practice is held constant throughout the study it is acceptable.

Gravimetrically, the most important diet by site varied (Table 8). Ephemeroptera was the most important taxa gravimetrically at Roland Creek composing 74.1% of the total weight of stomach contents collected at the site. Diptera composed 38.54% of the stomach content mass collected at Dry Creek. Hozomeen Creek had more amphipods by percent by weight (27.7%) than any other taxa, even though amphipods were not present in the upper reaches or during the June sample.

Ephermeroptera was the most gravimetrically important diet item during the May (32.1%) and June (33.6%) samples but not March or April (Table 8). The April event was dominated by amphipod mass at 43.4% of the total sample. During the March sample at Dry Creek amphipods only accounted for 10.1% of the total weight, in May amphipods were the second most important prey item gravimetrically (17.6%) but by June, when the lower reaches were inundated, they had disappeared from the diet completely. This emphasis is based almost solely on Hozomeen Creek; by May Dry Creek had no amphipods and percent by weight accounted for only 0.005% in Roland Creek. Comparatively amphipods accounted for 44.9% in Hozomeen during the month of May.

Ephemeroptera was the most commonly consumed diet item by weight in Rainbow Trout and Brook Trout and was the second most common by weight in Cutthroat Trout (Table 8). The most consumed diet item in Cutthroat Trout were unidentifiable insects with 32.0% of the total mass. Native Char were the only species in which Diptera (24.4%) was the most consumed diet item by weight but they were the second most consumed in Rainbow Trout at 24.6%. The lone Cutthroat Trout consumed 12.5% Diptera by weight. Of all Brook Trout

collected, Diptera only accounted for 6.7% of the total diet mass while Unidentified Insects (16.9%) and Plecoptera (12.2%) were more common.

Gravimetrically, Trichoptera was the most consumed diet item in only one sample (Table 8). Trichoptera composed 14.7% of the total diet items by weight during the June 21 sample when the pool elevation was at 1588.9 AMSL. Aside from this one variation, Ephemeroptera, Diptera, and Amphipoda were the most common by weight. Ephemeroptera was most common in the May 5<sup>th</sup> sample (43.3%) when the pool elevation was at 1521.3 AMSL and the June 2<sup>nd</sup> sample (45.0%) when the pool elevation was at 1572.3 AMSL. Diptera was the main diet item by weight during the first sample on March 29<sup>th</sup> when the pool elevation was at 1516.3 AMSL (63.2%). Diptera composed more of this sample by weight than any other taxa during any other sampling event. During the May 19 sample, when pool elevations were at 1561.3 AMSL, amphipods were again the most common at 44.9%.

# Frequency of occurrence

Diptera are the most frequently occurring forage for the juvenile trout and char in Ross Lake across all pooled samples (Table 8; Figure 17 and Figure 18). Seventy-five percent (75.4%) of stomachs contained Diptera as pupa, larvae, or both. Insects that were unidentifiable to any taxa level were the second most occurring stomach item occurring in 55.38% of all samples. Ephemeroptera was the third most frequently occurring item at 52.31%.

Diptera was the most frequently occurring prey item across all months (March: 100%; April: 66.7% [along with Ephemeroptera]; June: 76.92%) and most dates (3/29: 100%; 4/11: 66.7% [along with Amphipoda]; 5/19: 66.7%; 6/2: 82.4%; 6/21: 66.7%), sites (Dry: 89.29%; Hozomeen: 66.7%), and species (Native Char: 66.7%; Rainbow Trout: 86.11%; Cutthroat: 100%) (Table 8). Ephemeroptera was the most common forage in fish from Roland Creek (100%), on 5/5 at Dry Creek (85.71%), and in Brook Trout

(80.0%). Roland Creek was the only site to have a frequency of occurrence equal to 100% for any taxa across all samples (Figure 18). Ephemeroptera was present in every fish collected from Roland Creek.

Frequency of occurrence also shows the decline in Amphipoda abundance over time (Figure 18). Although Amphipoda was collected in the diets during the earlier sampling events, by June it was not consumed even once.

# *Index of Relative Importance*

The Index of Relative Importance allowed for ranking importance of prey by pool elevation, site, month, and species of fish and was the primary focus of this study. To emphasize the most important taxa overall, I identified major prey taxa as those with an average % IRI ≥5% in any factor and only considered these taxa in my analysis. It could be that a particular prey item was important to an individual fish, but to be considered major taxa the prey item had to retain importance for the given factor (month, date, species, etc.) Seven of seventeen taxa were considered major in at least one instance: Amphipoda, Diptera, Ephemeroptera, Plecoptera, Terrestrial Insects, Trichoptera, and Unidentified Insects. Sixty-three of the sixty-five fish sampled contained these major taxa. The two fish that did not contain any major taxa were removed from this analysis.

Distribution of % IRI differs across all major prey taxa (Kruskal-Wallis; p=0.0001, alpha=0.05). The % IRI was highest for Diptera (41.34%) followed by Ephemeroptera (27.53%) and Unidentified Insects (11.03%). Diptera was always an important taxon, exceeding 10% importance for all months. Diptera, Ephemeroptera, Terrestrial organisms, and unidentifiable organisms were major prey items in all four species of fish sampled (Table 9 and Table 10).

The most important prey items did not differ among fish species (% IRI, ANOSIM, p=0.689, Global R: -0.029; (Table 9 and Table 10; Figure 19). Diptera was the most important prey item for Native Char and Rainbow Trout, constituting nearly half of the diet. Ephemeroptera was the most important prey item for Brook Trout and the

single Cutthroat Trout sampled, representing about a third of each of their diets. Native Char had three prey taxa with %IRI greater than 5%, Rainbow Trout had four taxa, Brook Trout had five taxa, and the Cutthroat Trout had six taxa. This suggests that the introduced species of fishes are eating more of the important prey items than the native fishes.

Importance of major prey items was different at all three sites and cluster together using MDS (ANOSIM, p=0.001, Global R: 0.327; Figure 20 and Figure 21; Table 11 and Table 12). Dry Creek was different from Hozomeen Creek (p=0.001, R: 0.146) due to a higher importance of Diptera in the diets at Dry Creek and Amphipoda at Hozomeen Creek. Those two taxa contributed over 50% of the difference between the two sites (SIMPER, avg. dissimilarity: 74.83). Dry Creek differed from Roland Creek (p=0.001, R: 0.629) because juveniles at Roland Creek placed much more importance on Ephemeroptera than those at Dry Creek. Diptera were more important to juveniles at Dry Creek than Roland Creek. Ephemeroptera, Diptera, and the increased importance of unidentified insects at Dry Creek accounted for over 90% of the difference between the two sites (SIMPER, avg. dissimilarity: 82.97). Hozomeen and Roland Creeks also differed (p=0.001, R: 0.32). Ephemeroptera was much more important at Roland Creek than Hozomeen Creek while Diptera and Amphipoda were more important at Hozomeen Creek; the three taxa accounting for over 90% of the differences (SIMPER, avg. dissimilarity: 87.29). Ephemeroptera was the only taxa that exceeded 5% importance at Roland Creek (87.52%, Figure 21). Diptera was the second-most important at 4.48%. The % IRI at Dry Creek was highest for Diptera (61.12%) with unidentified insecta (11.08%) the second most important prey item. The prey items at Hozomeen Creek were closer in importance with Diptera the most important (31.65%) and Amphipoda the second (25.24%). In all cases Diptera accounted for differences in comparisons with Dry Creek while Ephemeroptera accounted for differences between Roland Creek and other sites, and Amphipoda explained differences with Hozomeen Creek.

Importance of prey differed by month (% IRI; ANOSIM, p=0.001, Global R: 0.217; Table 13 and Table 14; Figure 22). March and May (p=0.002, R: 0.265), March and June (p=0.001, R: 0.348), March and April (p=0.001, R: 0.594), May and April (p=0.011, R: 0.311), and June and April (p=0.002, R: 0.479) were different from one another. March had three taxa which exceeded 5% importance while April, May, and June each had five. June and April had the greatest dissimilarity of prey importance (IRI; SIMPER, avg. dissimilarity: 89.82; Table 15). This was due to the lack of Amphipods in the June samples, the importance of unidentified insects in the April samples, and the low importance of Ephemeroptera in the April sampling. Those two taxa accounted for over 55% of the difference between the two months. May and April were the second most dissimilar (IRI; SIMPER, avg. dissimilarity: 89.11; Table 15). Over 50% of the difference was accounted for in Amphipoda and unidentified insects. Both Amphipoda and unidentified insects were more abundant during the April sampling. May and June were the only two months that did not differ (p=0.191, R: 0.021). In all cases Diptera was responsible for differences during comparisons with March samples, Amphipoda during comparisions with April samples, and Ephemeroptera during comparisons with May samples. No taxa uniformly explained differences between June and other months.

Diet selection differed in all but two pair-wise tests on pool elevations (ANOSIM: p=0.001, Global R: 0.362; Table 16, and Table 17; Figure 23). I pooled dates to look for any effects related to the elevation of the reservoir. As the reservoir fills, the available stream habitat decreases. I expected to see the % IRI differ between pool elevations as fish had to become more opportunistic due to less available food and more competition. The sampling at Dry and Roland Creeks on May 5<sup>th</sup> at a pool elevation of 1521.3' compared to the June 2<sup>nd</sup> pool elevation of 1572.3' sample at the same locations and the 1520.0' pool elevation on April 11<sup>th</sup> at Hozomeen Creek compared to the May 19<sup>th</sup> Hozomeen Creek sample (1561.3) were the only two samples not statistically different from one another. The Hozomeen Creek sampling events would have been expected to be similar because the reservoir had not changed enough to alter the Hozomeen Creek sampling site in any way. I had

predicted the May and June samples at Dry and Roland to differ because almost the entire available stream habitat below full-pool had shifted to lacustrine by June.

Seasonality at Ross Lake affected prey importance as well (% IRI, ANOSIM; Global R: 0.352, p=0.001). Diptera and Amphipoda were more important prey items during the early season. This is as expected since Amphipods were not collected during any June samples and the importance of Diptera dropped from 43.2% during the early period to 23% in the late samples. Both of these species prefer slower moving water and were likely flushed out of the higher gradient reaches during the later sampling.

Reaches within the reservoir compared to those above did not differ (ANOSIM; Global R: -0.133, p-value=0.126). The habitat, substrate, and canopy cover above full pool visually differed from the lower reaches but neither benthic composition (ANOSIM; Global R: -0.13, p=0.752) or prey importance did.

# **Instantaneous Ration**

Stomach fullness, calculated by instantaneous ration (IR), was correlated to mean number of benthic macroinvertebrates collected (Spearman's 2-tailed; α=0.01: r=0.544; p<0.001, n=65) but did not follow the number of prey found in individual fish stomachs (Figure 24 and Figure 25). Brook Trout had the highest IR and the most items in their stomach. Native Char had the fewest items in their stomach by count, but the second highest IR echoing the trends at Hozomeen Creek where they had eaten fewer items weighing more and likely providing more caloric gains. As expected, stomach fullness followed benthos abundance trends with an increase from March to April and then a decrease during subsequent months (Figure 26, Figure 27, and Figure 28). Mean stomach fullness during different months did not follow trends for prey abundance in the diet (Figure 28 and Figure 29). Though not statistically significant (ANOVA, log transformed, p=0.749), April had the highest IR of all months sampled, directly related to the abundance of amphipods, while March had the lowest. In comparison, number of prey in the diet was highest in March, decreased in April, increased in May, and

decreased again in June. This high abundance of amphipods in April also explain some of why Brook Trout had the highest IR of all species of fish.

Stomach fullness was log transformed to meet assumptions of homogeneity of variances. It did not differ among sites (ANOVA, p=0.628), species (ANOVA, p=0.073), pool elevations (ANOVA, p=0.877), or reaches above full pool compared to those below full pool elevation (ANOVA, p=0.292). The instantaneous ration between reaches was unable to be transformed to meet ANOVA assumptions so analysis was unable to be completed. Stomach fullness again followed available benthos trends where Hozomeen Creek had the greatest number of prey items per fish stomach and Dry Creek had the smallest (Figure 30 and Figure 31). Trends for stomach fullness deviated greatly from the mean number of prey items per stomach at each site (Figure 31 and Figure 32). Hozomeen Creek had the least number of prey items per stomach than either of the two creeks. This means that fish captured at Hozomeen Creek had fewer organisms by number in their stomachs then those at Roland Creek, which had the most, but that those prey items accounted for more of the fish's overall weight, signifying a fuller stomach. I believe that this is intensified by the increased abundance of Amphipoda at Hozomeen Creek.

Although the instantaneous ration for species did not have differ, Brook Trout had the fullest stomachs in April and decreased feeding intensity over time while native Char increased and Rainbow Trout maintained feeding rates. This is likely due to the high abundance of Gammaridae (order Amphipoda) for Brook Trout to feed on during the April sample when the %IRI was over 50%, driving the April sampling instantaneous ration to high levels (Figure 24).

Instantaneous ration did not differ by site and month but some trends were present (ANOVA, fourth-root transformed, p=0.236,Figure 33). Hozomeen had the highest overall IR in April and decreased overtime. This decrease was the most dramatic across all sites because the IR was again the highest at Hozomeen in April, but had the largest variability, and was then the lowest of all samples in June. Dry Creek had the opposite trend with

the lowest IR occurring during the March sample and then a slight increase during subsequent months. Fish at Dry Creek had the fullest stomachs in June compared to other sites. Roland Creek, with only two samples, decreased from May to June.

## **Graphical Analysis of Stomach Contents**

Diet variability at each site, as determined in this study, is further supported by using the Costello (1990) graph, as modified in Amundensun et al (1996; Figure 34). Costello graphs have been used to two-dimensionally demonstrate prey importance and the feeding strategy of the predator, be it generalist or specialist. Amundensun et al (1996) modified the original Costello graph to include prey-specific abundance creating a visual representation of prey importance, feeding strategy, and niche width. A literature search shows Costello graphs, and those modified by Amundensun et al (1996), have been used to evaluate stomach content data of fish, including trout and char, throughout the world. By graphically representing feeding strategies, it is possible to see that predation on Amphipoda, although still rare taxa, were more selected for at Hozomeen Creek than Dry or Roland Creeks (Figure 35). The Costello graph illustrates that Diptera had a higher within phenotype component (WPC) than other prey taxon at Dry Creek. A high WPC indicates that Diptera are only occasionally consumed, but are eaten by many individual fish (Amundensen et al, 1996). This reinforces the results of the index of relative importance, which highlights Diptera as an important diet item, and also shows the benefits of graphically analyzing the data. The modified Costello (1990) graph allows determination of what makes it an important item; in this case it is consumed by numerous fish (Costello, 1990 cited by Amundensun et al, 1996). The differences in diet preference among months can also be visualized in the Costello graphs, supporting the index of relative importance results (Figure 36). Most of the prey items were generalized for and had no preferred niches, meaning fish did not actively seek out a given taxon and not all species of fish ate that prey item. The April Hozomeen Creek sample was unique because no Plecoptera or Trichoptera were present in any diets and Amphipoda was present in high numbers but because I did not sample each site during the same time

period, it is difficult to discern if the differences are from natural changes on the seasonal level or driven by changes in the pool elevation. Hirudinea and terrestrial organisms were more abundant in fish diets than the environment.

Based on trophic interactions, prey in the benthic community is expected to have a greater mean abundance than consumed abundance of the same taxon, and consumption of the more abundant forage is anticipated. Viewing the relative benthic abundance and diet abundance together shows that in most cases, juvenile trout are eating the abundant prey items. In all but two cases in the major taxa, the total abundance of benthic fauna exceeded taxa found in the diet (Figure 37). Terrestrial organisms were more abundant in fish diets than the environment. Terrestrial organisms are eaten while drifting and only organisms that have entered the channel drift would be in benthic samples, so it is expected that these would not be collected. Hirudinea had a much greater abundance in the diet than benthic sampling, which suggests it is either rare in the environment or selected for. Hirundinea did have an IRI of 10.7% during the April sampling at Hozomeen, but was not collected during any other samplings so was not considered a major taxa and was therefore not included in any further analysis.

Bray-Curtis hierarchical clustering using presence/absence of prey taxa at the Order level separates diet and benthos by the site level as well month and pool elevation (Figure 38, Figure 39, and Figure 40). This clustering shows taxa found in benthic samples are more similar to one another than to diets except in the case of April sampling (Figure 39). The April diet samples clustered more loosely with the benthos samples.

#### Discussion

Numerous studies have focused on dams and their effects both upstream and downstream, and a few studies have looked at daily fluctuations of reservoir levels associated with dams, but to the best of my knowledge no studies have examined effects related to inundation on the seasonal scale (Hall, 1971; Baxter, 1977; Stanton, 1977; Johnston, 1989; SCL, 1989; Brondi, 2006). Similar to the findings of others, juvenile trout and char diets were composed almost entirely of aquatic insects (Nakano et al, 1998; Gunkel et al, 2002; Wydoski and Whitney, 2003; Baxter et al, 2004; Quinn, 2005). The benthic macroinvertebrates found in the tributaries to Ross Lake reflect those commonly found in Pacific Northwest streams, and all four species of juvenile trout and char found in Ross Lake are known to eat the benthic macroinvertebrates that I found both in their environments and in their diets (Adams and Vaughn, 2003; Clapp, 2006; Edwards, 2008; and Cordell et al, 2012). Contrary to my original hypothesis that the seasonally inundated streams would be devoid of benthos and not suitable for the rearing of juvenile fish, my study suggests that the seasonally inundated reaches of streams in Ross Lake provide adequate habitat and forage for rearing and refuge for four species of trout and char. Additionally, composition of benthos and diet can differ with varying pool elevations, suggesting management of the reservoir could impact juvenile fish and forage. The benthic composition appears to be similar across sites, however the composition of prey consumed differed among sites. Neither benthic composition nor diet contents appear to be affected by reach location.

A survey of Ross Lake tributaries at low pool was concluded by Seattle City Light in 1989 to determine how seasonal fluctuations in lake level impact available spawning habitat for Rainbow Trout (SCL, 1989). This included Dry Creek, Roland Creek, and Hozomeen Creek. Seattle City Light postulated that the construction of Ross Dam inundated nearly all of the adequate spawning habitat for trout because these creeks above full pool have excessive gradient and inappropriate substrate. The presence of juvenile fish in my study indicates successful rearing in these streams. This suggests that adults are spawning in the upstream reaches and

juveniles are migrating down, or the juveniles are moving among streams via the lake and using non-natal streams for rearing. Because there are thriving populations of adults in the lake that present threats of predation, I believe that most of the juveniles rearing in a given stream emerged upstream and migrated down.

Based on photographic evidence and field recordings from SCL's assessment, Dry Creek appeared to have more active wood in the channel below full-pool, with two documented wood structures. These were determined to be no barrier to adults, but one total barrier to fish was present at elevation 1594' AMSL, 8' below full pool elevation (Figure 41; SCL, 1989). These wood structures and the barrier must have since washed out or been removed because there were no barriers or notable accumulations of wood during my study. Among the three creeks I sampled, juvenile trout and char appeared to most successfully rear below full pool in Dry Creek.

In the 24 years between the SCL assessment and my study, minor changes were evident in some habitat features. In Hozomeen Creek the results of my habitat survey of Hozomeen Creek below full pool appear to be very similar to SCL's 1989 assessment. Areas of large pools with large, medium, and small woody debris and silty sediments were present then as they are now. Two drops, determined not to be barriers to fish passage, were documented in 1989 that were not present during my 2013 site visits (Figure 42). Seattle City Light found that the alluvial fan at the mouth of the creek historically provided much of the spawning gravel in Hozomeen Creek, and the reservoir formed by Ross Dam greatly depleted spawning potential of this tributary.

Photographic documentation of Roland Creek suggests the substrate was composed of fewer fines in 1989 than 2013, with a similar lack of large woody debris in the channel and numerous stumps on the easily erodible banks (Ross Dam Construction Photographs, 1938-1948; SCL, 1989). Seattle City Light (1989) did not document any passage barriers during their survey and my findings agree with this (Figure 43). Available spawning sediment in Roland Creek was documented to be minimally affected by construction of the dam (SCL, 1989). However, based

on my fish collections potential rearing habitat in Roland Creek seemed to be less preferred than at the other sites sampled.

Juvenile trout and char require sufficient food supply for successful rearing and seasonal inundation of the streams could influence this. My study suggests adequate food, in the form of benthic macroinvertebrates, is present based on the presence of few fish with empty stomachs in the system. Stanton (1977) found that most aquatic insects were able to re-colonize habitat above a dam that alternated between lotic, lentic, and dry conditions in two to three weeks. Time available for re-colonization when the lotic habitat was available was the single most important factor in benthic composition near a fluctuating reservoir. Re-colonization by invertebrates can occur from downstream drift, upstream movement, random movement along and across the bottom, aerial dispersal (oviposition), or persistence in both environments (migration from within sediments; Lyman, 1955; Waters, 1965; Williams and Hynes, 1976; Lancaster, 1990; Williams and Williams, 1993; Winterbourn and Crowe, 2001; Jähnig et al, 2009; Anderson and Ferrington, 2013). Recolonization by downstream drift has been shown to be an important source of colonizers, providing for over 40% of new organisms to an area (Williams and Hynes, 1976). The yearly maximum for downstream drift density is often reached during winter high flows (White and Harvey, 2007). The lake level in Ross Lake decreases throughout the winter months, allowing for increased downstream drift facilitated by winter rains into the newly exposed stream segments. Oviposition from aerial migration can provide for almost 30% during the spring and autumn months in temperate regions, while upstream migration and movement from within the substrate have accounted for approximately 18.5% (Williams and Hynes, 1976). Downstream drift can be both active and passive and has been documented in such high densities that past research focused on how populations were able to maintain a source population in headwater streams in the face of it (Hynes, 1970; Townsend and Hildrew, 1976; Williams and Hynes, 1976; Williams and Williams, 1993). Behavioral drift, or active drift, driven

by organism characteristics and behaviors, is responsible for upstream migrations as well as downstream drift (Waters, 1965; Townsend and Hildrew, 1976; Walton Jr., 1980).

Environmental educators at Hope Mountain Center for Outdoor Learning, Chilliwack, British Columbia, have been collecting micro- and macroinvertebrate samples in the Skagit River and tributaries upstream of Ross Lake since 2010 (S. Denkers, Hope Mountain Centre for Outdoor Learning, personal communication). Similar to the Hope Mountain Center's 2013 findings, Ephemeroptera abundance was the greatest of all orders collected in my study. Hope Mountain Center found Ephemeroptera to be the most abundant benthic macroinvertebrate overall and also the most abundant in each of the upstream tributaries sampled by them, accounting for 48% of the total organisms collected (Doix, 2013). Baetidae (order Ephemeroptera) and Heptageniidae (order Ephemeroptera) were the two most abundant families across all sites in their study. In contrast to their findings, I found more Chironomidae (order Diptera) than other benthic macroinvertebrates, but Baetidae and Heptageniidae were the second and third most abundant, contributing to the similar order-level total of 41% Ephemeroptera.

Baxter (1977) found Chironomidae to be the first species to colonize following the installation of a dam, and my findings support that expectation. Davies (1976) reports Chironomidae to be the first to colonize new areas as well as successfully oviposit in lakes and along shorelines. Turner (2009) found Chironomidae to be the most abundant taxon in alpine lake outlets in the North Cascades. Numerous studies outside the park have found Chironomidae to be broadly dispersed in a variety of life stages throughout freshwater environments, including deep lakes and mountain streams. Of the over 15,000 known species, overall tolerance to environmental factors such as temperature, dissolved oxygen, and flow are documented to range from very sensitive to very tolerant, further ensuring their presence in Ross Lake tributaries (Merritt et al, 2008; McCafferty, 1998; Buffagni and Comin, 2000; Petts, 2000; Spilseth, 2008). Hope Mountain Center's 2013 sampling occurred in tributaries upstream of Ross Lake and they collected 4% Chironomidae compared to the 25% in my samples (Doix, 2013).

Buffagni and Comin (2000) found that although Chironomidae are widely distributed in fresh water, the relative abundance is greater in pool habitats compared to bedrock, riffle, and transitional zones so it may be that proximity to the lake along with the available pool habitat within the creeks provided preferred habitat for Chironomidae compared to those sites upstream of the lake. I surmise Chironomidae colonized the area from newly hatched larvae, retention in the area after the lake receded, and downstream drift based on the range of sizes from very small to pupae. The small-sized ones suggest they may have hatched in the immediate area (Davies, 1976; Waters, 1969).

Ephemeroptera are known to recolonize areas by passive downstream drift and by active drift both upstream and downstream (Pearson and Franklin, 1968; Madsen et al, 1977). The majority of Ephemeroptera recolonizing the stream channel likely comes from passive downstream drift from upstream habitats to the recently exposed stream area segments, but they may move upstream depending on habitat availability and density of individuals. Species in some families tend to actively drift more than others. Behavioral drift, which has been shown to provide the majority of downstream drift, is impacted by standing crop and has been documented as an important re-colonization tactic by Ephemeroptera (Pearson and Franklin, 1968; Madsen et al, 1977). Pearson and Franklin (1968) monitored movement of one species of Baetidae during a sudden decrease in water level. They reported seeing the organisms both swimming and crawling to deeper areas and found low mortality in the area. Baetidae, a very strong swimmer, is likely able to react to the changing water levels and actively migrate as the lake level changes to maintain suitable habitat (McCafferty, 1998). This is less likely for Heptageniidae as they are poor swimmers. However, Heptageniidae are found along lake shorelines and streams and are clingers so they may remain in the exposed stream channel as the lake recedes (Williams and Williams, 1993; McCafferty, 1998). Baetidae and Heptageniidae, the two most abundant families of Ephemeroptera in my study, are less tolerant of environmental stressors and less ubiquitous than Chironomidae, and are therefore likely more affected by changing water levels (McCafferty, 1998). This

supports my hypothesis that families within Ephemeroptera are not as able to adapt to the changing environment as Chironomidae and thus are found in smaller relative abundances.

Hope Mountain Center's sampling, which generally occurs in August or September, has not resulted in collection of any Amphipoda (Dolecki, 2010; Doix, 2011; Doix, 2012; Doix, 2013). This agrees with my findings that Amphipoda abundance in the creeks decreases as the reservoir fills. This may be due to habitat preferences. Amphipoda prefer slow moving water and may be able to persist in the lower gradient reaches near and in the lake but are flushed out of the higher gradient segments of stream. Amphipoda are probably not capable of recolonizing the lower reaches by migrating upstream from the lake during the winter months because they are poor swimmers (Pennak, 1978; Covich and Thorp, 1991; McCafferty, 1998).

Benthic composition did not differ according to pool elevation or reach location with respect to the full pool shoreline. Stanton's (1977) study of benthos in streams with daily inundation and exposure due to fluctuations in an adjacent reservoir, and Johnston's (1989) study of Ross Lake and its tributaries both found that reaches closest to the creek mouth had higher production and abundance of benthic macroinvertebrates than both the upstream reaches and areas within the lake adjacent to the creek mouth, but my study did not support those findings. It did support the notion that recolonization of benthic organisms can happen quickly (Williams and Hynes, 1976; Stanton, 1977; Brusven and Trihey, 1978). Stanton (1977) dealt with daily inundation which likely did not provide adequate time for re-colonization in areas further from the lake. Expanding the sampling timeline to include collection from full pool to low pool would allow for determination of the re-colonization direction, assuming the exposed channel is determined to be denuded of benthos at some point during the recession. It may be that stream reaches initially differ, but by the time the reservoir has reached the seasonal low, re-colonization has occurred and is therefore not reflected in my study.

Composition and abundance of available prey has been documented to change based on location within a stream or watershed, and this was supported by my study as not all streams had similar benthos. Turner (2009) suggested that the lack or comparatively low abundance of organisms at some sites compared to others in proximity is influenced by selective filters such as climate change impacts, the originating water source, flow regime, substrate, and temperature gradient, none of which were measured as part of this study. Most of my samples occurred within riffles of similar substrate in the given sample reach. Buffagni and Comin's (2000) findings suggest this is the most accurate location to sample and that benthic abundance is greater in riffle habitat than pool, bedrock, or transitional (fluctuating flows and low primary productivity) zones.

The rank of importance for diet items to fish differed at each site. If diet reflects abundance and availability in the environment, then this supports the contention that location of a tributary can affect the local food web (Welch, 2012). Others have also reported a changing diet based on location sampled within a reach (Gunkel et al, 2002; Jones et al, 2008). Nakano et al (1998) found that Brook Trout, Cutthroat Trout, and Bull Trout will create groups of mixed species while feeding. These mixed groups can then affect the consumption of available prey in an area as local as a pool. These "foraging microhabitats" could translate into different feeding preferences among sites as the individual juveniles interact with one another differently at each site, and have varying effects on prey availability and composition. Predators have also been found to impact the prey community and associated available prey by consumption, localized extinction, and training fearful prey that seeks hiding and may eventually migrate (Merrick et al, 2008; Orrock et al, 2008). This may explain the variation in Hozomeen Creek diet preferences. Hozomeen Creek has a larger abundance of Brook Trout, which may affect prey composition differently than streams with fewer of this introduced species (A. Rawhouser, North Cascades National Park, personal communication). Differences in diets among sites was further supported by Schoby and Keeley (2011) who found that the diets of Bull Trout were "considerably different" between tributaries and mainstem sites within the same watershed and in proximity to one another. These site differences could also be

caused by individual fish feeding habits as well as group behavior (Nakano et al, 1998). Fish abundance could explain these differences between species at the reach or microhabitat level but was not determined as part of this study.

Diet and stomach fullness at each site was likely impacted by the varying benthic assemblage among the sites.

Nakano et al (1999) found that Dolly Varden will alter their feeding behavior between drifting or benthic invertebrates dependent on available resources. Studies have also shown that predators can significantly impact distribution, behavior, and demography of their prey populations (Orrock et al, 2008; Lowery, 2009). Hozomeen Creek had the largest group of Amphipoda of any of the creeks, reflected in both fish diets and benthic samples. Although they can swim, they are generally found in shallow waters and are not able to retreat to interstitial spaces as well as other aquatic invertebrates, making them more susceptible to capture and thus are an important prey item (McCafferty, 1998; Baxter et al, 2005). It is unknown if this increased abundance in April was unique to Hozomeen Creek since this was a single-event phenomenon and the other sites were not sampled again until early May. It is clear that amphipods contributed to the high stomach fullness during April and to the differences in macroinvertebrate assemblages and fish diets between Hozomeen Creek and the other sites.

Stomach fullness varied among species. Brook Trout had the highest average instantaneous ration (a measure of stomach fullness) among the species. This could be because Brook Trout (and the single Cutthroat Trout) fed mostly on the more abundant Ephemeroptera while Native Char and Rainbow Trout competed for the less abundant Diptera. Although Diptera ranked third in overall importance in the diet of juvenile Brook Trout in my study, as determined by the IRI, other studies have shown Brook Trout can rely more heavily on Diptera, increasing the competitive pressure for food with native juvenile trout and char in Ross Lake (Wydoski and Whitney, 2003). Hilderbrand and Kershner (2004) reported competition among predators to the point of local extinction when food limitations occur. Brook Trout have been known to out-compete native fishes in western streams, to the point of driving Bull Trout to locations further upstream, and are highly opportunistic feeders

(Wydoski and Whitney, 2003; Reiman et al, 2006). Baxter et al (2004) found that the introduction of a nonnative trout can have impacts on the stream community that can be measured four trophic levels out and can include the terrestrial environment.

To compare prey availability with prey consumption I initially included Ivley's index of electivity (E) and Vanderploeg and Scavia's realized electivity (E\*) indices, but the results of these were opposite to those of IRI. In all cases IRI placed importance on taxa that E and E\* determined to be actively avoided. The same was true for those taxa IRI determined to be unimportant to fish diet; E and E\* determined they were actively selected for. One potential problem is that E can be affected by small sample size although E\* is not supposed to be (Vanderploeg and Scavia, 1979; Lechowicz, 1982; and Strauss, 1982). Authors of recent papers tend advocate the use of IRI as a good measure of dietary preference, even though it does not compare prey availability to consumption. Since I could find no studies that compared or used both IRI and E (or E\*), I removed the latter analysis from my study. That is not to say IRI may not be misleading. Ahlbeck et al (2012) found %IRI struggled with representing the true diet of benthivorous fish, routinely overestimating diets of small prey. Ahlbeck et al (2012) also found %IRI to be sensitive to small sample size which suggests the importance of a given prey item could have been overestimated in this study.

## Conclusion

Juveniles of Native Char, Rainbow Trout, Brook Trout, and Cutthroat Trout ate the same kinds of macroinvertebrates in three seasonally inundated tributaries to Ross Lake, but the composition of their diet was affected by location, month, and in some cases, the height of Ross Lake reservoir. Benthic macroinvertebrate abundance appeared to be sufficient to support rearing of these species. Assuming benthic macroinvertebrates are absent in newly exposed stream reaches as the reservoir level drops, re-colonization appears to occur consistently enough that abundance differences are not detectable at the reach or site level. The majority of recolonization likely originates in stream segments above the full pool elevation and drift downstream. Juvenile trout and char diets differ by site, which may be driven by channel morphology, prey abundance, and fish composition. Brook Trout, an introduced species in Ross Lake, was found to feed preferentially on Ephemeroptera, while the Native Chars and Rainbow Trout fed on the less abundant Diptera, in competition with one another. As Brook Trout abundance continues to increase and spread throughout the lake and into additional streams, benthic composition may shift and native juveniles may find increased competition for forage.

Many studies have been published on adult feeding strategies, especially in comparisons between species or environments. However, research on juvenile diets is less available, and to the best of my knowledge, research on prey availability and selectivity on seasonally inundated streams is non-existent. Further research on Ross Lake juvenile trout diet, the most important prey taxa, and the benthic community they rely on will result in a better understanding of fish stock dynamics and Ross Lake ecology and perhaps influence management of the fish stocks and lake levels in the future.

Table 1. Ross Lake reservoir elevation by sample date. Lake level was calculated by taking the elevation mean of the hourly stages per 24 hours

Date	Pool Elevation (feet Above Mean Sea Level [AMSL])
3/29/2013	1513.6
4/11/2013	1520
5/5/2013	1521.3
5/19/2013	1561.3
6/2/2013	1572.3
6/21/2013	1588.9

Table 2. Proportional substrate composition based on particle size for each creek with reaches combined. Subdominant sediment type was not always present at Hozomeen Creek due to the homogenous sediment of sand/silt.

		Dry Creek	Roland Creek	Hozomeen Creek
Dominant	Boulder	24%	0%	0%
	Cobble	36%	14%	2%
	Gravel	40%	50%	23%
	Sand and Silt	0%	36%	74%
Sub-	Boulder	4%	0%	0%
Dominant	Cobble	52%	50%	0%
	Gravel	24%	43%	7%
	Sand and Silt	16%	7%	16%

Table 3. Most abundant benthic macroinvertebrate taxa for each creek by sampling period. Sample 1: Dry and Roland Creeks: 3/29/2013, Hozomeen Creek: 4/11/2013; Sample 2: Dry and Roland Creeks: 5/5/2013, Hozomeen Creek: 5/19/2013; Sample 3: Dry and Roland Creek: 6/2/2013; Hozomeen Creek: 6/21/2014.

Family				
	OVERALL	Sample 1	Sample 2	Sample 3
Dry Creek	22% Baetidae	25% Baetidae	31% Chironomidae	26% Heptageniidae
Roland Creek	47% Heptageniidae	24% Heptageniidae	47% Heptageniidae	66% Heptageniidae
Hozomeen Creek	37% Chironomidae	39% Baetidae	35% Oligochaeta	68% Chironomidae
Order				
	OVERALL	Sample 1	Sample 2	Sample 3
Dry Creek	39% Ephemeroptera	33% Ephemeroptera	40% Ephemeroptera	52% Ephemeroptera
Roland Creek	71% Ephemeroptera	41% Ephemeroptera	80% Ephemeroptera	79% Ephemeroptera
Hozomeen Creek	37% Diptera	59% Ephemeroptera	35% Oligochaeta	68% Diptera

Table 4. Results from ANOSIM testing of benthic macroinvertebrate abundance at the family level during each date. (Global R=0.323, p-value = 0.005, alpha is 0.05). Significantly different pair-wise tests are shown in bold. Pool elevations were as follows: 3/29/2013: 1513.6 AMSL; 4/11/2013: 1520 AMSL; 5/5/2013: 1521.3 AMSL; 5/19/2013: 1561.3 AMSL; 6/2/2013: 1572.3 AMSL; 6/21/2013: 1588.9 AMSL.

Groups			Global R	p-value	Assessment
3/29/2013	VS.	4/11/2013	0.074	0.3	Similar
3/29/2013	VS.	5/5/2013	0.477	0.036	Dissimilar
3/29/2013	vs.	5/19/2013	0.296	0.2	Similar
3/29/2013	vs.	6/2/2013	0.333	0.143	Similar
3/29/2013	VS.	6/21/2013	0.167	0.3	Similar
4/11/2013	VS.	5/5/2013	0.159	0.25	Similar
4/11/2013	VS.	5/19/2013	-0.074	0.7	Similar
4/11/2013	VS.	6/2/2013	0.278	0.086	Similar
4/11/2013	VS.	6/21/2013	-0.083	0.6	Similar
5/5/2013	VS.	5/19/2013	0.231	0.125	Similar
5/5/2013	VS.	6/2/2013	0.438	0.032	Dissimilar
5/5/2013	VS.	6/21/2013	0.309	0.238	Similar
5/19/2013	vs.	6/2/2013	0.685	0.029	Dissimilar
5/19/2013	vs.	6/21/2013	0.167	0.4	Similar
6/2/2013	VS.	6/21/2013	0.643	0.067	Similar

Table 5. SIMPER results of overall percent dissimilarity and top five taxa contributing to differences in benthic macroinvertebrate composition among dates at all sites.

Таха	Mean Abundance First Date	Mean Abundance Second Date	% Contribution to Difference		
3/29/2013 vs. 5/5/2	013	Mean dissimilarity = 80.80			
Baetidae	5.67	46.00	23.53		
Heptageniidae	15.67	47.80	20.65		
Chironomidae	4.33	25.80	16.65		
Oligochaeta	4.00	14.60	9.58		
Unidentifiable Insecta	12.67	1.80	5.47		
5/5/2013 vs. 6/2/20	13	Mean dissimilarity = 68.00			
Heptageniidae	47.80	47.25	31.26		
Baetidae	46.00	9.50	21.50		
Chironomidae	25.80	4.50	14.10		
Oligochaeta	14.60	6.25	8.10		
Ephemerellidae	2.40	6.00	3.82		
5/19/2013 vs. 6/2/2	013	Mean dissimilarit	y = 76.33		
Oligochaeta	71.00	6.25	29.93		
Heptageniidae	21.00	47.25	20.40		
Chironomidae	66.00	4.50	18.10		
Baetidae	16.67	9.50	7.57		
Ephemerellidae	0.00	6.00	3.41		

Table 6. SIMPER results of overall percent dissimilarity and top five taxa contributing to differences in benthic macroinvertebrate composition among months.

Таха	Avg. Abundance First Month	Avg. Abundance Second Month	% Contribution to Difference		
March vs. May		Mean dissimilarity	y = 81.72		
Oligochaeta	4.00	35.75	19.81		
Chironomidae	4.33	40.88	17.74		
Baetidae	5.67	35.00	17.50		
Heptageniidae	15.67	37.75	17.29		
Unidentifiable Insecta	12.67	1.25	5.33		
May vs. June		Mean dissimilarity	y = 71.33		
Chironomidae	40.88	82.83	25.57		
Heptageniidae	37.75	38.00	21.35		
Oligochaeta	35.75	13.17	14.30		
Baetidae	35.00	15.33	13.90		
Gammaridae	9.75	0.00	3.60		

Table 7. SIMPER results by site for benthic macroinvertebrate families

Taxa	Mean Abundance Site 1	Mean Abundance Site 2	% Contribution to Difference			
Roland vs. Hozo	omeen	Mean dissimilarity = 76.63				
Heptageniidae	59.57	25.38	21.79			
Oligochaeta	5.86	51.38	19.47			
Chironomidae	6.29	84.75	19.37			
Baetidae	26.29	39.13	15.62			
Roland vs. Dry		Mean dissimilarity = 72.17				
Heptageniidae	59.57	11.6	28.75			
Baetidae	26.29	20.2	16.29			
Chironomidae	6.29	23.2	13.68			
Oligochaeta	5.86	13.8	8.33			
Hozomeen vs. I	Ory	Mean dissimilarity = 71.16				
Chironomidae	84.75	23.22	26.61			
Oligochaeta	51.38	13.8	17.18			
Baetidae	39.13	20.2	15.77			
Heptageniidae	25.38	11.6	12.02			

Table 8. Percent by number, percent by weight, and percent frequency of occurrence of most common forage item by site, month, pool elevation, and for all fish. An asterisk denotes when unidentified taxa were most abundant; the second-ranked taxon was reported in these cases. Pool elevations correspond to the following dates: 3/29/2013: 1513.6 AMSL; 4/11/2013: 1520.0 AMSL; 5/5/2013: 1521.3 AMSL; 5/19/2013: 1561.3 AMSL; 6/2/2013: 1572.3 AMSL; 6/21/2013: 1588.9 AMSL.

	% by Number	% by Weight	% Frequency of Occurrence
All Fish (n=65)	Diptera (31.38%)	Ephemeroptera (25.16%)	Diptera (75.38%)
By Site			
Dry (n=28)	Diptera (49.13%)	Diptera (38.54%)	Diptera (89.29%)
Hozomeen (n=24)	Diptera (23.05%)	Amphipoda (27.69%)	Diptera (66.67%)
Roland (n=13)	Ephemeroptera (77.06%)	Ephemeroptera (74.14%)	Ephemeroptera (100.00%)
By Month			
March (n=10)	Diptera (76.92%)	Diptera (63.16%)	Diptera (100.00%)
April (n=6)	Amphipoda (26.45%)	Amphipoda (43.13%)	Amphipoda (66.67%)
May (n=23)	Ephemeroptera (32.67%)	Ephemeroptera (43.27%)	Diptera (65.22%)
June (n=26)	Ephemeroptera (33.01%)	Ephemeroptera (44.88%)	Diptera (76.92%)
By Pool Elevation (AMSL)			
1513.6 ft (n=10)	Diptera (76.92%)	Diptera (63.16%)	Diptera (100.00%)
1520.0 ft (n=6)	Amphipoda (26.45%)	Amphipoda (43.43%)	Amphipoda (66.67%)
1521.3 ft (n=14)	Ephemeroptera (46.79%)	Ephemeroptera (43.27%)	Ephemeroptera (85.71%)
1561.3 ft (n=9)	Amphipoda (38.35%)	Amphipoda (44.88%)	Diptera (66.67%)
1572.3 ft (n=17)	Ephemeroptera (46.19%)	Ephemeroptera (44.99%)	Diptera (82.35%)
1588.9 ft (n=9)	Diptera (26.16%)	Trichoptera* (14.73%*)	Diptera (66.67%)
By Species			
Rainbow Trout (n=36)	Diptera (36.83%)	Ephemeroptera (31.01%)	Diptera (86.11%)
Native Char (n=18)	Diptera (33.77%)	Diptera (24.40%)	Diptera (66.67%)
Brook Trout (n= 10)	Ephemeroptera (28.78%)	Ephemeroptera (22.33%)	Ephemeroptera (80.00%)
Cutthroat Trout (n=1)	Ephemeroptera (35.71%)	Ephemeroptera (28.90%)	Diptera (100.00%)

Table 9. Prey taxa by species of fish by order of importance calculated by Index of Relative Importance (IRI).

Table 3. Fley (a)		ainbow Trout (n=			Native Char (n=19	-		Brook Trout (n=1	2)	Cı	itthroat Trout (n	=1)
Prey Item	%Weight	Freq of Occurrence	IRI	% Weight	Freq of Occurrence	IRI	%Weight	Freq of Occurrence	IRI	%Weight	Freq of Occurrence	IRI
Diptera	24.55	86.11	5285.37	24.40	66.67	3878.12	6.73	50.00	758.57	12.51	100.00	3394.28
Ephemeroptera	31.01	55.56	3413.82	14.84	27.78	796.18	22.33	80.00	4089.22	28.90	100.00	6461.62
Unidentified Insecta	17.24	61.11	1441.26	3.83	33.33	160.89	16.92	70.00	1975.91	32.00	100.00	3199.54
Terrestrial	7.72	50.00	868.49	6.20	22.22	321.01	0.87	30.00	123.77	14.42	100.00	2870.16
Trichoptera	3.07	27.78	243.05	8.66	16.67	231.61	10.55	50.00	1489.61	7.41	100.00	2169.08
Plecoptera	2.59	36.11	233.77	0.98	16.67	56.14	12.24	50.00	1092.74	2.20	100.00	1648.53
Incidentals	4.96	30.56	180.77	2.29	22.22	51.00	5.64	50.00	291.37	2.57	100.00	256.77
Amphipoda	6.94	13.89	140.63	23.97	33.33	1571.80	8.42	30.00	495.47	0.00	0.00	0.00
Collembola	0.19	13.89	26.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lepidoptera	0.83	8.33	9.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Oligochaeta	0.56	5.56	4.05	0.00	0.00	0.00	5.33	20.00	184.72	0.00	0.00	0.00
Coleoptera	0.20	8.33	3.78	0.00	0.00	0.00	0.16	10.00	18.31	0.00	0.00	0.00
Megaloptera	0.11	5.56	3.34	0.00	0.00	0.00	2.26	20.00	65.62	0.00	0.00	0.00
Arachnid	0.05	2.78	0.29	0.02	5.56	1.15	0.01	10.00	5.35	0.00	0.00	0.00
Gastropoda	0.00	0.00	0.00	0.09	5.56	1.55	0.43	20.00	26.46	0.00	0.00	0.00
Hirudinea	0.00	0.00	0.00	3.62	5.56	24.84	8.11	20.00	223.31	0.00	0.00	0.00
Unknown	0.00	0.00	0.00	11.11	11.11	246.91	0.00	0.00	0.00	0.00	0.00	0.00

Table 10.Index of relative importance and percent index of relative importance for all fish species sampled. Major prey taxa are those  $\geq$ 5% %IRI. The most important prey item is shown in a solid box and bold font, the second most important taxa is shown in a solid box, and the least important present prey taxa in a dashed box with italic font.

	Rainbow Trout (n=36)		Native	e Char (n	=18)	Brook Trout (n=10)			Cutthroat Trout (n=1)			
			%IRI			%IRI			%IRI			%IRI
Prey Item	IRI	%IRI	Major	IRI	%IRI	Major	IRI	IRI	Major	IRI	%IRI	Major
Diptera	5285.37	44.58	44.58	3878.12	52.83	52.83	758.57	7.00	7.00	3394.28	16.97	16.97
Ephemeroptera	3413.82	28.80	28.80	796.18	10.85	10.85	4089.22	37.72	37.72	6461.62	32.31	32.31
Unidentified Insecta	1441.26	12.16	12.16	160.89	2.19	L	1975.91	18.23	18.23	3199.54	16.00	16.00
Terrestrial	868.49	7.33	7.33	321.01	4.37		123.77	1.14		2870.16	14.35	14.35
Trichoptera	243.05	2.05	L	231.61	3.15		1489.61	13.74	13.74	2169.08	10.85	10.85
Plecoptera	233.77	1.97		56.14	0.76		1092.74	10.08	10.08	1648.53	8.24	8.24
Incidentals	180.77	1.52		51.00	0.69		291.37	2.69		256.77	1.28	<u>. L</u>
Amphipoda	140.63	1.19		1571.80	21.41	21.41	495.47	4.57		0.00	0.00	
Collembola	26.84	0.23		0.00	0.00	<u> </u>	0.00	0.00		0.00	0.00	
Lepidoptera	9.42	0.08		0.00	0.00		0.00	0.00		0.00	0.00	
Coleoptera	3.78	0.03		0.00	0.00		18.31	0.17		0.00	0.00	
Megaloptera	3.34	0.03		0.00	0.00		65.62	0.61		0.00	0.00	
Oligochaeta	4.05	0.03		0.00	0.00		184.72	1.70		0.00	0.00	
Arachnid	0.29	0.002		1.15	0.02		5.35	0.05	-1	0.00	0.00	
Unknown	0.00	0.00	j	246.91	3.36	.i	0.00	0.00		0.00	0.00	
Hirudinea	0.00	0.00		24.84	0.34		223.31	2.06		0.00	0.00	
Gastropoda	0.00	0.00		1.55	0.02		26.46	0.24		0.00	0.00	

Table 11. Prey taxa by site by order of importance calculated by Index of Relative Importance (IRI).

	Dry (n=28)			Hozomeen (	(n=24)		Roland (n=13)			
Prey Item	%Weight	Freq of Occurrence	IRI	%Weight	Freq of Occurrence	IRI	%Weight	Freq of Occurrence	IRI	
Diptera	38.54	0.89	782820.52	11.29	66.67	2289.05	4.05	61.54	773.45	
Unknown	17.93	0.61	141885.64	10.94	45.83	848.77	9.69	61.54	716.63	
Ephemeroptera	14.58	0.46	140592.60	10.98	33.33	681.00	74.14	100.00	15120.37	
Trichoptera	8.13	0.39	70060.82	5.77	16.67	242.94	1.03	30.77	84.18	
Terrestrial	6.26	0.46	62054.02	9.17	33.33	722.57	1.32	38.46	179.91	
Plecoptera	5.17	0.43	52359.94	2.35	16.67	78.12	2.64	46.15	263.19	
Incidentals	4.69	0.39	18422.89	5.98	37.50	266.29	0.31	7.69	7.93	
Collembola	0.07	0.11	772.70	0.21	8.33	17.14	0.00	0.00	0.00	
Coleoptera	0.12	0.07	323.91	0.07	4.17	3.18	0.28	7.69	2.15	
Oligochaeta	0.71	0.04	293.02	0.01	4.17	1.49	4.09	15.38	103.05	
Lepidoptera	0.07	0.07	270.53	0.00	0.00	0.00	2.15	7.69	17.82	
Megaloptera	0.06	0.04	164.35	0.94	8.33	11.39	0.17	7.69	5.20	
Amphipoda	3.60	14.29	106.01	27.69	37.50	1825.63	0.00	7.69	1.33	
Gastropoda	0.06	0.04	63.87	0.18	8.33	4.59	0.00	0.00	0.00	
Arachnid	0.01	0.04	47.72	0.00	4.17	0.93	0.13	7.69	2.25	
Hirudinea	0.00	0.00	0.00	6.09	12.50	100.07	0.00	0.00	0.00	
Unidentified Insecta	0.00	0.00	0.00	8.33	8.33	138.89	0.00	0.00	0.00	

Table 12. Index of relative importance and percent index of relative importance for all sites sampled. Major prey taxa are those comprising ≥5% %IRI. The most important prey item is shown in a solid box and bold font, the second most important in a sold box, and the least important prey taxa in a dashed box with italicized font.

	Dry (n=28)			Hozor	meen (n	=24)	Roland (n=13)		
			%IRI			%IRI			%IRI
Prey Item	IRI	%IRI	Major	IRI	%IRI	Major	IRI	%IRI	Major
Diptera	7828.21	61.12	61.12	2289.05	31.65	31.65	773.45	4.48	
Unknown	1418.86	11.08	11.08	848.77	11.74	11.74	716.63	4.15	J
Ephemeroptera	1405.93	10.98	10.98	681.00	9.42	9.42	15120.37	87.52	87.52
Trichoptera	700.61	5.47	5.47	242.94	3.36		84.18	0.49	
Terrestrial	620.54	4.85	L	722.57	9.99	9.99	179.91	1.04	
Plecoptera	523.60	4.09		78.12	1.08		263.19	1.52	
Incidentals	184.23	1.44		266.29	3.68		7.93	0.05	
Amphipoda	106.01	0.83		1825.63	25.24	25.24	1.33	0.01	
Collembola	7.73	0.06		17.14	0.24		0.00	0.00	j
Coleoptera	3.24	0.03		3.18	0.04		2.15	0.01	
Lepidoptera	2.71	0.02		0.00	0.00		17.82	0.10	
Oligochaeta	2.93	0.02		1.49	0.02		103.05	0.60	
Megaloptera	1.64	0.01		11.39	0.16		5.20	0.03	
Arachnid	0.48	0.00		0.93	0.01		2.25	0.01	
Gastropoda	0.64	0.00	I	4.59	0.06	l	0.00	0.00	
Hirudinea	0.00	0.00		100.07	1.38		0.00	0.00	
Unidentified Insecta	0.00	0.00		138.89	1.92		0.00	0.00	

Table 13. Prey taxa by month by order of importance calculated by Index of Relative Importance (IRI).

	March (n=10)			April (n=6)			May (n=23)			June (n=26)		
Prey Item	%Weight	Freq of Occurrence	IRI	%Weight	Freq of Occurrence	IRI	%Weight	Freq of Occurrence	IRI	%Weight	Freq of Occurrence	IRI
Amphipoda	10.08	40.00	831.13	43.43	66.67	4658.73	17.56	26.09	852.21	0.00	0.00	0.00
Arachnid	0.00	0.00	0.00	0.00	0.00	0.00	0.02	8.70	3.44	0.06	3.85	0.56
Coleoptera	0.15	10.00	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.27	11.54	14.27
Collembola	0.21	30.00	60.58	0.00	0.00	0.00	0.00	4.35	2.11	0.19	3.85	5.66
Diptera	63.16	100.00	14007.47	0.49	66.67	923.79	17.11	65.22	2716.39	14.41	76.92	2960.08
Ephemeroptera	0.16	10.00	2.52	3.61	33.33	439.23	32.09	65.22	4223.60	33.63	61.54	4101.17
Gastropoda	0.00	0.00	0.00	0.11	16.67	12.18	0.22	8.70	5.24	0.00	0.00	0.00
Hirudinea	0.00	0.00	0.00	13.52	33.33	620.30	2.83	4.35	15.21	0.00	0.00	0.00
Incidentals	0.48	10.00	4.84	0.27	33.33	158.07	2.32	26.09	60.64	8.42	46.15	405.39
Lepidoptera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.15	11.54	18.07
Megaloptera	0.00	0.00	0.00	3.77	33.33	182.27	0.00	0.00	0.00	0.16	7.69	6.41
Oligochaeta	0.00	0.00	0.00	0.04	16.67	23.80	3.17	8.70	40.36	0.01	3.85	0.49
Plecoptera	3.29	20.00	100.44	0.00	0.00	0.00	3.46	47.83	455.26	4.73	34.62	345.56
Terrestrial	9.57	40.00	524.20	0.75	16.67	79.39	6.53	39.13	592.88	6.23	46.15	793.81
Trichoptera	0.03	10.00	1.28	0.00	0.00	0.00	6.19	34.78	435.44	9.10	38.46	880.95
Unknown	12.88	50.00	894.37	17.34	33.33	1133.70	8.48	65.22	768.38	17.80	53.85	1234.86
Unidentified Insecta	0.00	0.00	0.00	16.67	16.67	555.56	0.00	0.00	0.00	3.85	3.85	29.59

Table 14. Index of relative importance (IRI) and percent index of relative importance (% IRI) for all months sampled. Major prey taxa are those with at least one metric  $\geq$ 5% %IRI. The most important prey item is shown in a solid box and bold font, the second most important prey item in a solid box, and the least important prey taxa in a dashed box with italicized font.

	March (n=10)		April (n=6)		May (n=23)			June (n=26)				
			% IRI			% IRI			% IRI			% IRI
Prey Item	IRI	% IRI	Major	IRI	% IRI	Major	IRI	% IRI	Major	IRI	% IRI	Major
Ephemeroptera	2.52	0.02		439.23	5.00	5.00	4223.60	41.53	41.53	4101.17	37.98	37.98
Diptera	14007.47	85.26	85.26	923.79	10.51	10.51	2716.39	26.71	26.71	2960.08	27.42	27.42
Unknown	894.37	5.44	5.44	1133.70	12.90	12.90	768.38	7.55	7.55	1234.86	11.44	11.44
Trichoptera	1.28	0.01		0.00	0.00		435.44	4.28		880.95	8.16	8.16
Terrestrial	524.20	3.19	j	79.39	0.90		592.88	5.83	5.83	793.81	7.35	7.35
Incidentals	4.84	0.03		158.07	1.80		60.64	0.60	L	405.39	3.75	L
Plecoptera	100.44	0.61		0.00	0.00		455.26	4.48		345.56	3.20	
Unidentified Insecta	0.00	0.00		555.56	6.32	6.32	0.00	0.00		29.59	0.27	
Lepidoptera	0.00	0.00		0.00	0.00		0.00	0.00		18.07	0.17	
Coleoptera	2.50	0.02		0.00	0.00		0.00	0.00		14.27	0.13	
Megaloptera	0.00	0.00		182.27	2.07		0.00	0.00		6.41	0.06	
Collembola	60.58	0.37		0.00	0.00		2.11	0.02		5.66	0.05	
Arachnid	0.00	0.00		0.00	0.000		3.44	0.03		0.56	0.01	
Oligochaeta	0.00	0.00		23.80	0.27		40.36	0.40		0.49	0.004	
Amphipoda	831.13	5.06	5.06	4658.73	53.02	53.02	852.21	8.38	8.38	0.00	0.00	!
Gastropoda	0.00	0.00	l	12.18	0.14		5.24	0.05		0.00	0.00	
Hirudinea	0.00	0.00		620.30	7.06	7.06	15.21	0.15		0.00	0.00	

Table 15. SIMPER results of overall percent dissimilarity and taxa contributing to at least 80% of the differences in diet composition between sample months that were determined to be dissimilar by ANOSIM.

Taxa	Mean Abundance First Month	Mean Abundance Second Month	% Contribution to Difference	Taxa	Mean Abundance First Month	Mean Abundance Second Month	% Contribution to Difference		
March and May	Mean dissim	ilarity = 81.29		March and April	Mean dissim	larity = 83.97			
Diptera	8.53	1.17	61.52	Diptera	8.53	1.90	38.63		
Ephemeroptera	0.00	1.81	76.85	Amphipoda	0.50	9.07	74.46		
Amphipoda	0.50	0.36	85.11	Unidentified Insecta	0.54	2.11	88.19		
March and June	Mean dissim	ilarity = 80.33		May and April	Mean dissimilarity = 89.11				
Diptera	8.53	1.09	63.98	Amphipoda	0.36	9.07	42.63		
Ephemeroptera	0.00	1.34	76.12	Unidentified Insecta	0.33	2.11	58.57		
Unidentifiable Insecta	0.54	0.48	83.74	Ephemeroptera	1.81	0.82	73.65		
May and June	Mean dissim	ilarity = 70.23		Diptera	1.17 1.90	86.7	74		
Ephemeroptera	1.81	1.34	34.14	June and April	Mea	n dissimilarity = 89.	82		
Diptera	1.17	1.09	59.21	Amphipoda	0.00	9.07	41.59		
Unidentified Insecta	0.33	0.48	69.03	Unidentified Insecta	0.48	2.11	59.42		
Terrestrial	0.25	0.32	77.95	Ephemeroptera	1.34	0.82	72.45		
Amphipoda	0.36	0.00	86.88	Diptera	1.09	1.90	84.99		

Table 16. Results from ANOSIM testing of Index of Relative Importance of prey taxa found in diet samples during each date. Pool elevations were as follows: 3/29/2013: 1513.6 AMSL; 4/11/2013: 1520 AMSL; 5/5/2013: 1521.3 AMSL; 5/19/2013: 1561.3 AMSL; 6/2/2013: 1572.3 AMSL; 6/21/2013: 1588.9 AMSL (Global R=0.362, p-value = 0.001, alpha is 0.05). Significantly different pair-wise tests are shown in bold.

Groups			R	p-value	Assessment
3/29/2013	VS.	4/11/2013	0.616	0.001	Dissimilar
3/29/2013	vs.	5/5/2013	0.604	0.001	Dissimilar
3/29/2013	vs.	5/19/2013	0.304	0.006	Dissimilar
3/29/2013	vs.	6/2/2013	0.533	0.001	Dissimilar
3/29/2013	vs.	6/21/2013	0.293	0.002	Dissimilar
4/11/2013	vs.	5/5/2013	0.600	0.001	Dissimilar
4/11/2013	vs.	5/19/2013	-0.043	0.600	Similar
4/11/2013	vs.	6/2/2013	0.613	0.002	Dissimilar
4/11/2013	vs.	6/21/2013	0.235	0.033	Dissimilar
5/5/2013	vs.	5/19/2013	0.437	0.002	Dissimilar
5/5/2013	vs.	6/2/2013	-0.001	0.369	Similar
5/5/2013	vs.	6/21/2013	0.309	0.003	Dissimilar
5/19/2013	VS.	6/2/2013	0.443	0.003	Dissimilar
5/19/2013	vs.	6/21/2013	0.207	0.021	Dissimilar
6/2/2013	VS.	6/21/2013	0.193	0.022	Dissimilar

Table 17. SIMPER results of overall percent dissimilarity and top three taxa contributing to differences in diet composition between sample dates for dates that were determined to be dissimilar by ANOSIM. Pool elevations are as follows: 3/29/2013: 1513.6 AMSL; 4/11/2013: 1520 AMSL; 5/5/2013: 1521.3 AMSL; 5/19/2013: 1561.3 AMSL; 6/2/2013: 1572.3 AMSL; 6/21/2013: 1588.9 AMSL. Only pair-wise tests with significant outcomes are shown below

Taxa	Mean Abundance First Date	Mean Abundance Second Date	% Contribution to Difference	Taxa	Mean Abundance First Date	Mean Abundance Second Date	% Contribution to Difference		
3/29/2013 vs 4/11/2013	Mean dissimila	rity = 84.94		3/29/2013 vs 6/21/2013	3/29/2013 vs 6/21/2013 Mean dissimilarity = 72.44				
Diptera	79.04	8.77	41.36	Diptera	79.04	23.87	40.02		
Amphipoda	8.19	41.65	23.87	Terrestrial	4.13	16.06	11.33		
Unidentifiable Insecta	7.2	16.95	12.6	Trichoptera	0.01	15.65	10.81		
3/29/2013 vs 5/5/2013	Mean dissimila	rity = 79.09		4/11/2013 vs 5/5/2013	Mean dissimila	rity = 72.44			
Diptera	79.04	19.36	40.96	Ephemeroptera	4.17	50.7	26.62		
Ephemeroptera	0.02	50.7	32.05	Amphipoda	41.65	0.01	23.14		
Unidentifiable Insecta	7.2	9.22	7.01	Unidentifiable Insecta	16.95	9.22	12.58		
3/29/2013 vs 5/19/2013	9/2013 vs 5/19/2013 Mean dissimilarity = 69.36				Mean dissimila	rity = 87.56			
Diptera	79.04	28.29	41.45	Ephemeroptera	4.17	48.57	26.63		
Amphipoda	8.19	44.94	32.04	Amphipoda	41.65	0	23.78		
Terrestrial	4.13	11.31	10.37	Unidentifiable Insecta	16.95	10.37	13.46		
3/29/2013 vs 6/2/2013	Mean dissimila	rity = 72.37		4/11/2013 vs 6/21/2013	Mean dissimila	rity = 88.16			
Diptera	79.04	25.62	38.49	Amphipoda	41.65	0	23.62		
Ephemeroptera	0.02	48.57	33.55	Unidentifiable Insecta	16.95	11.99	13.97		
Unidentifiable Insecta	7.2	10.37	9.24	Unknown	16.67	11.11	13.65		
5/5/2013 vs 5/19/2013	Mean dissimila	rity = 82.74		5/19/2013 vs 6/2/2013	Mean dissimilarity = 79.64				
Ephemeroptera	50.7	10.37	28.36	Ephemeroptera	10.37	48.57	28.72		
Amphipoda	0.01	44.94	27.15	Amphipoda	44.94	0	28.21		
Diptera	19.36	28.29	19.71	Diptera	28.29	25.62	19.07		
5/5/2013 vs 6/21/2013	Mean dissimila	rity = 82.74		5/19/2013 vs 6/21/2013	Mean dissimila	rity = 84.39			
Ephemeroptera	50.7	10.37	28.36	Amphipoda	44.94	0	26.62		
Amphipoda	0.01	44.94	27.15	Diptera	28.29	23.87	18.73		
Diptera	19.36	28.29	19.71	Terrestrial	11.31	16.06	13.97		
5/5/2013 vs 6/21/2013	6/5/2013 vs 6/21/2013 Mean dissimilarity = 76.42			6/2/2013 vs 6/21/2013	Mean dissimila	rity = 74.06			
Ephemeroptera	50.7	8	30.34	Ephemeroptera	48.57	8	30.66		
Diptera	19.36	23.87	19.2	Diptera	25.62	23.87	17.47		
Trichoptera	7.05	15.65	11.88	Trichoptera	6.49	15.65	12.58		



Figure 1. Location of North Cascades National Park in Washington State. Ross Lake National Recreation Area (NRA) shown on both sides of Highway 20. (Map courtesy of National Park Service [NPS])

## Upper Skagit River Basin: Past, Prevented Future, and Present

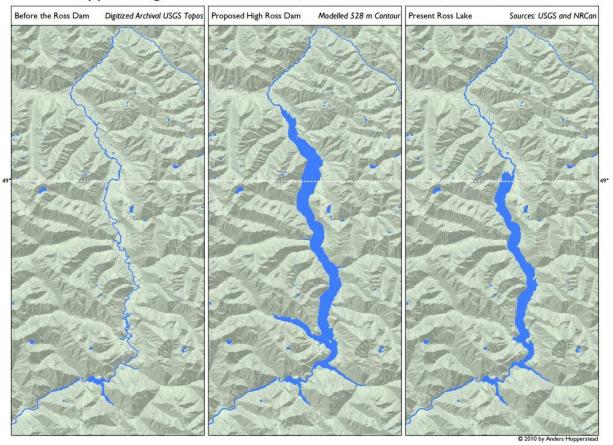


Figure 2. Comparison of the Upper Skagit before dam construction, present condition, and with 1970 proposed high dam. From Anders Hopperstead Maps.

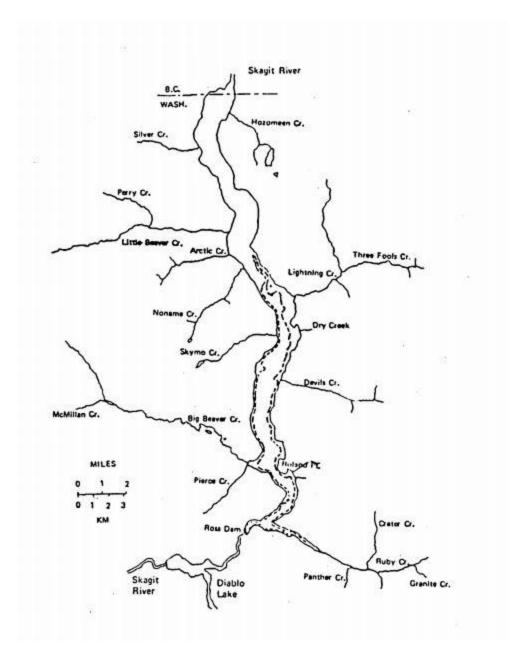


Figure 3. Ross Lake inundation footprint and major tributaries. Solid represent full-pool elevation and dashed line represents the winter low of 1475 feet above mean sea level. From Johnston, 1989.

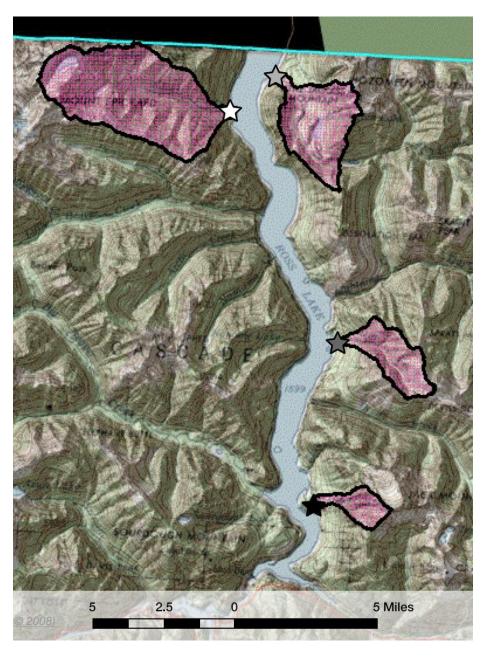


Figure 4. Watershed map of four tributaries to Ross Lake. White star (and only watershed on the eastern side) = Silver Creek; light grey star = Hozomeen Creek; dark gray star = Dry Creek; black star = Roland Creek. Map modified from StreamStats, 2012.

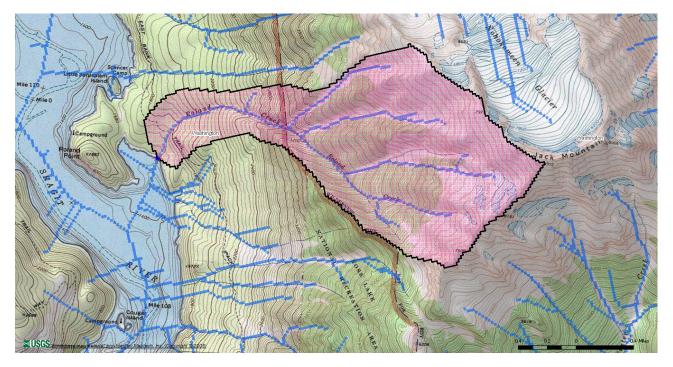


Figure 5. Roland Creek watershed boundary (StreamStats, 2012).

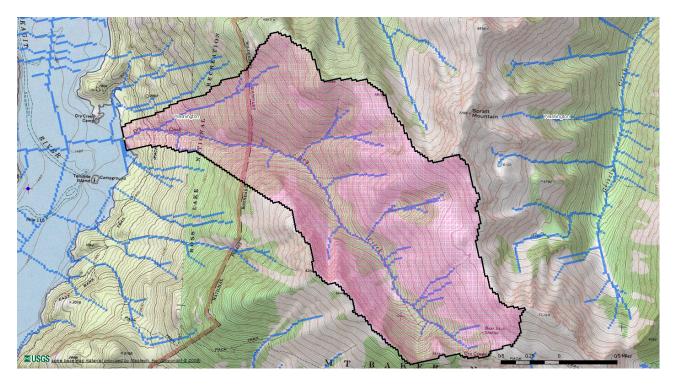


Figure 6. Dry Creek watershed boundary (StreamStats, 2012).

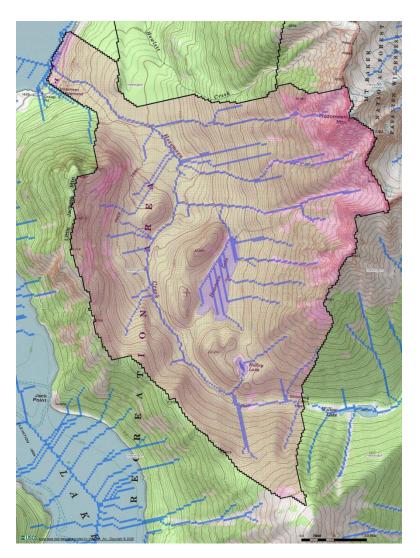


Figure 7. Hozomeen Creek watershed boundary (StreamStats, 2012).

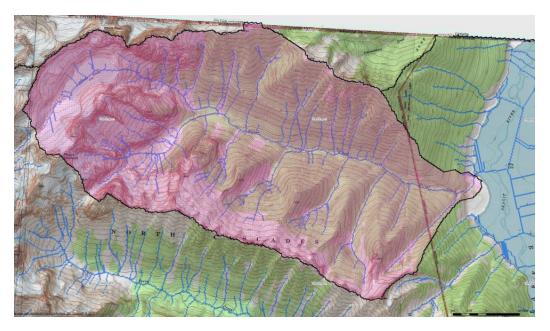


Figure 8. Silver Creek watershed boundary (StreamStats, 2012).

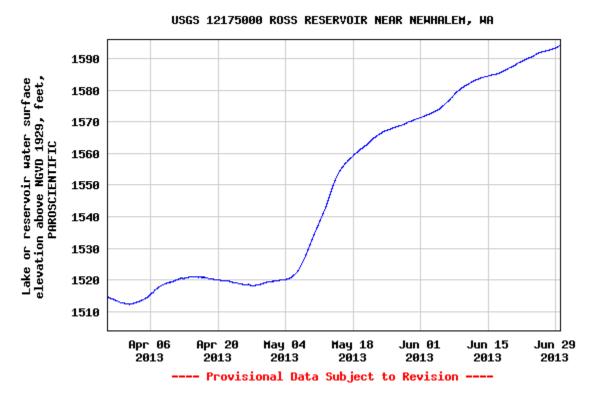


Figure 9. Ross Lake Level during season. Sampling season began March 29, 2013 and ended June 21, 2013.

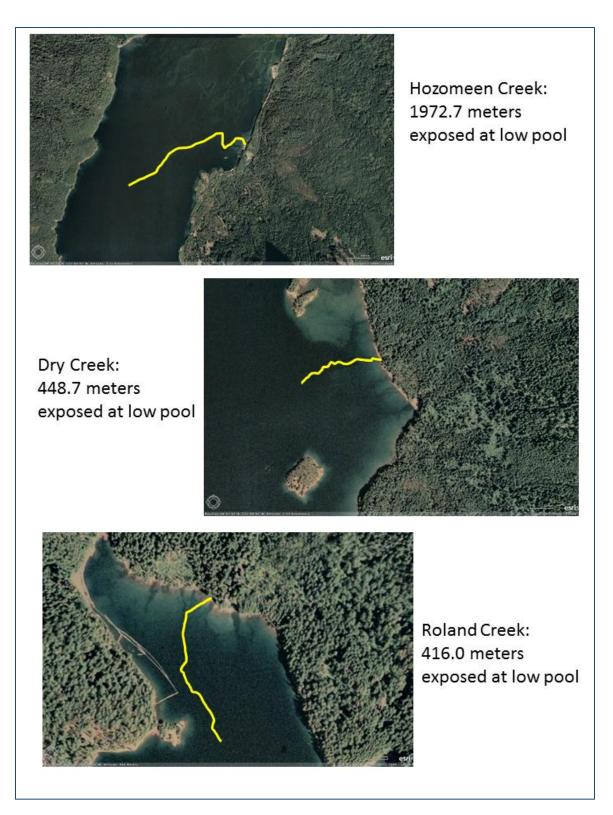


Figure 10. Additional creek exposed during low pool elevation periods based on GPS tracking during the initial site visit at each creek.

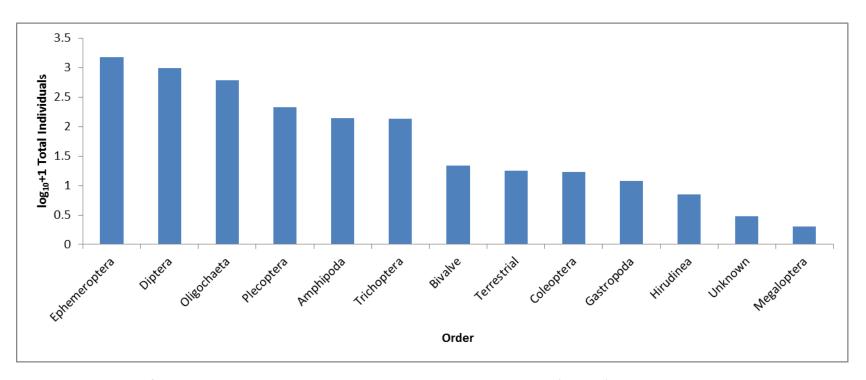


Figure 11.  $Log_{10}+1$  sum of individuals by order collected during benthic macroinvertebrate sampling (n=3,645). Ephemeroptera was most abundant at 1505 individuals (41% of total) across all sampling events.

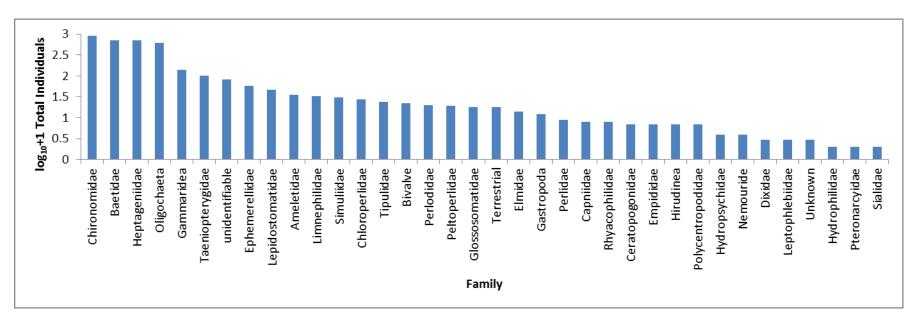


Figure 12. Log<sub>10</sub>+1 sum of individuals by family collected during benthic macroinvertebrate sampling (n=3,645). Chironomidae (order Diptera) was most abundant at 901 individuals across all sampling events.

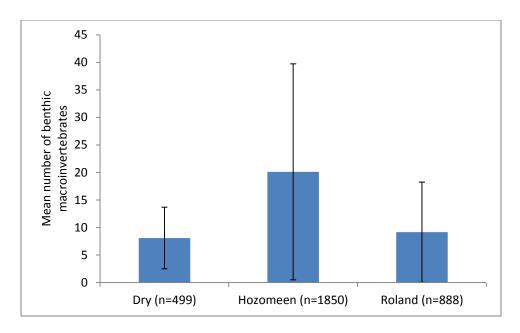


Figure 13. Mean number of benthic macroinvertebrates by site. Vertical lines represent standard error.

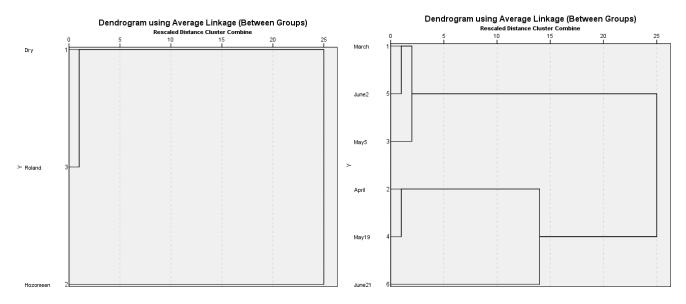


Figure 14. Bray/Curtis presence/absence of Benthic Macroinvertebrates across all sample events and sites. Sampling at Dry Creek and Roland Creek occurred on March 29 (except Dry Creek), May 5, and June 2. Sampling at Hozomeen Creek occurred on April 11, May 19, and June 21.

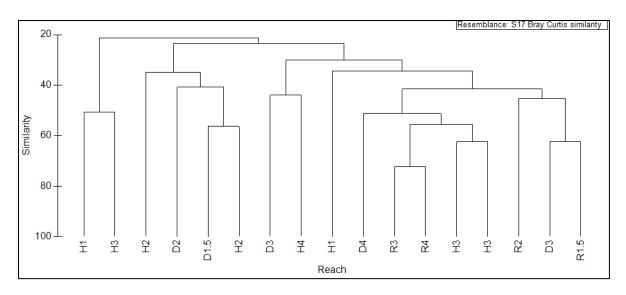


Figure 15. Bray-Curtis similarity plot for all benthos samples by stream reach. Letter indicates sample site (H= Hozomeen; D= Dry; R= Roland) and number denotes reach. Lowest reach: 1; lowest reach on second visit when reach 1 was inundated: 1.5; middle reach below full-pool: 2; upper reach above full-pool: 3; above full-pool reach: 4.

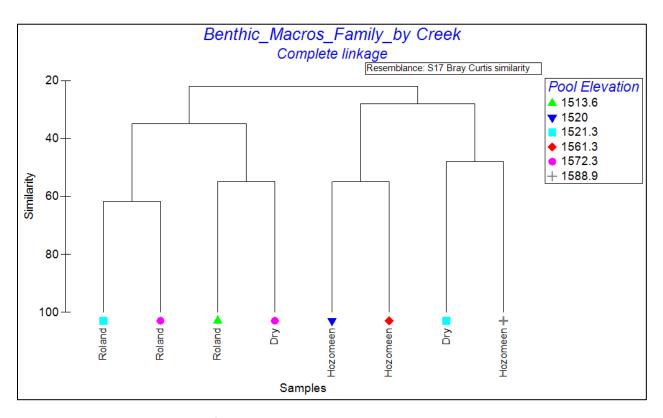


Figure 16. Bray-Curtis similarity plot for all benthos samples by stream and pool elevation.

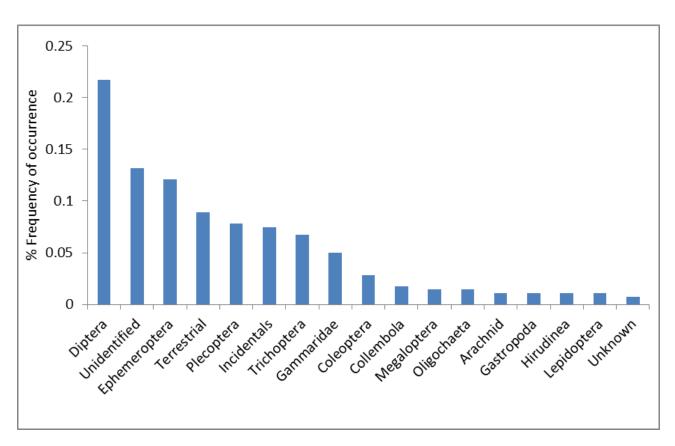


Figure 17. Frequency of occurrence of prey items to the lowest possible taxa from all fish (n=65). Unidentified represents insects unidentified to the Class Insecta level. Unknown represents those organisms unidentifiable even to the class level

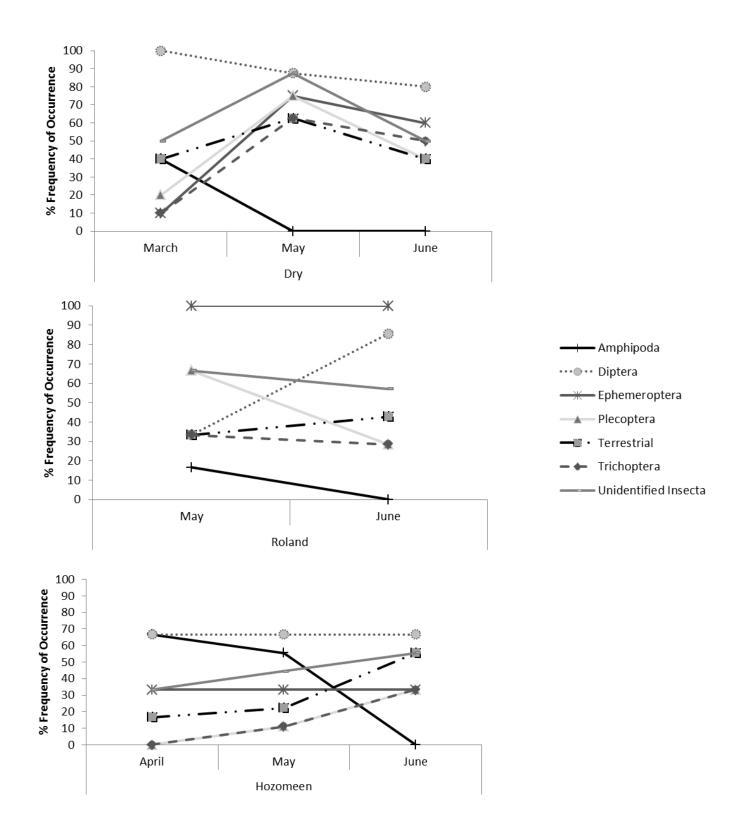


Figure 18. Percent frequency of occurrence of major food items for all diets sampled by month and site to lowest taxa.

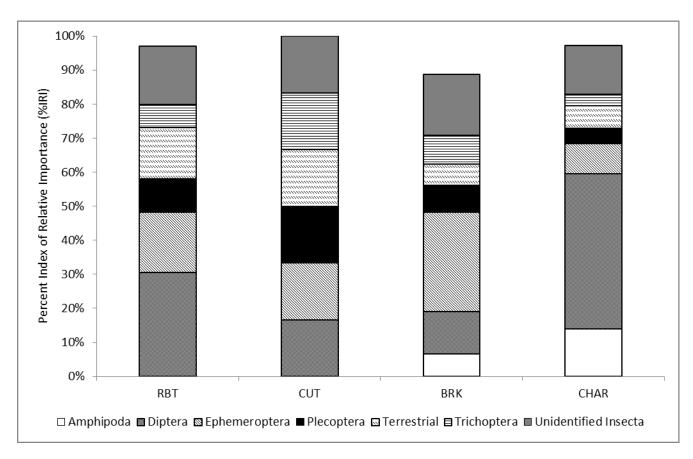


Figure 19. Major food items of Ross Lake juvenile trout by species. Food items are presented as percent of Index of Relative Importance (% IRI) for each site. Only those taxa considered major (% IRI greater than 5%) are presented so results will not always total 100%.

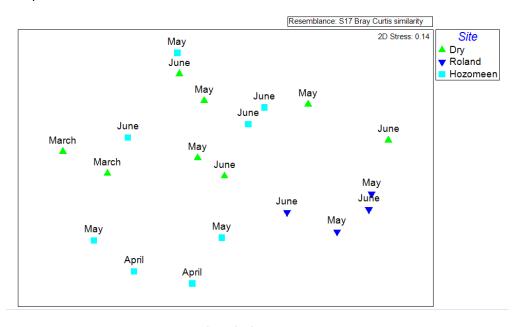


Figure 20. Multi-dimensional Scaling (MDS) of mean % IRI. Light blue square represents Hozomeen Creek, right-side up green triangle represents Dry Creek, and upside down dark blue triangle represents Roland Creek.

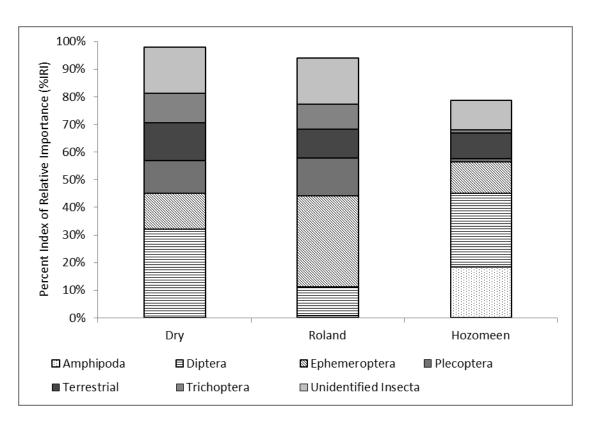


Figure 21. Major food items of Ross Lake juvenile trout by site. Food items are presented as percent of Index of Relative Importance (% IRI) for each site. Only those taxa considered major (% IRI greater than 5%) are presented so results will not always total 100%.

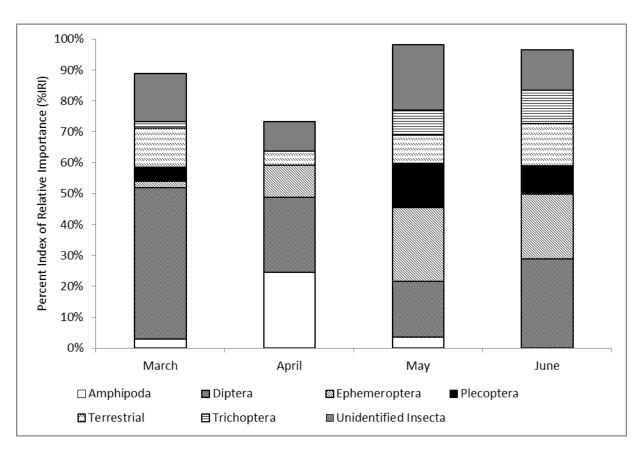


Figure 22. Major food items of juveniles in Ross Lake by month. Food items are presented as percent of Index of Relative Importance (% IRI) for each month. Only those taxa considered major (% IRI greater than 5%) are presented so results will not always total 100%.

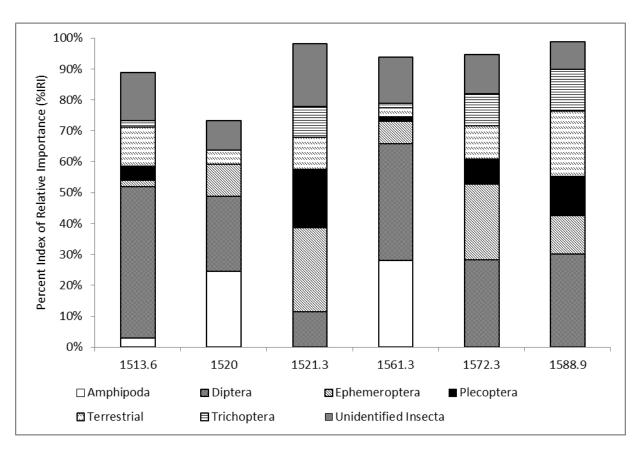


Figure 23. Major food items of Ross Lake juvenile trout by pool elevation. All elevations are reflected as feet above mean sea level. Food items are presented as percent of Index of Relative Importance (% IRI) for each site. Only those taxa considered major (% IRI greater than 5%) are presented so results will not always total 100%.

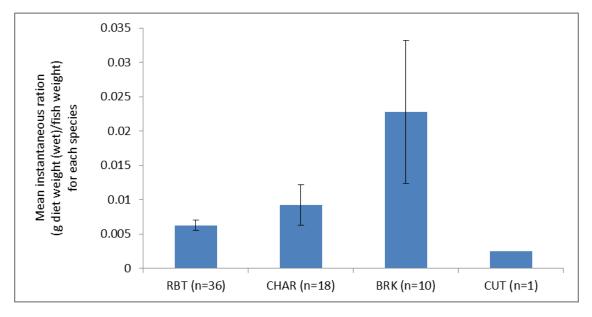


Figure 24. Mean instantaneous ration per fish by species. Vertical lines represent standard error. BRK= Brook Trout, CHAR = Native Char, RBT = Rainbow Trout, and CUT = Cutthroat Trout.

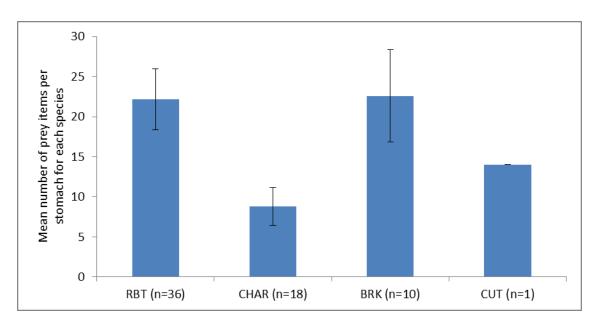


Figure 25. Mean number of prey items (invertebrates) in each fish stomach by species. Vertical lines represent standard errors. BRK= Brook Trout, CHAR = Native Char, RBT = Rainbow Trout, and CUT = Cutthroat Trout.

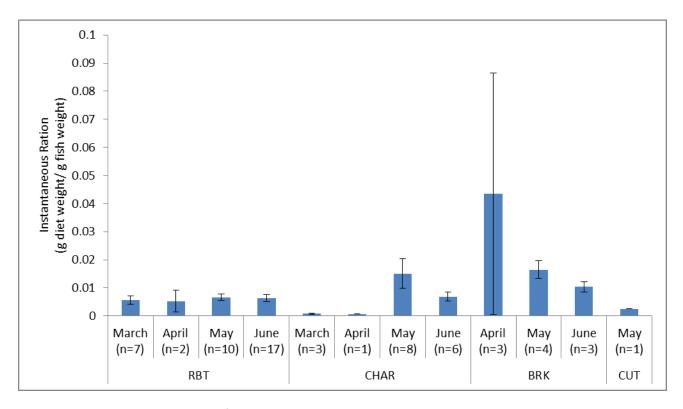


Figure 26. Mean instantaneous ration for all species by month. Vertical lines represent standard error. BRK= Brook Trout, CHAR = Native Char, RBT = Rainbow Trout, and CUT = Cutthroat Trout.

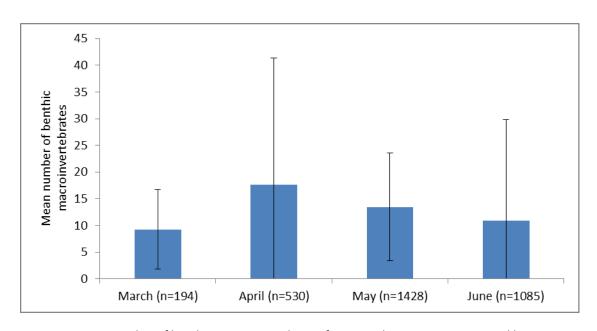


Figure 27. Mean number of benthic macroinvertebrates from March to June 2013. Vertical lines represent standard error. Samples collected in March at Dry Creek were not included in this analysis.

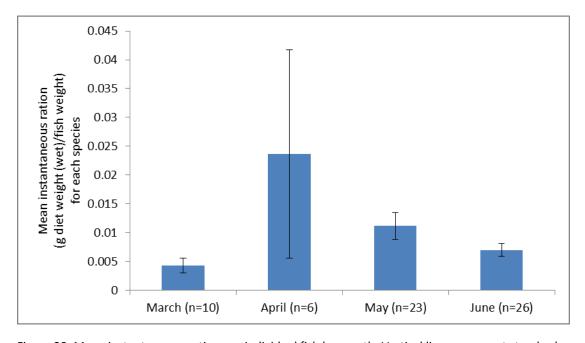


Figure 28. Mean instantaneous ration per individual fish by month. Vertical lines represent standard errors.

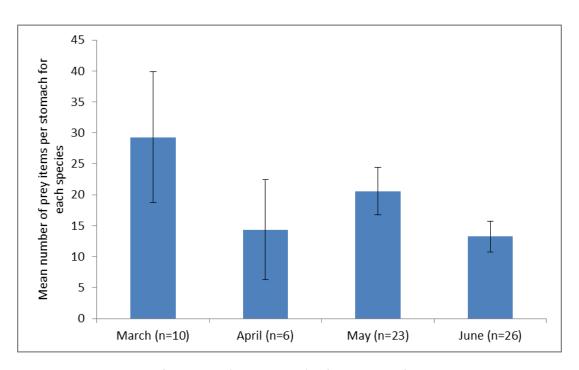


Figure 29. Mean number of prey items (invertebrates) in fish stomachs from March to June 2013. Vertical lines represent standard errors. March: n=10; April: n=6; May: n=23; June: n=26.

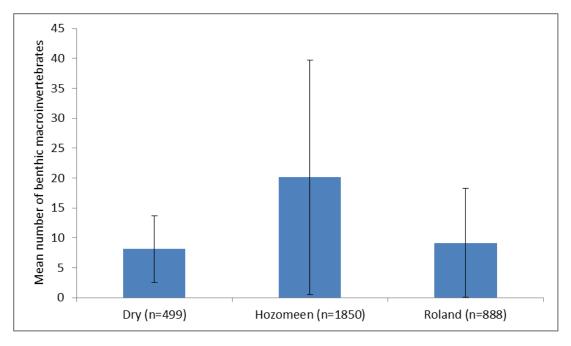


Figure 30. Mean number of benthic macroinvertebrates at each site. Vertical lines represent standard error. Samples collected in March at Dry Creek were not included in this analysis.

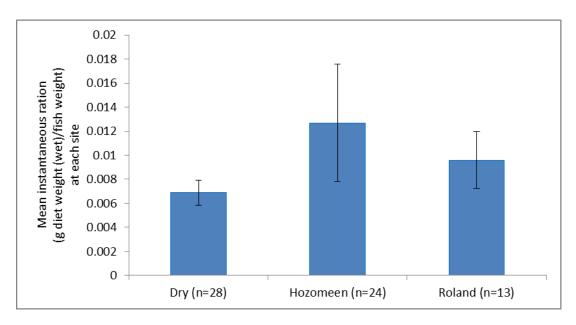


Figure 31. Mean instantaneous ration per fish by site. Vertical lines represent standard error.

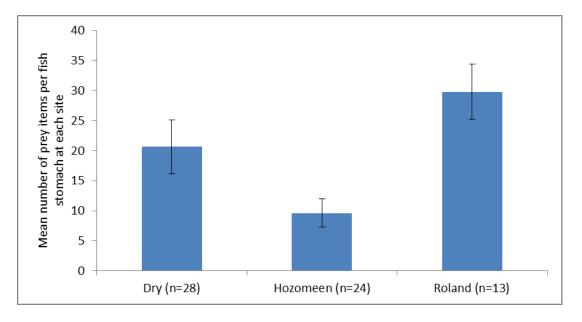


Figure 32. Mean number of prey items (invertebrates) in fish stomachs at each site. Vertical lines represent standard errors.

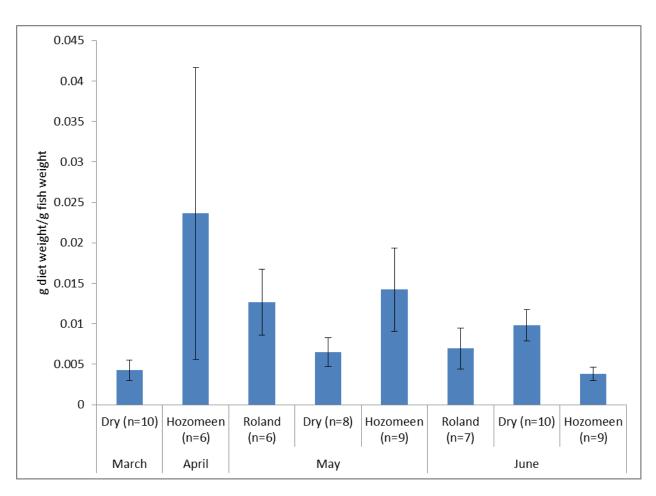
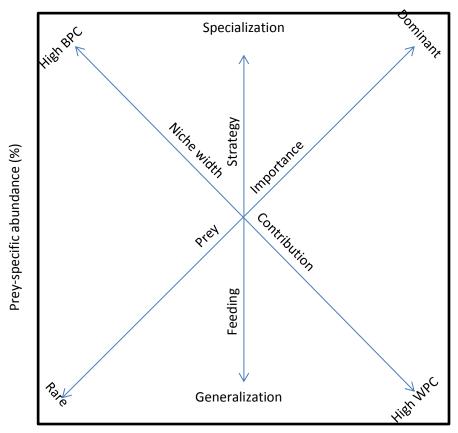


Figure 33. Mean instantaneous ration for all sites by month for the sampling period March through June at Ross Lake. Vertical lines represent standard error.



Frequency of occurrence

Figure 34. Amundensun et al (1996) modification to the Costello graph (1990) that graphically explains feeding strategy and prey importance. BPC = between-phenotype; WPC = within-phenotype.

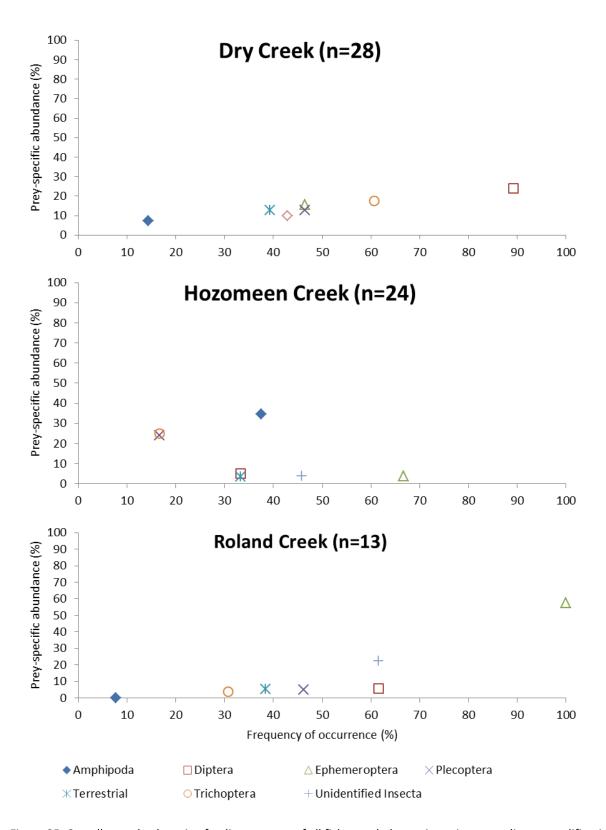


Figure 35. Costello graphs denoting feeding strategy of all fish sampled at a given site, according to modifications in Amunendson et al (1996).

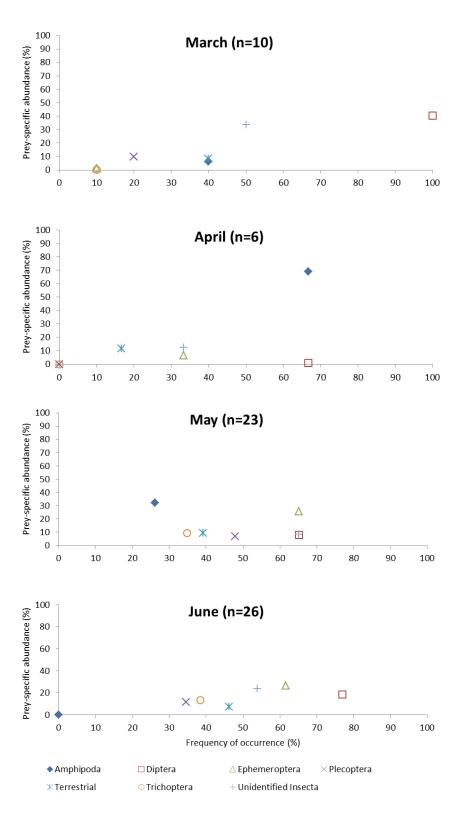
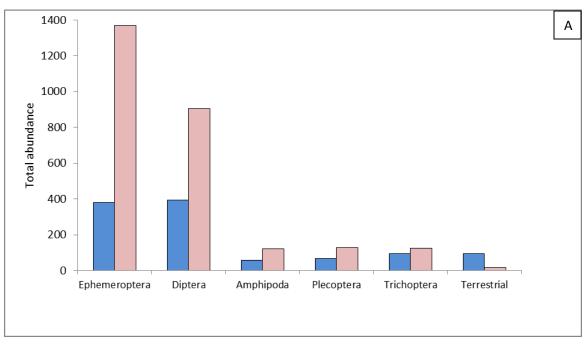


Figure 36. Costello graphs denoting feeding strategy of all fish sampled during a given month, according to modifications in Amundenson et al (1996).



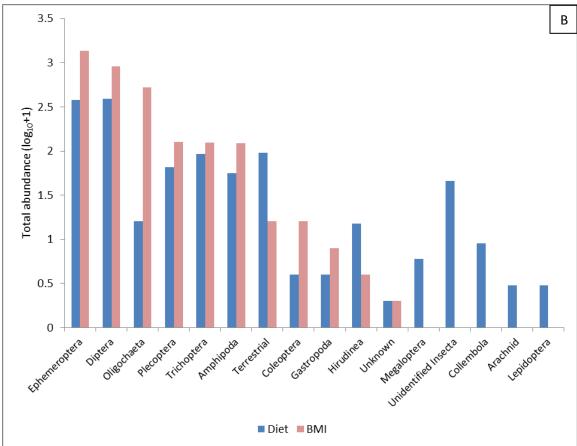


Figure 37. Total abundance of taxa collected in the diet and environment. A. Total relative abundance of major taxa (% IRI> 5), excluding unknown invertebrates. B. Log of all orders collected.

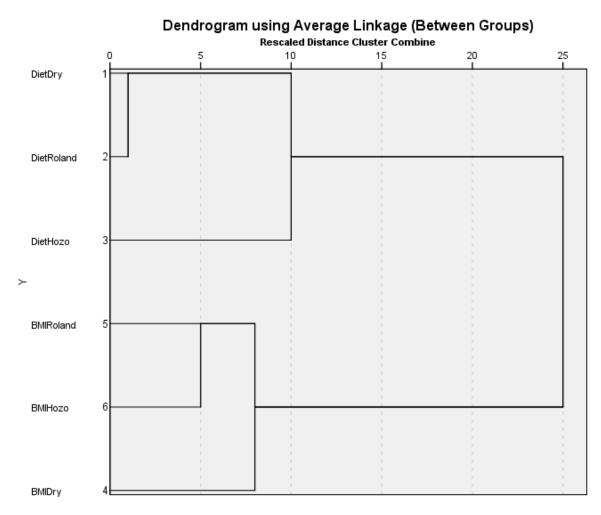


Figure 38. Bray-Curtis clustering on presence/absence of invertebrates found in stomach (Diet) and kick-net samples (BMI) for each site.

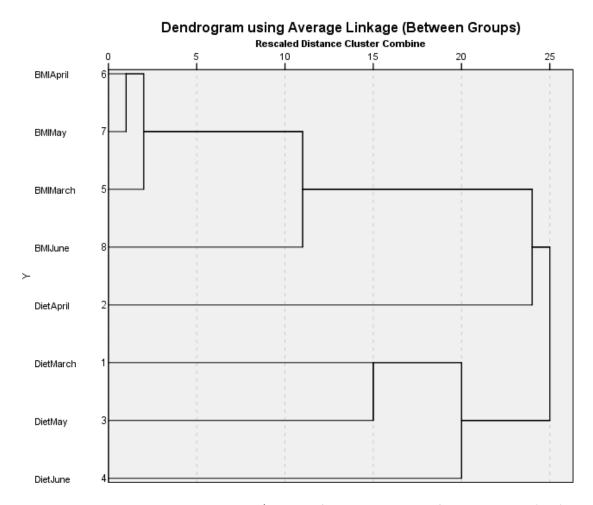


Figure 39. Bray-Curtis clustering on presence/absence of macroinvertebrates found in stomach (diet) and kick-net samples (BMI) for each month.

## Dendrogram using Average Linkage (Between Groups) Rescaled Distance Cluster Combine 0 5 20 25 B1520 9 B1521.3 B1561.3 10 7 B1513.6 B1572.3 11 B1588.9 12 1 D1513.6 D1588.9 6 3 D1521.3 D1561.3 D1572.3 5

Figure 40. Bray-Curtis clustering on presence/absence of macroinvertebrates found in stomach (diet; D) and kick-net samples (BMI) for each pool elevation. Elevations are shown following a B for benthos or D for diet samples and are in feet above mean sea-level.

D1520

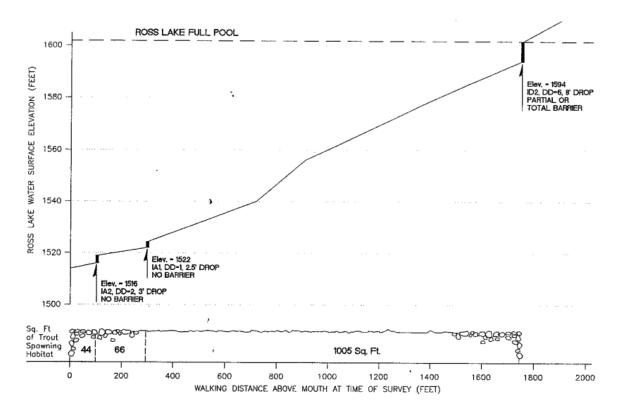


Figure 41. Dry Creek survey of potential barriers and spawning gravel from Ross Lake Tributary Stream Catalog (Seattle City Light, 1989).

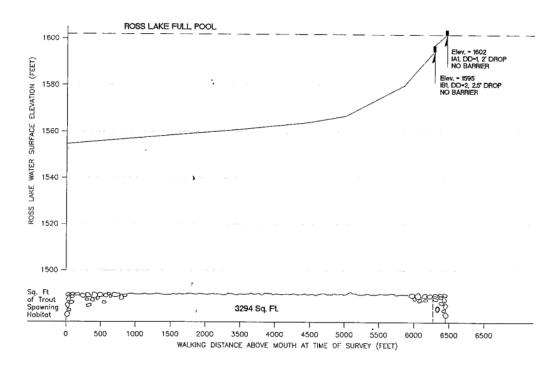


Figure 42. Hozomeen Creek survey of potential barriers and spawning gravel from Ross Lake Tributary Stream Catalog (Seattle City Light, 1989). Although they note 3,294 sq. feet of spawning gravel below full pool, field notes suggest this is marginal at best as the majority of the area was silty with 1-2 inches of deposition in a month.

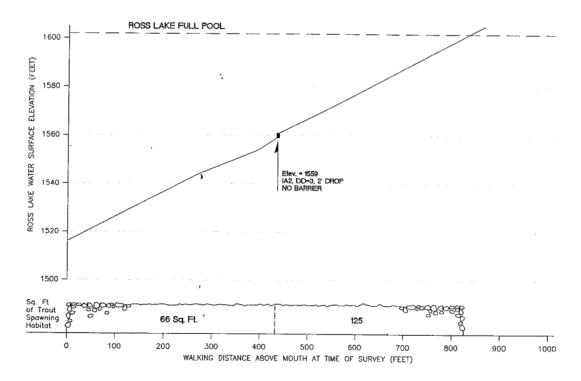


Figure 43. Roland Creek survey of potential barriers and spawning gravel from Ross Lake Tributary Stream Catalog (Seattle City Light, 1989).

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