

**SEASONAL AND AGE-BASED ASPECTS OF DIET
OF THE INTRODUCED REDSIDE SHINER
(*RICHARDSONIUS BALTEATUS*) IN ROSS LAKE, WASHINGTON**

By

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Accepted in Partial Completion
Of the Requirements for the Degree
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Master's Thesis

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Carmen Welch
July 17, 2012

**SEASONAL AND AGE-BASED ASPECTS OF DIET
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A Thesis
Presented to
The Faculty of
Western Washington University

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Abstract

This study investigates the introduced population of the Redside Shiner (*Richardsonius balteatus*) in Ross Lake, Washington. The Redside Shiner was introduced to Ross Lake around 2000 and in the summer months, can be found in densities of hundreds per cubic meter in the shallow areas of Ross Lake. Ross Lake is a protected thirty-five and a half kilometer long reservoir in North Cascades National Park with cold, clear water of exceptional quality. Fish native to Ross Lake include: Bull Trout, Dolly Varden and Rainbow Trout. It is a commonly held belief that the introduced Redside Shiner have no negative effect on the native fish in Ross Lake and that they benefit the native fish as a source of prey. However, previous studies in other lakes have reported reduced growth and survival of juvenile Rainbow Trout as a result of the introduction of the Redside Shiner. Considering the conflict about the potential effects of the introduced Redside Shiner in Ross Lake, the two main goals of this study were to determine what the Redside Shiner in Ross Lake consumes and to evaluate the potential threat of long-term impacts to the native fish in Ross Lake.

Samples were collected from three different locations in the lake across all seasons. Age was determined for 178 Redside Shiners and the stomach contents of 271 Redside Shiners were evaluated. Samples were collected to represent northern, middle and southern areas of Ross Lake. Collection occurred in the winter, spring, summer and fall. Age determination showed the samples consisted of Redside Shiners ages 0 to 6. Regardless of location, season and age, zooplankton and insects are the most important diet categories to the Redside Shiner in Ross Lake both in terms of frequency of occurrence and percent volume of total diet. They also consume oligochaetes, cestodes, algae and other unidentifiable incidental items such as wood, sediment and what appears to be plastics, however none are of much importance.

Based on my findings, the Redside Shiner likely competes with the native fish in Ross Lake for food. The competitive juvenile bottleneck theory explains the potential for a predator to be negatively impacted from its prey due to competition with its juveniles. Like the Redside Shiner in Ross Lake, the juvenile native fish may also depend primarily on aquatic insects and zooplankton. Unless food resources are partitioned spatially and seasonally, the competitive juvenile bottle neck theory holds merit in Ross Lake and direct competition between the introduced Redside Shiner and the native fish seems likely. Based on back-calculated ages of the Redside Shiner in Ross Lake, this population seems to be stable with the potential to persist in high numbers into the future, forecasting that the risk for competition may also persist into the future. Considering the potential for competition now and into the future, further research is required to generate information about the dietary habits of the juvenile native fish, their spatial distribution and how they use the different habitats in Ross Lake.

Acknowledgements

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Introduction

Following habitat destruction, the spread of alien species is the second greatest threat to biodiversity in the United States (Wilcove et al. 1998). Introduction of non-native species have long been problematic in fisheries and aquatic resource management. Introductions of fish to aquatic systems are of particular interest. Fish interact in a highly complex fashion, at multiple trophic levels, which can result in unanticipated changes in their new ecosystem (Wotton 1998). They interact with native fish through predation, competition for resources or even as prey. They can also influence other plant and animal communities, introduce and spread diseases to a system or cause changes to the trophic web within an aquatic system.

This study investigates the introduced population of the Redside Shiner (*Richardsonius balteatus*) in Ross Lake, Washington. Based on local knowledge and anecdotal information, the best estimate on the time of introduction was around 2000; however the Redside Shiner was not readily apparent in Ross Lake until 2003 or 2004 (J. Johnston (Ret.), Washington Department of Fish and Wildlife, M. Brondi and G. Cook (Ret.), North Cascades National Park, personal communications). The source of introduction of the Redside Shiner to Ross Lake is debated. Accidental bait bucket introduction and illegal introduction to establish a prey base for the sport fish in Ross Lake are two explanations. The third explanation involves an historical connection to the Fraser River, which is included in the native range of the Redside Shiner. There is speculation that the connection between the Fraser River and the Skagit River has allowed a slow migration of the Redside Shiner that finally presented itself in Ross Lake by 2004 (John Ridel, North Cascades National Park, personal communications). None of these theories has been tested or researched leaving the source of introduction unknown.

Since its introduction, the Redside Shiner has rapidly populated Ross Lake, raising a full range of concerns about their potential to influence or even threaten components of the Ross Lake ecosystem. The introduction of the Redside Shiner to Ross Lake can be considered a form of environmental

degradation that can play an important role in trophic conditions in the lake. Trophic conditions in lake systems are heavily influenced by biotic interactions (Wootton 1992). Elimination or reduction of food items, shifts in the diets of native fish, predation on native juveniles and eggs are some of the more obvious potential changes of concern that may result from the introduction of the Redside Shiner. Incidental data collection during routine surveys conducted by Washington Department of Fish and Wildlife (WDFW) and observations of anglers have already revealed that Redside Shiners have become a major diet item to all species of trout in Ross Lake. The Redside Shiner diet must be characterized to identify the role it occupies in the trophic web and how those roles link to other lake inhabitants. This information can begin to address the concerns of local managers that are related to resource competition with the native fish of Ross Lake, predation and changes to the trophic web

In addition to directly influencing the existing native fish, the introduction and substantial proliferation of the Redside Shiner in Ross Lake also raises questions and concerns about other trophic relationships. Fish are generally placed into specific trophic categories such as detritivore, herbivore, and carnivore. Typically, they are also omnivorous, exhibiting trophic flexibility and eating opportunistically (Wootton 1998). This flexibility creates ever changing trophic links, potentially causing a cascade of changes, down to and including inorganic nutrients. A diet study is the best way to identify what is being consumed by Redside Shiner in Ross Lake and begin the arduous process of identifying trophic links, patterns and changes.

Redside Shiners

Any information available about the Redside Shiner is because of its relevance to commercially and recreationally valued fish. In the family Cyprinidae, the Redside Shiner is a minnow that usually occurs in large schools, often in the thousands while foraging. It is generally considered a baitfish or forage fish, its value being described as prey for other fish species such as

trout, char and pike minnow. Additionally, Redside Shiners are consumed by piscivorous waterfowl such as mergansers and loons (Scott and Crossman 1973).

Distribution – The native range of the Redside Shiner occurs in North America, west of the Rocky Mountains. Post glacially it dispersed north from the Columbia Basin and then from the Fraser system east (McPhail and Lindsey 1970). The northern extent of the range is the Peace River in northwestern Alberta. The southern extent is northern Oregon (Wydoski and Whitney 2003). The Redside Shiner is considered native to several major drainages of Washington, Oregon and bordering states, including the Columbia River Basin, the Malheur Basin, and the Bonneville River Basin, the Mackenzie River Basin, Great Salt Lake tributaries and the Fraser River in Canada. They have also been introduced to the Colorado River system (McPhail and Lindsey 1970; Scott and Crossman 1973; Wydoski and Whitney 2003).

Habitat requirements - The Redside Shiner occupies both lakes and streams, ranging from small ponds and large lakes to irrigation ditches, small streams and larger rivers, including reservoirs. They use both littoral and pelagic areas and slow to moderately fast moving water. The Redside Shiner exhibits regular daily and seasonal migration patterns. They occupy shoreline habitats during the day and retreat to pelagic waters at night. During late spring, summer and early fall they occupy shoreline habitat and shallow waters, but from October to May they stay in deep water (Weisel and Newman 1951; Carlander 1969; McPhail and Lindsey 1970; Scott and Crossman 1973; Wydoski and Whitney 2003; Houston and Belk 2006). The depth of nightly and seasonal retreat is unknown and most likely variable depending on conditions. Redside Shiners generally occupy habitats with usual summer water temperatures that range from 13 to 20°C, but have previously been discovered in water as cold as 7°C and as warm as 24°C (Wydoski and Whitney 2003).

Physical characteristics – Redside Shiners have physical characteristics common to small minnows. The Redside Shiner has a deep and compressed body (Scott and Crossman 1973). They have

moderately sized heads, about one-quarter of their body length, large eyes and a small oblique mouth (McPhail 1970). Redside Shiners have dark olive or brownish backs with silvery bellies. The red coloring behind the gill cover gives Redside Shiners their names. Males and females exhibit dimorphism during spawning. Males become dark with more vivid crimson highlights and females are paler. They have small cycloid scales covered in a resilient epidermal tissue (McPhail 1970). The largest size reported is 180 mm, but the majority of reports and descriptions recorded 100 mm as the maximum length (Carlander 1969; McPhail 1970; Scott and Crossman 1973; Houston and Belk 2006). Among historical reports, the oldest Redside Shiner reported was 7 years old (Houston and Belk 2006).

Reproduction – Spawning, egg incubation and fry emergence are all temperature related. In Yellowstone Lake, Redside Shiners spawned from the middle of June through the first week of July, in waters ranging between 7 to 10°C (Biesinger 1961). In warmer water, 17 to 18°C, spawning occurs as early as April (Weisel and Newman 1951). Hatching estimates according to temperature are, 7 days at 21°C, 8 days at 18°C, 11 days at 15°C and 15 days at 12°C (Weisel and Newman 1951; Carlander 1959). Redside Shiners can start spawning in their second season of growth. They spawn at night over vegetation in lagoons, along lakeshores, and over gravel in inlet streams in groups of up to 30 individuals (Weisel and Newman 1951; Biesinger 1961; Carlander 1969; McPhail and Lindsey 1970; Wydoski and Whitney 2003). Males turn a more distinct crimson red and gold than the females. No territorial, courting or aggressive behaviors are displayed as females deposit eggs a few at a time on the substrate and males release milt. Non-adhered and stray eggs are commonly consumed by other Redside Shiners (Weisel and Newman 1951).

Diet – Smaller Redside Shiner feed on diatoms, copepods, ostracods, other small planktonic and benthic crustaceans and algae (Scott and Crossman 1973; Wydoski and Whitney 2003). Larger Redside Shiners feed on insects, mainly aquatic, and others that fall on the water surface. They also

eat algae, mollusks, fish eggs and small fishes (McPhail and Lindsey 1970; Wydoski and Whitney 2003). Dietary habits of adult and larger Redside Shiners in Pinanton Lake, British Columbia show differences in day versus night zooplankton consumption in the pelagic zone; *Daphnia* are eaten at night and algae are eaten during the day (Carlander 1969). Redside Shiners can be piscivorous, consuming small fish, such as other minnows including their own, and trout (McPhail and Lindsey 1970; Scott and Crossman 1973; Weisel and Newman 1951; Wydoski and Whitney 2003). Fish eggs as a dietary item are consistently reported, including Redside Shiner, sucker, and trout eggs (Carlander 1969; Scott and Crossman 1973; Weisel and Newman 1951; Wydoski and Whitney 2003). Stomachs of eighteen mature male and female Redside Shiner collected during a spawning event contained 78 of their own fish eggs or fish larvae (Weisel and Newman 1951).

Study Site

Geography - Ross Lake is a reservoir in the Ross Lake National Recreational Area (RLNRA) of North Cascades National Park (NOCA), Washington (Figure 1). It lies in the northeastern area of the Park, just west of the crest of the Cascade Range (Figure 1). Ross Lake is 35.5 km long, and on average 1.6 km wide, and extends into Canada. At the dam, the southernmost boundary of the lake, depths reach 106 to 122 meters (NPS 2010). At full pool, Ross Lake has a surface area 50 km² (Johnston 1989). Surface elevation of Ross Lake fluctuates from 488 to 450 meters (USGS 2010).

Ross Lake is the uppermost impoundment of the Skagit River, created by the Seattle City Light (SCL) hydroelectric project (Figure 2). Construction on Ross Dam started in 1937 and was finished in 1952 (Johnston 1989; NPS 2010). The upper Skagit River, now entirely contained in Canada, is the largest tributary to Ross Lake. Smaller tributaries, south to north on the west side include Silver Creek, Little Beaver Creek, No name Creek, Skymo Creek, and Big Beaver Creek (Figure 2). On the east side, north to south, tributaries are Lightning Creek, Dry Creek, Roland Creek, and Ruby Creek. Aside from functioning as a reservoir manipulated for power production, Ross Lake and the majority

of its watersheds are protected from development and other human related impacts, such as overuse, pollution, resource extraction and exploitation.

Dam operations and management for power production have effects on Ross Lake's morphology. Seattle City Light owns 4.6 km² of land in the RLNRA. In addition to the land they own, they are authorized to utilize federal lands for hydroelectric power generation including the operation of Ross Dam (NPS 2010). Operations on Ross Lake cause the surface elevation of the lake to fluctuate seasonally from a winter low of 450 meters to full pool at 488 meters (USGS 2010). This degree of rise and fall of Ross Lake creates a dynamic not often seen in lacustrine littoral zones.

The Skagit River Hydroelectric project produces 40% of Seattle's electricity, while maintaining an environment that is conducive to sustainable populations of salmon, char, and trout. Ross Lake is maintained at full pool from July through October to balance aesthetics and recreation. Higher lake levels are further maintained through the spawning seasons of Rainbow Trout in the spring, and native char in the fall to allow access to the tributaries for spawning and rearing (NPS 2010).

Ross Lake is a deep, cold, oligotrophic lake with exceptional water clarity (A. Rawhouser, North Cascades National Park, unpublished data). Temperature in Ross Lake is currently monitored by NOCA staff at four locations designated as Hozomeen, Little Beaver, Skymo Creek and Pumpkin Mountain stations (Figure 2). Summer surface temperatures are between 20 and 25°C (Figure 3). In the winter and early spring, they are between 5 and 10°C.

Native Fish Community - A protected watershed and cold clean water in Ross Lake provide habitat for genetically unique populations of the three native fish species, Bull Trout (*Salvelinus confluentus*), Dolly Varden (*Salvelinus malma*), and Rainbow Trout (*Oncorhynchus mykiss*) (Smith and Naish *in draft*). Ross Lake contains a robust population of native Bull Trout and Dolly Varden. The Rainbow

Trout of Ross Lake have a noted reputation in the regional angling community, which they pursue in this unique, historic recreational fishery.

Bull Trout - Native to Ross Lake, the Bull Trout is a vulnerable and threatened species. In 1999 the U.S. Fish and Wildlife Service (USFWS) listed Bull Trout as Threatened throughout the lower 48 states (USOFR 1999). Bull Trout are sensitive to specific habitat variables; the most relevant to the Skagit River system are migratory corridors, water temperature, cover, channel form, channel stability, and spawning and rearing habitat (USFWS 2004). The decline of Bull Trout can be attributed to the following conditions: habitat destruction and fragmentation due to dams, impoundments and water diversions blocking migratory corridors, degraded water quality, poor fisheries management and introduction of non-native species (USFWS 2004).

Bull Trout naturally exhibit patchy distribution and limited gene flow among populations, escalating the requirement for un-altered migratory corridors (Rieman and MacIntyre 1993). Genetically isolated populations of fish adapt to very specific habitat conditions (USFWS 2004). Changes or disruptions to these conditions can have detrimental effects on these locally adapted fish because there is no genetic mixing. In cases of declining and extirpated populations of genetically distinct fish, restoration may not be possible (USFWS 2004). Dams and other barriers exacerbate this genetic isolation, putting these populations at even greater risk (USFWS 2004). Bull Trout in the Skagit River, above the lowest impoundment, are less genetically diverse than populations below the impoundments (Smith and Naish *in draft*). The reduced genetic diversity and genetic dissimilation of Bull Trout in the upper Skagit River compared to the downstream populations indicate long periods of isolation, identifying their vulnerability.

In general Bull Trout are adapted to cold water, with a range of temperature requirements during different life stages. They require water temperatures below 15°C, and adults need temperature below

9°C for spawning. The ideal rearing temperature for juveniles ranges from 8 to 10°C (Wydoski and Whitney 2003; USFWS 2004). Ideal temperature for egg incubation is 2 to 4°C.

The Bull Trout in Ross Lake exhibit an adfluvial life history pattern, growing and maturing in the lake and migrating to tributaries to spawn (USFWS 2004). They spawn from mid-August through November. Snorkel surveys in the upper Skagit River suggest that Bull Trout may be increasing in numbers. From a baseline of 186 fish in 1998, Bull Trout have increased to; 957 in 2009, 1,650 in 2010 and 1,938 in 2011 (Anaka et al. 2010; D. Jesson, B.C. Ministry of Environment, unpublished data). Reasons for the increase in Bull Trout in the Skagit River are ultimately unknown. Managers speculate that the presence of Redside Shiner in Ross Lake provide an additional food source, increasing the robustness of the Bull Trout.

The diet of juvenile Bull Trout consists mostly of larval and adult aquatic insects and crustaceans. Other food items include worms, snails, clams, leeches, beetles, terrestrial insects, earthworms and amphibians. Sub-adult and adult Bull Trout feed exclusively on fish (Wydoski and Whitney 2003).

Dolly Varden - Dolly Varden are also native to Ross Lake, but so far have only been identified in Lightning Creek (Smith and Naish *in draft*). In general Dolly Varden and Bull Trout have similar habitat requirements and life history patterns, including dietary habits (Wydoski and Whitney 2003). Though they are genetically distinct, these two species are morphologically indistinguishable and require genetic analysis to differentiate individuals. The status of Dolly Varden is unknown; however Ross Lake's populations of native char are the only example in North America of Bull Trout and Dolly Varden co-existing in lacustrine habitat (E. Conner, Seattle City Light, personal communication). For management purposes Dolly Varden and Bull Trout are regarded as native char when they coexist (USFWS 2004).

Rainbow Trout - Like the native char, the native Rainbow Trout also require cool water and exhibit an adfluvial life history pattern. Rainbow Trout survive at water temperatures less than 21°C (Wydoski and Whitney 2003). The Rainbow Trout in Ross Lake spawn in the tributaries in the spring, with specific timing driven by changes in lake level. Access to the tributaries is dependent on surpassing the many barriers that are exposed in the drawdown zone when the lake level is low. From the winter low, lake level rises through the spring, typically allowing access to the tributaries starting in May or June.

Principal food items for both adult and juvenile Rainbow Trout include aquatic insects, amphipods, worms and fish eggs. Rainbow Trout less than 40 mm also feed on zooplankton. Juveniles rarely consume fish, but adult Rainbow Trout can be piscivorous (Wydoski and Whitney 2003).

Zooplankton has been discovered in the stomachs of both juvenile and adult Rainbow Trout from Ross Lake; Rainbow Trout larger than 40 mm consumed fish as well (Downen, unpublished data).

The Rainbow Trout fishery in Ross Lake has historical significance, with people returning year after year to fish, supporting the operation of a remote destination resort. With the increasing size of the Rainbow Trout, this fishery is becoming more popular (Downen 2011).

Other fish in Ross Lake - Ross Lake also has populations of Eastern Brook Trout (*Salvelinus fontinalis*) and Cutthroat Trout (*Oncorhynchus clarki*), both introduced from other previously stocked lakes nearby. During the 2006 fish community survey conducted by WDFW few Eastern Brook Trout were sampled, by 2008 they composed more than 50% by number of certain sample locations with increased distribution around Ross Lake (Downen 2011 and unpublished data). Much less is known about the Cutthroat Trout population in Ross Lake. During this study, in the January of 2010 Ross Dam sample they were captured along with Redside Shiners.

Brook Trout and Cutthroat Trout are adapted to similar habitat requirements and have similar diet habits as native char and Rainbow Trout. Eastern Brook Trout prefer clear and cool water, less than 20°C. In lakes the juveniles feed on zooplankton, and adults feed on zooplankton and midges. Adult Eastern Brook Trout will also feed on fish, shrimp, worms, snails, bees and beetles. Newly emerged Cutthroat Trout feed on zooplankton, and the fingerlings feed on aquatic insect larvae. Adult Cutthroat Trout diet items include adult insects, fish, snails and beetles (Wydoski and Whitney 2003).

Redside Shiner Introduction to Ross Lake

Ross Lake appears to be highly suited for the Redside Shiner. It meets all the environmental requirements reported for Redside Shiners, from shallow shoreline waters to deep pelagic water. In addition, the small and large tributaries to Ross Lake, with a variety of flow conditions, provide more potential habitat. Temperature in Ross Lake's shoreline habitat increases through the spring and summer (Figure 3), corresponding to the Redside Shiner's spawning and rearing thermal needs. Deep pelagic environments in Ross Lake are abundant, providing ample Redside Shiner winter refuge habitat.

Redside Shiner distribution and abundance shows an increase since discovery at the north end of the lake in 2003 or 2004. Since then the range has expanded south to the dam, and abundance has reached densities of hundreds per cubic meter in selected near shore habitats (Downen 2011).

Population estimates were formulated in 2006 and 2008 by snorkel surveys conducted by WDFW. In most of the lake the estimated numbers increased from 2006 to 2008 (Figure 4a and 4b).

Redside Shiners may have the potential to compete with native fish for food and other habitat requirements. According to the literature, both Redside Shiner adults and juvenile native fish consume aquatic insects as their most important food item. To a lesser degree, they both also consume terrestrial insects and mollusks. Preferred temperature ranges do overlap, indicating that

preferred habitats have the potential to overlap. Redside Shiners can occupy temperatures as low as 7°C, which is lower than the maximum temperature requirements for Bull Trout, Dolly Varden and Rainbow Trout, which are < 20°C. If juveniles leave the streams to utilize lake habitats within the same temperature ranges, there may be overlap in the habitat being used by each species of fish.

Growth and survival rates of juvenile Rainbow Trout associated with Redside Shiner introductions in other lakes have shown decreases. Possible advantages Redside Shiners have that could limit food resources for native trout are (Johannes and Larkin 1961; Vinyard and Yuan 1996):

- Redside Shiners can forage deeper into vegetated habitat consuming organisms, when trout cannot.
- Redside Shiners eat food items smaller than trout, potentially eliminating food sources before trout can access them. This also may reduce abundance of food available for trout.
- During summer, Redside Shiners and trout are associated with shoals that are heavily laden with zooplankton. However, Redside Shiners can concentrate directly above the shoals in warmer water and trout can only congregate near them in slightly deeper, cooler water. This also allows Redside Shiners to access prey before trout.
- Redside Shiners forage more meticulously, depleting food more in any given area.
- Redside Shiners may compete better than trout for zooplankton as water clarity decreases.

One goal of this project is to explore the common acceptance that has evolved with the introduction and increased presence of Redside Shiner in Ross Lake; which is that they are consuming resources that are unused by other fish in the lake (Resource Managers, Washington Department of Fish and Wildlife and North Cascades National Park, personal communication). Anecdotal information from

WDFW and anglers on Ross Lake provides evidence that the larger Rainbow Trout are readily consuming Redside Shiners. With this added information the original common acceptance surrounding the Redside Shiners continues to expand. In addition to assuming no resource competition, assumptions exist that attribute the increase in size of Rainbow Trout to the Redside Shiner introduction. This is perceived as an overall benefit to the native fish, with no concern for negative impacts due to resources overlapping or changes to the trophic web. Contrarily, Canadian biologist are presenting preliminary data that show increases in Bull Trout spawning in the Skagit River, and decreases in Rainbow Trout (D. Jesson, B.C. Ministry of Environment, unpublished data). They speculate that Bull Trout are successful at exploiting the Redside Shiners allowing them to dominate over Rainbow Trout in the Skagit River; the largest spawning tributary to Ross Lake for all native fish. Without further information and research, assumptions and speculations such as these cannot be confirmed or denied and management decisions and actions become limited and potentially insufficient. The objectives related to this goal are: 1) identify what the Redside Shiners in Ross Lake are consuming, 2) explore some of the patterns or differences in diet composition between sexes and among ages of Redside Shiners, 3) determine if there are any geographical or seasonal patterns in the Redside Shiner diet.

Another goal of this project is to start evaluating the long-term significance of the introduction of Redside Shiner to Ross Lake and potential for any long-term impacts to the native fish. The status of the Redside Shiner in Ross Lake is essentially unknown. However, the population seems to be increasing endlessly. Exploration of size and age structure, growth and mortality can provide information about population stability and help make predictions about future potentials. Objectives for this goal include: 1) determining Redside Shiner age over the maximum range of sizes using otoliths and scales, 2) make comparisons of the age structure of the Redside Shiner population in Ross Lake over time and space, 3) evaluate growth rates, 4) determine and compare mortality rates, 5)

and evaluate size structure geographically throughout Ross Lake and over the course of a growing season.

Methods

Sample Collection

Redside Shiner samples were collected in Ross Lake along a longitudinal gradient on a seasonal basis. Data from these samples were used to analyze size, age, growth, mortality and diet. The methods chosen for sampling ensured that random representative samples were collected for size class analysis, age determination and mortality and also that comprehensive samples were collected for age, growth and diet analysis. Graphical and statistical analysis allowed for comparisons of size, age, growth, mortality and diet across different locations in Ross Lake and over the time span of the study.

Locations and dates – Primary sample locations were established along the north to south axis of the lake to capture environmental gradients commonly present in reservoirs (Figure 5) (Wetzel 2001). When a large river is dammed, such as the Skagit River, depth, temperature and chemical gradients can form. Sample locations for this study were chosen to represent three main areas of Ross Lake the northern end, mid-lake and at the southern end, providing samples along the potential gradients that may exist in Ross Lake.

These primary locations were sampled in the winter, spring, summer and fall. Access, repeatability and distribution of Redside Shiners determined the specific sample locations. The northern samples consisted of one sample near Silver Creek in the spring and the rest near Hozomeen Creek. This change in sample location in the northern area of the lake was due to the fluctuations in lake level associated with power production operations. The sample location in the middle section of Ross Lake was near Lightning Creek. Collection locations at the southern end of Ross Lake included Roland

Creek and Ross Dam. Redside Shiner sampling events occurred seven times over the span of 11 months, November 2009 to September 2010 (Table 1).

Site characteristics – Two main environments were sampled. Sampling at the shallow sites Silver Creek, Hozomeen, Lightning Creek and Roland Creek was conducted in the littoral zone in areas with low angle lake bottom, using a beach seine. The substrate at Silver Creek, Lightning Creek and Roland Creek was mostly gravel and cobble with organic debris. The substrate at Hozomeen was dense submerged vegetation. Ross Dam was the only deeper water sample location. The samples collected near Ross Dam, using a fyke net, were always over water greater than 1 m, with little influence by substrate or vegetation.

Technique – After testing several methods of collection, beach seining and a modified Indiana style fyke net were the primary collection methods. During preliminary sample collection several methods were tested: trawling with an otter trawl; setting minnow traps at the shoreline, with and without bait; minnow traps in deep water with bait; beach seining; and trap netting with a fyke net. No fish were collected with the otter trawl and few fish were captured using the minnow traps. The beach seine and fyke net were the most successful collection methods. The seine was 4.5 m long by 1 m deep, with 5-mm bar mesh. Each seine pull was parallel to the shoreline for approximately 10 m in water less than 1 m deep. For collection in water greater than 1 m an Indiana style fyke net was set for 2 – 4 hours, except the Ross Dam sample in January which was set overnight. Modified to float, a 5-m lead was attached to the shore and the 1-m x 1-m trap frame, with 5-mm mesh, was pulled away from shore until taut.

Sample numbers – Random and comprehensive samples were collected for evaluation. The sample number target for the random sample was 100. If the initial sample of Redside Shiners was estimated to be near 100, total lengths of all the specimens were measured in the field. Samples with more than 100 Redside Shiners were haphazardly sub-sampled to a count of approximately 100 and also

measured for total length. Both approaches provided random representative samples for length frequency analysis, age determination and mortality (Anderson and Gutreuter 1983). Descriptive statistics and graphical comparisons were conducted using the statistical program R (Neumann and Allen 2007). The rest of each sample was checked to provide the compressive sample element to each sample. To ensure that size classes outside of the sub-sample would be represented in the age and diet analysis, the smallest and largest Redside Shiners were pulled from the original sample. This also provided an opportunity to identify any non-targeted fish species. To account for empty stomachs, the target was ten fish for every 10-mm size class (Davis and Savino 1994; Bowen 1996).

Sample preservation - The selected fish were euthanized, preserved and transported to the fish ecology lab at Huxley College of the Environment, Western Washington University. I euthanized fish with an overdose of MS222, according to the Journal of American Veterinary Association, (JAVMA 2000) and then placed them in a 10% buffered formalin solution, finally transferring them to ethyl alcohol until further processing (Bowen 1996).

Sample Analysis

Age - To determine ages of the Redside Shiners I used scales removed from the anterior surface of the body (Devries and Frie 1996). Fish from temperate regions have systematic patterns in the circuli of their scales that show irregularities when slow or ceased growth occurs in the winter because calcium is limited. These fluctuations in calcium vary the appearance of circuli showing small or re-absorbed circuli in the winter, creating an annulus, indicating a year of growth. Age determinations for Redside Shiners were used to evaluate length at age, growth and mortality.

Redside Shiner scales are covered with a highly resilient epidermal tissue that requires intensive cleaning to remove, so that annuli can be seen. I soaked scales in a 5% bleach solution, to remove the epidermal tissue (Whaley 1991). Larger, thicker and more robust scales were soaked for 12 to 24

hours, and small fragile scales needed a maximum of 1 hour, or a more dilute bleach solution. Using an Olympus SZ51 dissecting scope, the scales were further cleaned by hand with tweezers and a small brush and individually chosen to be glued with colored enamel between two slides for projection.

Otoliths, which also demonstrate systematic formation of annuli, were analyzed in conjunction with scales (Jearld 1983; Isely and Grabowski 2007). They were used to calibrate the scales for aging because scales are generally faster and easier to determine age, but when prepared properly otoliths can be more accurate. Age was determined for both scales and otoliths for approximately 15% of each sample used for age evaluation. These fish were used to create a set of guidelines to determine age for the remainder of the Redside Shiner in each sample using scales alone.

Growth and mortality statistics were developed using length at age values. Growth statistics were developed from the summer sample because the summer set of samples was the most complete (Ricker 1975). Length at age calculations were also applied to the representative length frequency distributions from spring and summer to evaluate Redside Shiner mortality in Ross Lake (Ricker 1975; Miranda and Bettoli 2007). In addition to determining the age from each scale, each annulus and the scale edge was measured so that length at age could be back-calculated. The Dahl/Lea method of direct proportion with a Fraser/Lee correction was used to back calculate length at age for the spring samples (Carlander 1969; Ricker 1975; Isely and Grabowski 2007). The pooling of samples and comparisons of length at age between groups, such as sex and ages, and comparisons among locations and over time were done graphically and with non-parametric statistical testing including Kruskal-Wallis and Wilcoxon tests.

Diet – The general dietary habits and morphological features of the Redside Shiner restricted the evaluation of stomach content. Minnows in the family Cyprinidae have an omnivorous diet that may include food items ranging from fish species to diatoms, making classification of diet items harder.

In addition to a large variety of diet items, pharyngeal teeth masticate and grind food beyond the ability to visually identify individual items (Hyslop 1980; Wootton 1998).

Individual fish selected for diet analyses were randomly chosen from each sample until all 5-mm size classes were represented by three guts containing contents. All diet data is based on fish that had some stomach contents. Empty guts were recorded but not used in diet evaluation.

Morphology again dictated the processing of samples for diet evaluations. Because Redside Shiners have no defined stomach, I removed the entire intestinal tract, from esophagus to anus, and preserved them in 99% alcohol. Due to the degree of grinding from pharyngeal teeth, wet weight of the entire gut content was the only measure of bulk that could be obtained, as opposed to volume or weight of individual diet items and entire diet categories. The intestinal tract was weighed before and after removal of contents. Weights were obtained using a calibrated Mettler Toledo, PR2003 DeltaRange precision scale to 0.001 g. I also re-measured length (mm), obtained a post preservation weight of the entire fish (g), determined sex and made notes on maturity and ripeness of each fish I processed for diet.

Stomach contents were removed and examined using an Olympus SZ51 dissecting scope. I categorized the diet items into taxonomic class, order or category. Contribution of each dietary category to the entire diet was determined qualitatively using the point method. The point method was originally developed to reduce the tedium involved in processing large amounts of material in samples using volumetric or numeric methods (Swynnerton and Werthington 1940; Hynes 1950). I adapted the point method to address the degree of grinding and inability to count or measure individual prey items. The point method awards points to each diet category proportional to its estimated contribution to the total stomach volume. For this study each dietary category was assigned a proportion of the total content.

I also determined fullness of each tract. Fullness was accounted for by dividing the entire intestinal tract into nine sections. Each section containing material was rated from zero to four, with zero being empty and four being full (Hyslop 1980). A full stomach was rated with the maximum of 36, a half full stomach would be 18 and a nearly empty would be less than 10.

Frequency of occurrence was based on the presence/absence determination of dietary items (Hynes 1950; Hyslop 1980; Bowen 1983; Chipps and Garvey 2007). The numbers of Redside Shiners that contained an item in a given dietary category were tallied, converted to proportions, and grouped based on age, sex, sample location and sample season. Using Excel graphical comparisons of groups and samples were used to identify any patterns.

To evaluate proportional volume, the proportion of total content assigned to each dietary category for each Redside Shiner were first adjusted according to the percent fullness of its intestinal track (Frost 1943). A full tract kept its original proportions; the proportions of a half full tract were reduced by half, etc. The adjusted proportions were adjusted and compared by age, sex, sample locations and sample seasons. To evaluate differences in proportional volume of dietary categories for these groups I used analysis of similarity (ANOSIM) and similarity percent (SIMPER) testing on Bray-Curtis similarity matrices. Proportional volumes were also compared with non-parametric multidimensional scaling (MDS) using Bray-Curtis similarity matrices. These are routines found in the program PRIMER v6.1 (Clark and Gorely 2006).

An ANOSIM is a randomized test performed on the Bray-Curtis similarity matrix. The difference between groups is measured by an R-value, which ranges between -1 and 1. Positive values indicate differences among groups, 0 indicates random grouping, and negative values represent samples that are more similar to samples of other groups than to samples of similar groups such as replicates, which may be caused by inappropriate sample designs (Chapman and Underwood 1999). When

ANOSIM returns a positive value for R ($P < 0.005$), a SIMPER will provide the contribution of each dietary category to the difference between groups (Clarke 1993).

The MDS constructs plots by rank order of the distances between the samples (Clarke 1993). The proximity of points within a sample on the plot is proportional to the degree of similarity. The associated stress value indicates scatter of points. A stress value < 0.1 indicates a plot that likely represents actual conditions and, a stress value > 0.2 indicates a plot where the locations of the points may be arbitrary (Clarke and Gorely 2006, cited in Penaluna 2006).

Results

To evaluate size, age, growth, mortality and diet of the Redside Shiner in Ross Lake seven visits were conducted from November 2009 to September 2010: November, January, May, June, August and twice in September (Table 1). A total of seventeen samples were collected during the seven visits and of those, twelve were used for data analysis. Size structure analysis was conducted with those twelve samples. Total length was measured for 2033 Redside Shiners. The same twelve samples were also used for age evaluation. Age was determined for a total of 178 Redside Shiners. The summer samples, a total of four samples, were used for growth evaluation. Mortality was evaluated for a total of eight samples, the spring and summer samples. And finally, diet was also evaluated for twelve samples. A total of 271 Redside Shiner intestinal tracts with contents were evaluated.

Size Structure

Analysis of size structure was conducted on beach seine and fyke net samples separately. Samples were compared by location and season. The beach seine samples from Silver Creek and the Hozomeen area in the north, Lightning Creek in the middle and Roland Creek at the south end of the lake were compared over time within each sample location and over location within each season. Fyke net samples from the southern boundary of the lake, near Ross Dam, were compared by seasons. These samples were chosen because the frequency of collections and methods for all these samples was consistent over the duration of this study, making comparisons across seasons and between sites consistent.

Redside Shiners ranged in size from 16 to 111 mm, though the majority of the samples had a predominance of small fish. The length-frequency histograms, with one-millimeter size class intervals, for all samples collected with the beach seine display high numbers of fish less than 60 mm

total length (Figures 6, 7 and 8). This is particularly evident in the samples from August and September; the majority of the fish were between 15 and 40 mm (Figures 7 and 8). All of the samples, except the sample from Lightning Creek in September, also included some larger fish, with the samples collected in May and June having the most (Figure 6).

Spatial comparisons by season show consistent uniformity in size characteristics in spring, and in summer (Figure 9A and 9B). Within spring and summer, the depth of the boxes show uniform variances among the samples and the whiskers show similar ranges in total length (mm). The samples collected in the summer and fall has decreasing median total lengths (dark central line in the boxes) from north to south. This decreasing trend potentially reflects a gradient in environmental conditions during the summer, most likely temperature as the northern portion of Ross Lake is shallow having warmer water than southern portions of the lake (Figure 9B). In the fall, the sample from Lightning Creek has a smaller median and it has much smaller range of lengths, indicating that the Lightning Creek sample was composed solely of small fish very closely related in size (Figure 9C).

Temporal comparisons within sample locations over the duration of the study indicate differences which coincide with beach seine and fyke net collection technique. Samples collected by beach seine in shallow water at Hozomeen, Lightning Creek, and Roland Creek, show decreasing median lengths over time, except from spring to summer at the north end of the lake where, the range of sizes decreased but the median did not (Figure 10). The median total lengths of the samples collected at Ross Dam, using a fyke net, remain similar over time, though the ranges do decrease (Figure 11).

Total length and weight of Redside Shiners were strongly linearly related across their size range when transformed to log₁₀ value. Regression analysis indicated that total length accounted for 99% of the

variation in weight ($r^2 = 0.99$; Figure 12). A population of fish with such strong relationship between length and weight, as determined by the high r^2 value, indicates a high degree of plumpness, or health.

Age, Growth and Mortality

Age - Redside Shiners collected during this study ranged in age from a few months old (young-of-the-year or age 0) to 6 years old (Table 2). Within age groups, mean length increased through the growing season from spring samples through fall with one exception; mean length of age 0 Redside Shiners decreased in length from summer to fall (Figure 13).

Samples collected within spring and within the summer, were pooled among sites. Lengths at age cohorts 1 – 4 for Redside Shiners collected in the spring were similar among sites (Kruskal-Wallis two-sample tests, see Table 3 for p-values; Figure 14). Similarly, lengths at age cohorts 0 – 3 for Redside Shiners collected in the summer do not differ among sites (Kruskal-Wallis two-sample test, see Table 4 for p-values; Figure 15). There were no age 4 fish in the summer sample and too few specimens collected in the older age cohorts 5 and 6 to compare length distributions among any season. Therefore, age analyses were conducted on pooled samples combined among sites within season, except age cohort 0 Redside Shiners collected in the fall, which were different (Wilcoxon test, $P = 0.00005$). Male and female Redside Shiners were also pooled among samples collected in both the spring and summer because length at age was not statistically different between the two sexes (Wilcoxon test, see Tables 5 and 6 for p-values; Figures 16 and 17). Redside Shiners caught in the fall were too immature to differentiate sex.

Standard methods of back-calculation underestimated length at age, requiring a correction factor. Back-calculation of the length at ages of the spring sample using the basic direct proportion method, also known as the Dahl/Lea method appeared to result in an underestimation of lengths at all ages based on the graphical difference between back-calculated length at age and actual lengths at age

(Figure 18). The Fraser-Lee correction method takes into account the delay in scale formation after hatching that is common in most scale-forming fish. The Fraser-Lee correction factor (y intercept or a) is derived by conducting a regression analysis on length at capture and scale radius at capture (Figure 19). When applied to the direct proportion equation, the resulting back calculated lengths at age are much closer to the actual length at age determined from the spring samples (Equation 1, Figure 20). When plotted by year these back calculations show consistency in length at age for the past five years (Figure 21). Mean lengths for ages 1 - 4 are clumped, with no individual age cohort diverging from the others.

$$\text{Equation 1. } L_i = a + (L_i - a)(S_i/S_c) \quad [a = 23.69]$$

L_i = Length at given year

a = y intercept

S_i = Scale radius at given year

S_c = Maximum scale radius at capture

Growth - In Ross Lake, Redside Shiner growth slows with age. This is reflected by comparing instantaneous rates of growth, which decrease as Redside Shiners age (Equation 2, Figure 22). Annual instantaneous growth rates decrease rapidly during the first 3 seasons of growth. After 3 seasons the changes become smaller, with less growth occurring after age cohort 3.

$$\text{Equation 2. } \textit{Instantaneous rate of growth} = \log_e l_2 - \log_e l_1$$

l_2 = Length at time two

l_1 = Length at time one

Mortality – To calculate mortality the length-based age structure of Redside Shiners was overlain on the representative samples to determine the proportion of fish at each age in the population. The small number of aged Redside Shiners were pooled within spring and within summer separately to estimate the age structure of the rest of the Redside Shiner population in spring and in summer (Tables 7 and

8). Age structure for fall samples was not assessed because they contain primarily age 0 and 1 Redside Shiners and mortality rates cannot be calculated.

The age-based instantaneous mortality rates calculated from spring samples and summer samples are similar. Instantaneous annual mortality rate (Z) was calculated as the absolute value of the slope of the regression line for the natural log of estimated number of fish at each age plotted against age in years. The instantaneous annual mortality rates of Redside Shiners collected in the spring is -0.8788 and in the summer is -0.8246 (Equation 3, Figures 23). Based on observation of year to year value, mortality is highest in the youngest fish, ages 1 - 2, and oldest fish, ages 4 - 6 (Figure 23).

$$\text{Equation 3. } N_t = (N_{t+1})e^{-Z}$$

N_t = Number of fish at age t

N_{t+1} = Number of fish age t plus 1

Z = Absolute value of slope of regression line

Diet

The variety of taxonomic groups consumed by Redside Shiners in Ross Lake included insects, microcrustaceans, various worm taxa, plants, algae, fish and debris. The only identifiable sub-category of insects were chironomids, otherwise insects were too masticated to be identified. Microcrustaceans were composed of ostromedusa, copepods, amphipods, isopods, and branchiopods. The branchiopods included the families; Daphniidae, Bosminidae, Chydoridae and Holopediidae. Algae were mostly diatoms. Fish included scales and eggs. Debris included all incidental items such as, sediment, wood particles and much other unidentified debris, including strings of what looked like blue tarp.

I condensed the diet items into categories: zooplankton, insects, cestodes, oligochaetes, algae, fish and incidentals. Zooplankton are all the microcrustaceans, insects refers to the all insects including *Chironomus*, oligochaetes is any segmented worm with the characteristics of Annelida, cestodes are

incidences of the parasite *Ligula intestinalis* as a diet item, algae refers to any green material, which is assumed to be diatoms, fish presents as fish scales and fish eggs, and incidental debris, which appeared to be sediment, wood and any of the other unidentifiable substances.

Overall a quarter of all the stomach samples looked at were empty and not used for diet evaluation. Proportions of empty guts did not vary by size, age or sex, but did vary by sampling method and season. In general, 12% of the fish in the beach seine samples had empty guts. The exception was the August Hozomeen beach seine sample; only half of the Redside Shiners captured there had contents in their guts. The fyke net samples had larger proportions of empty guts. Of the total Redside Shiners captured at Ross Dam in the spring, 90% had empty guts, 23% in August and 53% in January.

Frequency of occurrence - Sexes and ages were combined to evaluate frequency of occurrence differences between samples. I analyzed presence/absence of diet items and calculated the proportion of stomachs in which a particular diet category occurred within a given sample or group of Redside Shiners. Sample refers to the entire sample of fish collected in a given location during a given time, and group is the fish within a sample that share a characteristic such as age or sex. Sample sizes were not large enough to compare frequency of occurrence among groups of ages and sexes independently so, I pooled males and females to evaluate differences among age, and I pooled ages to evaluate between sexes.

Percent volume - In addition to frequency of occurrence, I evaluated the proportional volume of each of the diet category for a given sample or group of Redside Shiners. After accounting for fullness of guts, I calculated the proportion of the volume of a given diet category to the total volume for the entire diet for that sample. Using ANOSIM and SIMPER the sample size for analysis of proportional volume at each sample site was not large enough to compare ages and sexes independently, so I pooled sexes to evaluate age related differences in diet, and I pooled ages to evaluate sex related differences in diet.

Diet according to sex – Diet does not appear to differ between male and female Redside Shiners therefore can be pooled for further analysis. There are no strong patterns when evaluating frequency of occurrence by percent (Figures 24 to 26). At Lightning Creek, males consumed a larger variety of items than females in the spring and summer (Figure 24 and 25). As well, diet does not differ greatly between males and females when evaluating proportional volume (Figures 27 to 29). The only difference was found at Ross Dam in the summer, where proportions of insects and incidentals consumed produced a statistical difference between males and females (Table 9). Having incidental debris consumption contribute 25% of the dissimilarity between males and females reduces the impact of this statistical result, making it more important to pool the samples to compare ages.

Diet according to age – Diet among ages was evaluated specifically to determine if Redside Shiners of various ages are eating similar or different diet items. For frequency of occurrence, diets among ages of Redside Shiners were similar, and were pooled (Figures 30 to 32). At most sites during the summer, all ages of Redside Shiners (0 to 6) consumed zooplankton and insects at high frequencies. Redside Shiners of all ages also consumed incidental debris also. In the spring, cestodes and fish were only consumed by the older fish, > 2 years in age (Figure 30). In the summer zooplankton were consumed in less frequency after age 1 (Figure 31). Proportional volume of dietary categories among ages was similar enough to pool ages for further evaluation (Figures 33 to 35).

Diet among sites within seasons – Zooplankton and insects were the most frequently consumed diet categories in the spring. Zooplankton is the dietary category consumed at the highest frequency at all sites. Insects are the second highest at all sites followed by incidentals, fish, oligochaetes, and cestodes, all ranking differently among sites. No algae are consumed in the spring at any of the sites (Figure 36).

Within the typical results just presented, there are some additional frequencies of occurrence patterns between sites in the spring. The frequency of occurrence of zooplankton was 20% higher at Lightning

Creek and Ross Dam than at Silver Creek and Roland Creek. The opposite relationship is shown for insects. The frequency of occurrence for insect was 50% higher at Silver Creek and Roland Creek than at Lightning Creek and Ross Dam. Cestodes were only present in shallow water sample locations. Oligochaetes were only consumed at Silver Creek and Roland Creek. Fish, eggs and scales were present in a few fish guts at all sites as were incidental items, except at Lightning Creek.

Proportional volume of dietary categories within spring mimics the same pattern as described for frequency of occurrence for spring (Figure 36). Comparisons among all sites indicated that the composition of the diet in the spring at each site was uniquely different from the other three sites (ANOSIM, $P = 0.001$, Table 10). The proximity of points within samples on the non-parametric MDS is proportional to the degree of similarity, indicating separation among spring samples (Figure 37).

Although Ross Dam and Lightning Creek statistically differed, they were the most similar, with average dissimilarity value of 37%; compared to values of 92 % between Ross Dam and Silver Creek and Ross Dam and Roland Creek and the highest value, 95%, between Silver Creek and Lightning Creek (SIMPER). Proportional volumes of zooplankton and insects were primarily responsible for the differences between sites. Oligochaetes were secondarily responsible.

Summer samples were more similar among sites than in spring (Figure 38). Zooplankton and insects are the most frequently consumed dietary categories across all sites; an average of 70%, except zooplankton at Ross Dam, which is quite low (10%). Cestodes and oligochaetes are not consumed at any site, but algae, fish items and incidental items are at all sites (Figure 38). Frequency of occurrence of algae, fish and incidental dietary categories are lower than zooplankton and insects, at about 20%, and comparable across sites.

Proportional volume of dietary categories among sites within summer follows the same pattern as described for frequency of occurrence for summer (Figure 38). Proportional volumes of dietary categories during summer are graphically distinct, but not as clearly as spring (Figure 39). Hozomeen

and Ross Dam samples are the only comparison that is statistically similar (ANOSIM, $P = 0.001$, Table 11). In addition, average dissimilarity percentages are smaller in the summer than in the spring. Zooplankton, insects and incidental debris explain 95% of the dissimilarity in all cases.

Exclusively small fish collected in the fall show a distinct difference in diet between sample locations. A small size range of fish was collected at Hozomeen and Lightning Creeks. Cestodes, oligochaetes, algae and fish were not consumed at Lightning Creek, but only fish and cestodes are missing from the Hozomeen sample (Figure 40). Proportional volume was analyzed only for the Hozomeen sample (Figure 40). Zooplankton and insects accounted for most of the volume of the diet, with oligochaetes adding the final 10%. Algae and incidental debris are negligible.

Diet among season within sites – In the northern portion of the lake, Silver Creek and Hozomeen, zooplankton and insects are the most frequently consumed dietary categories, however zooplankton consumption decreases by season, and insects increase by season (Figure 41). Cestodes were only found in the spring and oligochaetes in the spring and fall, but not in summer. Algae were not present in the spring, spiked in frequency in the summer and dropped to a lower frequency in the fall. Fish items were present in the spring and summer, but not in the fall. Incidental items decreased in frequency from spring to fall.

Proportional volume of zooplankton and insects among seasons at the northern portion of Ross Lake does not match the frequency of occurrence results (Figure 41). Zooplankton and insects increase in proportional volume from spring to fall, as opposed to just the insects. Diets differed between spring and summer, and between spring and fall, but not summer and fall (ANOSIM, $p = 0.001$, Table 12). The graphic produced by the MDS depicts these differences well (Figure 42). Insects, zooplankton and incidentals make up 92% of the difference between spring and summer, but between spring and fall it was insects, zooplankton and oligochaetes (96%).

At Lightning Creek, mid-lake, zooplankton and insects are again the most frequent diet categories consumed. Even so the frequency of zooplankton decreased from summer to fall, and that of insects increased from spring to summer (Figure 45). Cestodes were present only in the spring, and algae were only present in the summer. Fish items increased from spring to summer and incidental debris decreased from summer to fall, and oligochaetes were not present at all. Zooplankton contributed most of the volume with the other categories contributing little, including insects (Figure 45). Proportional volume was similar between spring and summer, and no data was available fall (ANOSIM, $p = 0.511$).

In the south portion of the lake, at Roland Creek, as with all other sites, zooplankton and insects are the most frequently consumed dietary categories, as they increased from spring to summer (Figure 46). Cestodes and oligochaetes were only present in the spring, and algae were only present in the summer. Fish and incidental debris increased from spring to summer. Zooplankton and insects were also the dominate diet categories in terms of proportional volume, and were similar from spring to summer (ANOSIM, $P = 0.059$).

Diet characteristics vary greatly by seasons at Ross Dam, the most southern sample location. For both frequency of occurrence and proportional volume, Ross Dam had a different diet category that was dominant for winter, spring, and summer, in winter algae, in spring zooplankton and in summer insects (Figure 43). Proportional volume of diet categories are statistically dissimilar among seasons (ANOSIM, $p = 0.001$, Table 13). The proximity of points within each of the seasons indicates how similar the samples are within season (Figure 44). The sample separation on the MDS is strong reflecting how different they are. Dissimilarity values are 86% between spring and summer, 99% between spring and winter, and 95% between summer and winter.

Discussion

Diet

Contrary to the perception that the Redside Shiners are using food resources unused by other fishes, particularly the native fishes in Ross Lake, the most important dietary categories of the Redside Shiner are zooplankton and insects, in terms of both frequency of occurrence and proportional volume of the overall diet. In 90% of the samples from spring to fall, zooplankton was found in more than half of all the Redside Shiners evaluated. Lower than zooplankton, in 70% of the samples evaluated, but still especially important, insects were found in half of the Redside Shiners evaluated. Either zooplankton or insects were the dominant dietary categories, in terms of percent volume, in all of the samples evaluated. Food previously assumed to have been consumed such as algae and incidental debris occurred in fewer than 30% of the Redside Shiners evaluated, for any given sample, for both frequency of occurrence as percent and proportional volume.

In accordance with historical accounts, the diet results from this study indicate that Redside Shiners should be considered something other than omnivorous, because they mainly consume zooplankton and insects. The Redside Shiners studied in Yellowstone Lake were termed carnivorous, consuming midges, zooplankton and insects, with only traces of aquatic plants (Biesinger 1961). In other waters, Redside Shiners were principally insectivorous consuming primarily beetles, dipterans and other insects, but also few gammarids and traces of algae (Weisel and Newman 1951). In Pinanton Lake, British Columbia, algae contributed up to 69% of total volume of Redside Shiner diet, but only for one afternoon sample, otherwise they consumed mainly zooplankton and various types of insects (Johannes and Larkin 1961).

Feeding strategy and importance of diet categories can be assessed through graphical representation of frequency of occurrence and measurements of prey volume. Presented in Chipps and Garvey (2007), the Costello graph is a graphical model used to depict feeding strategy (Figure 47) (Costello

1990). When applied to the samples evaluated from Ross Lake in the spring and summer, zooplankton and insects are the only dietary items in the “dominant prey” area of the graph (Figures 48 and 49). Otherwise the other diet categories are generally categorized as “rare prey.” This reinforces the conclusion that zooplankton and insects are the most important diet categories for Redside Shiners. This graphical method will be particularly useful to compare changes in feeding strategy over time in Ross Lake.

Competition

The current premise about the proliferation of Redside Shiners in Ross Lake being a benefit to native fish as an additional food source with no evidence of consequences is not accurate. In a size-structured community, one in which larger individuals consumes smaller ones, such as the community in Ross Lake, predators can be affected by competition between their juveniles and their potential prey. According to the “competitive juvenile bottleneck theory”, competition with prey may result in reduced recruitment and growth rates of the juveniles of the predators (Byström et al 1998). For example, in the experimental Abborrtjärn Lakes in Sweden, the prey fish roach (*Rutilus rutilus*) were introduced into to natural populations of perch (*Perca fluviatilis*), a predator (Byström et al 1998). The introduced fish did not affect larval perch, but they did affect juvenile perch. Larval perch were defined as young of the year in their first month of growth. Fish that had grown beyond that first month, through the first growing season were considered juveniles. Towards the end of the growing season, growth and condition of the juvenile perch were negatively affected. These negative effects were mainly due to diet shifts at the end of the growing season resulting in direct competition between the roach and perch for food. Ultimately, starvation and over winter and spring mortality increased in juvenile perch.

The results of this study and the information that is known about the diets of the trout species in Ross Lake all lead to my conclusion that the native fish in Ross Lake may be susceptible to competition

between juveniles and Redside Shiners. Although, Redside Shiners in Ross Lake show geographic differences and seasonal shifts in diet, zooplankton and insects are the primary dietary categories consumed during every life stage. Juvenile Bull Trout, Rainbow Trout, non-native Brook Trout and Cutthroat Trout also feed on zooplankton and insects. Unless food resources are partitioned spatially and seasonally, then direct competition seems likely.

Other reports suggest that Redside Shiners have had negative impacts on the existing fish in systems where they have been introduced. Generally in cold water systems, introductions of Redside Shiner often times result in competition and are considered undesirable (Wydoski and Bennet 1981). After the Redside Shiner was established in Paul Lake British Columbia, amphipods, previously a food item heavily relied upon by Rainbow Trout, were diminished to the point of being negligible in trout diets (Johannes and Larkin 1961). Ultimately, competition for food with Redside Shiner resulted in reduced growth and survival of the Rainbow Trout juveniles (Larkin and Smith 1954).

The exclusive diet of algae during the winter may increase the competitive effect. It takes a diet item that was assumed to have been consumed by the Redside Shiner in lieu of diet items important to the native fish, and turns it against the native fish instead. All fish species in Ross Lake experience harsh conditions during winter. Temperatures decrease, reaching 5° C, and resources become less abundant. Redside Shiner are using algae during the winter when the other fish in Ross Lake are not. Previous studies attributed wider distribution and better likelihood to survive periods of food shortage between two species of fresh-water sticklebacks, *Gasterosteus aculeatus* and *Pygosteus pungitius*, to algae consumption in the winter. During periods of food shortage the fresh-water stickleback *G. aculeatus* ate algae, whereas *Pygosteus pungitius* did not, suggesting that *G. aculeatus* may have been more likely to survive a food shortage which would have given it an advantage over *P. pungitius* (Hynes 1950). The Redside Shiners consumption of algae during the winter may give them increased

over winter survival and increased health, likely to exacerbate the competitive juvenile bottleneck theory.

Presence of multiple species of additional introduced fish increases the uncertainty surrounding the potential impacts of the Redside Shiners to Bull Trout and Rainbow Trout in Ross Lake. In addition to Redside Shiners, Ross Lake has populations of non-native Brook Trout and Cutthroat Trout. Scientific surveys conducted on Ross Lake suggest Brook Trout may be increasing in distribution, population size and growth (M. Downen, Department of Fish and Wildlife, unpublished data). In a small lake in Sweden, controlled experiments with Perch (*Perca fluviatilis*), Ruffe (*Gymnocephalus cernuus*) and Roach (*Rutilus rutilus*) showed that manipulation of densities of Ruffe, a benthic insectivore, and Roach, a planktivore, affected the growth of the Perch (Bergman and Greenberg 1994). In this study, the Perch experienced decreased growth because of dietary overlap with each of the two specialized feeders. Introductions and increases of additional fish such as Brook Trout and Cutthroat Trout, in Ross Lake, could potentially increase the demand for certain food items, especially insects. Because Redside Shiners are prolific consumers of zooplankton, Bull Trout and Rainbow Trout may experience the adverse effects of overlapping diets among several species.

Diet complexities

The difference in diet of the Redside Shiner in the Ross Dam sample, compared to the other locations may be explained by the habitat differences at the collection site. Silver, Lightning and Roland Creek samples were all collected in shallow water less than 1m deep, with gravel to cobble substrate, high levels of particulate matter and no woody debris. The Ross Dam site had deeper water greater than 1m deep with no bottom substrate influencing the samples. The differences in habitat can lead to differences in distribution and abundance in diet items available to Redside Shiners in Ross Lake; as the different diet at Ross Dam indicates. This means that the Redside Shiners in Ross Lake are exploitative feeders.

Additional differences may be based on temperature, bathymetry, location to different tributaries and longitudinal location in the lake. The proximity of all the sample locations to tributaries adds the elements of localized hydrologic regimes, temperature regimes. Each tributary uniquely affects the local food web (Wetzel 2001). Inherent characteristics in a reservoir environment may also contribute to spatial differences in Redside Shiner diets. Generally the three distinct zones in a reservoir are a riverine zone, a zone of transition and a lacustrine zone (Wetzel 2001). Each zone has complex and dynamic hydrologic, physical and chemical characteristics that could influence biota including the prey of Redside Shiner. Evidence of diets shifting over location has been well documented. Diets of the bream differed statistically between two differing basins within Lake Balaton (Biró et al 1991). The diets of five co-existing cyprinids differed between two contrasting basins (Vinni et al 2000).

Variability in use of lacustrine zones may also affect feeding. For some of the Redside Shiner samples, combinations of zooplankton, insects, oligochaetes, sediment and small particles of wood, in one fish gut, indicate that several habitats are being exploited. Zooplankters are water column dwelling macro-crustaceans. However, the insects and oligochaetes, which were too masticated to identify beyond basic class or order, in combination with sediment and wood, suggest benthic feeding. This provides evidence of small scale diet complexity in addition to large scale factors identified by the different diet characteristics at Ross Dam.

Age, Growth and Future Persistence

The maximum estimated age of Redside Shiners in Ross Lake was 6 years. Houston and Belk (2006) and Scott and Crossman (1973) both report 7 and 8 years. Wydoski and Whitney (2003), however, report that few live beyond 5 years.

The length at age of Redside Shiners in Ross Lake support Houston and Belk's (2006) contention that the growth rates of Redside Shiners run counter to the typical pattern of decreasing length at age with

increase in northern latitudes. Length at age is comparable, if not a bit larger than populations of Redside Shiners studied in Utah, Idaho and Wyoming, especially at greater ages (Houston and Belk 2006) and smaller than populations reported farther north in British Columbia (Carlander 1969; Scott and Crossman 1973). Compared to a population from Yellowstone Lake, the population of Redside Shiner in Ross Lake is slower growing but has greater longevity (Biesinger 1961). Reduced growth after the first year is common among all reports.

Back-calculated length at age of Redside Shiners in Ross Lake indicates that the introduced fish will most likely continue to be stable in Ross Lake, confirming that there is potential for long-term impacts to the native fish. Much like the literature and presence of large number of Redside Shiners currently in Ross Lake suggest the stability in the back-calculated lengths at age presents evidence that Ross Lake provides a suitable environment. The low variability in mean total length at each age in every year class of Redside Shiners indicates a pattern of consistent growth going back five years. Projecting this into the future, assuming Redside Shiners do proliferate at densities present today or maybe even increase allows the risk of trophic changes, competition and negative impacts to native fish to continue.

Parasites

Ligula intestinalis was detected in Redside Shiners during this study. *L. intestinalis* is a cestode parasite common to minnows. Older Redside Shiners are often bloated by the presence of *L. intestinalis* causing direct and indirect death (Scott and Crossman 1973). During this study *L. intestinalis* was found during three seasons, in the spring at Silver and Roland Creeks, in the summer at Hozomeen and again in the fall at Hozomeen. It was found in 6 of the 271 Redside Shiners evaluated for diet. The smallest Redside Shiner infected was 46 mm and 1 year old. Though the Redside Shiner in Ross Lake are infected with *L. intestinalis* the other native and non-native fish are ultimately not at risk for direct infection. It was discovered in less than 1% of Rainbow Trout in Lake

Ototoa, New Zealand, making it likely that fish in Ross Lake other than the Redside Shiner are not going to be infected (Weekes and Penlington 1985).

L. intestinalis infection in the Redside Shiner in Ross Lake is one factor that could have an impact contrary to the speculations created from the back-calculated results, which may still lead to negative impacts to the native fish of Ross Lake. Direct infection is not a threat to the native fish in Ross Lake. However, an increase in infection rates and mortality due to infection may cause indirect effects by altering the Redside Shiner population in terms of mortality and abundance. The Redside Shiner has been integrated into the Ross Lake food web being consumed by adult trout, more importantly adult Bull Trout and Rainbow Trout. The degree to which the adult native fish depend on the introduced and integrated Redside Shiner population is under question. Alterations such as an increase in mortality and reduction in population could reduce or even remove a food source that the native fish have become dependent on.

The speculated result of increased mortality and decreased population and concern about effects to native fish in Ross Lake originate from the complicated life history of *L. intestinalis*. The life cycle of *L. intestinalis* has four parts: parasite eggs pass out of an avian host in feces; free swimming coracidia are ingested by copepods; copepods are consumed by fish; and fish are consumed by birds (Hoole et al. 2010). Any one of these parts can change, and increase the spread of *L. intestinalis* and presumably increase mortality, ultimately reducing Redside Shiner abundance in Ross Lake.

The literature suggests that in a minnow population the size of the Redside Shiners in Ross Lake, *L. intestinalis* would typically be found at a higher frequency than was discovered. One factor that could aid this population in reaching the rates suggested in the literature is the presence and number of birds on Ross Lake. Casual observation by NOCA resource managers, suggests a recent (2010) and noticeable increase in birds on Ross Lake since the introduction of Redside Shiners (G. Cook, NOCA (Ret.), personal communication). An increase in birds widens one of the links in the complicated

lifecycle of the parasite potentially leading to increases in infection. Fish bloated and weakened by *L. intestinalis* will not only be at risk to direct mortality but also mortality due to bird consumption, overall resulting in increased mortality. If advanced far enough and spread fast enough this could result in changes to first the Redside Shiner population and then to the native fish. Again, confirming the potential for negative impacts to native fish in the long-term.

Size Structure

The observed decreases in mean total length, which is atypical in repetitive sampling, of Redside Shiners over the course of the growing season may be an effect of migratory patterns. Typically when sampling the same location over the duration of the growing season you may see an increase in the mean total length as fish grow. This is the case in many other studies. The mean total length of the minnow, *Barbus anoplus*, increased with every sampling event over the course of each year in a reservoir in South Africa (Cambray and Bruton 1985). As described in the introduction, Redside Shiners have daily, seasonal and life history migration patterns related to size or maturation or predator avoidance and seasonal patterns related to spawning and hatching. Larval and juvenile Redside Shiners tend to occur closest to shore and larger Redside Shiners occupy water slightly off shore (Scott and Crossman 1973). Beach seining was efficient enough to collect several age classes of Redside Shiners, however, it may have selected for the larval and juvenile Redside Shiners that would have been seeking refuge, or in the case of the fall samples just emerging. Because trap nets select for larger sized fish, perhaps using the fyke net in combination with beach seining would have collected samples that are more representative and show seasonal growth (Laarman and Ryckmann 1982).

Conclusion

The Redside Shiner in Ross Lake, introduced in the early 2000s and reaching extreme densities today has the potential to threaten the native fish in Ross Lake. Redside Shiners predominantly feed on zooplankton and insects, which are the same items necessary for juvenile Bull

Trout and juvenile Rainbow Trout growth and survival. In addition to the Redside Shiner, Brook Trout and Cutthroat Trout have been introduced to Ross Lake, and their juveniles also feed on insects and zooplankton. However, the dynamics of all of the introduced populations of fish in Ross Lake are under studied. The ability for Redside Shiners to use algae as a food item during the winter may increase their fitness over the winter reducing mortality. This advantage could keep the Redside Shiner population intact for many years to come, further exacerbating the competitive juvenile bottleneck theory. Back calculated length at age supports the notion of Redside Shiner stability in Ross Lake over time. Redside Shiner growth from 2005 to 2010 reveals consistent length at age for all ages sampled. The absence of much variation or a potential trend indicating reduced growth or fitness over time suggests that the population has sufficient environmental resources such as food, spawning habitat and refuge to persist at high numbers for many years. A better understanding of potential competition and the specific mechanisms that could be involved in competition will be hard to identify for the native fish of Ross Lake without more details on the complexities and patterns of the diets of all the fishes in Ross Lake; especially the native juveniles.

Table 1. Redside Shiner collection effort on Ross Lake, November 2009 to September 2010. This summarizes all the effort for the duration of this study. Sample events with no fish captured are indicated and samples that were analyzed are indicated.

| Month, Year | Capture Methods | | | |
|--------------------------|---|--|-------------------------------|------------------------------|
| | Otter trawl | Beach seine | Fyke net | Minnow traps |
| November, 2009 | Hozomeen ⁰ Lightning Creek ⁰ Roland Creek ⁰ Ross Dam ⁰ | | Ross Dam ⁰ | Ross Dam Dry Creek |
| January, 2010 | | Lightning Creek ⁰ Dry Creek ⁰ Big Beaver Creek ⁰ Roland Creek ⁰ | Ross Dam ^{1,2,5} | Ross Dam |
| May, 2010 | | Silver Creek ^{1,2,4,5} Lightning Creek ^{1,2,4,5} Big Beaver ⁰ Roland Creek ⁰ | Ross Dam ^{1,2,4,5} | Roland Creek |
| June, 2010 | | Roland Creek ^{1,2,4,5} | | |
| August, 2010 | | *Silver Creek ⁰ Hozomeen ^{1,2,3,4,5} Lightning Creek ^{1,2,3,4,5} Big Beaver Roland Creek ^{1,2,3,4,5} | Ross Dam ^{1,2,3,4,5} | Lightning Creek ² |
| Early September, 2010 | | Hozomeen ^{1,2,5} | Hozomeen | |
| Late September, 2010 | | Silver Creek Hozomeen ^{1,2,5} Lightning Creek ^{1,2,5} Roland Creek | | |

⁰No fish captured, *Present but could not capture

Analyses conducted - ¹ Size frequency, ² Age, ³ Growth, ⁴ Mortality, ⁵ Diet

Table 2. Age, length and weight of Redside Shiners collected in Ross Lake. Redside Shiners, age cohorts 0 - 6 were collected in spring, in summer and in fall. Samples from spring and summer were pooled regardless of collection method, either beach seine or fyke net.

| | Seasons of Growth | N | Total Length (mm) | | Weight (g) | |
|-------------------|-------------------|----|-------------------|-----------|------------|-------------|
| | | | Mean | Range | Mean | Range |
| Age 0 - August | 0+ | 8 | 32 | 29 - 36 | 0.26 | 0.15 - 0.37 |
| Age 0 - September | 1 | 19 | 28 | 19 - 36 | 0.22 | 0.05 - 0.38 |
| Age 1, Spring | 1 | 17 | 37 | 31 - 41 | 0.42 | 0.26 - 0.63 |
| Age 1, Summer | 1+ | 32 | 46 | 35 - 64 | 1.01 | 0.35 - 2.47 |
| Age 1, Fall | 2 | 12 | 53 | 46 - 61 | 1.48 | 0.84 - 2.22 |
| Age 2, Spring | 2 | 22 | 52 | 42 - 60 | 1.19 | 0.53 - 1.86 |
| Age 2, Summer | 2+ | 10 | 61 | 55 - 67 | 2.01 | 1.41 - 2.65 |
| Age 2, Fall | 3 | 2 | 67 | – | 3.07 | 3.02 - 3.12 |
| Age 3, Spring | 3 | 21 | 65 | 58 - 75 | 2.64 | 1.52 - 4.14 |
| Age 3, Summer | 3+ | 8 | 71 | 64 - 79 | 3.42 | 2.18 - 4.81 |
| Age 3, Fall | 4 | 1 | 77 | – | 3.73 | – |
| Age 4, Spring | 4 | 19 | 75 | 62 - 84 | 4.22 | 1.83 - 5.65 |
| Age 4, Summer | 4+ | 1 | 81 | – | 3.17 | – |
| Age 4, Fall | 5 | – | – | – | – | – |
| Age 5, Spring | 5 | – | – | – | – | – |
| Age 5, Summer | 5+ | 3 | 89 | 85 - 91 | 6.50 | 6.14 - 7.09 |
| Age 5, Fall | 6 | – | – | – | – | – |
| Age 6, Spring | 6 | 1 | 92 | – | 6.53 | – |
| Age 6, Summer | 6+ | 2 | 102 | 101 - 102 | 9.14 | 8.74 - 9.74 |

Table 3. Mean total length (mm) of Redside Shiners at age were similar among sites for samples collected from Ross Lake in spring, 2010 (Kruskal-Wallis rank sum test, alpha = 0.05). Silver Creek, Lightning Creek and Roland Creek were beach seined. Ross Dam was captured by fyke net. Male and female Redside Shiners are combined (Figure 16, Table 6).

| Age | Mean Total Length (mm) | | | | p-value |
|-----|------------------------|-----------------|--------------|----------|---------|
| | Silver Creek | Lightning Creek | Roland Creek | Ross Dam | |
| 1 | 38 | 36 | 36 | 35 | 0.3709 |
| 2 | 52 | 55 | 50 | 52 | 0.3201 |
| 3 | 65 | 65 | 63 | 67 | 0.7553 |
| 4 | 76 | 76 | 75 | 75 | 0.9594 |
| 5 | – | – | – | – | – |
| 6 | – | – | – | 92 | N/A |

Table 4. Mean total length (mm) of Redside Shiners at age were similar among sites for samples collected from Ross Lake, in summer, 2010 (Kruskal-Wallis rank sum test, alpha = 0.05). Silver Creek, Lightning Creek and Roland Creek were beach seined. Ross Dam was captured by fyke net. Male and female Redside Shiners are combined (Figure 17, Table 7).

| Age | Mean total length (mm) | | | | p-value |
|-----|------------------------|-----------------|--------------|----------|---------|
| | Hozomeen | Lightning Creek | Roland Creek | Ross Dam | |
| 0 | 36 | 32 | 31 | — | 0.2763 |
| 1 | 47 | 49 | 49 | — | 0.6205 |
| 2 | 59 | 64 | — | 63 | 0.3757 |
| 3 | — | 68 | — | 76 | 0.0990 |
| 4 | — | — | — | 81 | N/A |
| 5 | — | — | — | 89 | N/A |
| 6 | — | — | — | 102 | N/A |

Table 5. Lengths at age cohort were similar between male and female Redside Shiners from Ross Lake in spring, 2010 (Wilcoxon tests).

| Age | Males | | Age | Females | | p-value |
|-----|-------|------------------------|-----|---------|------------------------|---------|
| | N | Mean Total Length (mm) | | N | Mean Total Length (mm) | |
| 2 | 10 | 53 | 2 | 7 | 54 | 0.8192 |
| 3 | 11 | 66 | 3 | 11 | 65 | 0.7749 |
| 4 | 8 | 75 | 4 | 19 | 77 | 0.3970 |

Table 6. Lengths at age cohorts were similar between male and female Redside Shiners from Ross Lake in summer, 2010 (Wilcoxon test).

| Age | Males | | Age | Females | | p-value |
|-----|-------|------------------------|-----|---------|------------------------|---------|
| | N | Mean Total Length (mm) | | N | Mean Total Length (mm) | |
| 1 | 19 | 50 | 1 | 9 | 46 | 0.08011 |
| 2 | 19 | 60 | 2 | 3 | 61 | 0.79280 |
| 3 | 6 | 71 | 3 | 2 | 69 | 1 |
| 4 | 1 | 81 | 4 | — | — | N/A |
| 5 | 2 | 88 | 5 | 1 | 90 | 1 |
| 6 | 2 | 102 | 6 | — | — | N/A |

Table 7. Age distribution in a sample of Redside Shiners captured in spring, 2010 from Ross Lake. Subsample (X) was used to establish the age-length relationship, which was then applied to total number of fish (Sample(Y)) in each size class to determine proportions of captured fish in each age class (Totals) for Sample (Y). Samples among sites were pooled.

| Size-class (mm) | Subsample (X) | Age-groups in X | | | | | | Sample (Y) | Calculated age representation in Y | | | | | |
|-----------------|---------------|-----------------|-----------|-----------|-----------|----------|----------|------------|------------------------------------|------------|-----------|-----------|----------|----------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 |
| 30-34 | 4 | 4 | – | – | – | – | – | 54 | 54 | – | – | – | – | – |
| 35-39 | 9 | 9 | – | – | – | – | – | 54 | 54 | – | – | – | – | – |
| 40-44 | 8 | 5 | 3 | – | – | – | – | 86 | 54 | 32 | – | – | – | – |
| 45-49 | 4 | 1 | 3 | – | – | – | – | 34 | 9 | 26 | – | – | – | – |
| 50-54 | 7 | – | 7 | – | – | – | – | 49 | – | 49 | – | – | – | – |
| 55-59 | 8 | – | 7 | 1 | – | – | – | 39 | – | 34 | 5 | – | – | – |
| 60-64 | 11 | – | 1 | 9 | 1 | – | – | 25 | – | 2 | 21 | 2 | – | – |
| 65-69 | 11 | – | – | 9 | 2 | – | – | 31 | – | – | 25 | 6 | – | – |
| 70-74 | 8 | – | – | 4 | 4 | – | – | 37 | – | – | 19 | 19 | – | – |
| 75-79 | 5 | – | – | 3 | 2 | – | – | 36 | – | – | 22 | 14 | – | – |
| 80-84 | 5 | – | – | – | 5 | – | – | 13 | – | – | – | 13 | – | – |
| 85-89 | – | – | – | – | – | – | – | 5 | – | – | – | – | 5 | – |
| 90-94 | 1 | – | – | – | – | – | 1 | 3 | – | – | – | – | – | 3 |
| Totals | 81 | 19 | 21 | 26 | 14 | 0 | 1 | 466 | 170 | 143 | 91 | 54 | 5 | 3 |

Table 8. Age distribution in a sample of Redside Shiners captured in summer, 2010 from Ross Lake. Subsample (X) was used to establish the age-length relationship, which was then applied to total number of fish (Sample(Y)) in each size class to determine proportions of captured fish in each age class (Totals) for Sample (Y). Samples among sites were pooled.

| Size-class (mm) | Subsample (X) | Age-groups in X | | | | | | | Sample (Y) | Calculated age representation in Y | | | | | | |
|-----------------|---------------|-----------------|-----------|-----------|----------|----------|----------|----------|------------|------------------------------------|------------|-----------|-----------|-----------|-----------|----------|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| 25-29 | 3 | 3 | - | - | - | - | - | - | 4 | 4 | - | - | - | - | - | - |
| 30-34 | 4 | 4 | - | - | - | - | - | - | 51 | 51 | - | - | - | - | - | - |
| 35-39 | 4 | 1 | 3 | - | - | - | - | - | 150 | 38 | 113 | - | - | - | - | - |
| 40-44 | 8 | - | 8 | - | - | - | - | - | 149 | - | 149 | - | - | - | - | - |
| 45-49 | 10 | - | 10 | - | - | - | - | - | 52 | - | 52 | - | - | - | - | - |
| 50-54 | 6 | - | 6 | - | - | - | - | - | 14 | - | 14 | - | - | - | - | - |
| 55-59 | 8 | - | 4 | 4 | - | - | - | - | 17 | - | 9 | 9 | - | - | - | - |
| 60-64 | 6 | - | 1 | 4 | 1 | - | - | - | 17 | - | 3 | 11 | 3 | - | - | - |
| 65-69 | 5 | - | - | 2 | 3 | - | - | - | 16 | - | - | 6 | 10 | - | - | - |
| 70-74 | 1 | - | - | - | 1 | - | - | - | 9 | - | - | - | 9 | - | - | - |
| 75-79 | 3 | - | - | - | 3 | - | - | - | 10 | - | - | - | 10 | - | - | - |
| 80-84 | 1 | - | - | - | - | 1 | - | - | 13 | - | - | - | - | 13 | - | - |
| 85-89 | 1 | - | - | - | - | - | 1 | - | 10 | - | - | - | - | - | 10 | - |
| 90-94 | 2 | - | - | - | - | - | 2 | - | 4 | - | - | - | - | - | 4 | - |
| 95-99 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 100-104 | 2 | - | - | - | - | - | - | 2 | 3 | - | - | - | - | - | - | 3 |
| Totals | 64 | 8 | 32 | 10 | 8 | 1 | 3 | 2 | 519 | 93 | 339 | 26 | 31 | 13 | 14 | 3 |

Table 9. Results from ANOSIM testing of proportional volume as % compositions of dietary items among ages and between sexes for each sample (alpha = 0.05).

| | | Hozomeen | | Lightning Creek | | Roland Creek | | Ross Dam | |
|---------------------|----------|----------|--------|-----------------|--------|--------------|--------|----------|--------|
| | | Age | Sex | Age | Sex | Age | Sex | Age | Sex |
| Spring ^a | Global R | 0.03 | -0.037 | 0.179 | 0.163 | -0.019 | 0.021 | 0.221 | -0.064 |
| | p-value | 0.222 | 0.684 | 0.054 | 0.059 | 0.645 | 0.260 | 0.093* | 0.649 |
| Summer | Global R | 0.221 | -0.209 | 0.076 | -0.251 | -0.054 | -0.148 | -0.011 | 0.116 |
| | p-value | 0.050 | 0.984 | 0.247 | 0.847 | 0.673 | 0.946 | 0.487 | 0.027* |
| Fall | Global R | 0.211 | -0.067 | | | | | | |
| | p-value | 0.050 | 0.647 | - | - | - | - | - | - |

^aSilver Creek, near the Hozomeen area.

* Statistically significant relationships indicating dissimilarity.

Table 10. Results from the ANOSIM testing of proportional volume as % of dietary items among spring sample sites. All pairs are significant. (Global R = 0.370, p-value = 0.001, alpha is 0.05)

| Groups | | | Global R | p-value | Assessment |
|-----------------|-----|-----------------|----------|---------|------------|
| Ross Dam | vs. | Roland Creek | 0.228 | 0.003 | Dissimilar |
| Ross Dam | vs. | Lightning Creek | 0.167 | 0.006 | Dissimilar |
| Ross Dam | vs. | Silver Creek | 0.410 | 0.001 | Dissimilar |
| Roland Creek | vs. | Lightning Creek | 0.272 | 0.001 | Dissimilar |
| Roland Creek | vs. | Silver Creek | 0.478 | 0.001 | Dissimilar |
| Lightning Creek | vs. | Silver Creek | 0.526 | 0.001 | Dissimilar |

Table 11. Results from the ANOSIM testing of proportional volume as % of dietary among summer sample sites. All but one pair are significant. (Global R = 0.222, p-value = 0.001, alpha is 0.05)

| Group | | | Global R | p-value | Assessment |
|-----------------|-----|-----------------|----------|---------|----------------|
| Hozomeen | vs. | Lightning Creek | 0.315 | 0.002 | Dissimilar |
| Hozomeen | vs. | Roland Creek | 0.125 | 0.019 | Dissimilar |
| Hozomeen | vs. | Ross Dam | 0.009 | 0.392 | Not dissimilar |
| Lightning Creek | vs. | Roland Creek | 0.131 | 0.017 | Dissimilar |
| Lightning Creek | vs. | Ross Dam | 0.451 | 0.001 | Dissimilar |
| Roland Creek | vs. | Ross Dam | 0.19 | 0.002 | Dissimilar |

Table 12. Results from the ANOSIM test of proportion of total volume of dietary items of northern samples sites by season. (Global R = 0.310, p-value = 0.001, alpha is 0.05)

| Seasons | | | Global R | p-value | Assessment |
|---------|-----|--------|----------|---------|----------------|
| Spring | vs. | Summer | 0.288 | 0.001 | Dissimilar |
| Spring | vs. | Fall | 0.425 | 0.001 | Dissimilar |
| Summer | vs. | Fall | 0.049 | 0.134 | Not dissimilar |

Table 13. Results from the ANOSIM testing of relative volume percent of dietary items ANOSIM test of Ross Dam samples by season. (Global R = 0.770, p-value = 0.001, alpha is 0.05)

| Seasons | | | Global R | p-value | Assessment |
|---------|-----|--------|----------|---------|------------|
| Spring | vs. | Summer | 0.513 | 0.001 | Dissimilar |
| Spring | vs. | Winter | 0.959 | 0.001 | Dissimilar |
| Summer | vs. | Winter | 0.806 | 0.001 | Dissimilar |

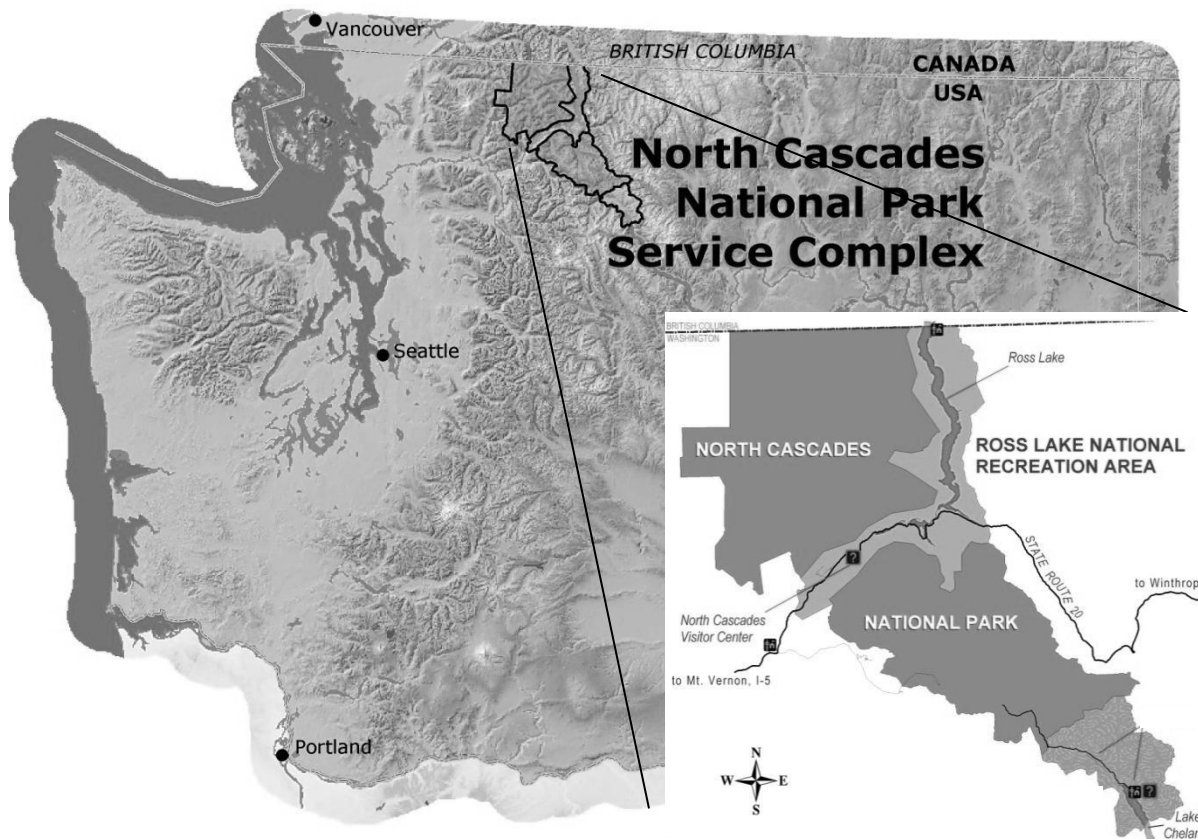


Figure 1. Location of NOCA in Washington State (main map), and Ross Lake in NOCA and RLNRA (expansion). (North Cascades National Park 2003, 2007)

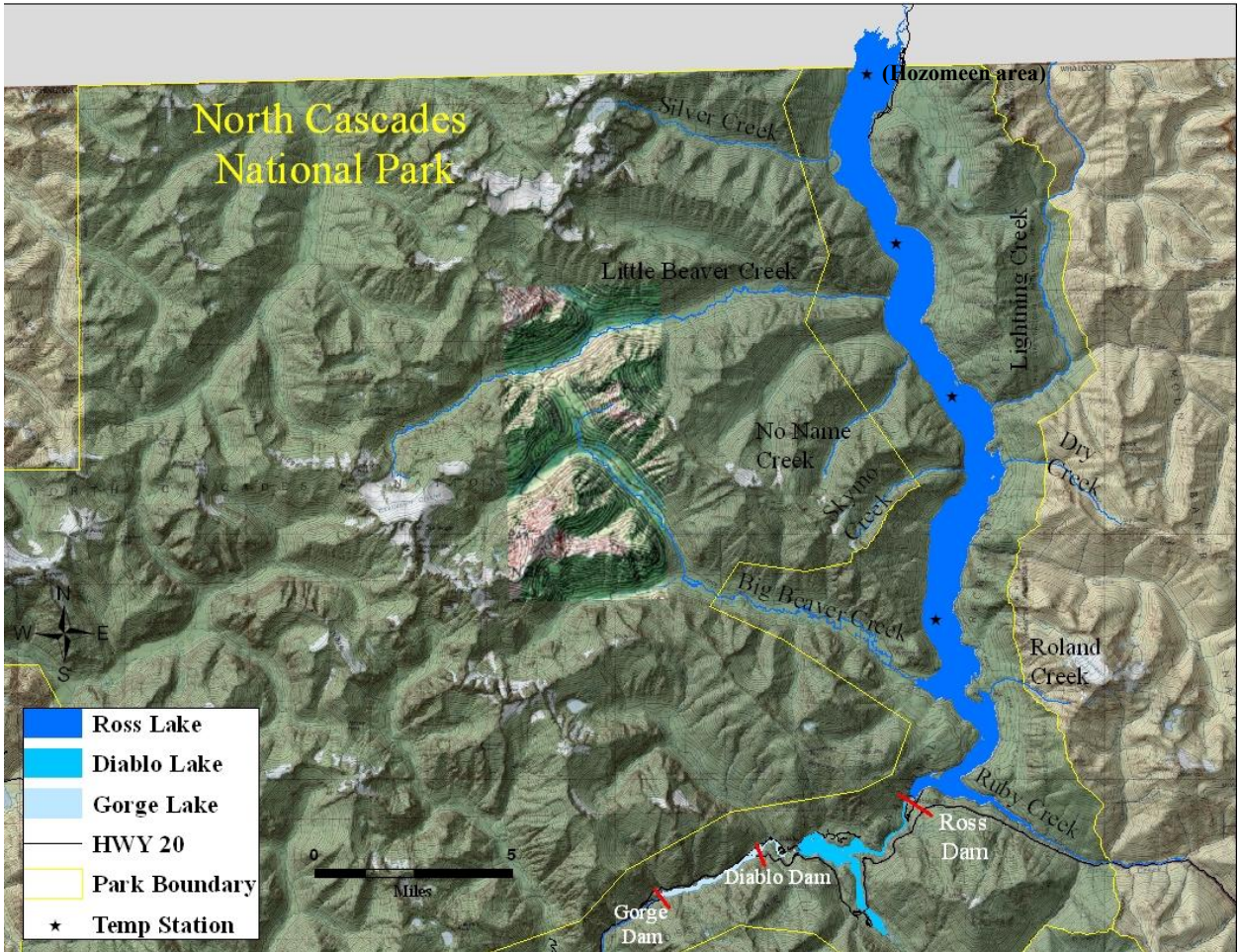


Figure 2. The three impoundments of the Skagit River, formed by Gorge Dam, Diablo Dam and Ross Dam, which compose the Skagit River Hydroelectric Project and major tributaries to Ross Lake. (Temperature monitoring stations being serviced by NOCA are starred, from north to south they are: Hozomeen, Little Beaver, Skymo and Pumpkin Mountain.)

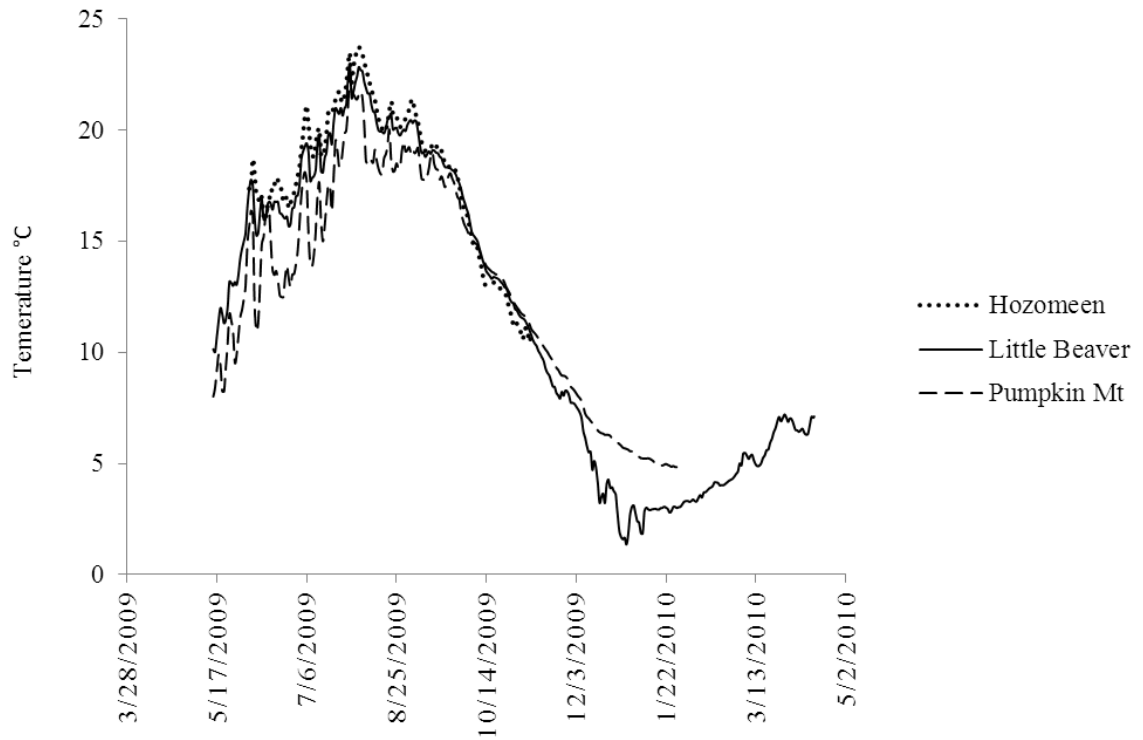


Figure 3. Ross Lake surface temperatures at Hozomeen, Little Beaver and Pumpkin Mountain from May 2009 to May 2010. No data is available for the Skymo station because it was tangled in submerged snags and not recovered. (unpublished data, North Cascades National Park)

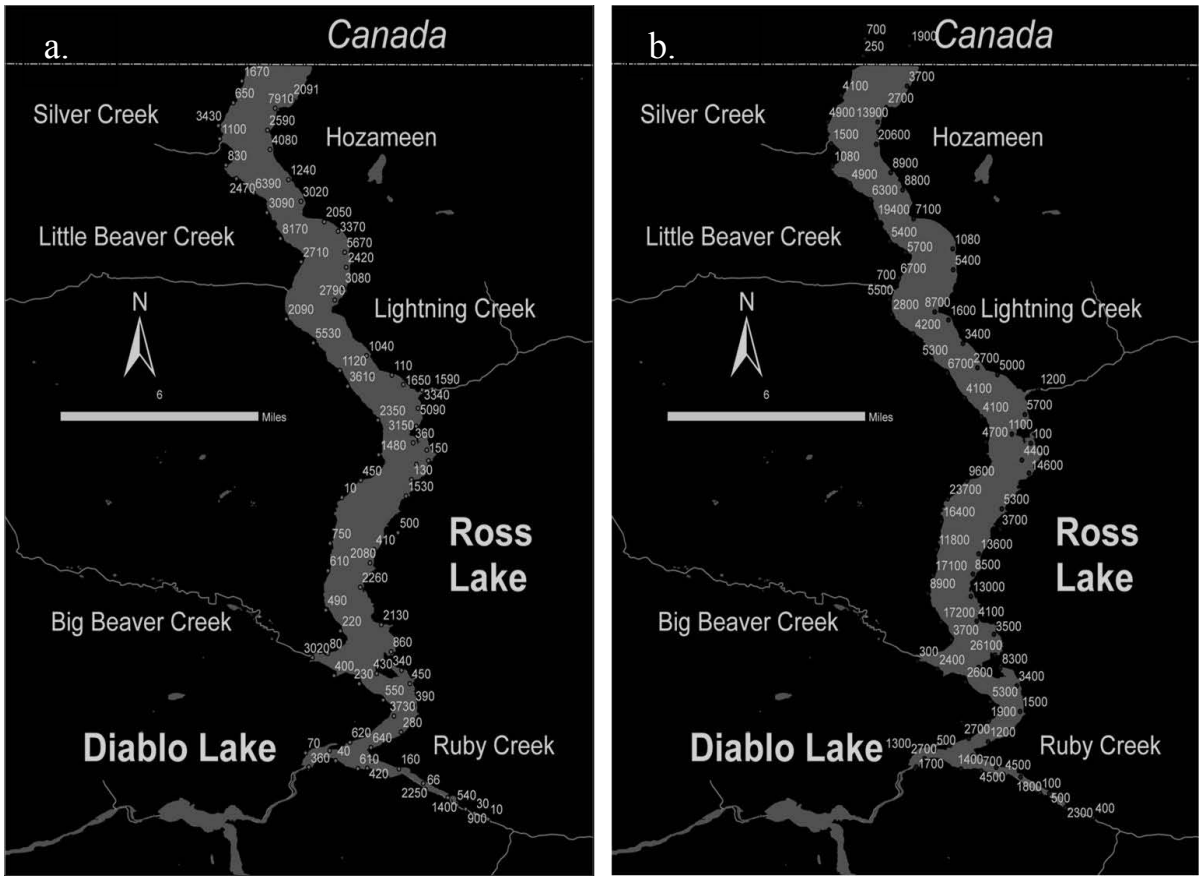


Figure 4a and 4b. Results from snorkel survey estimates of Redside Shiner abundances in Ross Lake, (a, 2006; b, 2008) conducted by WDFW. (Adapted from Downen, unpublished)

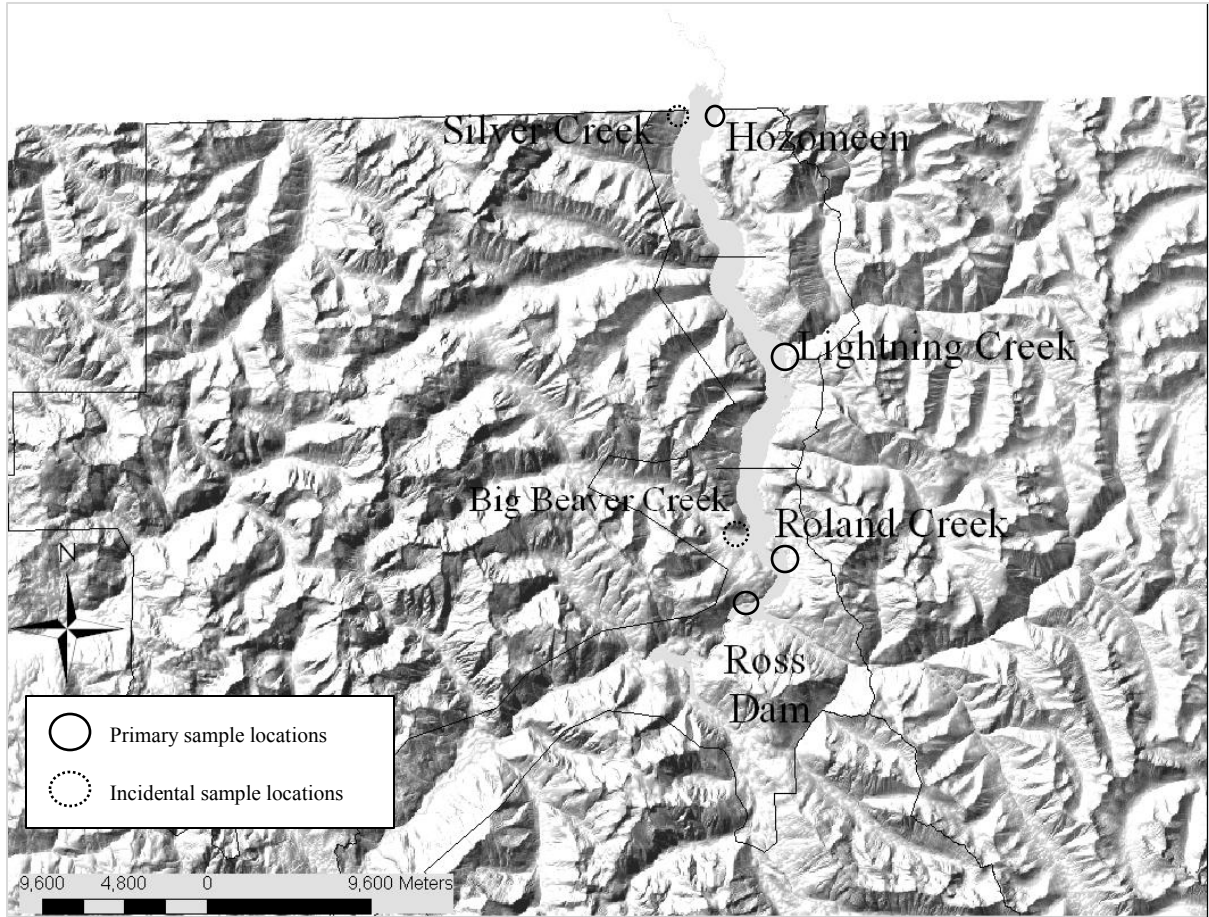


Figure 5. Redside Shiner collection locations on Ross Lake, from November 2009 to November 2011. Solid circles locate the primary collection locations that were repeatedly sampled and dashed circles are locations incidentally sampled. The northern, middle and southern parts of the lake are separated by horizontal bars.

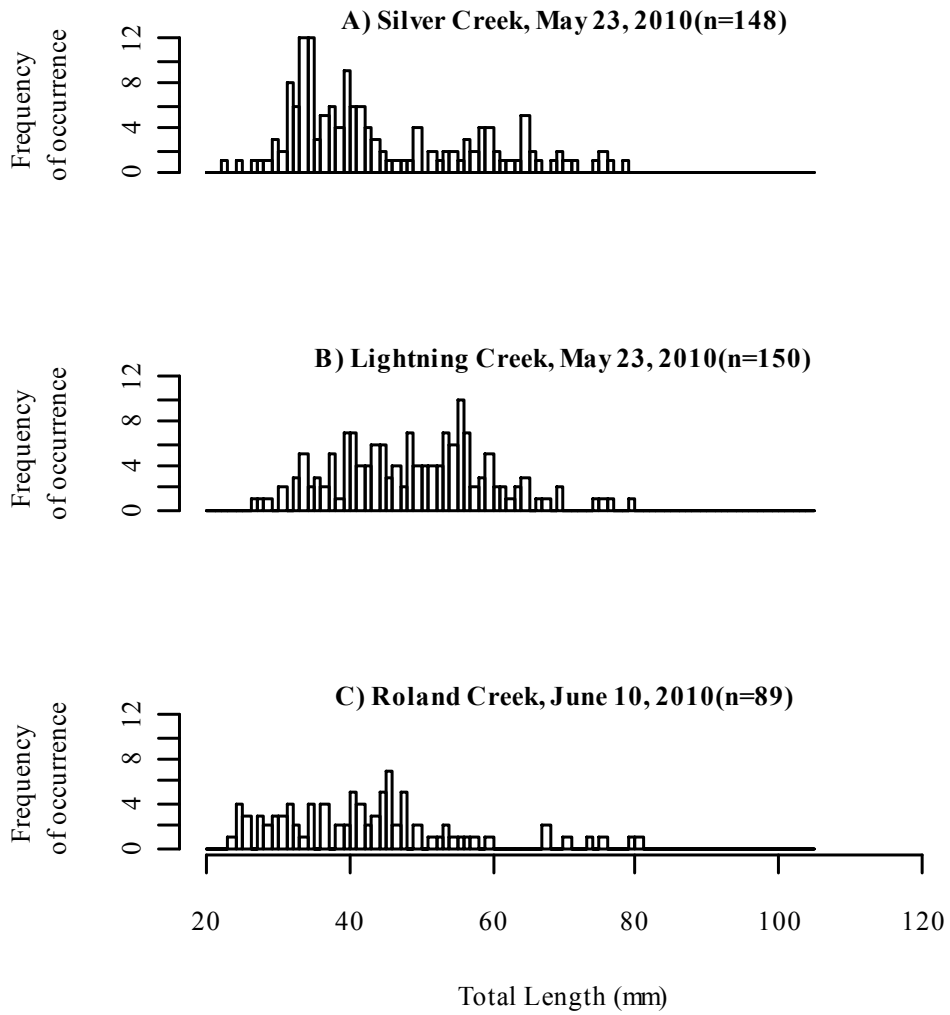


Figure 6. Length-frequency distribution based on 1-mm groupings for samples collected in the spring using beach seine: A) Silver Creek; B) Lightning Creek; C) Roland Creek.

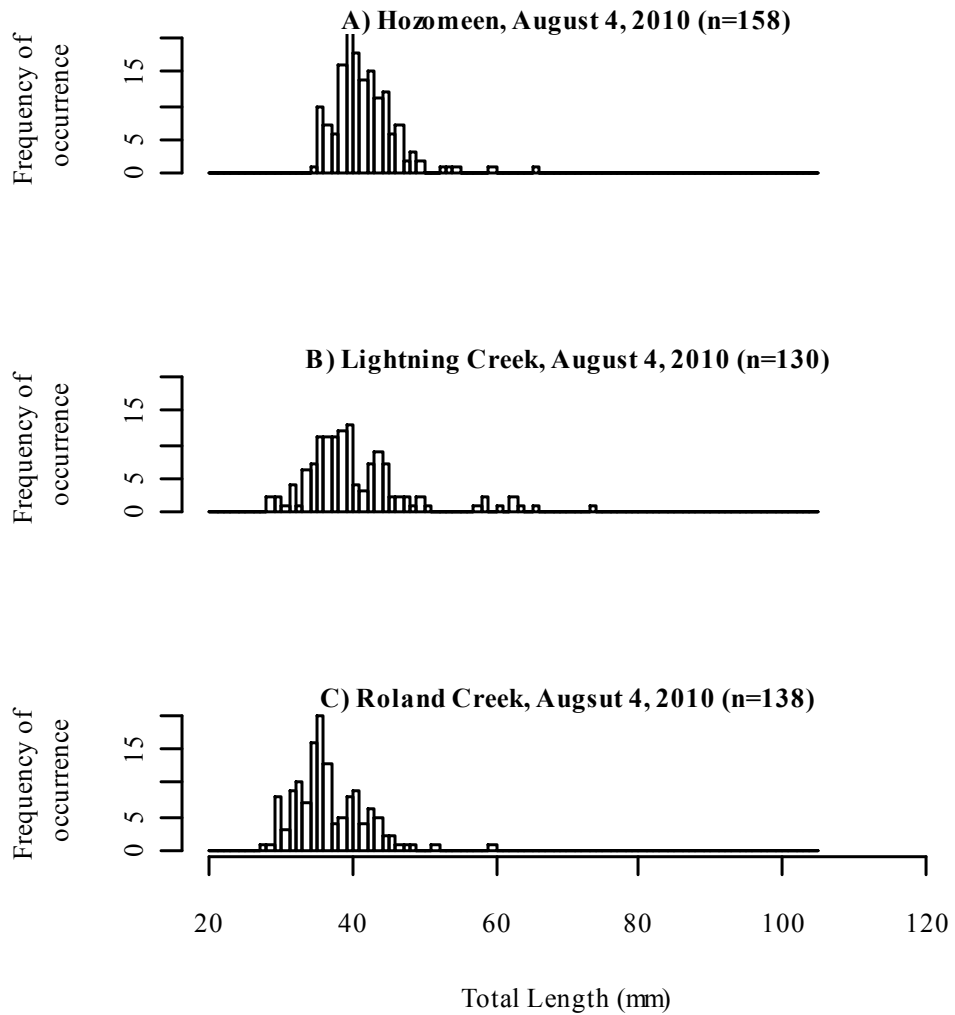


Figure 7. Length-frequency distribution based on 1-mm groupings for samples collected in the summer using beach seine: A) Hozomeen; B) Lightning Creek; C) Roland Creek.

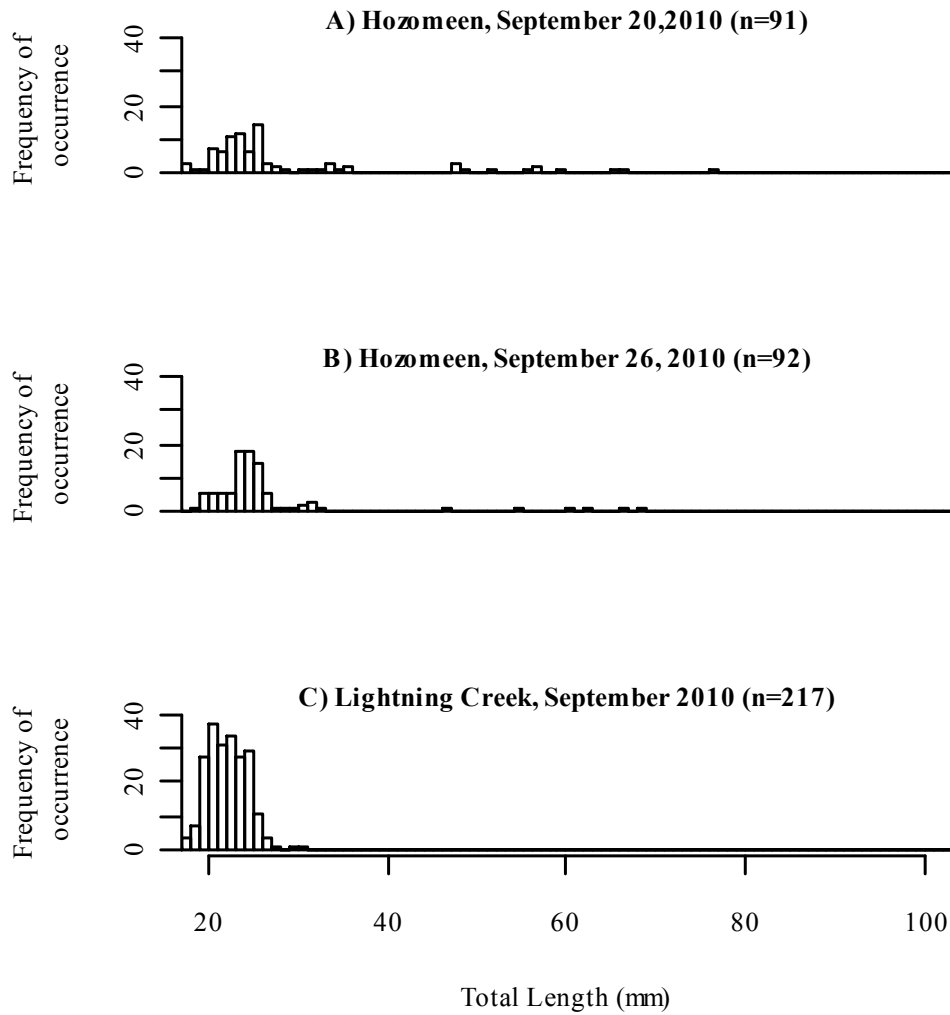


Figure 8. Length-frequency distribution based on 1-mm groupings for samples collected in the fall using beach seine: A) 1st Hozomeen sampling event; B) 2nd Hozomeen sampling event; C) Lightning Creek.

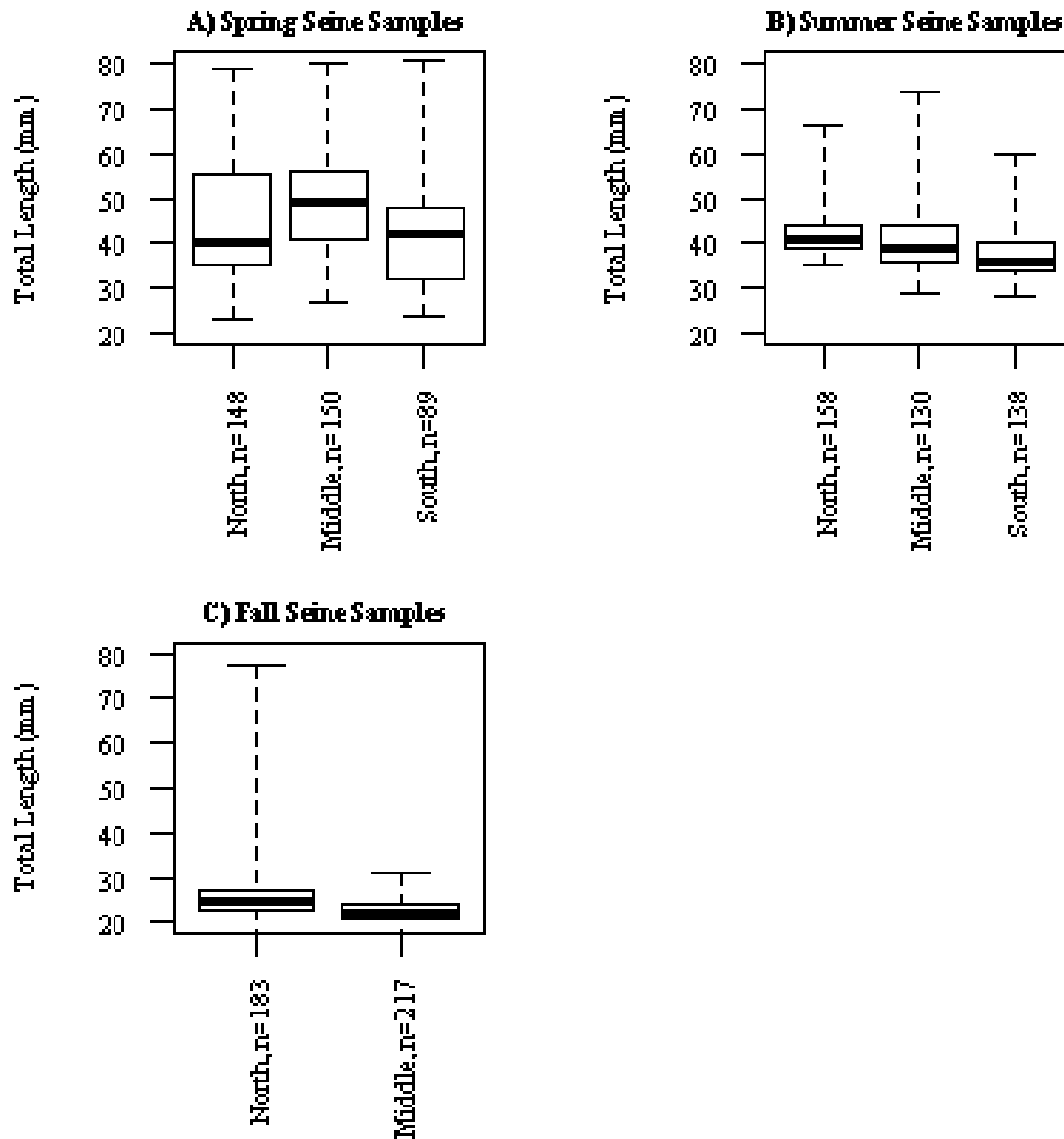


Figure 9. Box plots comparing the length distribution of Redside Shiners from each of the sample locations along the north-south axis of the lake: **A)** Northern spring sample collected from Silver Creek, May 23, 2010; middle one from Lightning Creek, May 23, 2010; and southern one from Roland Creek, June 10, 2010; **B)** All summer samples were collected August 4, 2010, from Hozomeen, Lightning Creek and Roland Creek; **C)** Collected on September 20 and 26, 2010, fall Hozomeen samples were pooled and Lightning Creek was collected on September 26, 2010. There is no fall Roland Creek sample, (C).

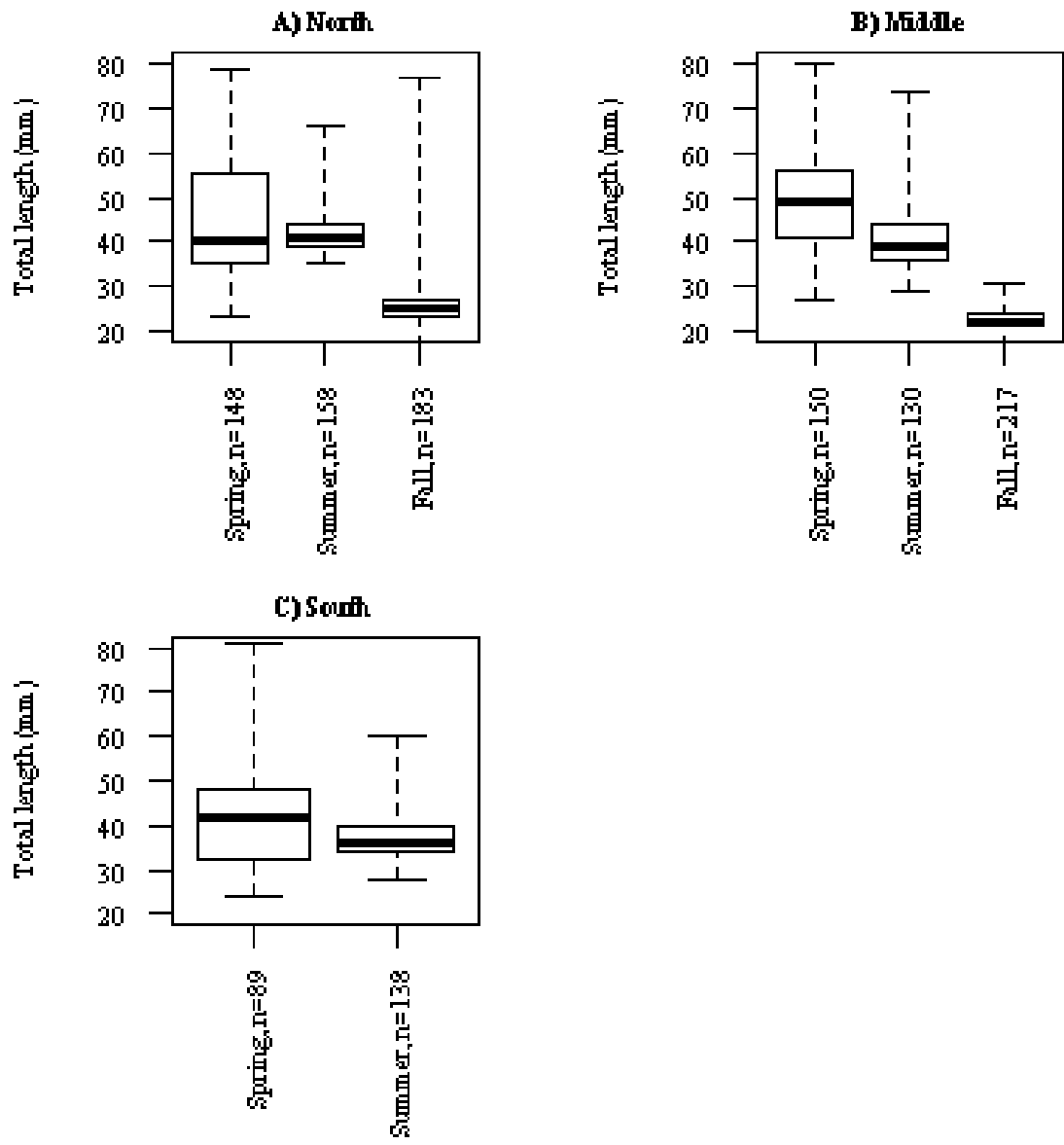


Figure 10. Box plots comparing the length distribution of Redside Shiners from each of the seasons sampled; spring, summer, and fall: **A)** The sample representing spring was from Silver Creek, and samples from summer and fall were from Hozomeen; **B)** Samples from spring to fall collected at Lightning Creek; **C)** Samples from spring and summer collected from Roland Creek. There was no sample collected from Roland Creek in the fall (C). Dates correspond to ones given in Figure 9 description.

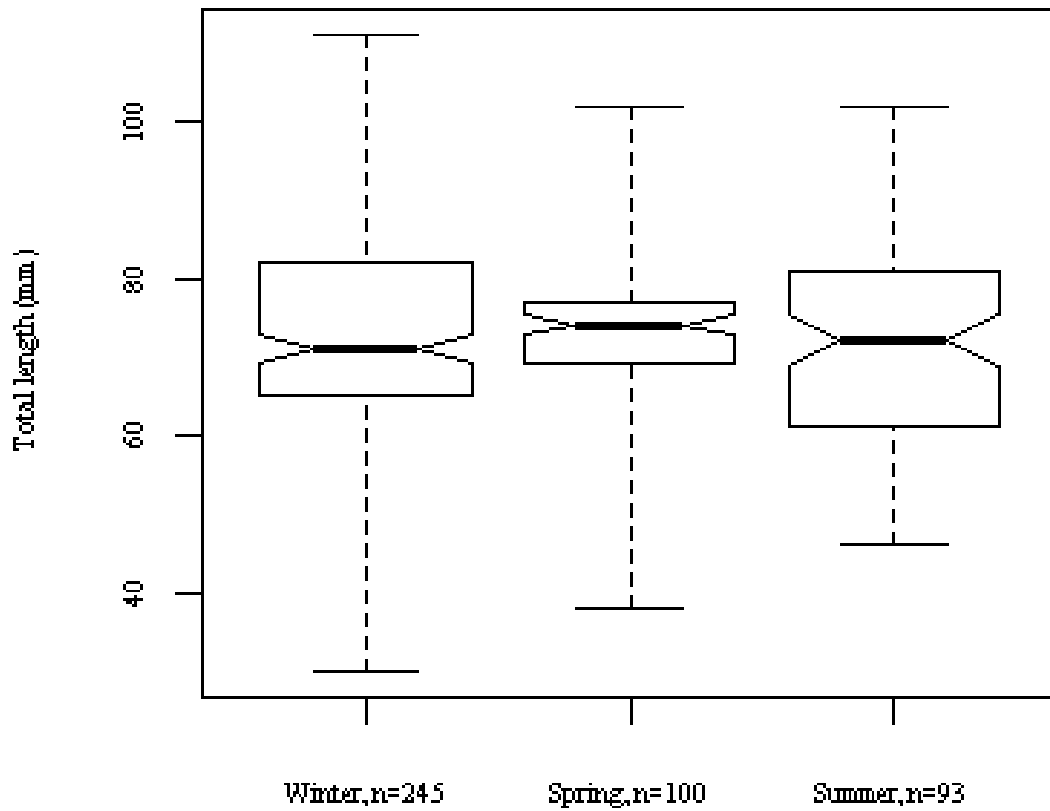


Figure 11. Samples collected by fyke net set in water deeper than 3 meters near Ross Dam in January, May and August.

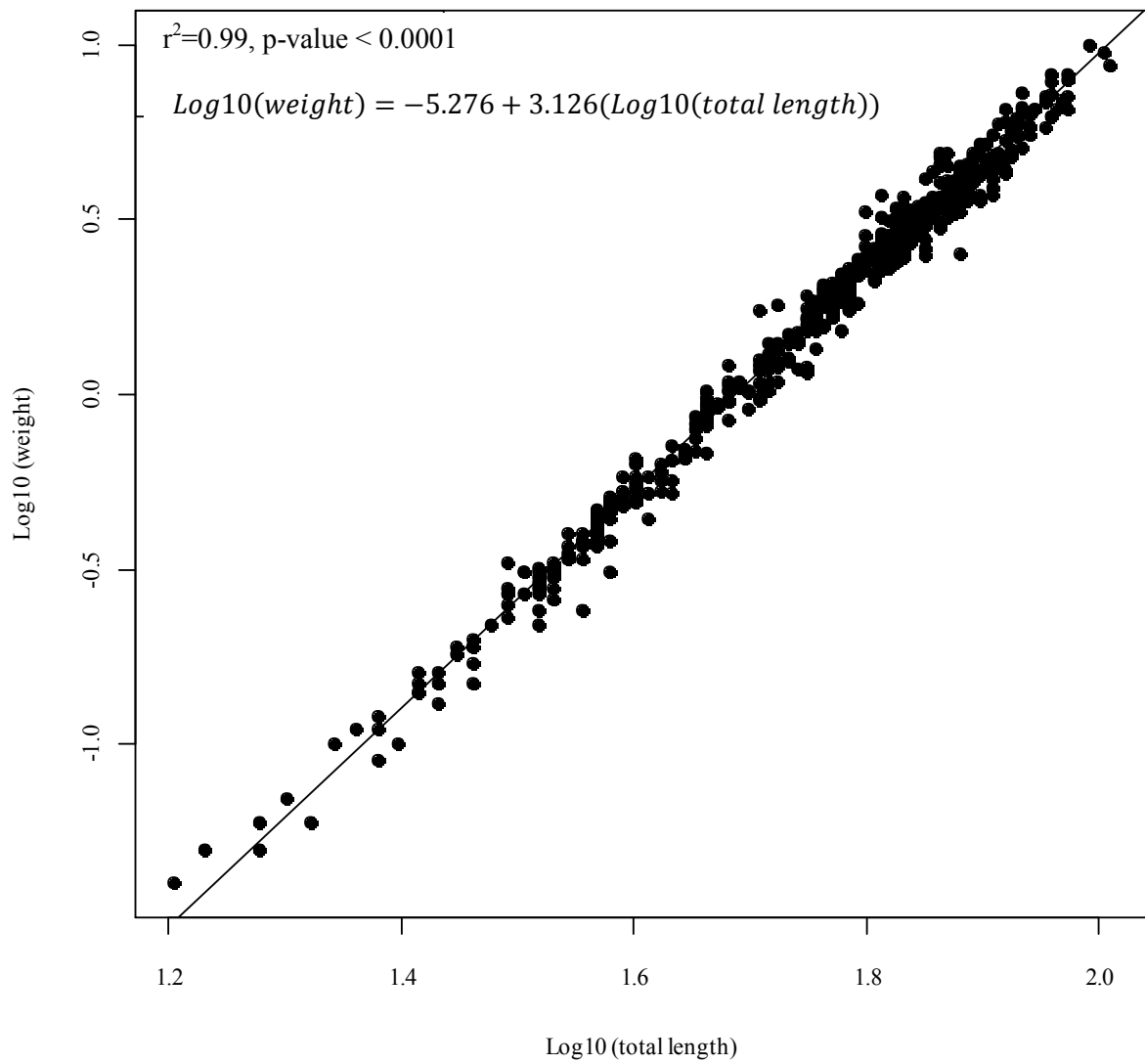


Figure 12. Log10 transformed total lengths (mm) and weights (g) for all samples of Redside Shiners collected from Ross Lake from January, 2009 to September, 2010.

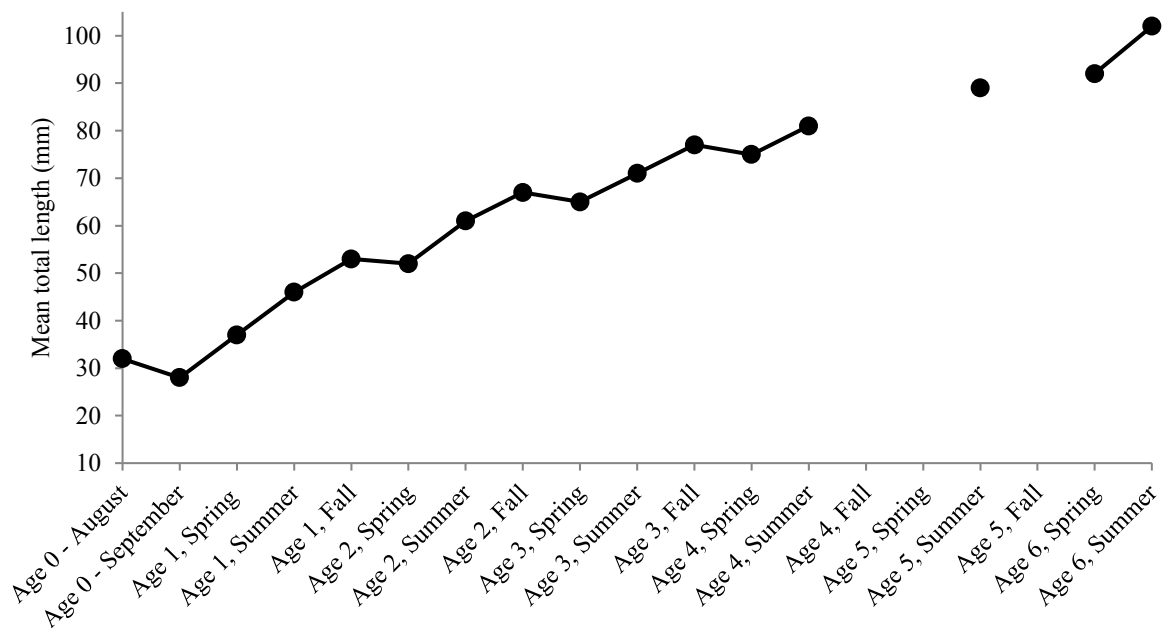


Figure 13. Length at age of Redside Shiners from Ross Lake. Age cohorts 0 - 6 are arranged according to their capture times, spring to fall, sample were pooled across sites. N ranged from 2 – 32.

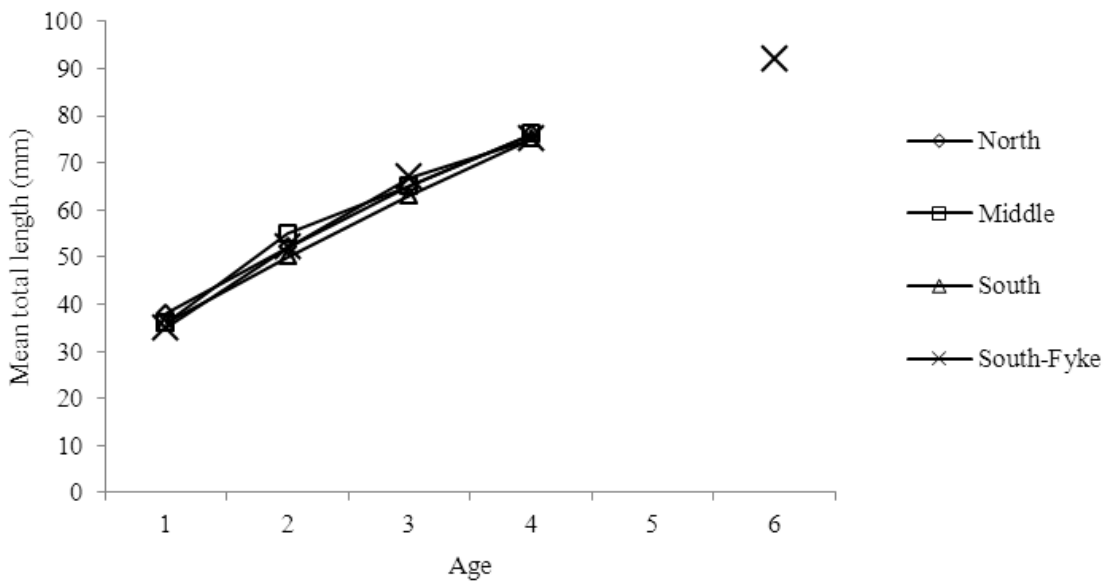


Figure 14. Length at age cohorts 1 - 4, for Redside Shiners sampled from Ross Lake in spring, 2010. The northern, middle and southern samples were beach seined. The sample from Ross Dam, in the southern area of the lake, was captured by a fyke net. Males and females are pooled. No 5 year old Redside Shiners were present (Figure 16, Table 6).

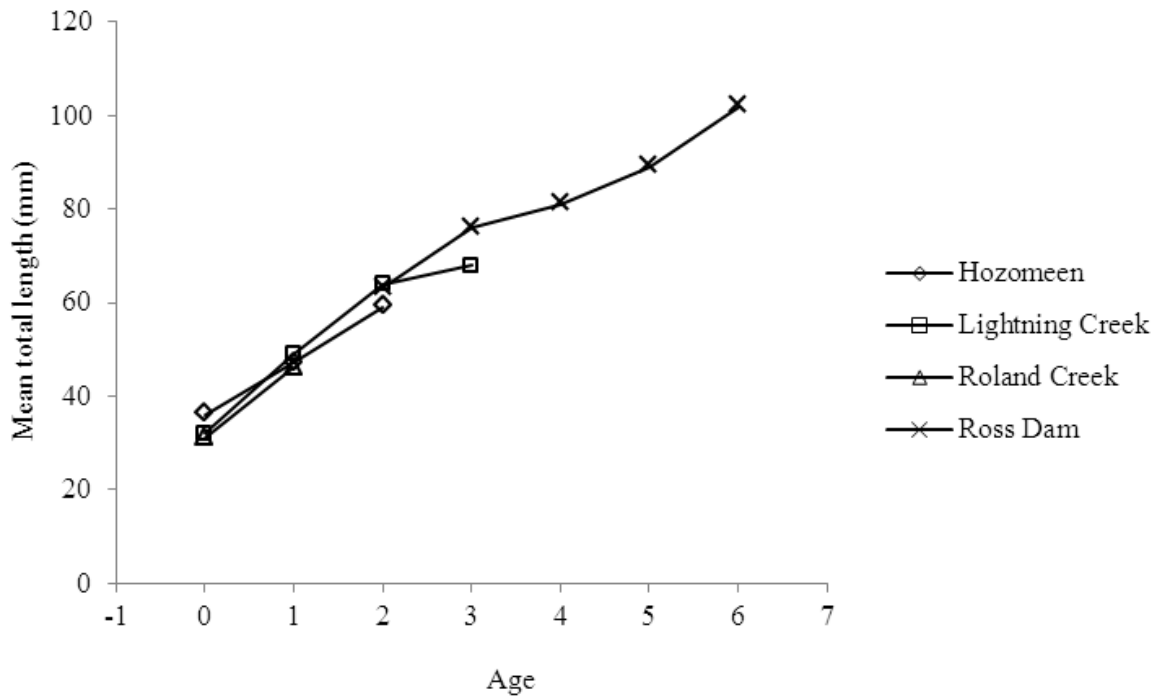


Figure 15. Length at age cohorts 0 - 3 for Redside Shiners sampled from Ross Lake in summer, 2010. Hozomeen, Lightning Creek and Roland Creek were beach seined. Ross Dam was captured by a fyke net. Males and females are pooled (Figure 17, Table 7).

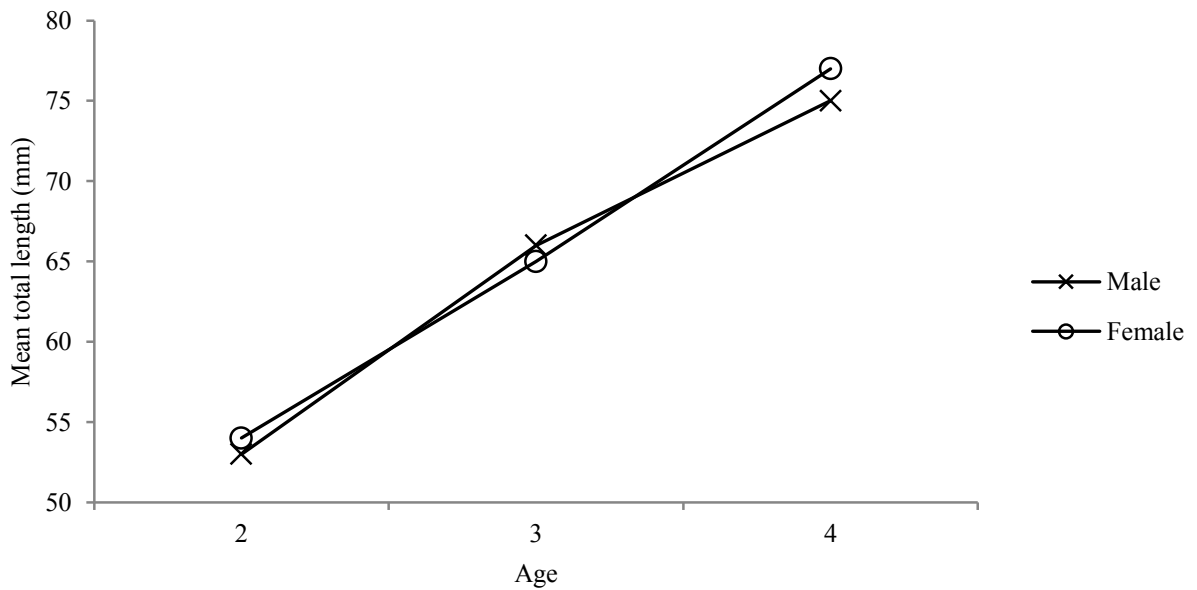


Figure 16. Length at age cohort by sex for Redside Shiners sampled in spring, 2010 from Ross Lake.

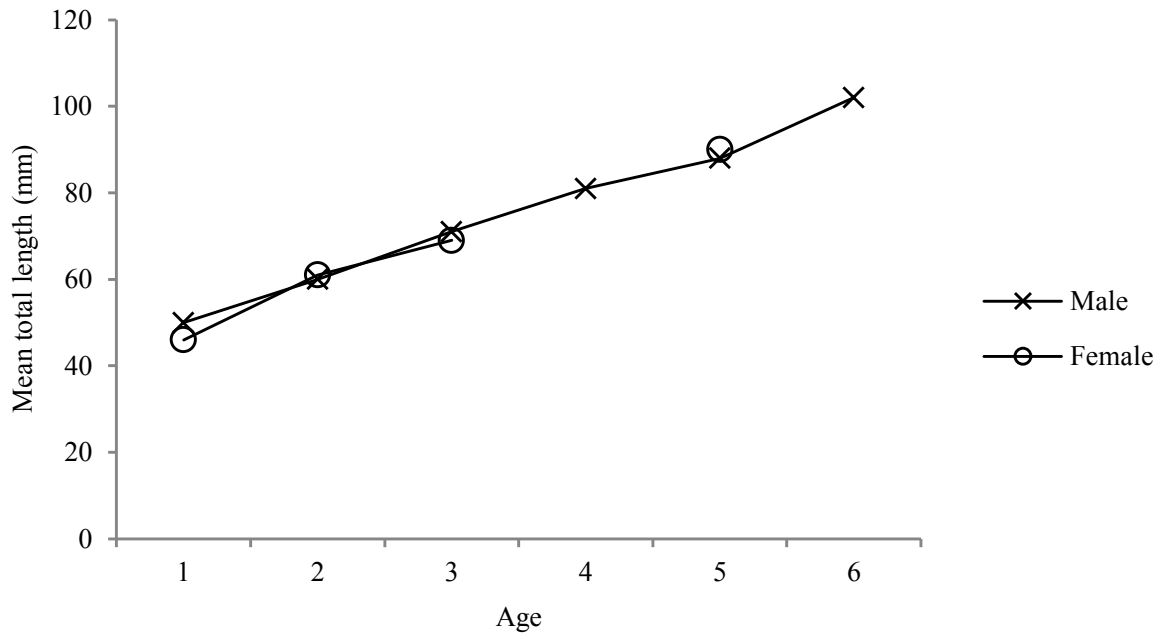


Figure 17. Length at age cohorts by sex for Redside Shiners sampled in summer, 2010 from Ross Lake.

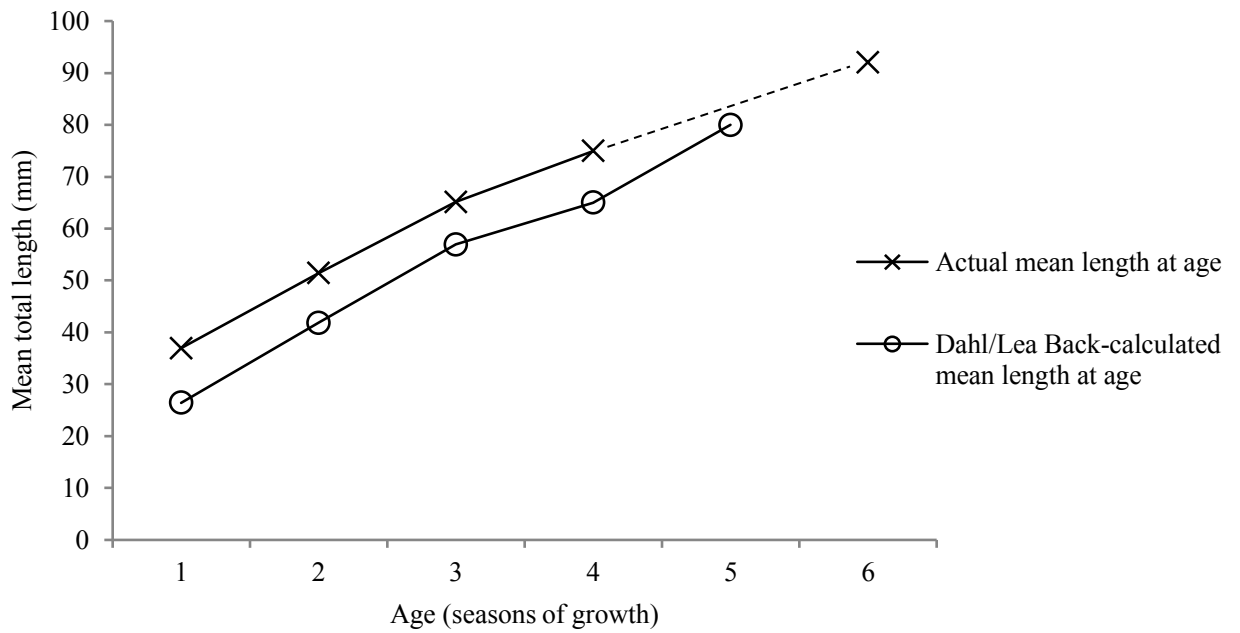


Figure 18. Actual length at age cohorts vs. back calculated length at age (Dahl/Lea method) for Redside Shiners, from Ross Lake. Samples are pooled among sites.

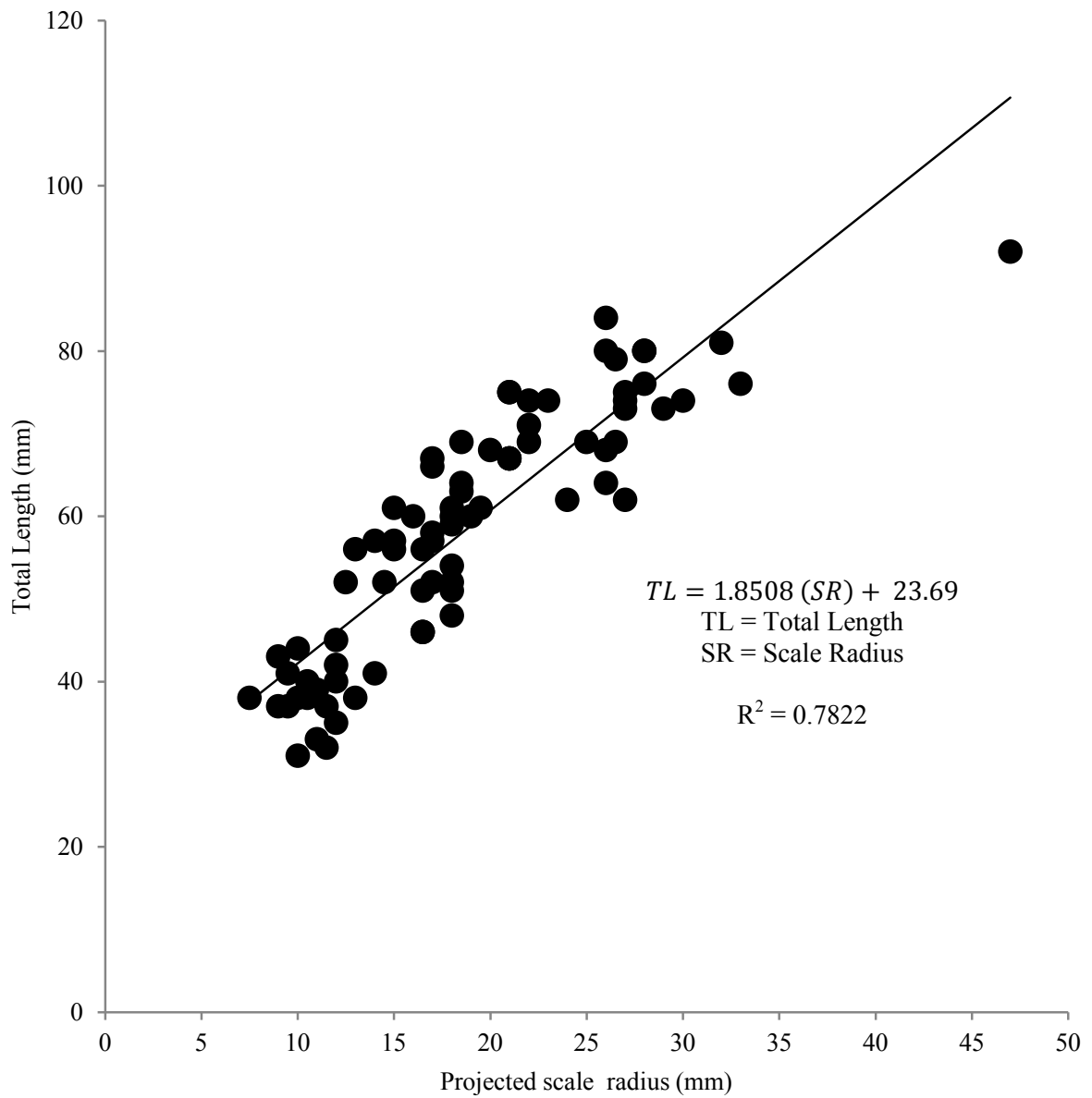


Figure 19. Regression analysis of actual length at capture vs. scale radius, the Fraser/Lee correction (y intercept) is 23.69 mm.

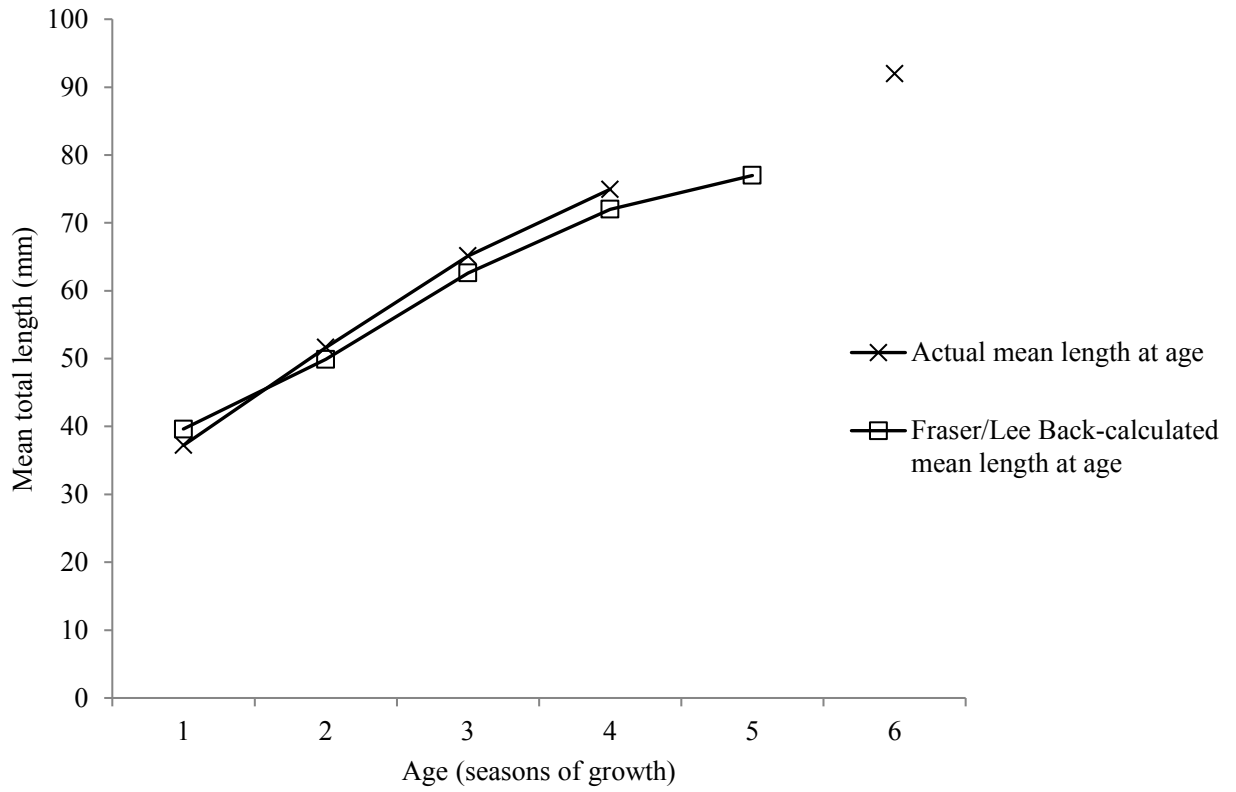


Figure 20. Actual length at age cohorts vs. back calculated length at age after applying the Fraser/Lee correction to back-calculated length to Redside Shiners, from Ross Lake. All spring samples are pooled.

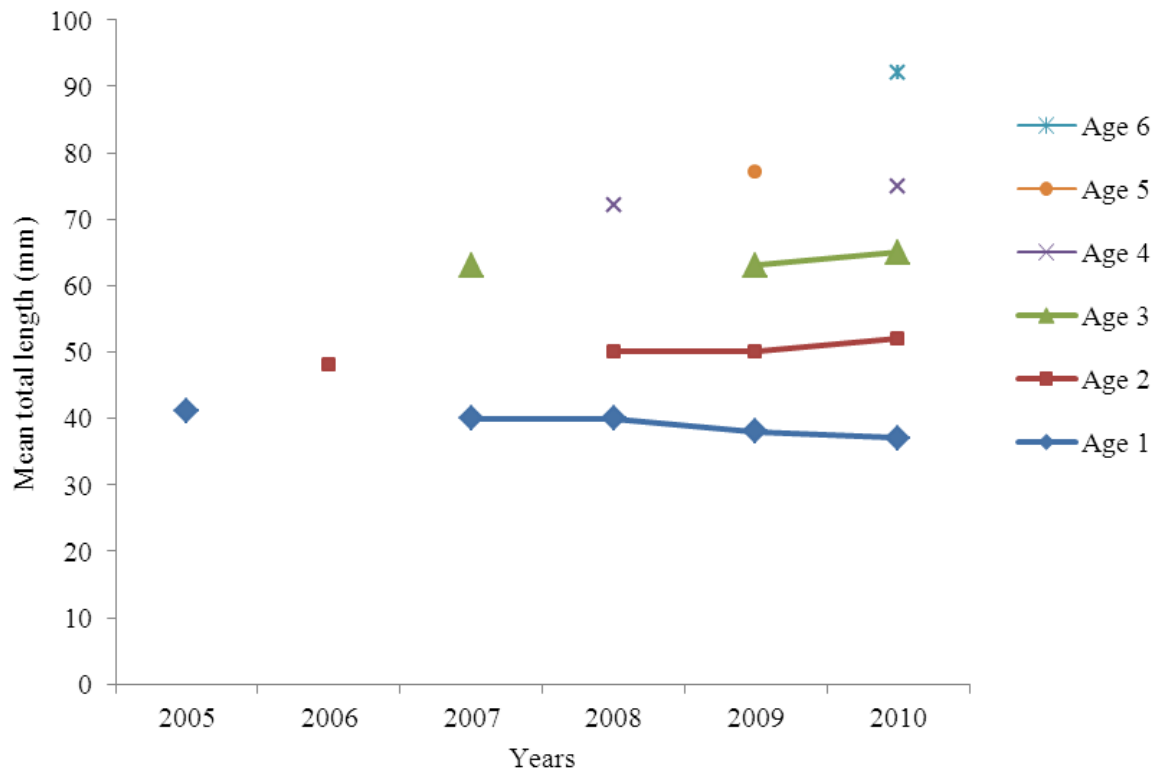


Figure 21. Back calculated mean total lengths (with Fraser/Lee correction) at age for Redside Shiners from Ross Lake, by year. Year 2010 is actual length at ages.

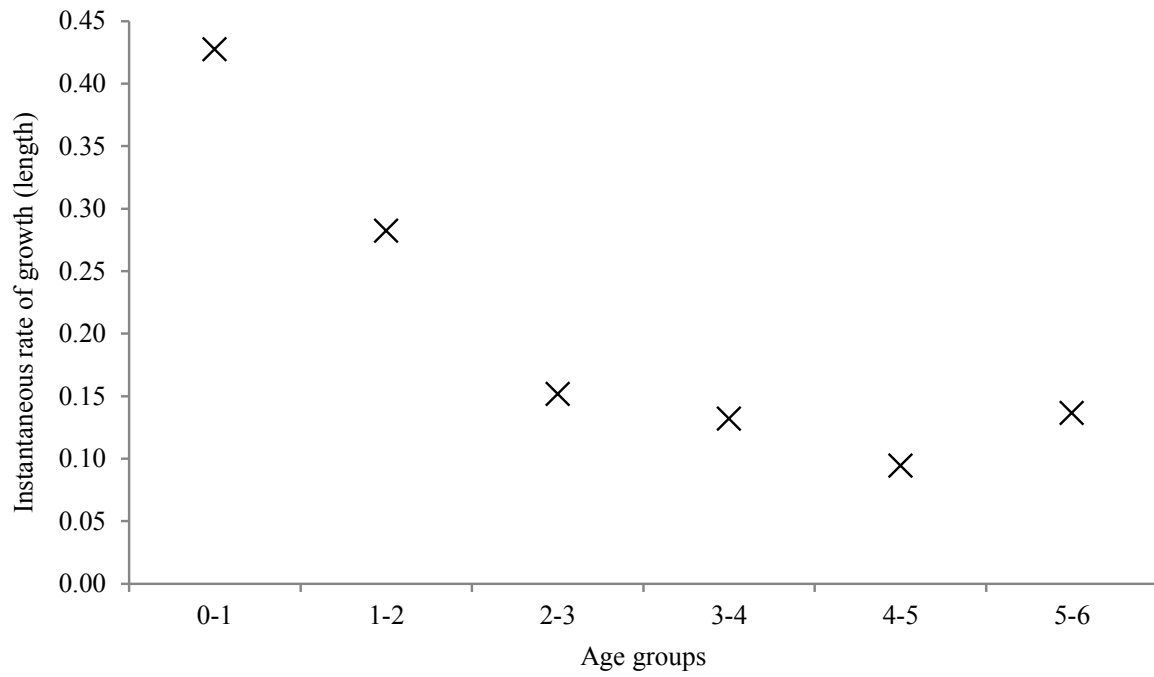


Figure 22. Length-based instantaneous rates of growth for Redside Shiners from Ross Lake, based on actual age structure and lengths of all fish captured during summer, 2010.

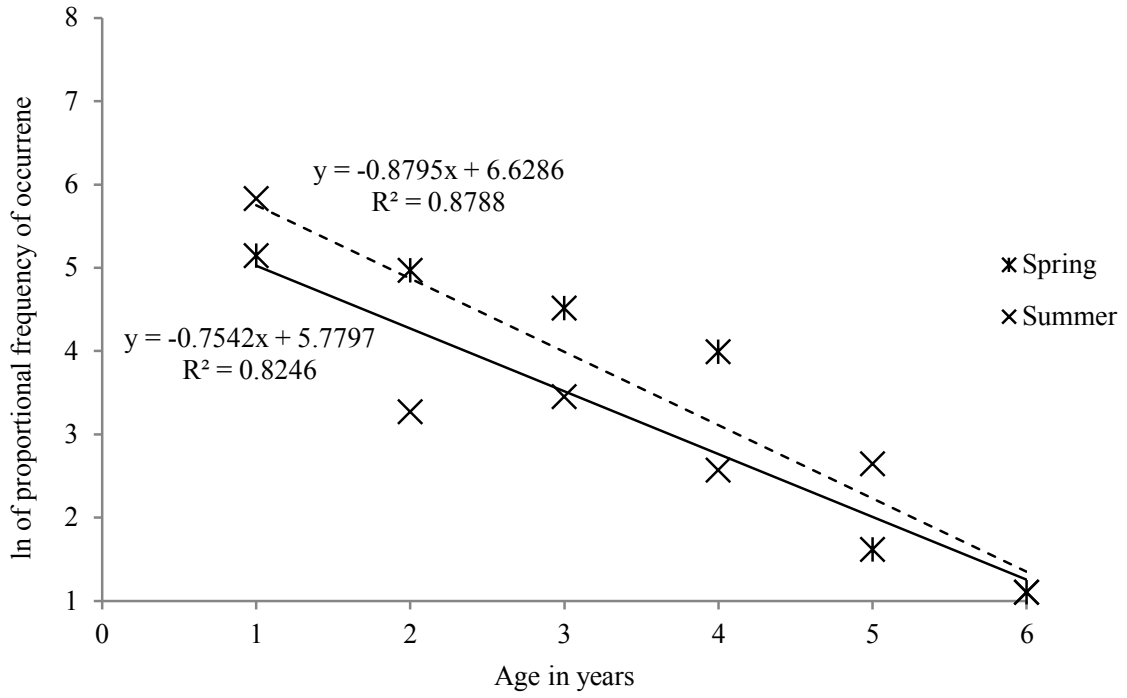


Figure 23. Instantaneous annual mortality rate of Redside Shiners obtained with linear regression of natural log of proportional frequency of occurrence vs. time (age) to determine; samples use are from Ross Lake in spring and in summer, 2010.

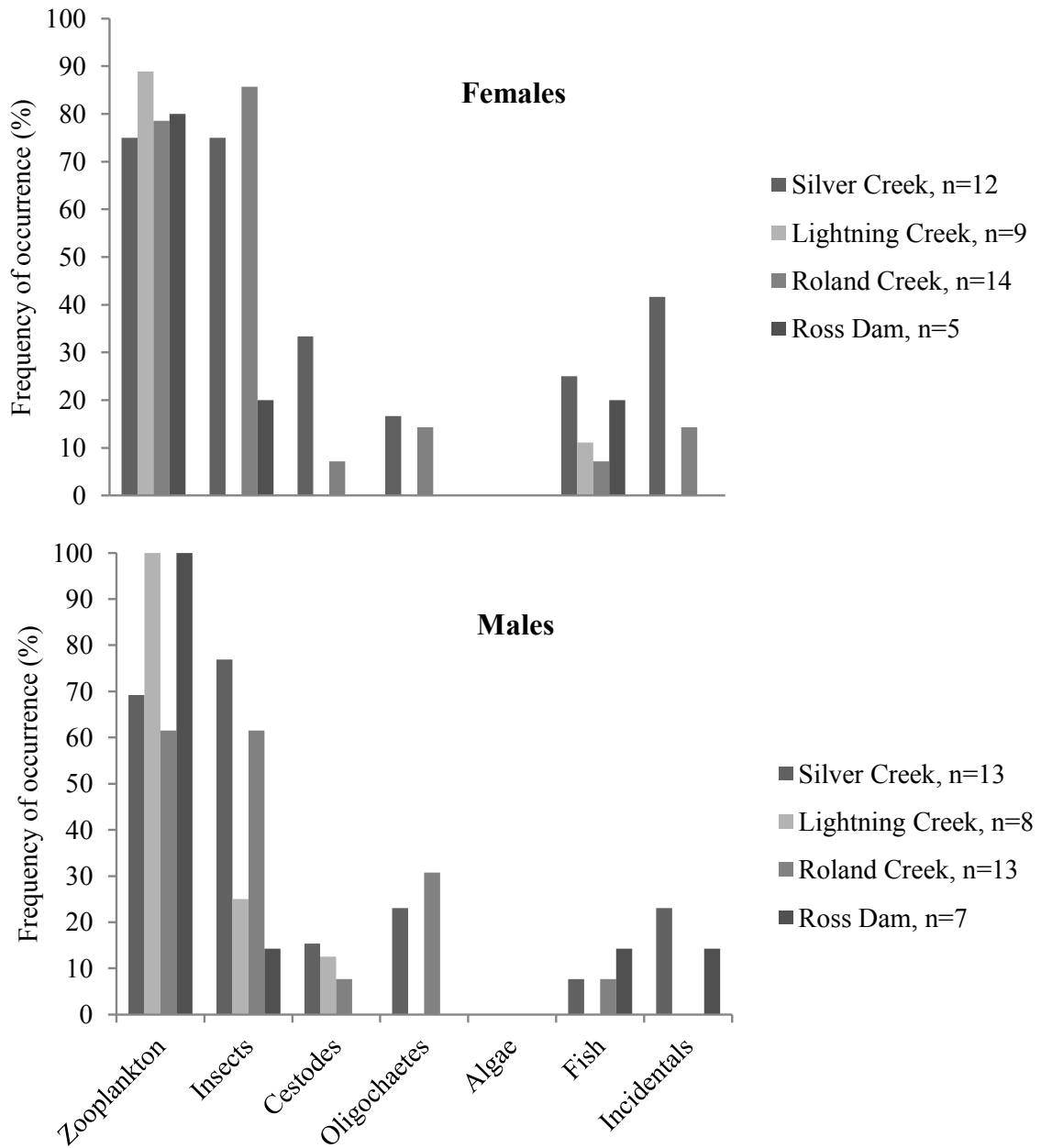


Figure 24. Frequency of occurrence as % of stomachs with given diets items for female and male Redside Shiners, from Ross Lake, in spring, 2010.

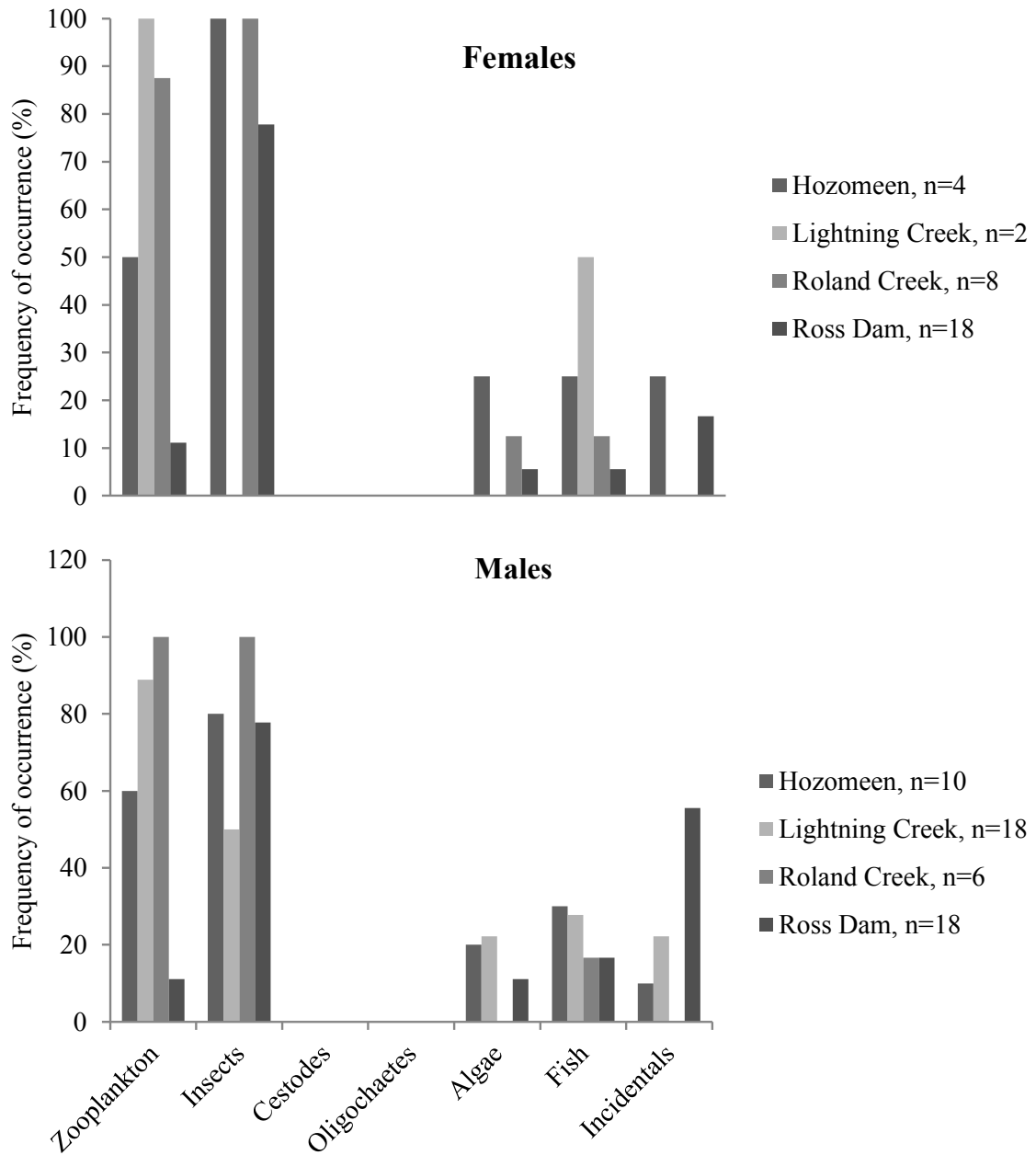


Figure 25. Frequency of occurrence as % of stomachs with given diets items for female and male Redside Shiners, from Ross Lake, in summer, 2010.

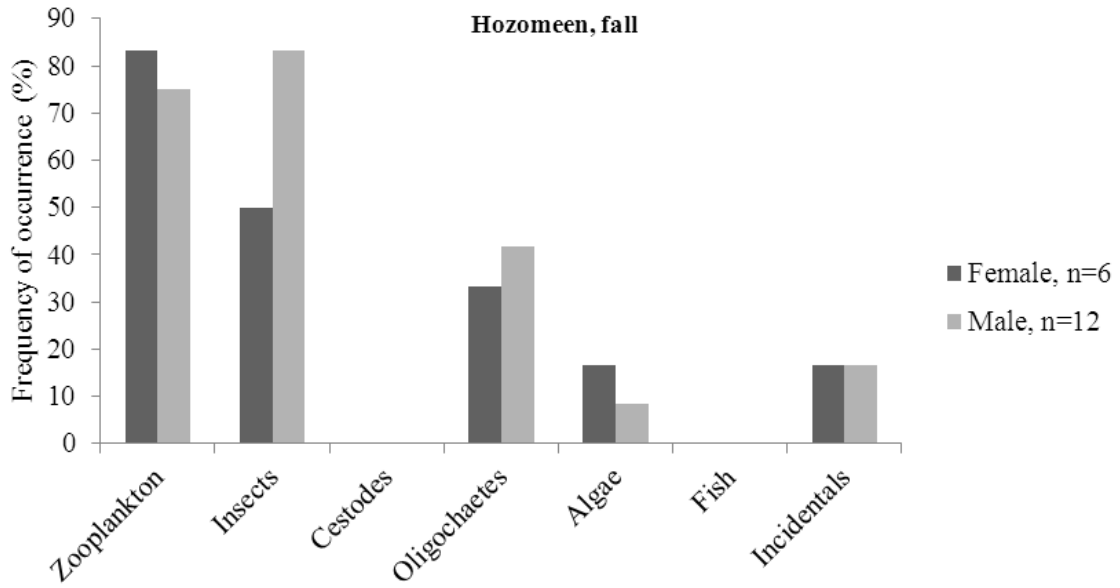


Figure 26. Frequency of occurrence as % of stomachs with given diet items for female and male Redside Shiners taken from Ross Lake at the Hozomeen site, in fall, 2010.

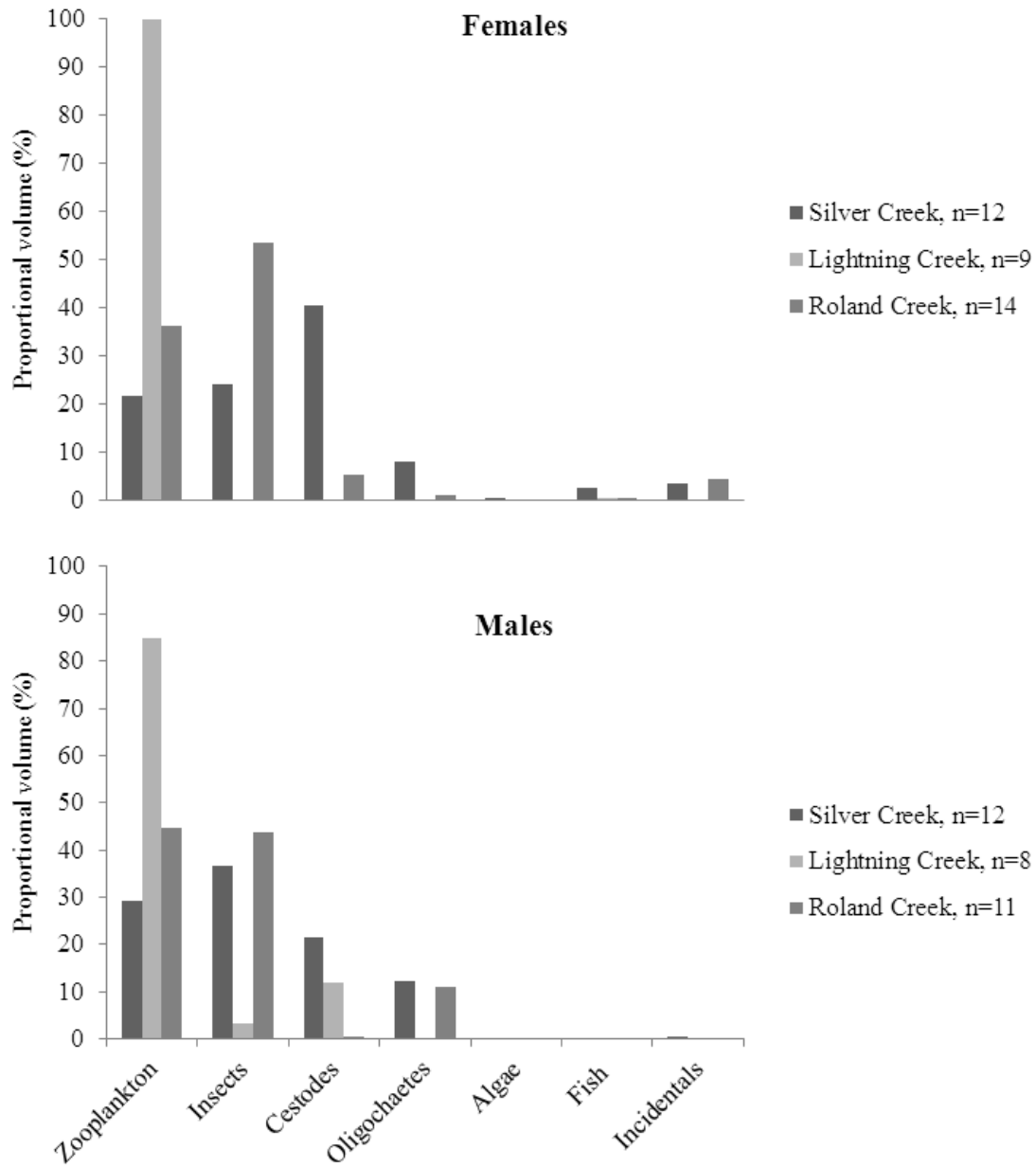


Figure 27. Portion of total volume of dietary items for female and male Redside Shiners, from Ross Lake, in spring, 2010.

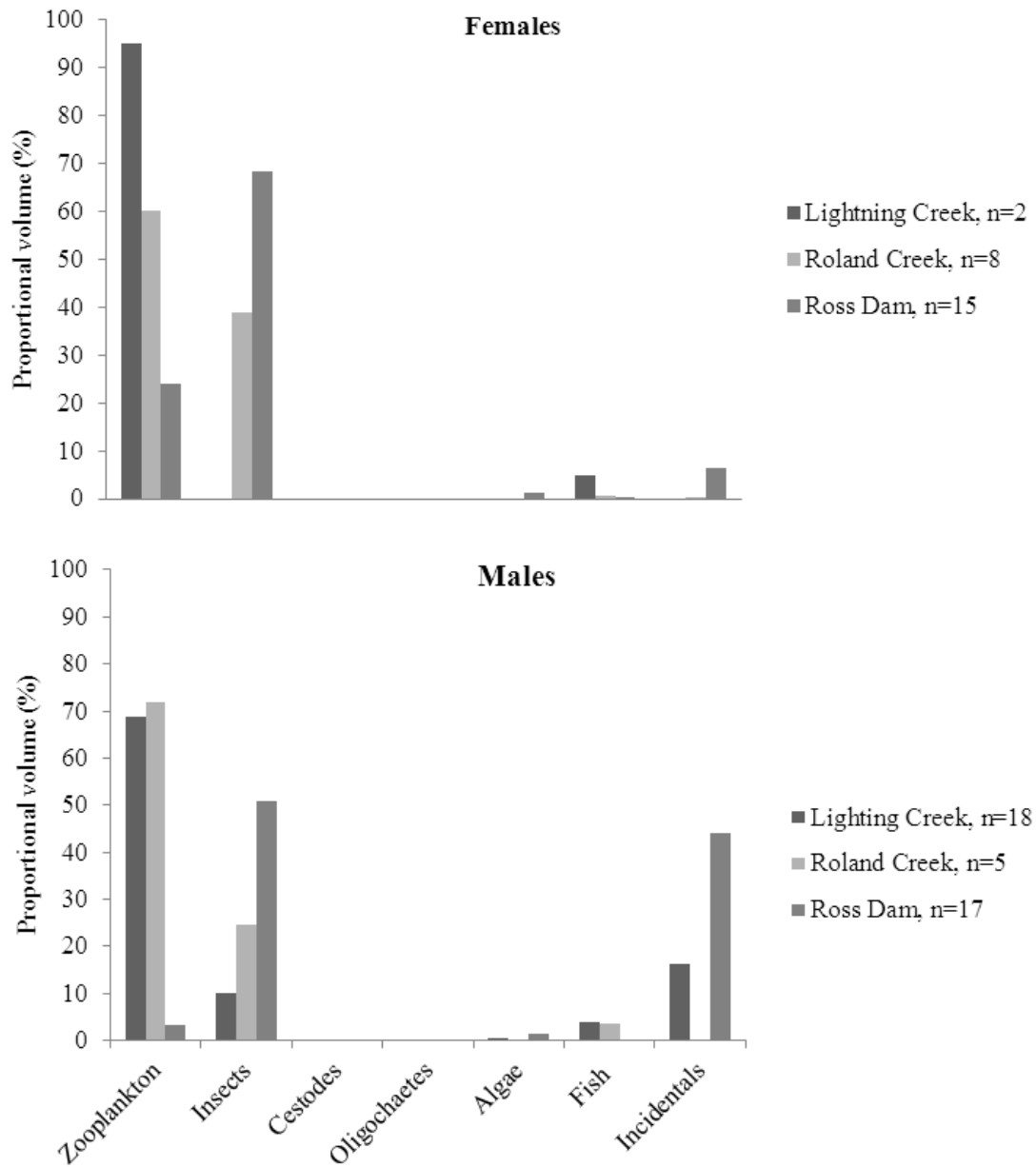


Figure 28. Portion of total volume of dietary items for female and male Redside Shiners, from Ross Lake, in summer, 2010.

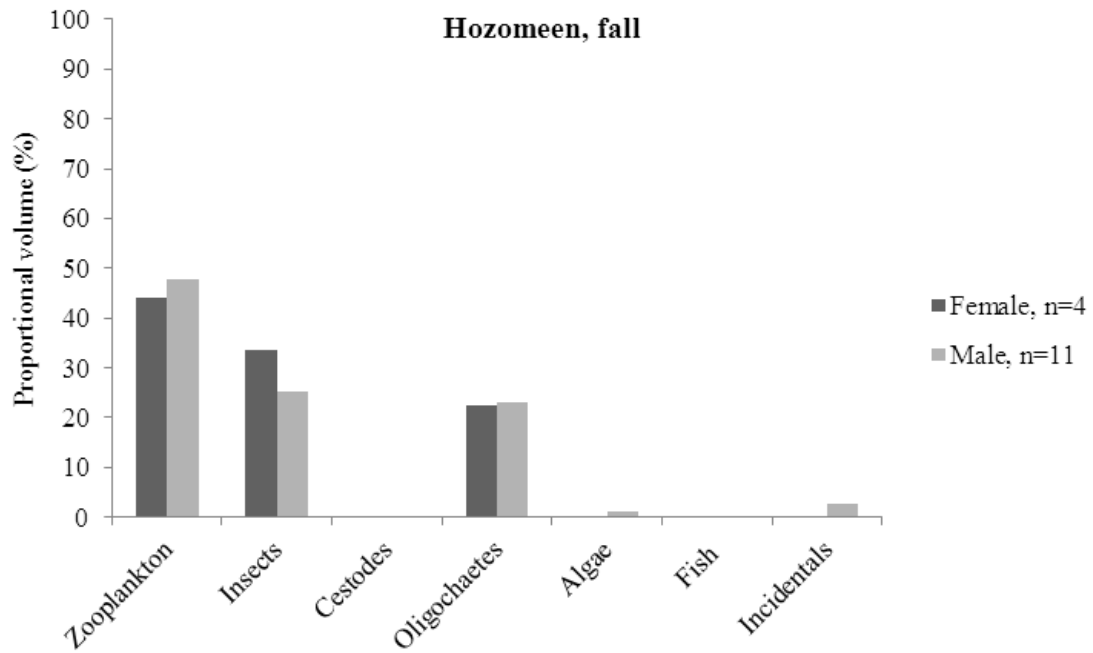


Figure 29. Portion of total volume of dietary items for female and male Redside Shiners, from Ross Lake at the Hozomeen site, in fall, 2010.

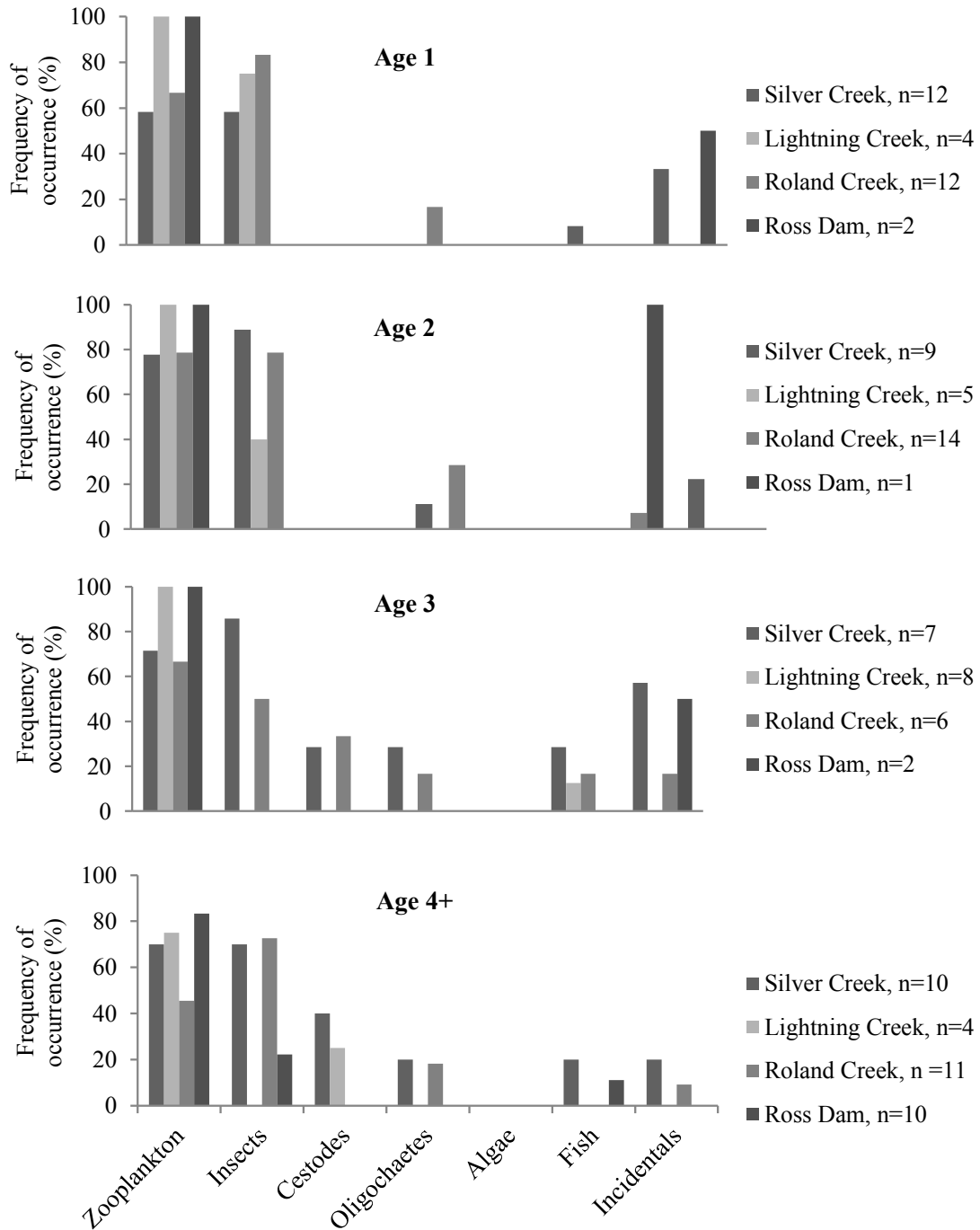


Figure 30. Frequency of occurrence as % of stomachs with given diet items for ages 1 to 4+ Redside Shiners, taken from Ross Lake, in spring, 2010. Four plus (4+) represent the average for fish 4 years and older, for this sample, Ross Dam was the only sample location with fish ages 5 and 6.

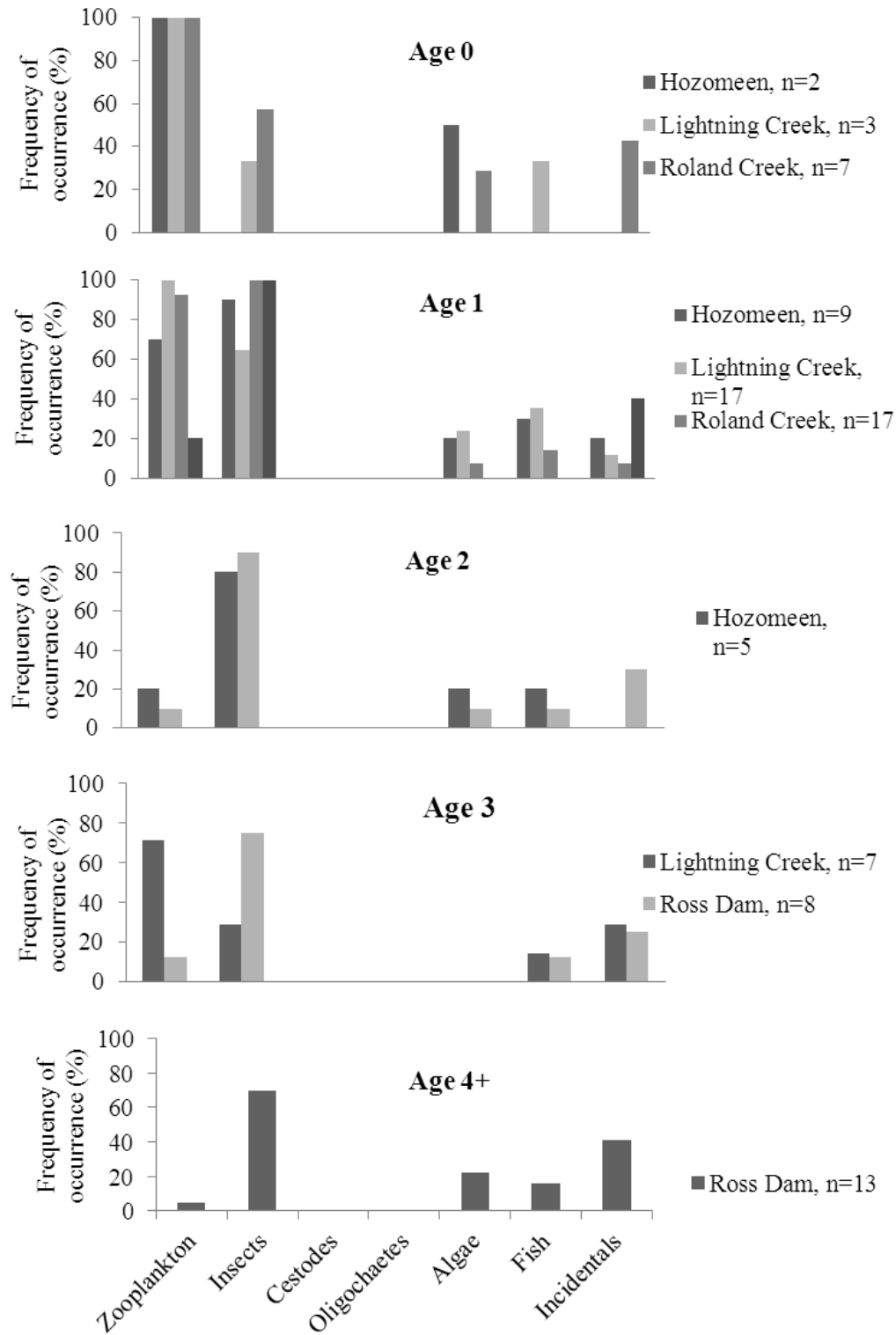


Figure 31. Frequency of occurrence as % of stomachs with given diet items for ages 0 to 4+ Redside Shiners, taken from Ross Lake, in summer, 2010. Four plus (4+) represent the average for fish 4 years and older, for this sample, Ross Dam was the only sample location with fish ages 5 and 6.

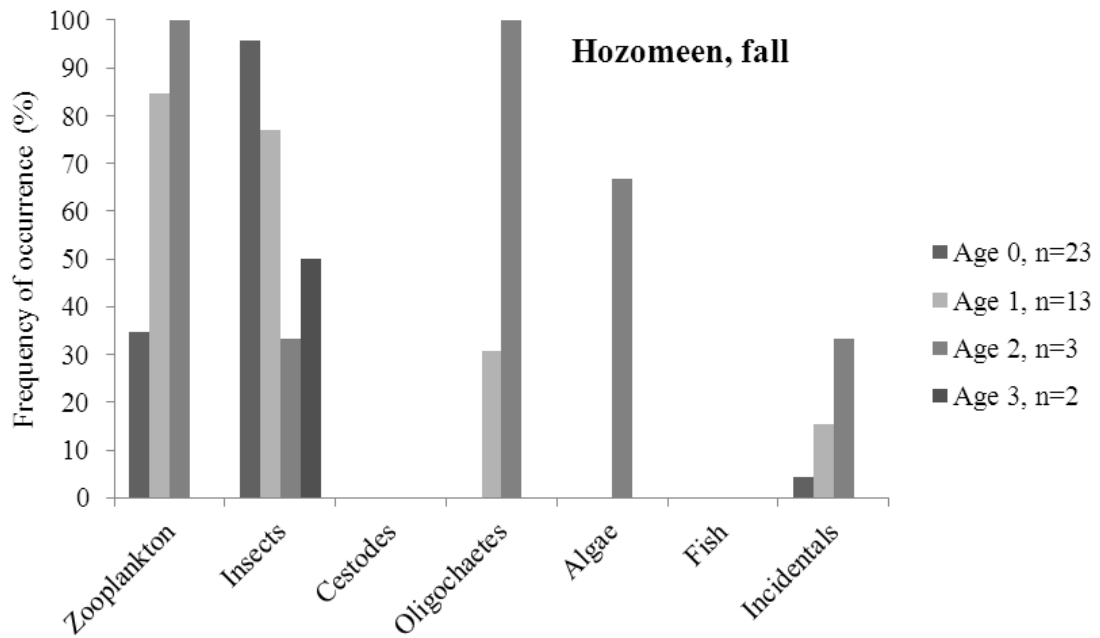


Figure 32. Frequency of occurrence as % of stomachs with given diets items for ages 0 to 3 Redside Shiners, from Ross Lake at the Hozomeen site, in fall, 2010.

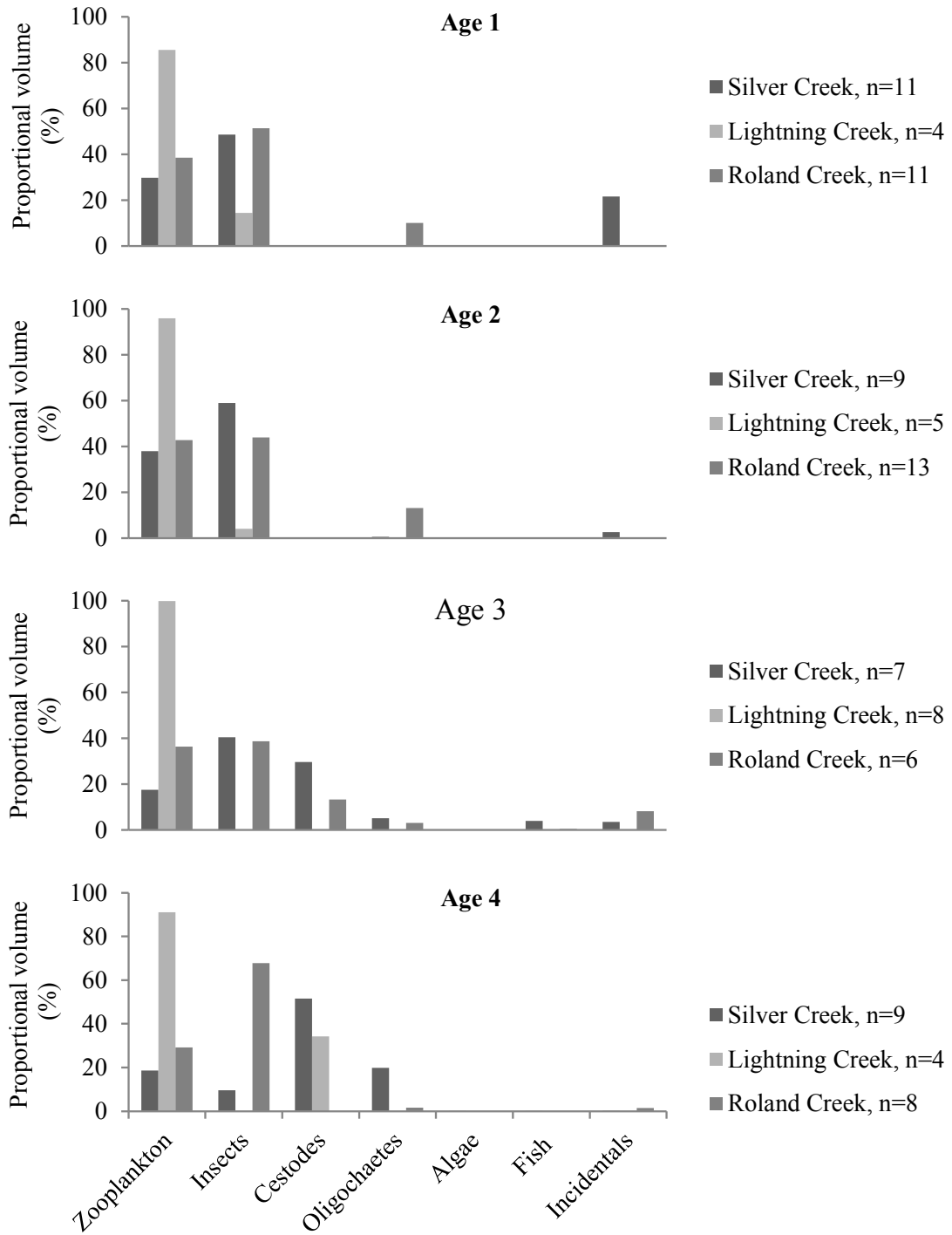


Figure 33. Portion of total volume of dietary items for Redside Shiners ages 1 to 4, from Ross Lake, in spring, 2010.

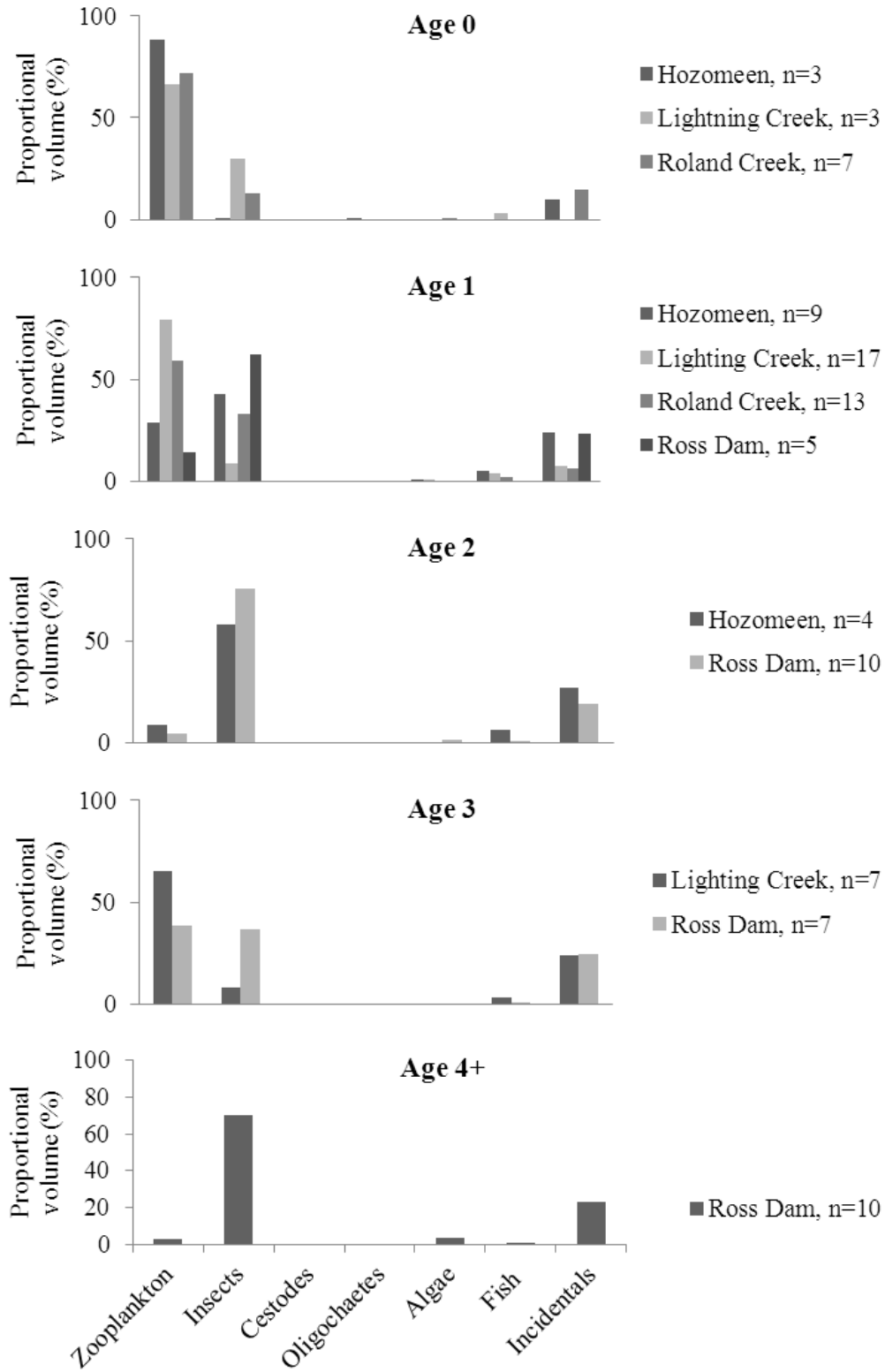


Figure 34. Portion of total volume of dietary items for Redside Shiners ages 0 to 4+, from Ross Lake, in summer, 2010. Four plus (4+) represent the average for fish 4 years and older, for this sample, Ross Dam was the only sample location with fish ages 5 and 6.

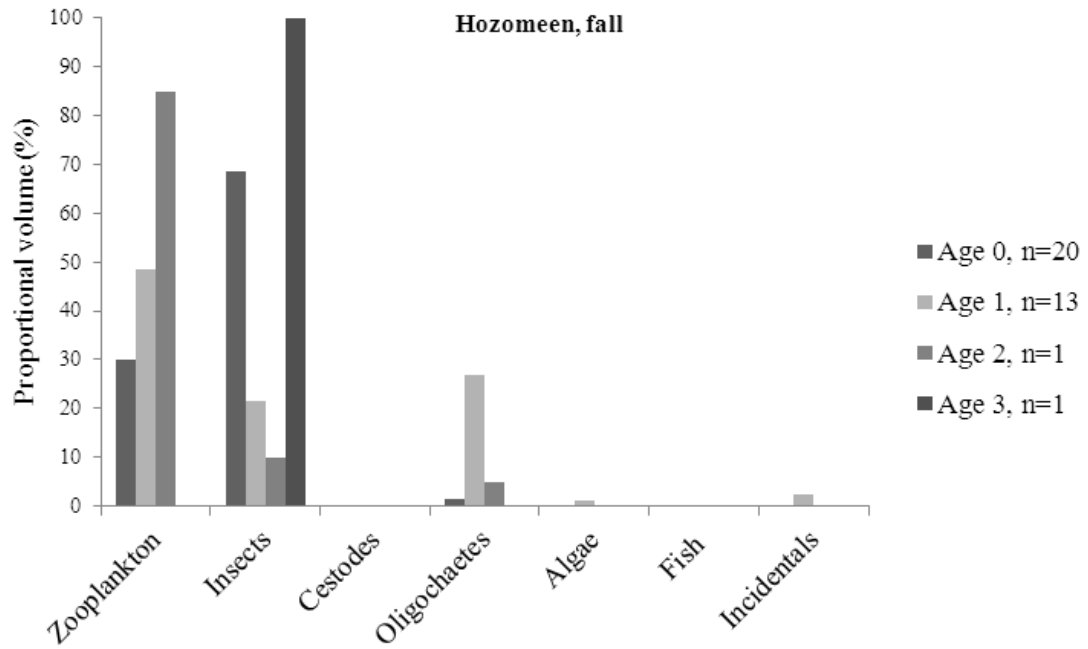


Figure 35. Portion of total volume of dietary items for Redside Shiners ages 0 to 3, from Ross Lake at the Hozomeen site, in fall, 2010.

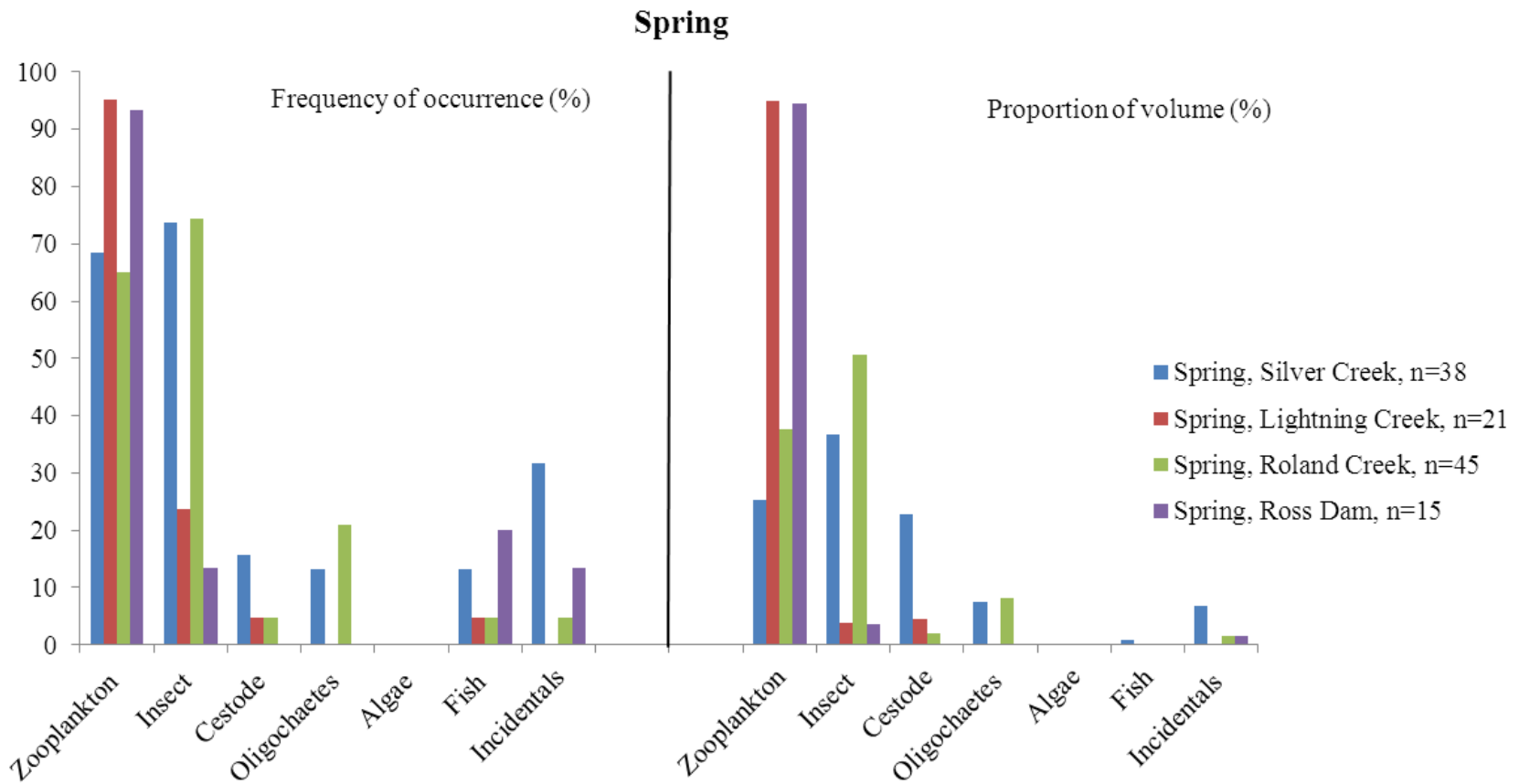


Figure 36. Frequency of occurrence and proportion of total volume of dietary items for the samples collected from Ross Lake, in spring, 2010. All samples were collected using a beach seine, except Ross Dam, where a fyke net was used.

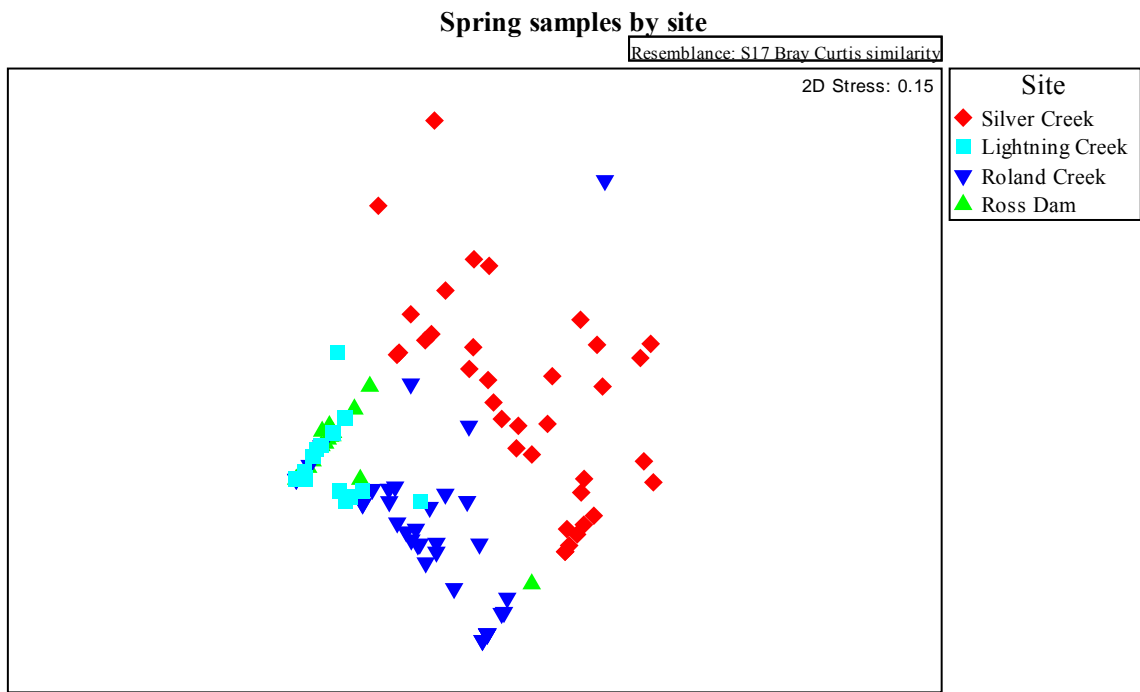


Figure 37. Graphical output of non-parametric multidimensional scaling of spring samples, by proportion volume of dietary items.

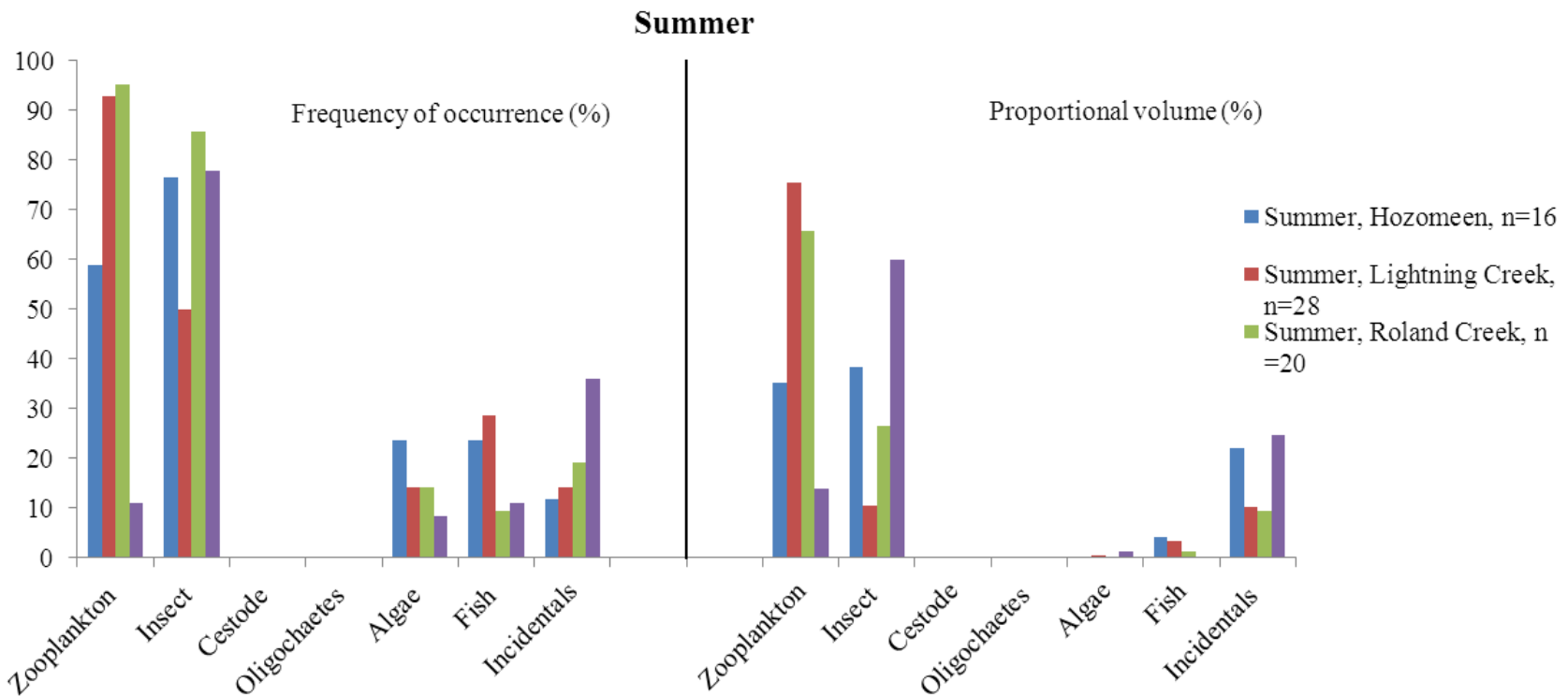


Figure 38. Frequency of occurrence and proportion of total volume of dietary items for the samples collected from Ross Lake, in summer, 2010. All samples were collected using a beach seine, except Ross Dam, where a fyke net was used.

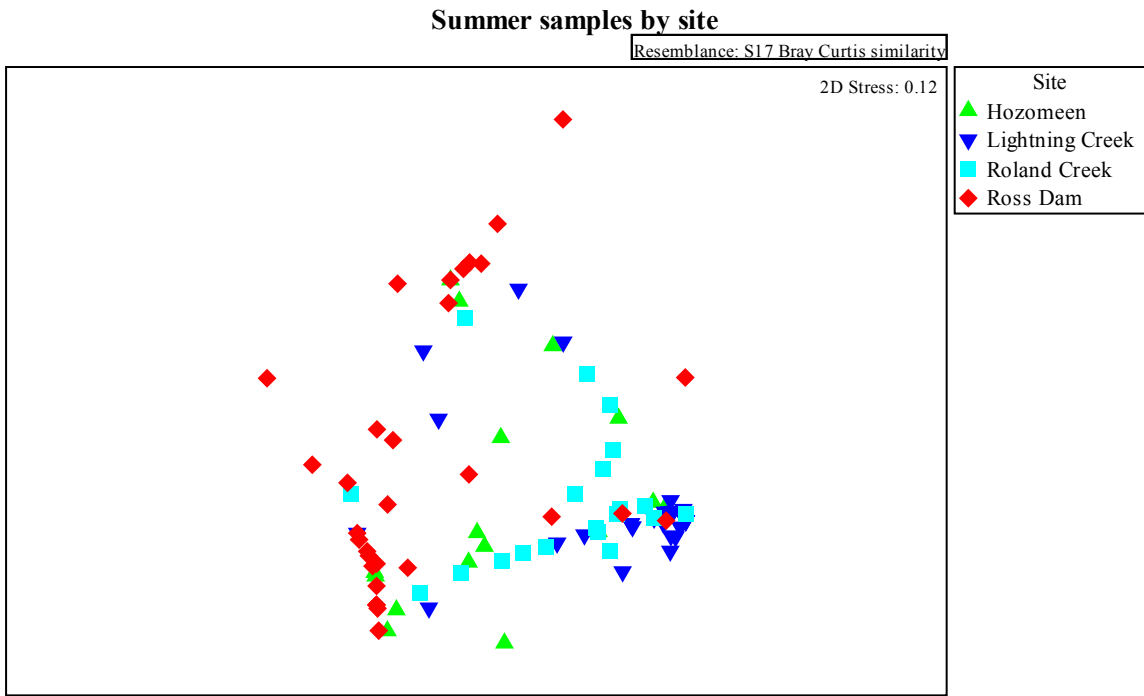


Figure 39. Graphical output of non-parametric multidimensional scaling of summer samples by proportional volume of dietary items.

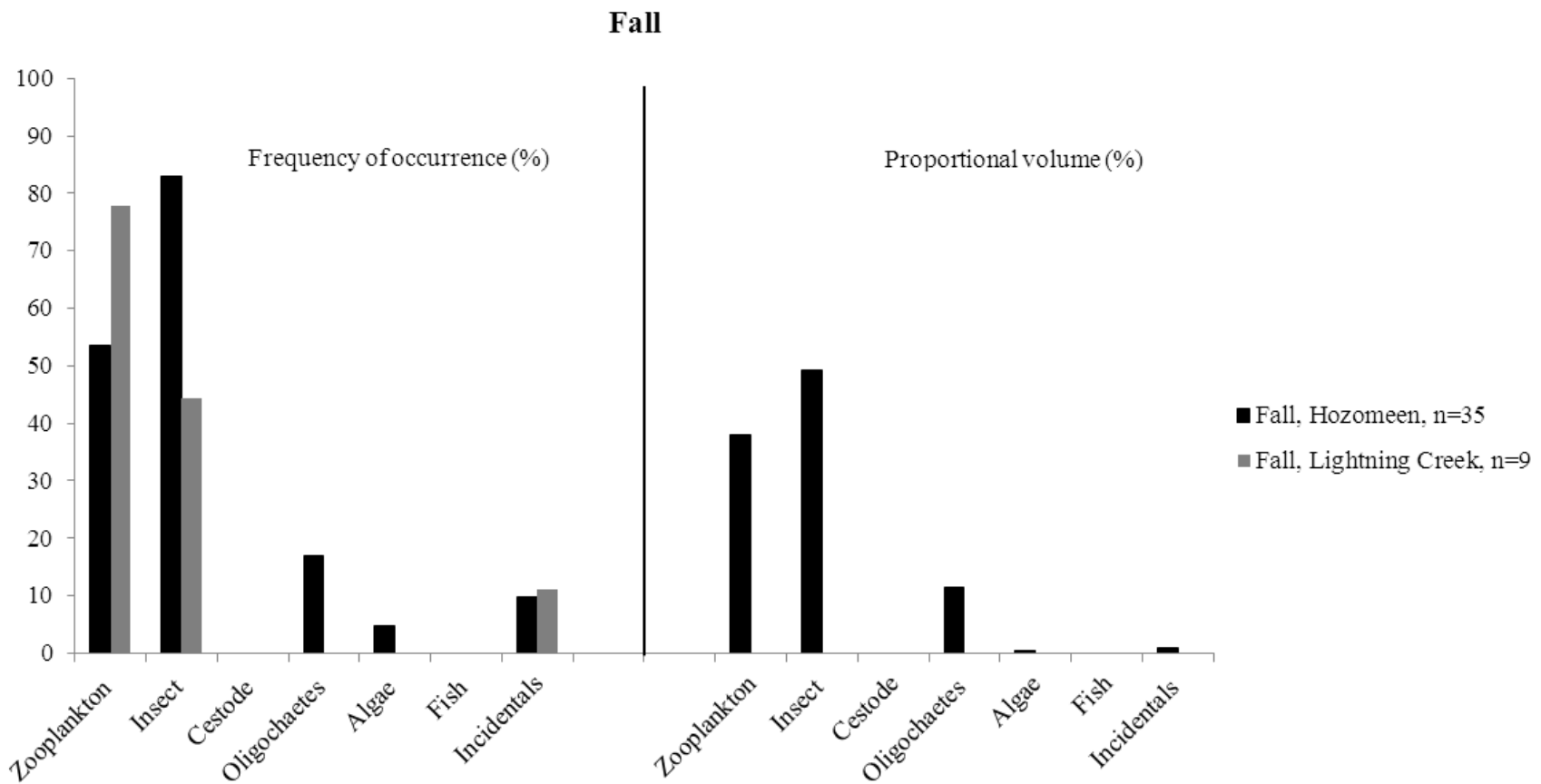


Figure 40. Frequency of occurrence as % and proportion of total volume of dietary items for the two fall samples collected from Ross Lake in 2010. All samples were collected using a beach seine. Volume was not analyzed for the Lightning Creek sample.

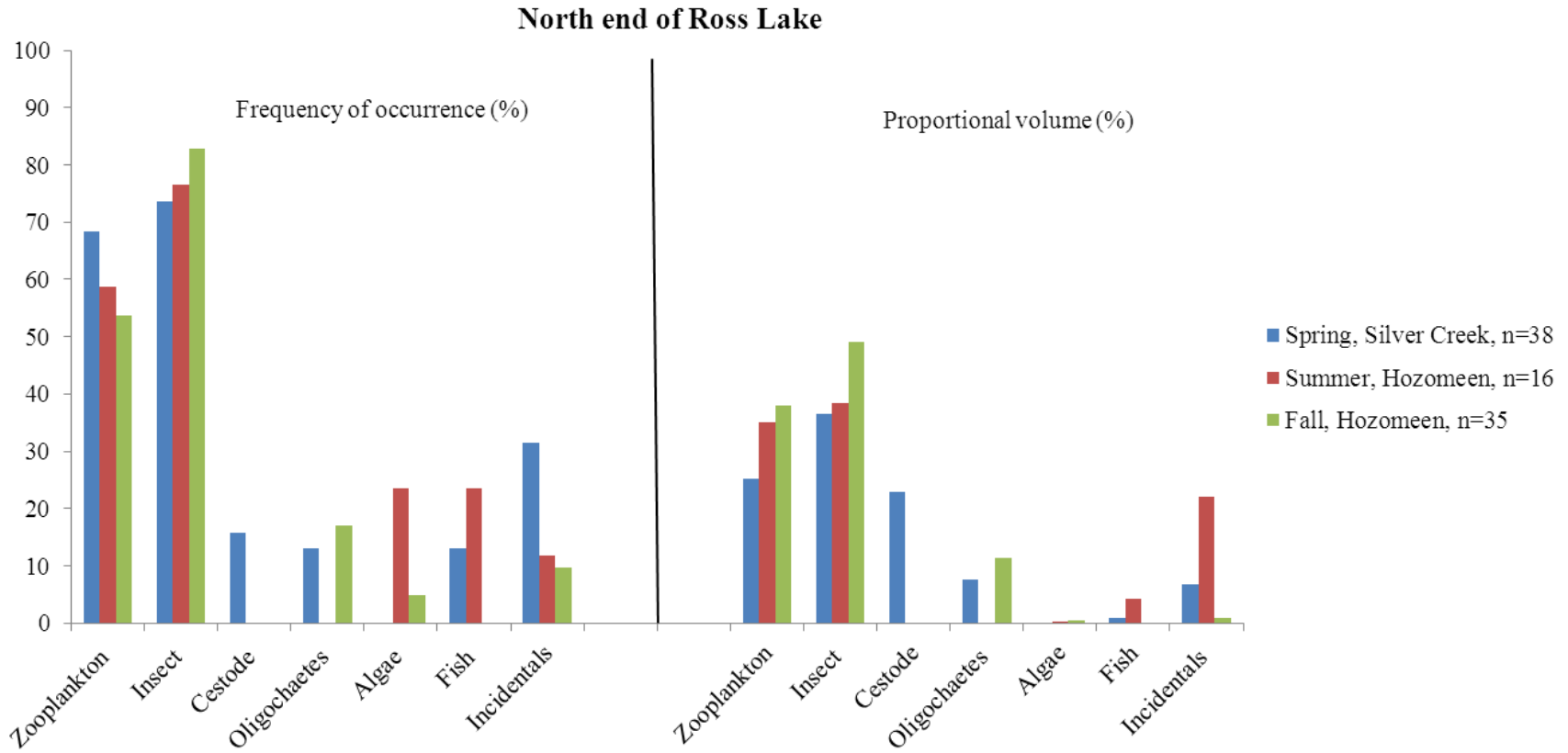


Figure 41. Frequency of occurrence as % and proportion of total volume of dietary items for samples collected at the north end of Ross Lake, at Silver Creek in spring, and at Hozomeen in summer, and in fall. A beach seine was used for all three collection events.

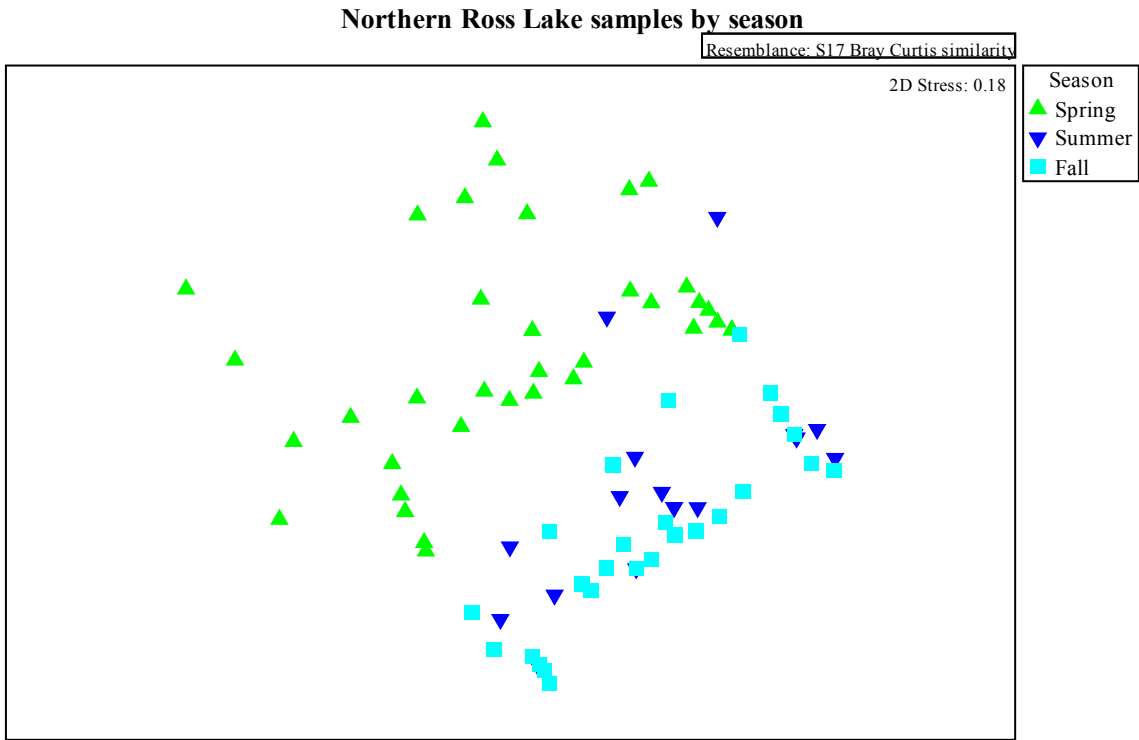


Figure 42. Graphical output of non-parametric multidimensional scaling of samples from northern Ross Lake, by proportion volume of dietary items.

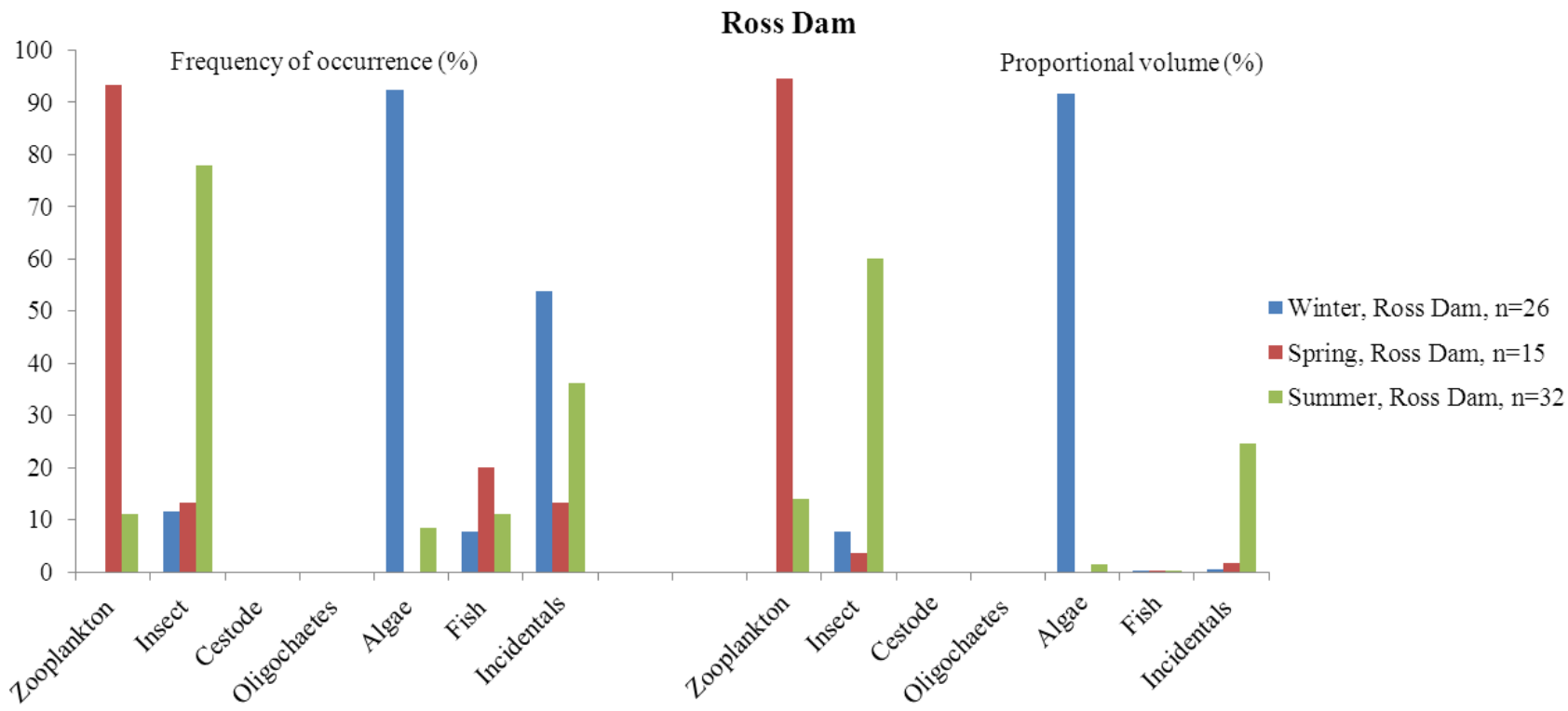


Figure 43. Frequency of occurrence as % and proportion of total volume of dietary items for samples collected at the south end of Ross Lake, at Ross Dam, in winter, in spring and in summer, 2010. A fyke net was used to for all three samples.

Ross Dam samples by season

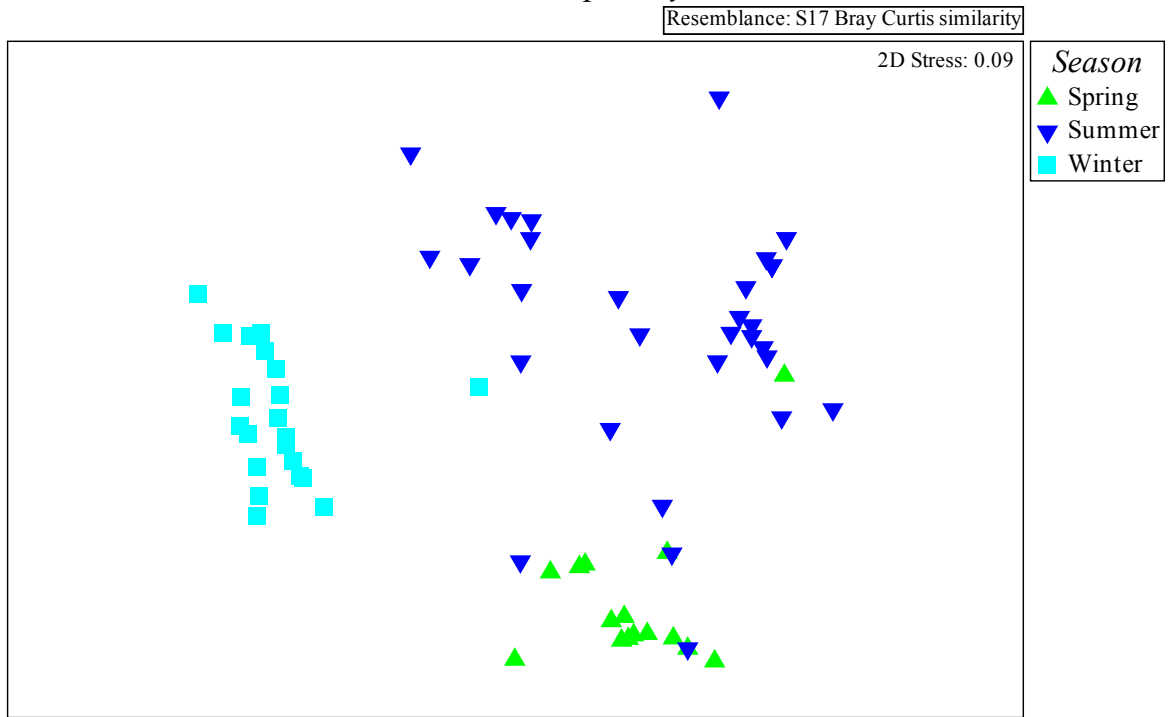


Figure 44. Graphical output of non-parametric multidimensional scaling of samples Ross Dam, by proportion of total volume of dietary items.

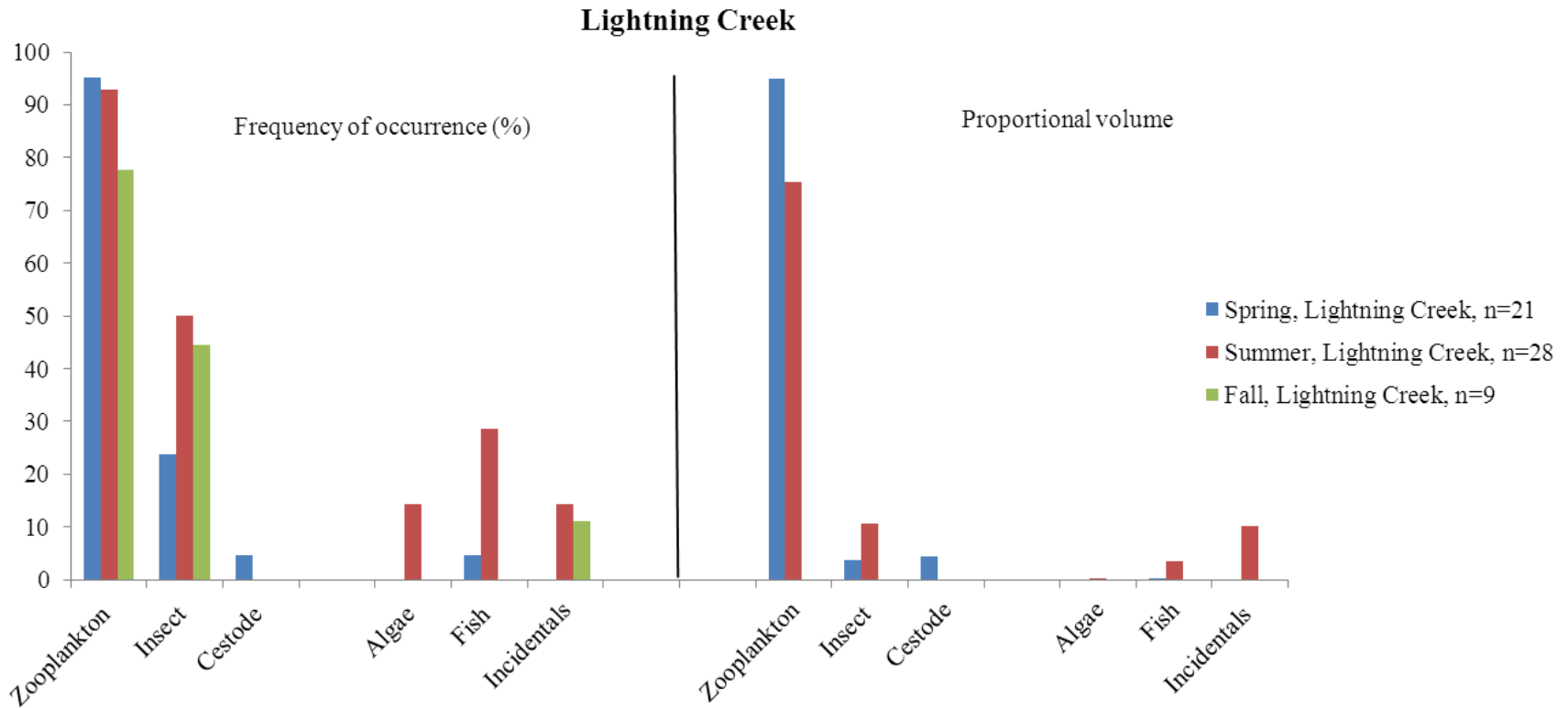


Figure 45. Frequency of occurrence as % and proportion of total volume of dietary items for samples collected at Lightning Creek, in spring, in summer, and in fall. A beach seine was used for all three collection events. Volume was not analyzed for the fall Lightning Creek sample.

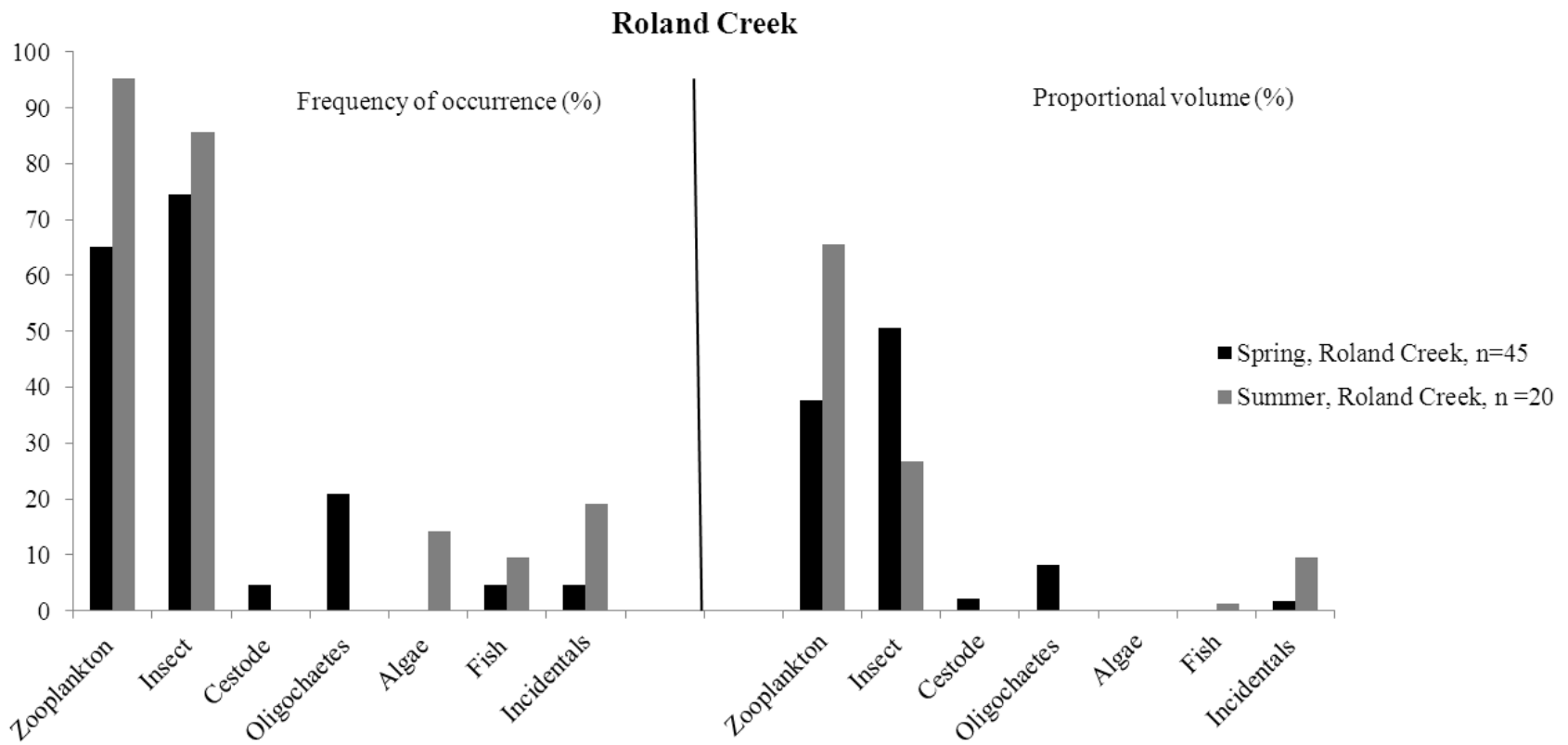


Figure 46. Frequency of occurrence as % and proportion of total volume of dietary items for samples collected at Roland Creek, in spring and in summer, 2010. A beach seine was used for both collection events.

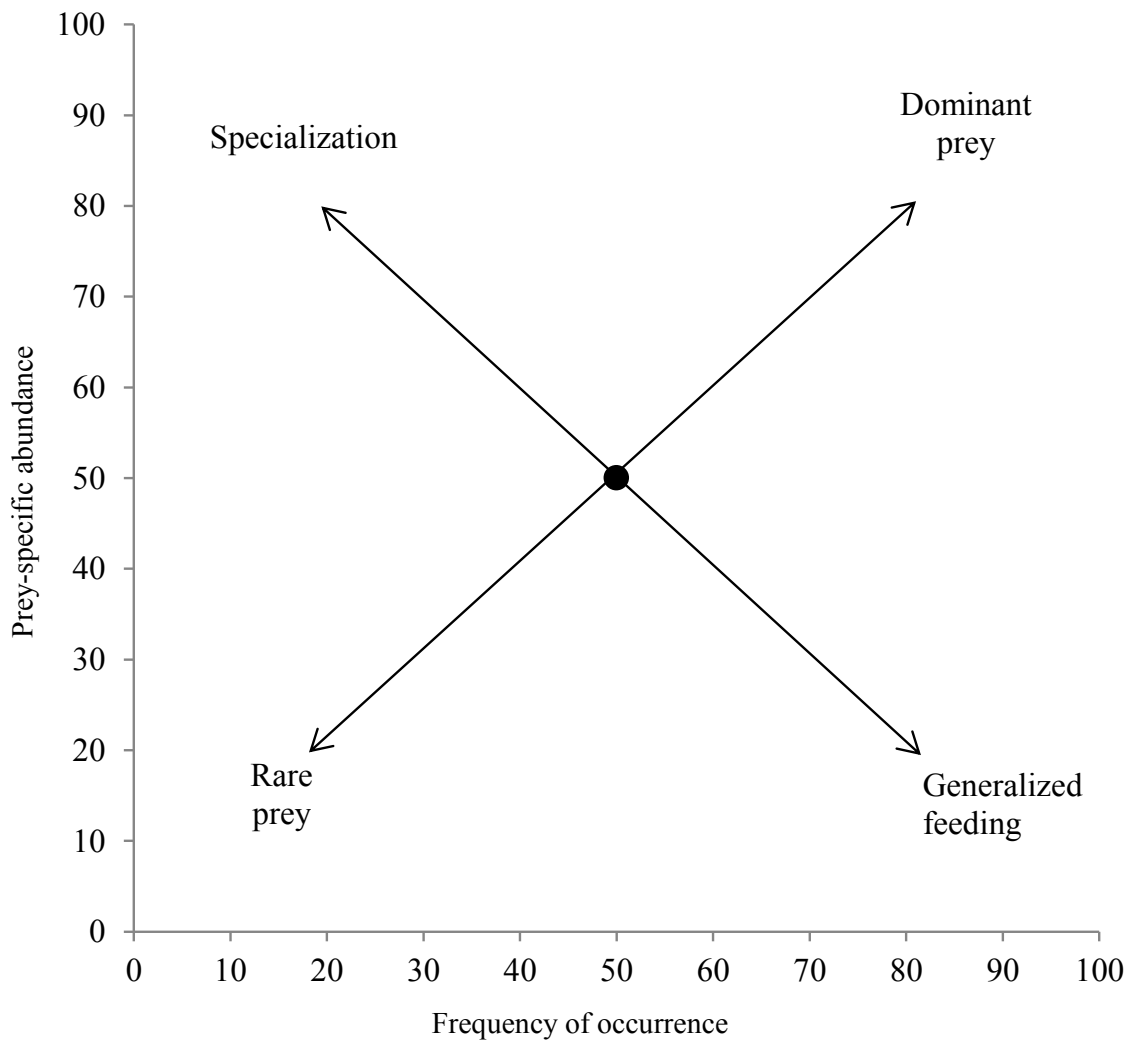


Figure 47. This Costello graph is a visual depiction of feeding strategy (reproduced from Chipps and Garvey 2007, originally in Costello 1990).

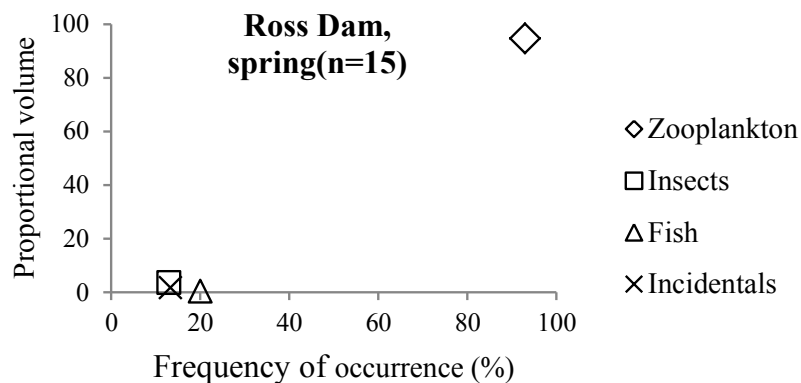
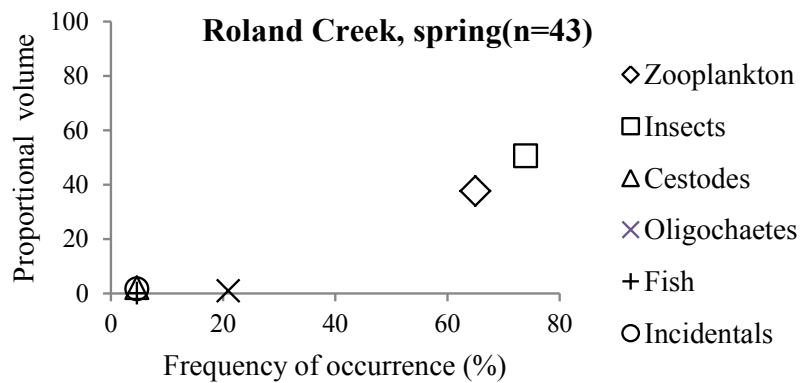
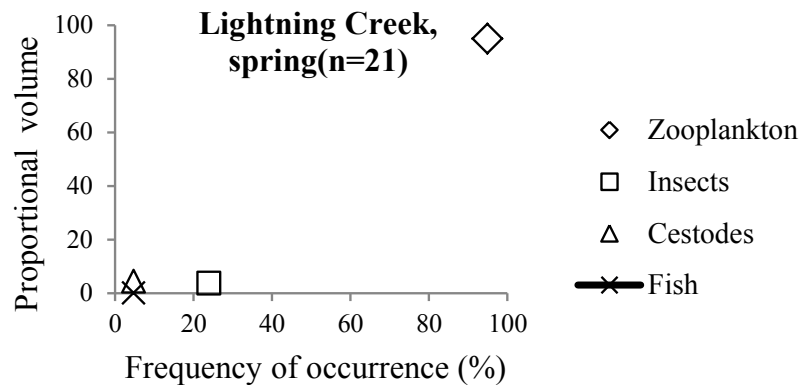
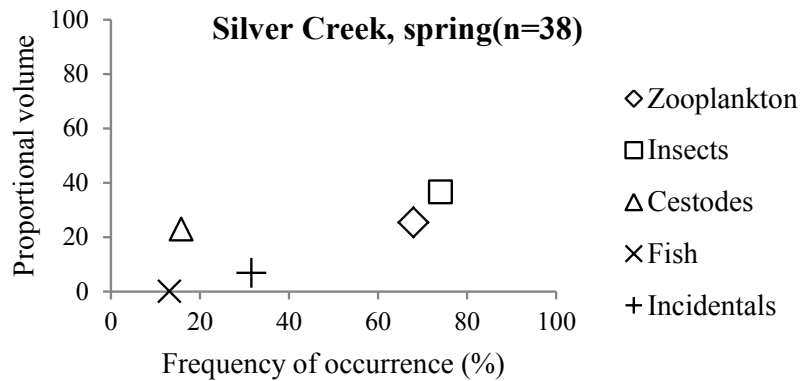


Figure 48. Diets of Redside Shiner from samples collected from Ross Lake in the spring, plotted, according to Amundson et al (1996) on a Costello graph to determine feeding strategy.

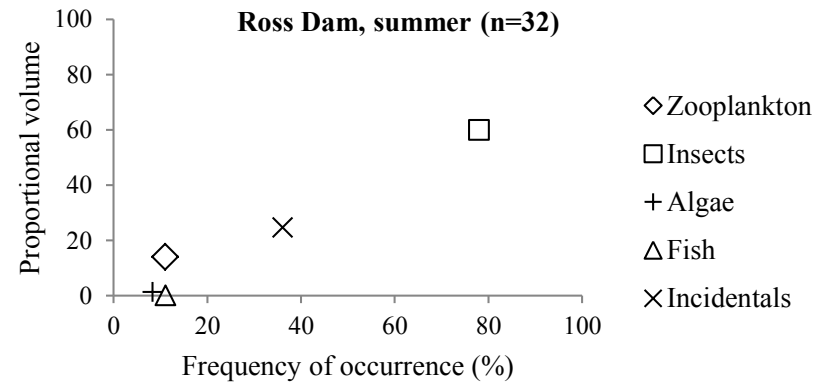
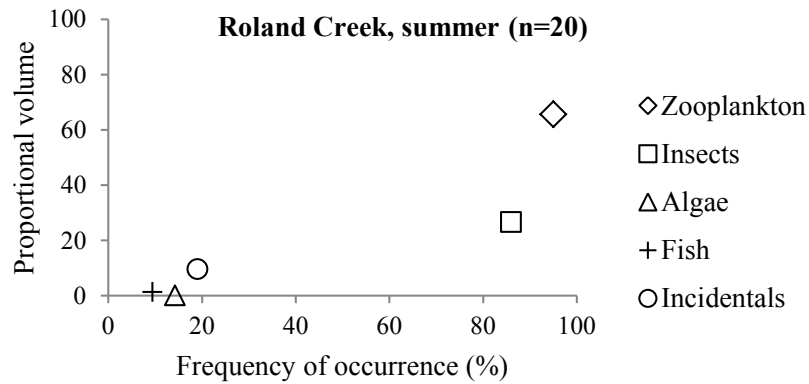
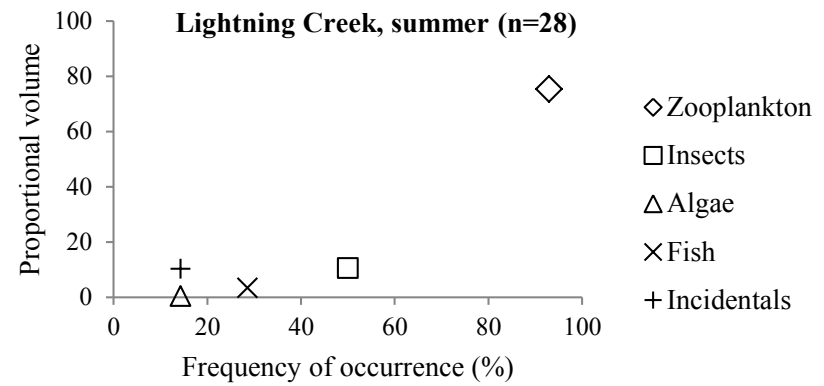
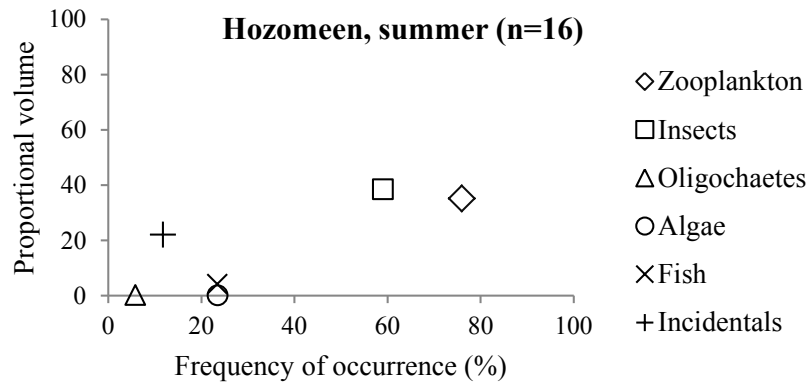


Figure 49. Diets of Redside Shiner from samples collected from Ross Lake in the summer, plotted according to Amundson et al (1996) on a Costello graph to determine feeding strategy.

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