Rainbow-Cutthroat Trout Hybrids (*Oncorhynchus clarki lewisi*) in Alberta: A Study of Diet Composition and Ontogenic Shift

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Introduction:

The current understanding of how rainbow-cutthroat hybrids affect the distribution of Westslope cutthroat trout in Alberta is lacking, and the loss of the Westslope cutthroat could have dramatic effects. Cutthroat trout are important prey for avian and mammalian predators and it has been found that river otters and grizzly bears rely on migrating cutthroat trout (Crait and Ben-David 2006; Middleton et al 2013). Replacement of native cutthroat trout with non-native species can also have negative effects on the stream and riparian ecosystems, since non-native trout species can out-compete the cutthroat trout and therefore significantly reduce benthic and emergent macroinvertebrates (Benjamin et al 2013). A reduction in emergent insects could then affect the populations of riparian predators that rely on them. The effects of hybridization of cutthroat populations can have consequences for terrestrial ecosystems, as well as the aquatic systems.

The Alberta populations of the Westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and rainbow-cutthroat hybrids have received no attention in regards to dietary habits and this research will provide an insight into the natural history of the hybrids. Previous research has focussed largely on American populations of cutthroat trout and is often directed at the genetic differences between the pure cutthroat and the hybrids. While the diet of other subspecies of cutthroat trout has been studied to a limited degree, we cannot assume these descriptions will be the same for Alberta populations of Westslope cutthroat or the hybrids. Understanding the dietary habits of a population allows for more informed conservation policies, such as protecting riparian zones. Changes to the invertebrate food supply will have an effect on the migration capabilities of the trout populations, but also on the survivability of individual trout.

In Alberta the Westslope cutthroat trout is the only native subspecies of the cutthroat trout, and is currently listed as threatened under Alberta's Wildlife Act (The Alberta Westslope Cutthroat Trout

Recovery Team 2013). A number of factors are influencing the decline of this subspecies throughout its native range including the introduction of invasive species, pollution and other disturbances to their habitat, and exploitation through overfishing. The latter of these issues can be addressed with proper policy and management, but the former has become a major contributor to the disappearance of the Westslope cutthroat.

Across the native range of the Westslope cutthroat trout, numerous species have been introduced including the rainbow, brown and brook trout (The Alberta Westslope Cutthroat Trout Recovery Team 2013). Of these three species, the rainbow trout represent the greatest threat due to introgressive hybridization and potential competition. Rainbow trout (*Oncorhynchus mykiss*) readily hybridize with the native Westslope cutthroat, with the offspring being fertile. The hybrids can then backcross with pure Westslope cutthroat and rainbow trout, or breed within the hybrid cohort, which can lead to the rapid spread of hybrids within a watershed. When compared against the rainbow trout and rainbow-cutthroat hybrids, the pure Westslope cutthroat appears to be competitively superior in cooler, high elevation headwaters, but competitively inferior in the warmer waters. As a result, the range of the Westslope cutthroat has been largely restricted to these cooler waters, which has left populations isolated and more susceptible to stochastic events (Yau and Taylor 2013). This raises concerns since global climate change has the potential to further restrict the Westslope cutthroat range if watersheds increase in temperature.

Unfortunately, while the Westslope cutthroat is threatened with extinction, there has been little research devoted to understanding the life history. Specifically, the diets of the Albertan populations of Westslope cutthroat trout and the rainbow-cutthroat hybrids have not been described in detail. Current trends among the literature of closely related species such as the rainbow trout, brook trout, brown trout and other cutthroat subspecies show that terrestrial insects may play a dietary role as important as aquatic insects, especially during summer months. There has also been very limited research into the ontogenetic shift in diet. This data is important to understanding the life history of the entire cohort. Exploring these factors together will help describe the dietary patterns of the species and aid in proper conservation and management policies.

The purpose of this research is to describe the diet composition of the rainbow-cutthroat hybrids in Camp Creek, with a comparison between terrestrial and aquatic invertebrate input, as well as the relative importance of each group to the various age groups within the hybrid cohort. I hypothesize that terrestrial macroinvertebrates will compose one quarter to one third of the diet based on previous research, and that the young individuals will have a less diverse diet composition than the older individuals, due to limits in dietary selection.

Materials and Methods:

The samples were collected from Camp Creek in the summer of 2004 by Dr. Janowicz. There are one hundred and two hybrid trout collected from Camp Creek. Along with the fish samples, benthic kick samples were also collected. Five benthic samples were collected at various locations along the creek. Since invertebrate drift samples were not taken, the availability of terrestrial prey cannot be compared to the diets. The benthic samples were analysed first by using a dissecting microscope and keyed with Aquatic Invertebrates of Alberta by Hugh Clifford. The invertebrates were identified to the lowest taxonomic level possible, but at least to order level. Digital pictures were taken of each new specimen and used to build a library for future comparison. After identification the invertebrates were sorted by family and stored in 70% ethanol.

The fish stomachs have been previously removed from the fish and preserved in 70% ethanol. In order to analyze the contents, the stomachs were dissected and flushed with distilled water to remove the contents. The cuts were made along the length of the stomach to the pyloric caeca, which prevented contents from the intestines from being included in the analysis. The total contents were then filtered through 125mm filter paper and then weighed to the nearest 0.01 gram. All intact invertebrates were identified to the lowest possible taxonomic level using a Technival 2 dissecting microscope. Body parts and partially digested items, when they could be identified with confidence, were also identified to the lowest possible taxonomic order and sorted. Specimens were also classified as terrestrial and aquatic groups, depending on whether they are the result of secondary terrestrial or aquatic production. The identified invertebrates and parts were then filtered, weighed, and stored in 70% ethanol.

Analysis of the stomach contents was conducted according to methods outlined by Hyslop (1980). Stomachs lacking contents were recorded and omitted from calculations of prey occurrence. Percent occurrence of prey items was calculated based on a proportion of numbers of individual taxonomic groups over total numbers.

% Occurrence= (Number of individuals in taxa)/(Total number of macroinvertebrates) * 100

The proportion of aquatic and terrestrial input to the total diet was calculated.

P_a= (Total aquatic macroinvertebrates)/(Total number of macroinvertebrates)

P_t= (Total terrestrial macroinvertebrates)/(Total number of macroinvertebrates)

where P_a= Proportion of aquatic macroinvertebrates

where P_t= Proportion of terrestrial macroinvertebrates

A simple presence-absence analysis was also done to compare the orders and families found in the stomach and benthic samples, which was followed by a percent overlap calculation.

% Overlap= (Number of taxa in benthic samples)/(Number of taxa in stomachs)*100

In order to analyze the potential ontogenetic shift in diet, the age data previously collected by Dr. Janowicz will be used. The age groups that will be used for the analysis are the juveniles (age 0-3) and adults (age greater than 3). These will be used to calculate proportional similarity index based on the formula provided by Bozek and others (1994).

$$PSI = 1 - 0.5\left(\sum_{i-1}^{s}\left|P_{ij} - P_{ik}\right|\right)$$

where, P_{ij} and P_{ik}, are the proportions of the food resource (i) used by size classes j (juveniles) and k (adults), and s are the total number of resource categories used by each size class.

This calculation will provide the proportion of diet overlap between age groups.

Results:

The stomachs were found to have a larger variety of taxa present compared to the benthic samples (Table 1). The percent overlap of macroinvertebrate orders was 35.7%, and percent overlap of families was 36.4%. Among the orders present in the stomachs that were absent from the benthic samples were Coleoptera, Hymenoptera, Hemiptera, Lepidoptera, Lumbriculida, Megaloptera, Odonata and Orthoptera. Within the orders that overlapped between the benthic and stomach samples, there were generally more variable families present in the stomach samples than in the environment. There were seven families within Diptera present in the stomach samples but absent in the benthic samples. Within Ephemeroptera there was one family present in the stomach samples but absent in the benthic samples, and in Plecoptera there were two families present in the stomach samples but absent in the benthic samples. Lastly, within Trichoptera there was one family present in the benthic samples that was absent in the stomach samples.

The proportion of aquatic and terrestrial macroinvertebrate input to the diets was calculated. Aquatic invertebrate input was 85% and terrestrial invertebrates made up 15% of the total diet. Furthermore, the proportion of the major orders present was calculated for Plecoptera (28%), Ephemeroptera (21%), Diptera (20%), Hymenoptera (14%), and Trichoptera (6%). At 93%, order Hymenoptera made up the largest proportion of the terrestrial input. The percent composition of these five orders was calculated for each age and fork length group of fish and three orders, Trichoptera, Ephemeroptera and Diptera did not appear to differ. Percent composition of Hymenoptera seemed to increase as age and fork length increased, and percent composition of Plecoptera seemed to decrease as age and fork length increased (Figure 3a and 3b).

There was a significant difference between the numbers of invertebrates present in the stomachs across the various ages (F[8,32]=2.24, p=0.000198). The proportion of aquatic invertebrate input to the diet is not significantly different across the various ages of fish (F[8,93]=1.514, p=0.163). However, age 9 appears to have a significantly lower proportion of aquatic invertebrates than age 1 (Figure 5). Across the various age groups, the proportion of terrestrial input did not differ significantly (F[8,93]= 1.601, p=0.135).The variation in numbers of invertebrates eaten and proportion of aquatic invertebrates was the highest within the age 8 and age 9 groups. There was a significant correlation (r=0.565) between the proportion of aquatic and terrestrial input to the diet (Figure 6). Also, there was complete diet overlap between the juveniles (age 0-3) and adults (age 4-9), since the PSI between the groups was 1. After expanding the age groups to age 0-3, 4-6 and 7-9, the PSI between each of the groups was 1, which still indicated a complete diet overlap.

Table 1. Presence-absence of Orders and Families of invertebrates in benthic and stomach samples.

Presence is marked by X.

Taxa	Benthic	Stomach
Acari		X
Araneae	X	X
Pisauridae	X	X
Coleoptera		X
Carabidae		X
Chrysomelidae		X
Curculionidae		X
Dysticidae		X
Hydraenidae		X
Hydrophilidae		X
Unknown Terrestrial		X
Diptera	X	X
Ceratopogonidae		X
Chironomidae	X	X
Culicidae		X
Empididae		X
Psychodidae		X
Ptychopteridae		X
Simulidae	X	X
Stratiomyidae		X
Tipulidae		X
Ephemeroptera	X	X
Baetidae	X	X
Ephemerellidae	X	X
Heptageniidae	X	X
Siphlonuridae		X
Hemiptera		X
Gerridae		X
Notonectidae		X
Unknown Terrestrial		X
Hymenoptera		X
Formicidae		X
Lepidoptera		X
Lumbriculida		X
Megaloptera		X
Sialidae		X
Odonata		X
Orthoptera		X
Plecoptera	X	X
Chloroperlidae		X
Perlidae	X	X
Taeniopterygidae		X
Trichoptera	X	X
Brachycentridae	X	X
Glossosomatidae	X	
Hydropsychidae	X	X
Limnephilidae	X	X
Rhyacophilidae	X	X

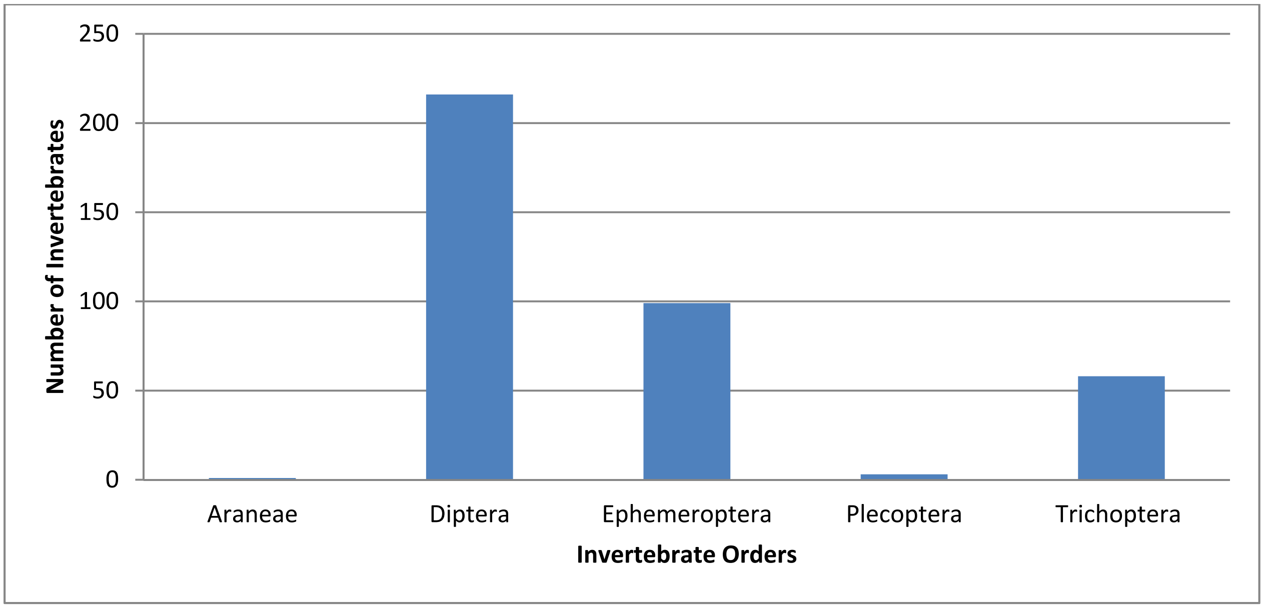

Figure 1. Number of aquatic invertebrates found in benthic samples by numbers.

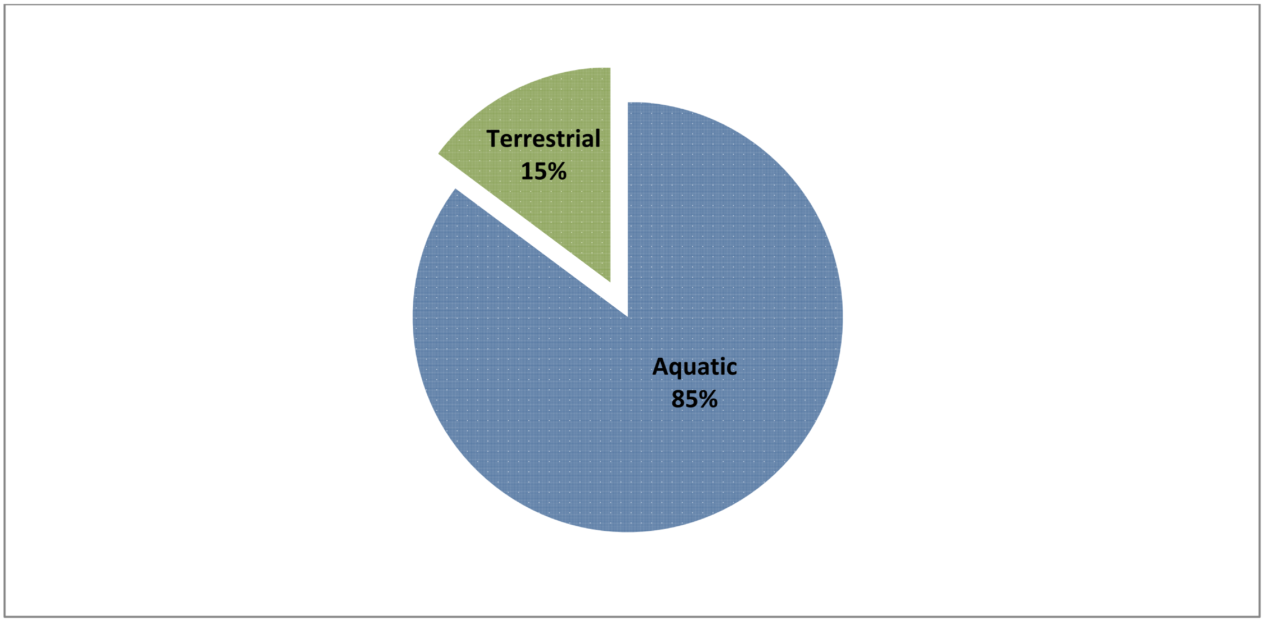

Figure 2. Proportion of aquatic and terrestrial invertebrate input to the total diet.

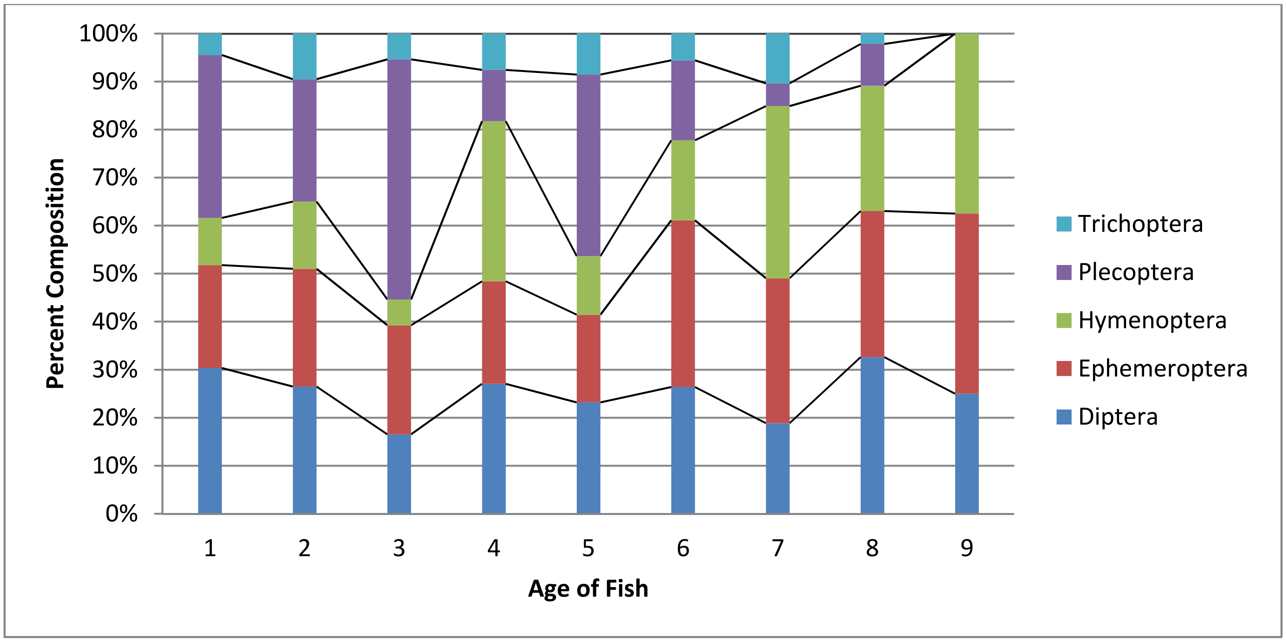

Figure 3a. Percent composition of macroinvertebrate orders to the diet of fish compared to age.

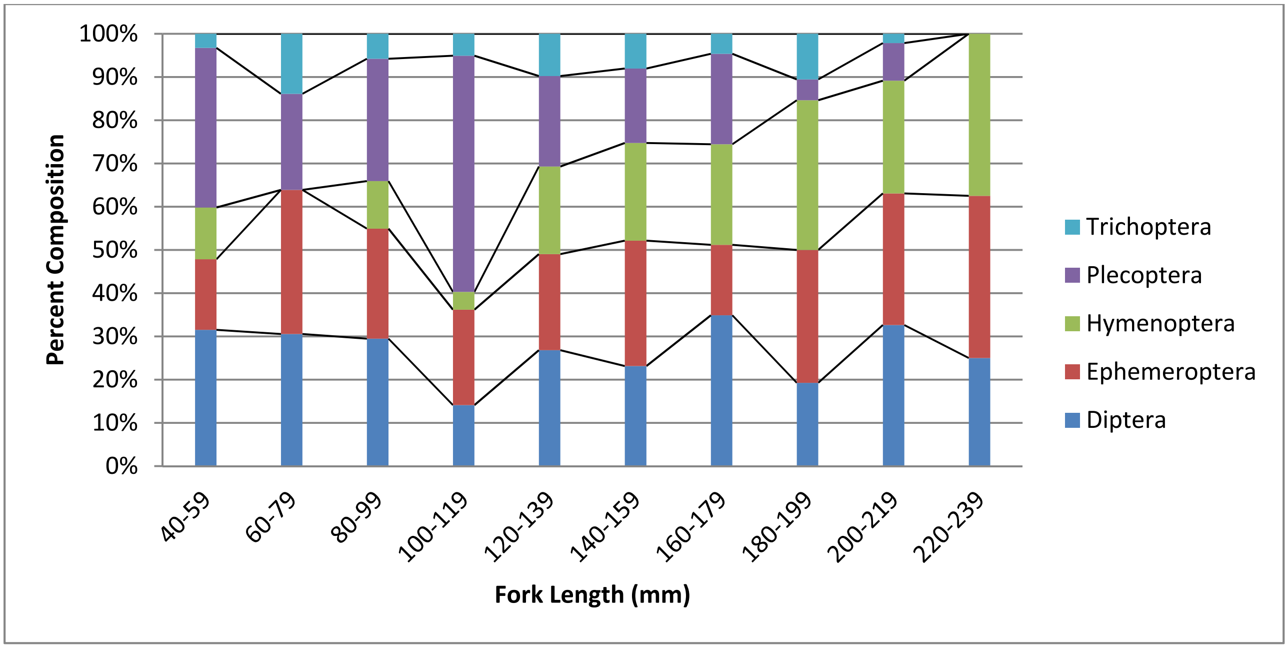

Figure 3b. Percent composition of macroinvertebrate orders to the diet of fish compared to fork length (mm).

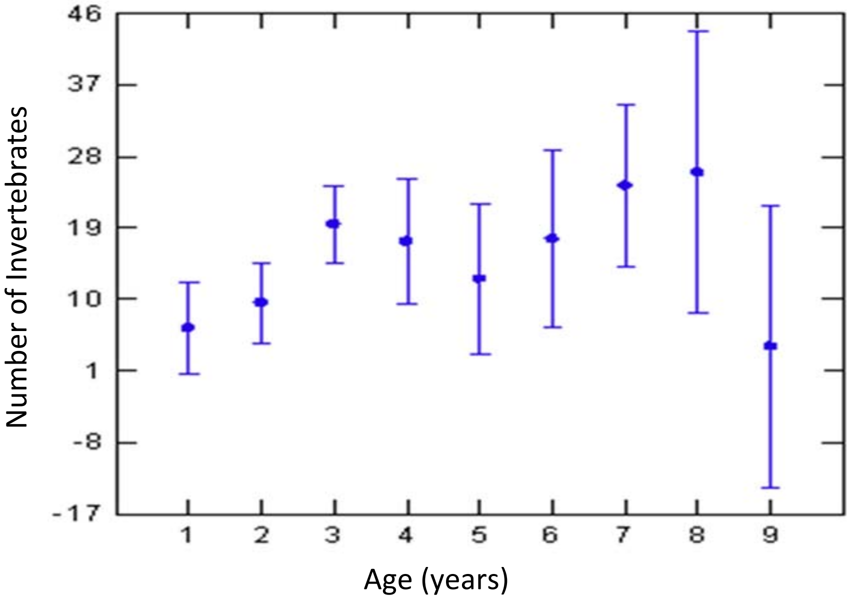

Figure 4. Number of invertebrates found in the stomachs of Westslope cutthroat trout of various ages. Error bars indicate standard deviation.

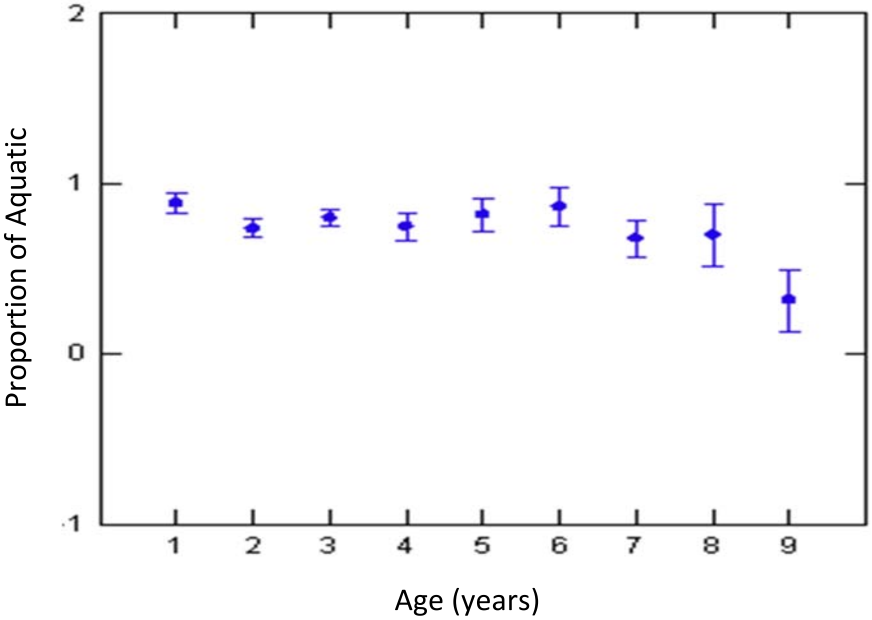

Figure 5. Proportion of aquatic input to the diets of rainbow-cutthroat hybrids of various ages. Error bars indicating standard deviation.

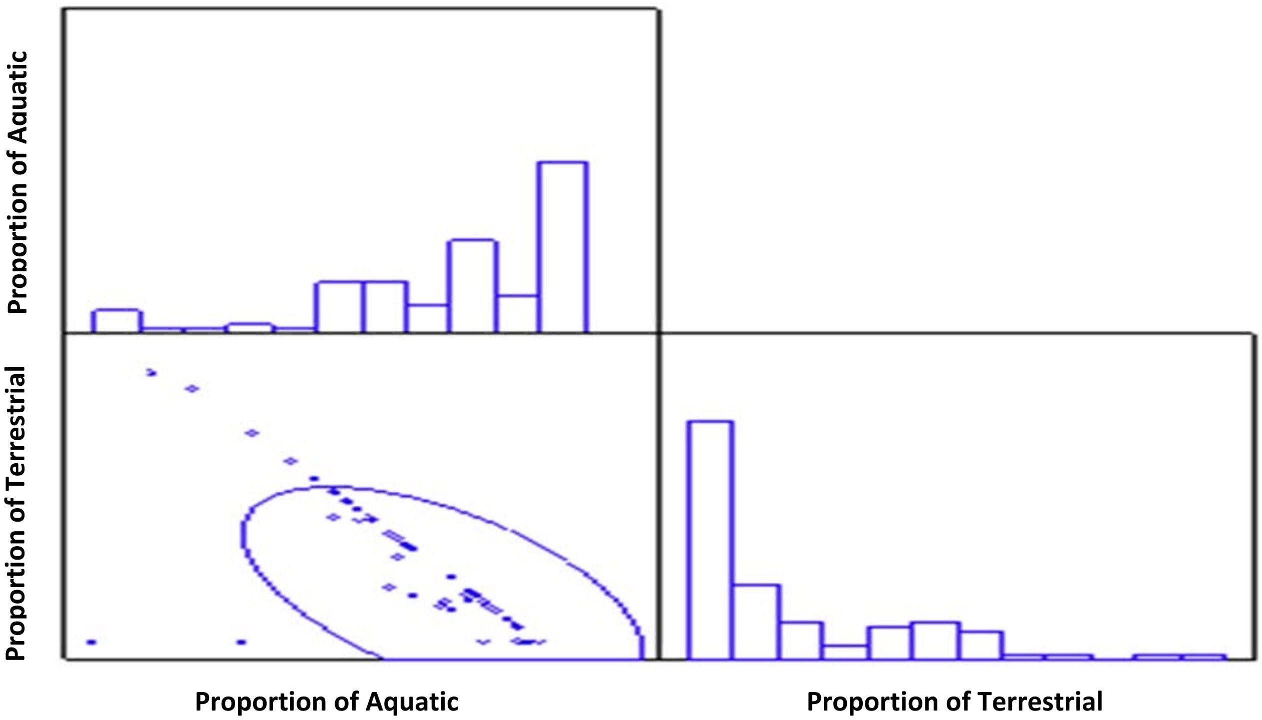

Figure 6. Correlation of proportional input of aquatic and terrestrial invertebrates to the fish diet (r=0.565).

Discussion:

There has been very little research dedicated to the diet of the Westslope cutthroat in Alberta, but other subspecies have been studied. Diptera, Trichoptera, Plecoptera and Ephemeroptera were found to be the largest components of macroinvertebrate drift in Rocky Mountain streams during summer months (Allan 1987). While this study looked at drift as opposed to benthic samples, the presence of the orders is still relevant since these aquatic macroinvertebrates inhabit the benthic zone before ending up in the drift. It was found that those four orders were also the most abundant in the benthic samples in this study, with Diptera being the most abundant. However, Trichoptera had the highest diversity of families present in the environmental samples (Table 1). This could be potentially due to a wider range of ecological niches available to Trichopteran families. Competitive exclusion within the other orders could limit the diversity of families present, and lead to a few dominant families.

Aquatic Ephemeroptera, Diptera and Trichoptera typically dominate the stomach contents of rainbow trout (Angradi and Griffith 1990; Riehle and Griffith 1993). Due to the close relation and similar foraging behaviours between rainbow and Westslope cutthroat trout, we can compare previous research on the rainbow trout to what was found in the stomach samples. Research done on a British Columbian population of Westslope cutthroat trout showed that they mainly fed on Diptera, Ephemeroptera, Plecoptera, and Trichoptera (Bennett 2004). These studies support the findings that the majority of the aquatic input to rainbow-cutthroat hybrid diet is made up of Plecoptera (28%), Ephemeroptera (21%), Diptera (20%), and Trichoptera (6%). While Plecoptera made up less than 1% of the total invertebrates in the environmental samples, it contributed the most to the fish diets which indicates that the fish were preferentially selecting Plecopterans. This can also be seen by the variety of Plecoptera families present in the stomachs as compared to the benthic samples.

While aquatic invertebrates would be expected to contribute the most to rainbow-cutthroat hybrid diet, terrestrial invertebrates may be important as well. Terrestrial insect populations are typically the greatest during the summer when vegetation biomass peaks, and thus the input of terrestrial insects into the stream food web also peaks during this time (Nakano and Murakami 2001). They found that 46.3% of rainbow trout summer diet was comprised of terrestrial insects. This is because aquatic insects typically emerge as adults in spring and so the aquatic macroinvertebrate biomass is low throughout the summer months. The input of terrestrial arthropods was found by Cloe and Garman in 1996 to be the highest during the summer months, and as such were the most important to salmonid diets during these months.

These previous studies were conducted on various salmonid species and show that insectivorous fish could potentially rely on terrestrial insects during the summer months when aquatic macroinvertebrate biomass is low. It is possible that the same is true for the Westslope cutthroat trout.

In a study of the cutthroat trout in the Colorado River in 1997, Young and others found that while terrestrial Hymenoptera, Coleoptera and Lepidoptera made up 14% of the macroinvertebrate drift, they comprised more than 31% of the cutthroat diet. This study was conducted during the late summer and shows that cutthroat trout preferentially selected for terrestrial insects during these months. Similar results were found by Wipfli in 1997, when cutthroat trout in Alaska were studied. It was shown that terrestrial invertebrates were an important food source for cutthroat trout, and depended heavily on the type of riparian vegetation with young growth forest providing more terrestrial invertebrate input. Another study in 2005 by Romero and others showed that terrestrially derived invertebrates made up 35% of coastal cutthroat diet.

However, the terrestrial invertebrate input to the rainbow-cutthroat hybrid diet was found to be roughly 15% of the total diet, with Hymenoptera making up 93% of the terrestrial input. Due to the lack of drift samples we cannot determine the abundance of terrestrial invertebrates in the environment, but based on the percent composition and correlation analysis it seems that the older fish select for terrestrial invertebrates, specifically Hymenoptera. Variation in the riparian vegetation likely determines the abundance of terrestrial invertebrates and so future work with an additional five streams may increase the proportion of terrestrial input to the diets. The percent composition analysis shows that fish of age 7 and older, and larger than 180mm in fork length appear to shift from eating Plecoptera to Hymenoptera. This corroborates with the correlation analysis which shows that as the proportion of aquatic invertebrate input decreases, terrestrial invertebrate input increases, and the proportion of aquatic invertebrates in the diet decreases. This could be due to different feeding strategies between age groups, and that trout typically have flexible feeding strategies based on prey size and availability (Sánchez-Hernández and Cobo 2015). Predator avoidance may also play a role in prey selection, since the younger fish may be less inclined to feed near the surface and would thus not have access to the terrestrial invertebrates in the drift.

The number of invertebrates in the stomachs was significantly different across the ages with the trend being generally positive to age 8. Given that older, larger fish would have a higher energy requirement, it follows that there would be a higher number of food items in the stomachs. This relationship does not reveal any potential shift in diet, however since it is generally positive. From the proportion of aquatic invertebrate input, there appears to be a shift at age 7 where the hybrids began consuming less aquatic prey in favor of terrestrial invertebrates. However, the proportions of aquatic and terrestrial invertebrate input were not found to be significantly different across ages. Furthermore, the PSI indicated a complete diet overlap between the juvenile and adult age groups. This suggests that there was no significant shift in the composition of the rainbow-cutthroat hybrid diets. Sánchez-Hernández and Cobo (2015) failed to find significant ontogenic shifts when studying brown trout (*Salmo trutta*) when they investigated prey size across various age and length groups. It is possible that due to the opportunistic feeding behaviour and flexible feeding strategies of trout in general, simply investigating numbers of prey was not enough to reveal clear ontogeny.

In conclusion, rainbow-cutthroat hybrids were found to predominantly feed on Plecoptera, Diptera, Ephemeroptera, and Trichoptera, which are the four major aquatic taxa reported in previous studies on rainbow and Westslope cutthroat trout. Hymenoptera was found to be the largest contributor to the terrestrial invertebrate input to the diet, but the proportions of terrestrial and aquatic input were not found to differ significantly across the age groups. There was a strong correlation between the proportions of aquatic and terrestrial invertebrate inputs, showing that as the proportion of aquatic input decreased, the proportion of terrestrial input increased. However, we could not find a clear shift in the diet of the rainbow-cutthroat hybrids. This could be based on the limitation of only using numbers of prey items, and further analysis using prey item length and weight could reveal a clearer shift. Future work which will include an additional five streams will increase the stomach sample size and potentially increase the statistical variation across the ages.

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