THE ECOLOGICAL CONSEQUENCES OF HYBRIDIZATION BETWEEN NATIVE WESTSLOPE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKII LEWISI*) AND INTRODUCED RAINBOW TROUT (*O. MYKISS*) IN SOUTH WESTERN ALBERTA

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B.Sc., Okanagan University College, 2005

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfillment of the
Requirements for the Degree
[MASTERS OF SCIENCE]

Biological Sciences Department
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

Dedication

This thesis is dedicated to Daniel James Robinson for instilling in me a love and respect for nature.

Abstract

This thesis addresses the issue of hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*), giving strong consideration to their differing glacial refugia during the Wisconsin Glaciation. We hypothesize that having more recently derived from an anadromous form *O. mykiss* will possess life history characteristics more typical of a highly anadromous species. This hypothesis would also predict hybrids to be intermediate in these characteristics. In a comparison of growth rates and survivorship (Chapter 2) *O. clarkii lewisi* were found to employ a slower growing, longer lived strategy than *O. mykiss*, with hybrids typically being intermediate. Additionally, *O. mykiss* were also found to have aerobic and anaerobic metabolic capacities superior to *O. clarkii lewisi* in a first time comparison of these species (Chapter 3). These results support the glacial refuge hypothesis, but furthermore provide a potential explanation of the establishment of the elevational gradient commonly observed in hybridization studies. It would seem likely that *O. mykiss* would require more productive reaches being a faster growing, shorter lived species with higher metabolic costs. This study confirmed the gradient of *O. mykiss* persisting at lower elevations, trending through a hybrid zone to pure *O. clarkii lewisi* in headwater reaches and above migratory barriers (Chapter 2). A similar gradient was also reported when considering only the hybrid population, supporting the notion that habitat preference is under some genotypic control. The importance of migratory barriers was found to decrease with elevation suggesting potential additional limiting factors. Hybrid individuals were also found to be intermediate in morphological characteristics (Chapter 4). The confidence in differentiating between pure and non-pure *O. clarkii lewisi* was

found to increase with the number of *O. mykiss* alleles (degree of hybridization) an individual possessed. Morphological-based identification was found to be an efficient, cost-friendly, preliminary assessment tool that could be useful in limiting the number of sites needing detailed genetic assessment.

Acknowledgements

I would very much like to thank Dr. Joseph Rasmussen for being more than I had ever hoped for as a supervisor. Dr. Rasmussen's honest desire to mentor developing researchers is evident in each one of the endless conversations he has with his students. Another important cog in this research is Dr. Daniel Heath (GLIER) whose laboratory provided the genetic analyses. I would also like to thank Dr. Alice Hontela and Dr. Martin Lalumière, my supervisory committee, and Dr. Robert Behnke, my external examiner, for their honest interest and input towards this project. This project would not have been half of what it was without the support from Ryan MacDonald, Corinna Robinson, Crystal Waters and Jim Robinson in the field and the rest of the Rasmussen and Hontela labs, especially "Red". Thanks to Dr. Patrice Couture (INRS, UQ) and Dr. Ken Rodnick (ISU) for advise on the metabolic assays, Daryl Wig for services as the Regional Fisheries Biologist covering my study area and Jon Carter and Jon Underwood from the Government of Alberta's hatchery program. This project was funded by NSERC grants to myself and Dr. Rasmussen, Trout Unlimited Canada's Cold Water Conservation Fund and an Alberta Conservation Association (ACA) Biodiversity Grant.

Finally I would like to acknowledge the love and support from my wife, Corinna Robinson, who has directly improved this project with help in the field, the lab and everywhere else, Mya Isabella Robinson for reminding me to view nature with the simplicity and enthusiasm of a three-year old, and most recently Nate "The Great" for reasons unknown yet.

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1. The specific issue of hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*O. mykiss*): literature review

Abstract

Species introductions are legitimate, modern threats to native ecosystems worldwide. While the commonly recognized mechanisms of impact (predation, competition, disease transmission and habitat manipulation) remain valid, hybridization, introgressive and otherwise, has been recognized as the most significant threat to certain ecosystems. Specifically, the introduction of rainbow trout (*Oncorhynchus mykiss*) has been identified as a main factor in the extinction, extirpation and genetic pollution of multiple cutthroat subpopulations, namely the westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) (Behnke 2002).

This thesis offers a perspective on this situations that is strongly based on the differing glacial refugia occupied by these sister taxa during the Wisconsin Glaciation. While *O. clarkii lewisi* and several other salmonid species (bull trout, *Salvelinus confluentus*; lake trout *Salvelinus namaycush*; white fish, *Coregonus clupeaformis* and *Prosopium williamsoni*) sought refuge in proglacial lakes, *O. mykiss* and other anadromous pacific salmon typically sought refuge in the Pacific Ocean through their anadromous forms. I hypothesize that having more recently derived from an anadromous form *O. mykiss* will possess characteristics more typical of a highly migratory species, than the more

anciently land-locked *O. clarkii lewisi*; the characteristics, high growth and low survivorship, have been further enhanced through years of domestication in hatchery programs. We discuss the implications of this differentiation on the likelihood of hybridization and the establishment of sympatry in situations where *O. clarkii lewisi* and *O. mykiss* experience anthropogenic coexistence. There are relatively few occurrences of historic range overlap between these two species since post-glacial recolonization. It is apparent that wherever *O. mykiss* had access to reaches below migratory barriers *O. clarkii lewisi* are not found. From this, predictions can be made of the relative competitive abilities of these two species, and furthermore on the chances of long-term success of genetically pure *O. clarkii lewisi* populations.

Species introductions

The spread of invasive species is rapidly becoming one of the major environmental problems of our time, and freshwater biota are among the most endangered elements of the world's fauna (Ricciardi and Rasmussen 1998; Ricciardi and Rasmussen 1999). While effects of habitat destruction and pollution can often be mitigated or restored, exotic invaders are generally permanent since their extirpation is rarely possible. Allendorf *et al.* (2004) describe the persistent nature of hybridization as a unidirectional, "genomic ratchet." Although many of the "classic" invaders have arrived inadvertently from remote areas like the Pontocaspian (Ricciardi *et al.* 1998), some of the greatest adverse impacts result from the intentional extension of ranges of our native species to augment fisheries. As a prime example, rainbow trout (*Oncorhynchus mykiss*), native primarily to Pacific regions of northern hemisphere continents, now exist across North America and at many locations in the southern hemisphere (Behnke 2002). The introduction of *O. mykiss* has been identified as a main factor in the extinction, extirpation and genetic pollution of multiple cutthroat subpopulations, namely the westslope cutthroat trout (*O. clarkii lewisi*) (Behnke 2002).

Either intentionally or accidentally, the last half a century has seen a significant acceleration in the rate of exotic species introductions. Between the periods of 1851-1960 and 1961-1995 new species establishments in the San Francisco Bay and Delta have increased from one every 55 weeks to one every 14 weeks (Cohen and Carlton 1998). Outside of their native ranges many introduced species may become naturalized, often irreversibly altering the natural ecosystem. While we commonly think of introduced

species adversely affecting native populations through predation, competition, disease transmission and habitat manipulation (Taylor 2003), hybridization is now recognized as a significant threat to native ecosystems. Introductions of exotic species into aquatic ecosystems and the sensitivity of these systems to invasions have been well documented and contribute to the estimated 5% higher extinction rates of freshwater fauna when compared to terrestrial species (Ricciardi and Rasmussen 1999).

Hybridization: its role, extent and consequences

There are wide-ranging views on the importance of hybridization in evolution (See Burke and Arnold 2001). One perspective is that natural hybridization (introgressive) is an important mechanism for the exchange of genetic information among taxa (Verspoor and Hammer 1991), acting as a path through which beneficial adaptations may be incorporated into the genome of closely related taxa. We can further illustrate this by discussing the intraspecific equivalent to hybridization. Commonly referred to as straying, intraspecific hybridization provides a way in which ecologically adaptive traits can spread throughout a population of individuals who typically have strong homing instincts. This typically results from the breakdown or failure of homing mechanisms and, as with hybridization, species usually possess instinctive behaviours that limit its extent. This is one of the key underpinnings or components underlying the argument that habitat fragmentation can increase the risk of extinction of species (Riemann and Dunham 2000). In the same way that straying passes beneficial traits throughout a population (among populations) hybridization may do the same between groups at higher taxonomic levels.

Hybridization also plays a significant role in speciation. In situations of increased hybrid fitness, new species may arise from novel genetic combinations that are better adapted to certain environmental conditions than either parental taxon (Rhymer and Simberloff 1996). Alternatively, a reduction of fitness in a hybrid zone can lead to the evolution of isolating mechanisms between sympatric species. If a stable hybrid zone should develop then gene flow becomes limited and divergence ensues (Barton and Hewitt 1989).

Hybridization is a naturally occurring event that is especially common in freshwater fishes, the extent of which has been under study for over 50 years (Hubbs 1955). More recently genetic analyses have shown that natural hybridization occurs more commonly than morphological studies would suggest (e.g. Rhymer and Simberloff 1996; Docker *et al.* 2003; Rubidge and Taylor 2005). Thus, the subject of hybridization has become controversial, both in regard to taxonomic and evolutionary issues (Barton 2001), especially in situations where anthropogenic influences are involved. The consequences of a hybridization event are variable and unpredictable, and may include homogenization of taxa, pre-reproductive barrier reinforcement, extirpation of a parental taxa or speciation (Arnold 1992, Rahel 2002), with one effect frequently increasing the risk of another (Rhymer and Simberloff 1996).

The native ranges of Oncorhynchus clarkii lewisi and Oncorhynchus mykiss

This review will be based around the effects of rainbow trout (*O. mykiss*) introduction into the native range of westslope cutthroat trout (*O. clarkii lewisi*) through hybridization. As early as 1988, Allendorf and Leary (1988) considered these effects to be the main threat facing this subspecies of cutthroat trout.

O. clarkii lewisi and *O. mykiss* are closely related species that have been identified as sister taxa in several molecular phylogenetic studies (Allendorf and Leary 1988; Crespi and Fulton 2004). Both are considered to have evolved in northwestern North America, with estimates of divergence between six (Stearley and Smith 1993) and two million years ago (Behnke 2002). As this period saw the onset of the Pleistocene glaciation (beginning 2.5 mya), a period of repeated glaciation and deglaciation in the northwestern Cordillera, it is important to consider their native ranges within the context of the events that occurred during the final recession of the Wisconsin glaciation (10 000 mya).

Throughout the Pleistocene proglacial lakes formed when advancing ice would dam a major drainage. These lakes acted as refugia for *O. clarkii lewisi*, bull trout (*Salvelinus confluentus*) and lake trout (*Salvelinus namaychus*), while *O. mykiss* and pacific salmon typically sought refuge in the Pacific Ocean through their anadromous forms (Behnke 2002). The most notable refuge for *O. clarkii lewisi* was Glacial Lake Missoula (Taylor *et al.* 2003) that formed in northern Montana when ice dammed the Clark Fork River. An apparent exception for *O. mykiss* is the Athabasca rainbow trout; the only *O. mykiss*

population naturally existing east of the Continental Divide. The distribution of this population is most logically explained by an inland refuge with molecular evidence existing to support this (Carl *et al.* 1994).

As the glaciers receded the lacustrine water was released leaving behind a network of streams that became inhabited by species present in the glacial lakes. The significance in terms of the development of the *O. clarkii lewisi* and *O. mykiss* native ranges is that while *O. clarkii lewisi* were already inland as streams developed and became open for recolonization, *O. mykiss* had to return inland by migrating up large river networks where major barriers were often present. Thus, as a result of different Pleistocene histories *O. clarkii lewisi* became established in river systems much farther inland, and even east of the continental divide, whereas *O. mykiss* were generally restricted to rivers with direct coastal links and no migratory barriers (Figure 1-1).

From the native ranges we see that *O. clarkii* subspecies and *O. mykiss* are almost entirely allopatric, excluding the area where coastal cutthroat trout (*Oncorhynchus clarkii clarki*) have coexisted and likely coevolved with steelhead (anadromous *O. mykiss*) (Behnke 2002). As a result of the restricted overlap between ranges of *O. clarkii lewisi* and *O. mykiss* ranges, primarily restricted to six drainages in Oregon, Idaho and Washington (Behnke 2002), it is reasonable to hypothesize that in higher-order reaches *O. mykiss* have displaced or prevented the recolonization of *O. clarkii lewisi*, with migratory barriers being the saving grace for many *O. clarkii* subspecies. Supporting this is molecular evidence presented by Taylor *et al.* (2003) that upper South Thompson River

O. clarkii lewisi are more likely remnants of a larger Fraser River population that has been replaced by inland-progressing *O. mykiss*, than descendants brought over from the Columbia River drainage via headwater capture. The persistence of *O. clarkii clarki*, the only cutthroat subspecies that is truly sympatric with *O. mykiss*, is attributed to pre-reproductive isolation resulting from differentiated spawning habitat preference between it and *O. mykiss* (Behnke 2002; Ostberg *et al.* 2004).

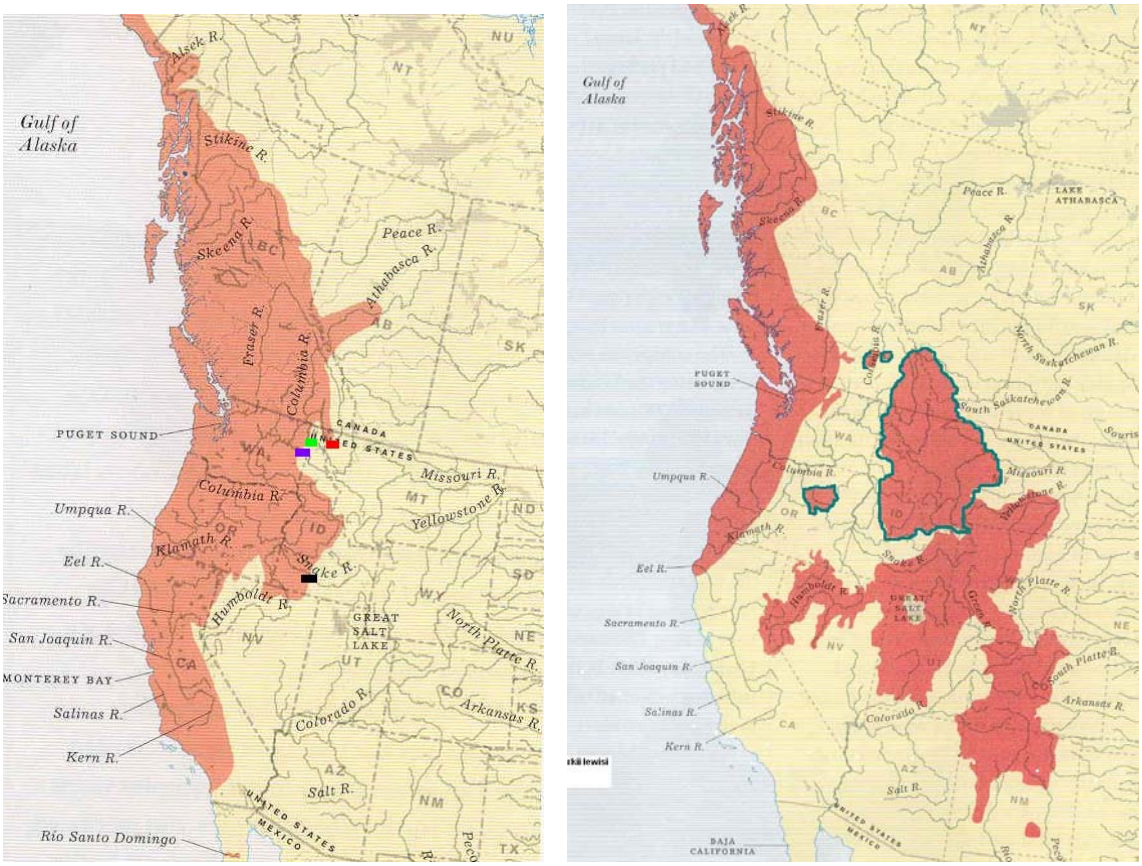

Figure 1-1: Native ranges of *O. mykiss* (left) showing major migratory barriers (red=Kootenay Falls on Kootenay River, green=Albeni Falls on Pend'Oreille River, purple=Spokane Falls on Spokane River and black=Shoshone Falls on Snake River) and *O. clarkii lewisi* (right) showing *O. clarkii lewisi* range outlined in blue. Modified from Behnke (2002).

Due to human activities, the native ranges of both *O. clarkii lewisi* and *O. mykiss* have been altered. For over 100 years *O. mykiss* have been actively stocked into many parts of

native cutthroat ranges where they readily hybridize with the native populations, while *O. clarkii lewisi* were rarely moved outside of its natural boundaries (Behnke 2002). Many studies have failed to report any evidence in support of negative hybrid selection (Rubidge et al. 2001; Hitt et al. 2003; Weigel *et al.* 2003), commonly reporting broad hybrid zone existing over a wide range of environmental conditions. Opportunity for negative hybrid selection becomes even more unlikely when addressing potential mechanisms of selection, with respect to these two species.

Pre-reproductive hybrid selection

Loss of pre-reproductive isolation

Negative pre-reproductive (prezygotic) selection would logically favour the maintenance of species separation. We will present two main pre-reproductive factors involved in selection and how their failure to act results in the extensive hybridization observed between introduced *O. mykiss* and native *O. clarkii lewisi*. The first deals with coevolution between rainbow and cutthroat trout, and its role in the evolution of pre-reproductive isolation. Since *O. clarkii lewisi* and *O. mykiss* have not coexisted historically, to the extent that *O. mykiss* and coastal cutthroat trout have (Behnke 2002), prezygotic barriers of this kind are generally not observed between *O. clarkii lewisi* and *O. mykiss*. Although it is accepted that hybridization in areas of natural sympatry exists, its extent is not yet clear. Based on genetic analysis from over 30 years of data Leary *et al.* (1995) found that when *O. clarkii lewisi* and *O. mykiss* are naturally sympatric hybridization beyond F1 hybrids was rare (Allendorf *et al.* 2004). This absence of

introgression (back-crossing) could be interpreted either as an indication that F1 hybrids are not fertile, or more likely, that hybridization is so rare that introgressive backcrosses were below detectable limits and did not turn up during sampling. However, Campton and Kaeding (2005) argue that natural hybridization is more extensive than Allendorf *et al.* (2004) indicate. While the potential role of prezygotic isolating mechanisms in limiting hybridization seems likely, it has not yet been clearly demonstrated.

Although it has long been recognized that there is very little overlap between the native ranges of *O. clarkii lewisi* and *O. mykiss*, the potential significance of this has received little attention. The rarity of natural sympatry likely indicates that the postglacial inland spread of *O. mykiss* has resulted in an almost complete replacement of *O. clarkii lewisi* and/or prevention of establishment of *O. clarkii lewisi* below major migratory barriers (Figure 1-1). This implies that historically *O. clarkii lewisi* and other subspecies of *O. clarki* as well, have not been able to coexist with *O. mykiss*. If this is indeed true, it may have much more significant long-term implications for the persistence of *O. clarkii lewisi* populations, currently affected by hybridization, than does the present day frequency of natural hybridization.

It is clear however that increases in the level of hybridization are observed whenever stocking extends the range of *O. mykiss* into the native range of *O. clarkii lewisi* (Allendorf and Leary 1988; Rubidge *et al.* 2001; Hitt *et al.* 2003). Furthermore, stocking increases the occurrence of hybridization as well as the geographic span of the hybrid zone in areas where *O. clarkii* subspecies and *O. mykiss* are naturally sympatric (Docker

et al. 2003; Weigel *et al.* 2003). In a hybridization study of anadromous *O. mykiss* (steelhead) and *O. clarkii clarki*, Docker *et al.* (2003) found that streams in which *O. mykiss* populations had been supplemented by stocking had a much higher frequency of hybridization (53%) compared to unaltered streams (9%).

Hawkins and Foote (1998) describe hybridization as "the breakdown of isolating mechanisms between two reproductively isolated species or populations." Often, isolating mechanisms are the result of differences in locally adaptive behaviours. When hybridization occurs the local genome may become altered, potentially resulting in the loss of local adaptations (Allendorf *et al.* 2004). Local adaptations are attributes or behaviours that have been shaped by local exogenous pressures to increase fitness. Fry emergence (Hawkins and Foote 1998), time of spawning (Leary *et al.* 1989) and rearing strategies (Rasmussen *et al.* 2003) are examples of traits that become locally adapted. Furthermore, they have all been shown to be genetically based and therefore maintained through reproductive isolation.

The creation and/or supplementation of fisheries by hatchery programs involves introduction of fish that possess instinctive behaviours and life-history traits that may or may not be well suited to the local environment. In other words, fish collected from one waterbody, reared under artificial conditions and stocked into a new region are likely to be instinctively "lost". This is not to say that the traits of the introduced individuals will always be selective against—indeed they may often be favoured. If the traits of hatchery

introduced fish were always disadvantageous enough to be selected against, hybridization would likely not be the conservation issue it is today.

A common argument presented in support of supplemented fisheries is one based on the following fundamental truth of adaptive evolution; natural selection can only work with whatever mutations it is presented with. In other words, although the fish being favoured by selection may be the most locally adapted, it does not mean that further superior variations of the same behaviour or trait do not exist elsewhere; mutations may simply not yet have occurred. The argument, therefore, is that regardless of which individual is native, the one with the traits that best fit the local environment (most locally adapted) will outperform the other. Although true in principle, this assumption becomes complicated when temporal variation is taken into account. When considering the consequences of lost local adaptations, one must question the regularity at which they are required. The benefit of an interim higher fitness may be offset by the loss of adaptations required to deal with exceptional environmental conditions (Allendorf *et al.* 2004).

It should also be recognized that mate selection by fish can lead to traits that are maladaptive to survival. Mate selection in salmonids is well documented to be dependant on morphological characteristics, such as body size, colouration and aggressiveness (Fleming and Gross 1994; Berjikian *et al.* 2001; Foote *et al.* 2003). Characteristics selected for are commonly thought to be indications of individual health and fitness. When non-native fish are introduced they may possess sexually desirable characteristics but also traits that are maladaptive in other aspects. An example of this is the observed

reduction in egg size of chinook salmon (*Oncorhynchus tshawytscha*) populations supplemented by hatchery programs (Heath *et al.* 2007). This may be explained by selection for hatchery females, which were found to produce smaller eggs. The maternal decision between egg size and number has been described as a trade-off between "quantity and quality" (Einum and Fleming 2000) and is likely adapted to be stream specific. By introducing hatchery stock the risk of selecting a potentially maladaptive egg size is also introduced.

Another common concern with introductions is the potential introduction of individuals with inherently lower disease resistance. Evidence exists suggesting stock specific resistance to specific pathogens (Buchanan *et al.* 1983).

To further address the importance of local adaptive, pre-reproductive barriers in maintaining species boundaries, it is helpful to explore examples of potential isolating factors. General temperature preferences and attributes of spawning (timing with respect to discharge and temperature, habitat and water depth and velocity) are strong, potentially isolating environmental variables. When contrasting these factors between *O. clarkii lewisi* and *O. mykiss* we find large overlap in preference and behaviour, therefore promoting the possibility of hybridization.

General temperature preference should be considered a large-scale isolating mechanism as significant distances must typically be travelled in order to see major changes in stream temperature. In a laboratory experiment, Bear (2005) found strong similarity in

optimal growth temperature of *O. clarkii lewisi* and *O. mykiss* (13.6°C and 13.1°C respectively), with *O. mykiss* having a broader temperature range in which growth occurred. Furthermore, it was found that *O. mykiss* have a higher ultimate upper incipient lethal temperature. This implies that, although *O. mykiss* may potentially outperform *O. clarkii lewisi* at higher temperatures, they are able to function similarly throughout the optimal temperature range of *O. clarkii lewisi*. This reduces the likelihood of larger scale isolation of these species.

As literature on *O. mykiss* spawning characteristics within inland, low-order streams is almost nonexistent I will mainly contrast the following two studies. Schmetterling (2000) documented the characteristics of a fluvial *O. clarkii lewisi* population in the Blackfoot River of western Montana. Although two of the four subject streams were known to be hybridized (<8%) it will be used to represent *O. clarkii lewisi* behaviour. Muhlfeld (2002) documented the characteristics of a native *O. mykiss* population in the East Fork Yaak River drainage, a tributary to the Kootenay River entering downstream of the Kootenay Falls (Figure 1-1).

Water temperature, along with discharge, can form a pre-reproductive barrier specifically with regards to spawning timing. Both of these factors influence the timing of spawning migration as well as the act itself. A study by Magee *et al.* (1996) found *O. clarkii lewisi* migration to begin when the mean daily temperature was 7 to 9^{o}C, with peak spawning occurring at approximately 8^{o}C. Similarly, Muhlfeld (2002) found *O. mykiss* spawning in a headwater tributary during mean daily water temperatures of 6.0 to 8.2°C. Streams to

which *O. clarkii lewisi* are native exhibit an annual hydrograph dominated by a peak discharge occurring between April and June. The timing of this peak has been found to be related to timing of spawning. Schmetterling (2000; estimated from Fig.3) found *O. clarkii lewisi* to spawn on the descending limb of the hydrograph, approximately 11 days post peak discharge and extending over 12 days. Similarly again, Muhlfeld (2002) found *O. mykiss* spawning to start 10 days after peak discharge extending over 18 days. Flows during Muhlfeld's spawning period decreased through the range of 2.1-1.5 m^3/s.

Initially, a difference in spawning site selection is apparent with respect to channel morphology. While Muhlfeld (2002) found *O. mykiss* preferring pool tailouts (80%) Schmetterling (2000) located the majority of redds upstream of glide tailouts (75%). Supporting this is the difference in mean water depth where the redd was located (28 cm and 12.9 cm, respectively). Although differing by stream morphological classification both of these habitats are "tailouts" and are therefore hydrologically similar. Tailouts are areas that exhibit a relatively quick decrease in stream bed elevation and therefore promote interstitial water flow. The selection of spawning areas with high hyporheic (interstitial) flow is a well known trait of many salmonids (Behnke 2002). When comparing redd water velocity between studies (40–70 cm/s Muhlfeld (2002) and 56 cm/s Schmetterling (2000)) we see that both *O. clarkii lewisi* and *O. mykiss* are selecting similar flows. Overlap in water velocity selection will further increase in streams where either pools or glides (runs) are limited. Both studies had pool and glide categories but the basis on which differentiation was made is unknown and may overlap to some extent.

Although Magee *et al.* (1996) found *O. mykiss* at higher proportion in lower reaches during spawning migrations, suggesting a possible isolation due to substrate preference, the role of spawning substrate will not be discussed in this section because it is dependant on fish body size and therefore variable among streams (Kondolf 2000).

Assuming these studies represent typical temperature preference and spawning characteristics of both parental lineages, it is apparent that these genetically similar sister species are similar in many behavioural aspects. Furthermore, we see no evidence or reason to suspect the existence of previously existing, pre-reproductive barriers when these two species are anthropogenically sympatric. Supporting this are the results of Rubidge and Taylor (2004) and Weigel *et al.* (2003). By plotting the frequency of genotypes one can assess the presence or absence of pre-reproductive isolation. A unimodal hybrid zone suggests no selection against hybrids and therefore an absence of isolation, while a bimodal distribution shows selection for either parental genotype, potentially from a pre-reproductive aspect (Rubidge and Taylor 2004). In areas of anthropogenic allopatry Rubidge and Taylor (2004) found a unimodal distribution whereas Weigel *et al.* (2003) found a bimodal distribution in an area of natural sympatry that has been affected by stocking. The results of the later study present a unique situation where both natural and introduced *O. mykiss* coexist with *O. clarkii lewisi*. The persistence of the bimodal distribution in the presence of stocking may indicate that genes responsible for the isolating mechanisms of the native *O. mykiss* have introgressed into stocked *O. mykiss*, as introgression will occur here as well. This could effectively introgress co-evolved local adaptations into stocked *O. mykiss* before they have the

chance to hybridize with *O. clarkii lewisi*, thus reducing the likelihood of *O. clarkii lewisi* x *O. mykiss* production.

Genetic relatedness

The breakdown of pre-reproductive isolation was presented as one of two main rationales of why introduced *O. mykiss* and native *O. clarkii lewisi* readily hybridize. The other factor that increases the probability of hybridization between naturally allopatric *O. clarkii lewisi* and *O. mykiss* is the degree of relatedness of the taxa.

Hybridization appears more likely to occur between closely related taxa (Table 1-1). With respect to *O. clarkii lewisi* and *O. mykiss* this fact arguably originates with the events that caused the original speciation. The relatively recent divergence between and within *O. mykiss* and *O. clarkii* lineages likely resulted from geographic isolation rather then the sympatric evolution of pre-reproductive barriers. Assuming no significant difference between environments inland, allopatric populations of both taxa would likely have continued with many behaviours indicative of their common ancestor, as is seen between steelhead and *O. clarkii clarki* (Hawkins and Quinn 1996). Re-establishing sympatry will then present an opportunity for interbreeding to occur since isolating mechanisms had not evolved in allopatry, producing fit offspring. Feguson *et al.* (1985) write that developmental impairment increases with genetic distance and that the distance required for developmental complication should be greater than the separation between *O. clarkii lewisi* and *O. mykiss*. The genetic relatedness hypothesis can be supported by

the near infertility of "tiger trout", the resultant hybrid of brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) (Brown 1966).

Table 1-1: Examples of hybridizing species producing viable offspring indicating offspring fertility and whether observed in wild populations or only in hatchery settings.

Species	Fertile offspring	Wild populations	References
O. mykiss x O. clarkii lewisi	Yes	Yes	Allendorf and Leary 1988; Rubidge *et al.* 2003
O. mykiss x O. clarkii bouvieri	Yes	Yes	Henderson *et al.* 2000, Campbell *et al.* 2002
O. mykiss x O. clarkii clarki	Yes	Yes	Docker *et al.* 2003
O. clarkii lewisi x O. clarkii bouvieri	Yes	Yes	Allendorf and Leary 1988
O. mykiss x O. clarkii lewisi x O. clarkii bouvieri	Yes	Yes	Allendorf and Leary 1988; Behnke 2002
Salvelinus confluentus x S. fontinalis	Yes	Yes	Leary *et al.* 1985; Spruell *et al.* 2001
Salvelinus alpinus x S. fontinalis	Yes	Yes	Hammer *et al.* 1991
Salvelinus alpinus x S. namaycush	Yes	Yes	Hammer *et al.* 1989
Salvelinus confluentus x S. malma	Yes	Yes	Baxter *et al.* 1997; Redenback and Taylor 2003
Salmo salar x S. trutta	Yes	Yes	Garcia-Vazquez *et al.*2003
Salvelinus fontinalis x S. namaychush (Splake)	Yes	No	Buss and Wright 1958; Behnke 2002
Salvelinus fontinalis x Salmo trutta (Tiger Trout)	Rare	No	Brown 1966; Behnke 2002
O. mykiss x Salvelinus fontinalis	No	No	Buss and Wright 1958
O. mykiss x Salmo trutta	No/Rare	No	Buss and Wright 1959

Given the relatively high degree of genetic relatedness between *O. clarkii lewisi* and *O. mykiss* (Crespi and Fulton 2003) it is not unexpected that hybridization is extensive wherever they are anthropogenically sympatric. Although estimates of lineage divergence are as ancient as six million years ago, dendrograms show *O. mykiss* to be more closely related to *O. clarkii lewisi* then any other *O. clarkii* subspecies (Behnke 2002). It should

be noted that multiple factors exist to determine whether or not hybridization will occur following species introductions. Evidence of this is the fact that introduced *O. mykiss* will not readily hybridize with the Athabasca rainbow trout, a sub-population of its own taxa, but will with *O. clarkii lewisi* of another species (Taylor *et al.* 2007).

Post-reproductive hybrid selection

From a pre-reproductive standpoint there is no apparent reason why hybridization and resultant fertile offspring should not exist following the mating of *O. clarkii lewisi* and *O. mykiss*. The lack of pre-reproductive isolation and high genetic relatedness of these species strongly favour the occurrence of hybridization. However, the concept of a unimodal versus bimodal genotype distribution previously discussed (Rubidge and Taylor 2004; Weigel *et al.* 2003) underscores the importance of a second aspect, namely post-reproductive selection. Although it is commonly believed that randomly generated hybrids are less fit on average (Barton 2001), hybridization occasionally results in offspring of neutral effect or even increased fitness (Burke and Arnold 2001; Edmands and Timmerman 2003). Therefore, post-reproductive selection should be an important contributing factor to the shape (unimodal vs. bimodal) of the genotypic distribution.

Loss of fitness

Fitness loss can be further divided into intrinsic and extrinsic outbreeding depression. Intrinsic outbreeding results from selection against hybrids resulting from genetic incompatibility (endogenous selection) (Allendorf *et al.* 2004). Complications can result from meiotic irregularities (e.g. chromosome number differences) or the creation of a

maladaptive hybrid genotype, evident during development or later, physiologically (Burke and Arnold 2001). It is expected that two populations, producing fully functional offspring when breeding intraspecifically, may possess mechanisms that restrict interspecific breeding, thus strengthening specific boundaries. This mechanism can lead to the evolution of "complimentary genes" which are more likely to have derived post-divergence as opposed to existing in a common ancestor (Orr 1995).

With regards to chromosome numbers, *O. clarkii lewisi* and *O. mykiss* are within range of other hybridizing pairs producing fertile offspring (Table 1-2). The previously mentioned near infertility of "tiger trout" (*Salvelinus fontinalis* and *Salmo trutta*) exemplifies the idea that even with similar chromosome numbers, 84 (Behnke 2002) and 80 (Hartley and Horne 1984), respectively, other factors are involved in determining offspring success.

Table 1-2: Chromosome numbers of species hybridizing to produce fertile offspring from Behnke (2002) unless otherwise noted.

Species	Respective chromosome numbers
O. mykiss x O. clarkii lewisi	58-64 and 66
O. mykiss x O. clarkii bouvieri	58-64 and 64
O. mykiss x O. clarkii clarki	58-64 and 68
O. clarkii lewisi x O. clarkii bouvieri	66 and 64
S. confluentus x S. fontinalis	78 and 84
S. alpinus x S. fontinalis	78 and 84
S. alpinus x S. namaycush	78 and 84
S. confluentus x S. malma	78
S. salar x S. trutta (Hartley and Horne 1984)	56 (N. America) or 58 (Europe) and 80

Extrinsic outbreeding depression occurs when there is selection against hybrids due to environmental factors (exogenous selection) (Allendorf *et al.* 2004). Typically, parental genotypes are segregated by differentiated habitat-specific success, creating a hybrid zone in between two habitat types. Logically then, as long as habitats preferred by both

parental taxa, as well as sufficient intermediate habitat exist, exogenous selection against hybrids will be lessened. This would mean that extrinsic outbreeding depression is likely to result when there is no intermediate habitat present with characteristics most suitable to the hybrid offspring. However, in cases where suitable intermediate habitat exists, a hybrid zone featuring a wide range of hybrid types is typically present (Hitt *et al.* 2003; Rubidge and Taylor 2004). If the absence of intermediate habitat did in fact explain extrinsic outbreeding depression, than only certain hybrid types would be selected (Barton and Hewitt 1989). Burke and Arnold (2001) write that hybrids typically become established in habitats quite different from either parental taxon. Results of *O. clarkii lewisi* x *O. mykiss* hybridization studies commonly show an elevational gradient with pure *O. clarkii lewisi* populations persisting at high elevation, and mainly hybrids and *O. mykiss* at lower elevation reaches (Rubidge *et al*. 2001; Hitt *et al.* 2003; Weigel *et al.* 2003). In Chapter 2 we investigate this gradient with the idea that species-specific environmental (habitat) preference is an important component in the establishment of hybrid zones downstream from pure *O. clarkii lewisi* populations.

Hybrid vigour

Increased hybrid fitness, also termed hybrid vigour or heterosis (Rhymer and Simberloff 1996), can result in spite of a considerable reduction in hybrid success. Epafanio and Phillipp (2001) found that even a very slight fitness advantage to hybrids in F_2 and further generations may result in the establishment of a hybrid population if pre-reproductive mechanisms (assortative mating) are not complete. This has been explained by the fact that "the production of hybrids is unidirectional" (Allendorf *et al.* 2004). In

other words, for a pure individual to create pure offspring it must find a pure conspecific individual, whereas a hybrid will always produce hybrid offspring regardless of its mate.

It is commonly thought that, in general, the fitness of hybrids will be less than or equal to that of their parental genotypes. However, in certain situations hybrids will outperform parental strains (Burke and Arnold 2001). Indeed, Rhymer and Simberloff (1996) argue that hybrid vigour is actually documented more frequently than outbreeding depression. Hybrid selection specific to O. clarkii lewisi and O. mykiss has received little study with no known studies of wild populations being found to date. The laboratory experiments that do exist show mixed results (Table 1-3). Results from Bear (2005) are included in the following table even though hybrid survival was not a focus of the study.

Table 1-3: Summary of laboratory studies of developmental and survival rates of *O. clarkii* spp, *O. mykiss* and reciprocal hybrids.

Reference	Results	Species
Hartman 1956	No difference compared to wild success.	*O. clarkii clarki* and *O. mykiss* (steelhead)
Ferguson *et al.* 1985	No evidence of developmental impairment in hybrids.	*O. clarkii lewisi* and *O. mykiss*
Bear 2005	Negative hybrid selection during development (approximately 3% survival).	*O. clarkii lewisi* and *O. mykiss*

Elevational gradient: The role of life-history strategies

It would seem apparent that neither pre- nor post-reproductive selection are likely to exhibit strong pressure against hybrids, supporting the documented extent and rate-of-spread of hybridization in the wild (Hitt *et al.* 2003; Rubidge *et al.* 2001). This fact, along with life-history strategies, becomes important when describing drainage-scale distribution of hybridization. In addition to the native ranges previously discussed, it seems likely that differing glacial refugia (inland vs. coastal) may also have produced differences in life-history strategies and physiological characteristics between *O. clarkii lewisi* and *O. mykiss*. Furthermore, it can be argued that life-history strategy then plays an important role in determining habitat preference of *O. clarkii lewisi* and *O. mykiss*, ultimately determining their relative distributions within a stream network.

Studies of hybridization commonly report an elevational gradient of *O. mykiss* dominating low-elevations, pure *O. clarkii lewisi* populations persisting in headwater reaches and an intermediate hybrid zone. Reason exists to suspect that this segregation initially results from the preferential downstream movement of *O. mykiss*, where they presumable outcompete *O. clarkii lewisi* (Paul and Post 2001). Once established, a combination of superior competitive ability and the lack of hybrid selection allows the advance of *O. mykiss* alleles upstream, displacing *O. clarkii lewisi* much like the postglacial recolonization of inland streams. This results in the formation of the above-noted gradient of diminishing *O. mykiss* allele abundance with increased elevation.

Support for this hypothesis may be found by comparing attributes typically indicative of anadromous and nonanadromous lifecycles, specifically growth, survivorship and metabolic rates. In Chapter 2, we compare life-history strategies to determine if *O. mykiss* possess a faster growing/lower survivorship strategy typical of anadromous individuals. In Chapter 3, we hypothesize that because of a more recent divergence from an ancestral anadromous form, inland *O. mykiss* will possess a higher metabolic rate than that of the more anciently landlocked *O. clarkii lewisi*. The basis of this hypothesis is that anadromous forms typically possess a higher metabolism since they are migratory and complete their life cycle on a much larger spatial scale (Lahti *et al.* 2001, 2002; Morinville and Rasmussen 2003). It is further expected for *O. mykiss* to possess higher growth rates within this study area as they have originated from hatchery stock and likely have had this trait selected for.

Combining these hypotheses predicts that *O. mykiss* will require reaches of sufficient productivity (low elevation) to satisfy an elevated metabolism and still maintain a competitive growth rate. This would further require *O. mykiss* to be competitively superior to *O. clarkii lewisi* at low elevations to secure enough of the available food resources. Should species-dependant preference exist then one would predict hybrid habitat preference and competitive ability to be intermediate of either parental taxon, as their intermediate geographic location would indicate. Under this hypothesis, one would also expect to see a secondary gradient within the hybrid population, as higher-degree hybrids (%RT alleles) would show stronger downstream preference.

Conservation of O. clarkii lewisi *populations*

In Canada two distinct populations of *O. clarkii lewisi* exist, one in British Columbia and one in Alberta. These populations represent the northern extent of the historic range. In British Columbia *O. clarkii lewisi* are designated as a blue listed species by the Ministry of Environment (MOE) (BCMOE 2007). The MOE website defines the blue list to include "*any ecological community, and indigenous species and subspecies considered to be of special concern in British Columbia*." The federal government's Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recognizes both the British Columbian (Special concern) and Albertan (Threatened) populations (COSEWIC 2007). This website defines threatened as "*a wildlife species likely to become endangered if limiting factors are not reversed*," and special concern as "*A wildlife species that may become a threatened or an endangered species because of a combination of biological characteristics and identified threats*." COSEWIC acts as an advisory body for the federal Species At Risk Act (SARA) and any designation by COSEWIC will be considered when assessing the need to add a species to the legal listing under SARA.

In the United States the main *O. clarkii lewisi* populations exist in Montana, Idaho and a small population in Wyoming, with each state recognizing the threatened status of this subspecies. Montana Fish, Wildlife and Parks gives *O. clarkii lewisi* a State Rank of "S2" defined as "*At risk because of very limited and potentially declining numbers, extent and/or habitat, making it vulnerable to global extinction or extirpation in the state*" (MFWP 2007). The Idaho Fish and Game department list *O. clarkii lewisi* as "Vulnerable (S3)", defined as "*at moderate risk because of restricted range, relatively few*

populations (often 80 or fewer), recent and widespread declines, or other factors that make it vulnerable to range wide extinction or extirpation" (IFG 2007). The population existing in Wyoming have a Heritage Rank of "S1" by the Wyoming Natural Diversity Database (WYNDD), University of Wyoming (WYNDD 2007). The S in this code specifies this listing to be specific only to the portion of the population inhabiting the State and is defined as "*Critically imperilled because of extreme rarity (often <5 extant occurrences) or because some factor makes it highly vulnerable to extinction*."

Some Federal recognition of *O. clarkii lewisi* exists in the United States as well. The Montana Fish, Wildlife and Parks website also gives the designations of the United States Forest Service (sensitive "Any species for which the Regional Forester has determined there is a concern for population viability within the state, as evidenced by a significant current or predicted downward trend in populations or habitat") and the Bureau of Land management (sensitive "Any species proven to be imperilled in at least part of its range and documented to occur on BLM lands").

In this lengthy documentation of the *O. clarkii lewisi* listings by several government bodies we see that all provinces and states in which *O. clarkii lewisi* populations exist, as well as some bodies at the federal level, have listed *O. clarkii lewisi* with their respective "threatened" designations. Furthermore, we see that this subspecies remain unlisted under endangered species acts both in Canada (SARA) and the U.S. Endangered Species Act (ESA) in the United States.

Currently, *O. clarkii lewisi* are under review for inclusion under SARA (Rubidge and Taylor 2005). To date no formal decision has been made. In 1997 a formal petition was made to list *O. clarkii lewisi* under the ESA (Allendorf *et al.* 2004). On April 14, 2000, the United States Fish and Wildlife Service (USFWS) issued a news release stating that upon a 12 month review, sparked by the 1997 petition, "*biologists found the species is not threatened*" and the *O. clarkii lewisi* populations do not "*warrant listing as a threatened or endangered species under the Endangered Species Act* (USFWS 2000)." It is unclear as to whether hybridized populations were separated from pure populations as this news release does not specifically mention the issue of hybridization. In September 2002 the USFWS reopened a public commenting period on the new status of *O. clarkii lewisi* following a court order, one that also stressed the inclusion of the issue of hybridization (USFWS 2002). The results of this re-visitation supported the April 2000 decision (USFWS 2003).

The management of *O. clarkii lewisi* populations is complicated for reasons common to many threatened species. Debates on the best conservation approach of threatened species are often strenuous as all aspects from defining biological and ecological significance to considering potential rehabilitative measures and the extent of further human intervention need to be considered. Further complicating this specific issue is the fact that hybridization creates a continuous spectrum of individuals from pure *O. clarkii lewisi* to pure *O. mykiss*. Therefore, the extent to which an individual is hybridized must be defined within the management plan.

Identification of areas affected by hybridization is mandatory for the successful implementation of management plans. Historically, hybrids have been distinguished from either parental taxon using genetic or morphological techniques. Recently, genetic analysis has become a widely used, effective method of identifying individual genotypes, primarily as a result of the development of the polymerase chain reaction (PCR) (Allendorf *et al.* 2004). This method allows for the quick amplification of isolated target sequences. In hybridization studies target sequences, or markers, are chosen for their observed interspecific differences in PCR-fragment length. Earlier genetic techniques commonly exploited variation in fragment lengths that arose from "short tandem repeats" or microsatellites (Avise 2004). Microsatellites are typically di-, tri- or tetra-nucleotide repeats that result in much shorter fragment lengths than minisatellites (a source of variation utilized in RFLP analyses). Ideal markers would show opposing dominant or diagnostic (fixed) expression in both parental genomes and therefore have relatively minor or no overlap in fragment length. However, the concern of overlap was still common. Recently, techniques have evolved from mini- and microsatellites to intron-based markers (e.g. IKAROS) and those discovered through amplified fragment-length polymorphisms (AFLPs). For most salmonids, this has lead to the development of a number of fixed markers and has essentially removed the concern of fragment overlap between species as the markers are typically fixed. The resultant increase in confidence has lead to the preference of molecular genetics over morphologically-based detection methods with regards to individual genotypic identification (Allendorf *et al.* 2004). The notion that genetic-based identification is superior to morphological assessment appears

to be well substantiated (e.g. Allendorf *et al.* 2004); however, the potential utility of morphological approaches as an adjunct to molecular genetics has not been considered.

Conclusion

Hybridization resulting from *O. mykiss* introductions is identified as a major factor threatening many trout native to North America. The expansion of the native range of *O. mykiss* has contributed to the extinction of the yellowfin and Alvord cutthroat trout and the near replacement of the greenback and Bonneville cutthroat (Behnke 2002). The Apache trout (*Oncorhynchus apache*) of the American southwest are also threatened by hybridization resulting from *O. mykiss* introductions (Dowling and Childs 1992).

Although variable, estimates of Montana *O. clarkii lewisi* populations approximate pure populations remaining in only 10% of their native range (Allendorf 2004). Estimates of 8-10% historical range occupancy are reported for *O. clarkii lewisi* in Idaho and similarly, 8-20% of their entire native range (Sheppard *et al.* 2003). With no pre- or post-reproduction isolation apparent, close genetic relatedness and the past interactions implied by native ranges it would appear that *O. clarkii lewisi* are being seriously challenged by introduced *O. mykiss*. Sheppard *et al.* (2003) stress the need for a dual-purpose management approach that limits further hybridization as well as protects remaining *O. clarkii lewisi* populations.

2. The distribution and life-history of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) x rainbow trout (*O. mykiss*) hybrids in the Upper Oldman River: how important are barriers?

Abstract

Studies of hybridization between *O. clarkii lewisi* and *O. mykiss* commonly report the development of a genotypic gradient, with pure *O. mykiss* at lower elevation reaches, pure *O. clarkii lewisi* populations in headwater reaches and hybrids distributed intermediately. The objectives of this study were: to determine the status and structure of hybridization within the study area and specifically describe the genetic gradient of the hybrid population along the elevational gradient, to compare life-histories of *O. clarkii lewisi* and hybrids within the study area and to assess the effect of migratory barriers on the advance of hybridization and in the development of the upstream gradient. This study confirmed the expected distribution, but furthermore described a similar gradient in the mean number of *O. mykiss* alleles possessed by hybrids. On average *O. mykiss* exhibited a significantly faster growing, lower survivorship life-history strategy compared to *O. clarkii lewisi*, with hybrids being intermediate. We found an inverse affect of elevation on the effectiveness of migratory barriers in limiting the upstream spread of hybridization, supporting the notion that the influence of other limiting factors (e.g. temperature) increases with elevation. A hypothetical temperature threshold (7.25°C) to hybridization was presented in an attempt to explain why some high elevation reaches with no physical barriers were found to still hold genetically pure *O. clarkii lewisi*, despite extensive *O. mykiss* stocking, which began over 80 years of. It is suspected that differences in life-history strategy, and potentially temperature preference, play an

integral role in habitat selection and competitive ability of these two species, potentially explaining the observed gradient.

Introduction

Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) populations of the Oldman and Bow River basins in southern Alberta represent the north eastern extent of the historic range of this species. In 2005 the entire Albertan *O. clarkii lewisi* population was recognized as "threatened" by COSEWIC. As is the case throughout the range of *O. clarkii lewisi* (Allendorf *et al.* 2004), hybridization with introduced *O. mykiss* is believed to be a major cause for the decline in abundance of this species in Alberta (COSEWIC 2007).

Studies of hybridization between *O. clarkii lewisi* and *O. mykiss* commonly report the development of a genotypic gradient, with pure *O. mykiss* at lower elevation reaches, pure *O. clarkii lewisi* populations in headwater reaches and hybrids distributed intermediately (Rubidge et al. 2001; Hitt et al. 2003; Weigel *et al.* 2003). A genotypic gradient could result from reduced hybrid fitness (outbreeding depression), leading to a narrow hybrid zone, occupied mainly by F1 hybrids, and a bimodal genotype frequency histogram indicating disruptive selection against hybrids (Rubidge and Taylor 2004). However, most studies involving hybridization with introduced *O. mykiss* report a broad hybrid zone containing a wide spectrum of backcrossed hybrids and a relatively low number of F1 hybrids (Rubidge et al. 2001; Hitt et al. 2003). Using genotype frequency histograms Rubidge and Taylor (2004) showed the majority of populations to be unimodal-skewed to either pure parental genotype. From this it would appear that outbreeding depression is not strong enough to prevent hybridization in these situations.

Throughout the eastern slopes of the Rocky Mountains, Paul and Post (2001) found that *O. mykiss* preferentially move to lower elevations regardless of stocking location, where they presumably outcompete *O. clarkii lewisi* and become established (Paul and Post 2001). This suggests that the genotypic gradient may be initiated by the preference of *O. mykiss* for low elevation reaches, and is not simply the result of most introductions having occurred at low elevation (Weigel *et al.* 2003; ASRD 2005).

O. clarkii lewisi and *O. mykiss* differ in zoogeographical history, with *O. mykiss* being anadromous and having utilized coastal refugia, while *O. clarkii lewisi* utilized inland proglacial lakes, during the Pleistocene. From this, combined with years of domestication in hatcheries, we would expect the habitat preferences and life-history of introduced *O. mykiss* to differ considerably from that of *O. clarkii lewisi*, and reflect to some degree, the anadromous habits of its wild ancestors. *O. mykiss* should therefore exhibit a faster growth/lower survivorship life-history strategy reflecting the higher energy demands of anadromous life that can be supported by the rich marine food supply (Gross 1987). Thus the rainbow trout's preference for low elevation reaches shown by Paul and Post (2001) likely reflects their higher food requirements. On the other hand, life in the unproductive and turbid waters of an inland proglacial lake, would likely have led to a slow growth/high survivorship, low energy demanding, non-migratory life-cycle for *O. clarkii lewisi*. While such adaptations would likely equip these fish for survival in cold, unproductive streams with a very short growing season, we should expect them to be competitively inferior to rainbow trout in productive low elevation reaches.

Given the differences in life-history between *O. clarkii lewisi* and *O. mykiss* it seems likely that hybrids would possess a suite of characteristics intermediate to both parental genotypes. These could include life-history strategies (growth rate and survival), metabolic rates and competitive abilities, all of which could influence habitat preference. In this way one would expect hybrids to occupy intermediate positions along the genotypic gradient, and perhaps avoid the upper reaches allowing pure *O. clarkii lewisi* populations to persist. Therefore, following the initial establishment of *O. mykiss* in their preferred environment, hybridization can then advance upstream by producing hybrids with intermediate characteristics. The extent of upstream movement would then be limited by a combination of behaviour/ecology and migratory barriers.

Many studies report barriers to be the only factor truly limiting the spread of hybridization (Hitt *et al.* 2003; Weigel *et al.* 2003; Rubidge and Taylor 2005). This would certainly be true if the barrier was located within the hybrid zone, which is within the zone of hybrid preference. However, were hybrids to avoid high elevations, barriers at that level might have less influence. Therefore, it would seem likely that the upstream advance of hybridization is influenced by a combination of migratory barriers and hybrid preference.

Objectives and Hypotheses

In this chapter we will describe the distribution of *O. clarkii lewisi*, *O. mykiss* and hybrids along the elevational gradient of the Upper Oldman River and its tributaries, investigate possible migration barriers and assess their potential role in restricting the upstream

advance of hybridization. We will also attempt to compare the life-history strategies through the growth rates, age-specific structure and mortality rates of *O. clarkii lewisi*, *O. mykiss* and hybrids. We will investigate the following four objectives and related hypotheses in this portion of this study:

1.) To determine the status and structure of hybridization within the study area.

Ha1: Hybridization will be extensive in the study area following an elevational gradient.

2.) To describe the genetic gradient of the hybrid population and the genotypic frequency histograms along the elevational gradient.

Ha2: The mean proportion of *O. mykiss* alleles (%RT) carried by hybrid individuals will decrease with elevation; genotype frequencies will be skewed towards *O. mykiss* at low elevations and *O. clarkii lewisi* at high elevations, and F1 hybrids will be rare.

3.) To compare life-histories of *O. clarkii lewisi* and hybrids within the study area.

Ha3a: Individual specific growth rate will be directly related to %RT.

Ha3b: %RT will decrease with age, i.e. individuals carrying more *O. mykiss* alleles will show a lower survivorship.

4.) To assess the affect of migratory barriers on the advance of hybridization and in the development of the upstream gradient.

Ha4: The effectiveness of a migratory barrier in decreasing the occurrence of *O. mykiss* alleles upstream of a barrier will be higher a low elevations.

Methods

Study area

This study was conducted in the upper Oldman River basin of south western Alberta (Figure 2-1). The study area consisted of the Oldman River and Crowsnest River drainages upstream of the Oldman River reservoir (11N 722800E 5495850N), located at the confluence of these two streams. The Oldman River and tributaries, together with the Bow River, represent the north eastern limit of the historic *O. clarkii lewisi* range, and one of the few *O. clarkii lewisi* populations to become established on the east slopes of the Rocky Mountains (Behnke 2002). This implies isolation from all other *O. clarkii lewisi* populations for approximately 10 000 years. With the exception of the Athabasca River population, *O. mykiss* are absent east of the Rocky Mountains. Documented stocking of *O. mykiss* into the study area dates back to1926 and continues into the 1970s, with the exception of Crowsnest Lake which is stocked to date (Crowsnest drainage) (ASRD 2005) (Table 2-1).

Table 2-1: Combined stocking records for the Oldman and Crowsnest basin from 1926-2003.

Species	# introduced individuals	Stock
O. clarkii lewisi	943 810	Job Lake, AB
O. mykiss	1 478 811	Shasta, Arlee, Montana, and Donaldson*

* original *O. mykiss* stock used is uncertain; however, these strains are likely to have been stocked in Alberta (Underwood *pers comm.*)

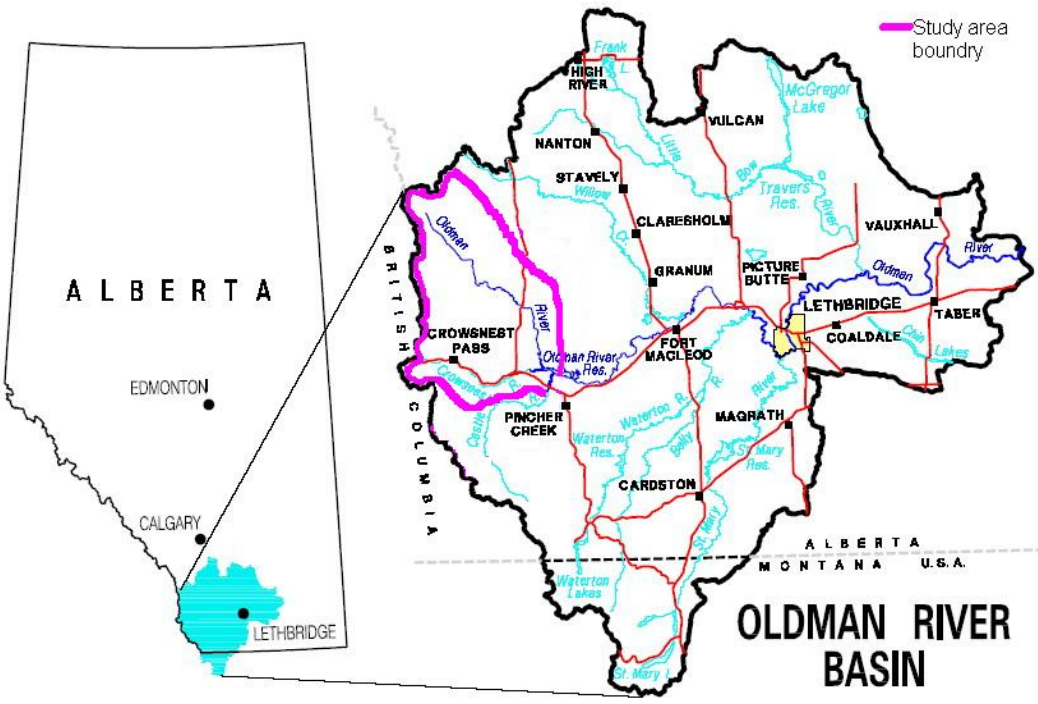

Figure 2-1: Overview map showing study area outlined (Courtesy of Oldman Watershed Council)

Between July and August of 2005 and 2006, fish from 23 sites were sampled primarily by a one-pass, open-ended backpack electrofishing method (Smith Root LR-24 electrofisher), with supplemental samples from angling and seining. Sampling occurred after peak discharge had subsided. All fish were genetically identified as described in Chapter 4. However, in short an adipose fin clip was taken from all fish handled, which was stored in 95% ethanol for DNA extraction and genetic analysis using three diagnostic co-dominant nuclear species markers (OCC16, OCC36 and IKAROS), and one mitochondrial marker (ND3) in the molecular assessments. All four have been previously reported in the literature (Ostberg *et al*. 2002; Baker *et al.* 2002; Docker *et al*. 2003; Ostberg *et al*. 2004). A minimum of 30 fish per site were sampled for genetic analysis as per Allendorf *et al.* (2004). Mean %RT was calculated for each site and tested for significant interannual variation using a paired t-test. For site sampled in both years the overall site mean was calculated as the site average of both years.

Sites were located throughout 10 streams and were selected to ensure sufficient elevational and stream order coverage (Table 2-2). Elevation was obtained either in the field using a Brunton MNS® handheld GPS or derived from Softmap Plus® mapping software when sampling occurred at known locations. This mapping program was also used to derive stream order at a 1:50 000 scale. Multiple regression was used to assess the relationship of the degree of hybridization to elevation and stream order. Distribution of %RT (histograms) was acquired within each site to identify any major gaps signifying differentiated selection among genotypes.

Table 2-2: Summary of site UTM coordinates (NAD 83), elevation and stream order. * indicates sites sampled in both 2005 and 2006.

Stream	Site	Zone	Easting	Northing	Elevation (m)	Order
Oldman River	Reservoir	11	722800	5495850	1097	6
Byron Creek	By1*	11	691870	5491960	1234	3
Gold Creek	Go1	11	687580	5496560	1280	3
Oldman River	Ol0	11	697450	5526450	1311	6
Gold Creek	Go2	11	688610	5498640	1341	3
Oldman River	Ol1	11	690790	5528000	1402	6
Racehorse Creek	Ra1*	11	687560	5526290	1417	5
Dutch Creek	Du0*	11	684250	5530950	1471	4
Daisy Creek	Da1*	11	686310	5522440	1478	4
Racehorse Creek	Ra2*	11	681020	5522770	1501	4
Daisy Creek	Da2	11	686000	5520670	1524	3
Dutch Creek	Du2*	11	678790	5531650	1539	4
Vicary Creek	Vi1*	11	682790	5519790	1539	3
Dutch Creek	Du1*	11	674600	5530100	1631	4
North Racehorse	NR*	11	675120	5523370	1646	3
Livingstone River	Li1*	11	683650	5552050	1661	4
Dutch Creek	Du3*	11	670840	5528780	1676	3
Beaver Creek	Be1*	11	683500	5553000	1684	1
Honeymoon Creek	Ho1*	11	675150	5545250	1692	3
Vicary Creek	Vi2	11	680520	5514350	1692	2
South Racehorse	SR*	11	672790	5516370	1707	3
Oldman River	Ol2*	11	672200	5548800	1722	3
Livingstone River	Li2*	11	683450	5556250	1722	4

Life-history strategies: Survival rates

We used two approaches in order to assess age-specific hybrid survival rates. The first approach involved analyzing the effects of age on the proportion of *O. mykiss* alleles. We analysed this over the entire population with linear regression and also using multiple regression to assess any elevational effects. Elevation was included as an additional independent variable since mean age tended to increase with elevation, and *O. mykiss* alleles tended to decrease along the elevational gradient. Age-specific *O. mykiss* allele frequency was also analyzed by three elevational classes (1 – low, 2 – medium and 3 - high). These classes are represented by the similar number of sites and present significantly different degrees of hybridization (Table 2-3). The second approach was age-specific proportion of hybrids in the population. As highlighted by Allendorf *et al.* (2004), both the number of hybrids within a population and the extent of hybridization can increase without experiencing an increase in the proportion of *O. mykiss* alleles. This analysis was used to identify any trends in the degree of hybridization of the average individual. Fish were aged with a combination of otoliths and scales. For both analyses age-classes 1-10 were used. In certain situations analyses were rerun to determine if low sample sizes above age 6 confounded any results.

Table 2-3: Elevational classes used in survivorship analyses and their respective mean elevations, elevational ranges, mean degree of hybridization (±%SE) and number of sites incorporated.

Elevational class	Mean elevation (m)	Elevation range (m)	Mean (%RT)	SE mean	# sites
1 – low	1334	1280-1402	30%	10%	4
2 - medium	1589	1417-1501	7%	1%	4
3 – high	1678	1524-1692	3%	1%	5

In addition to hybrid specific approaches we calculated the mortality coefficients for both *O. clarkii lewisi* and hybrids throughout all sites that showed hybridization (i.e. not broken into elevational classes). The low occurrence of *O. mykiss* throughout the study area precluded the assessment of this genotype during this analysis.

Life-history strategies: Specific growth rate

Specific growth rate (SGR) was estimated through scale back-calculation and used to assess, in part, the strategies of *O. clarkii lewisi*, *O. mykiss* and hybrids throughout the range of elevations (Equation 2-1). This was done using multiple regression with a design of elevation, age and %RT for predictor variables and SGR as the dependant. The effect of %RT on SGR was also analyzed by age within each degree of hybridization class for ages 2-6. Due to the strong representation of first and second year classes in one lower elevation site (Byron Creek = By1), it was excluded from the hybrid survival analyses as it possessed only first and second year fish. However, for SGR analyses site By1 was included, resulting in minor changes to the various characteristics represented by elevational class 1 (Table 2-4). ANOVA analysis was used to assess the effect of genotype within both classes and ages. Back-calculations were performed on two radii per scale and two scales per fish. Condition factor (K) was used to convert the length-at-age estimates produced from back-calculations in order to be able to calculate SGR.

$$SGR = \sum_{x=1}^{n} \ln\left(\frac{Wx+1}{Wx}\right) / n-1$$

Equation 2-1: Equation used to calculate SGR, where W= weight, x= age and n=total age at time of sampling.

Table 2-4: Elevational classes used in SGR analyses and their respective mean elevations, elevational ranges, mean degree of hybridization (±%SE) and number of sites incorporated, used in SGR analyses.

Elevational class	Mean elevation (m)	Elevation range (m)	mean (%RT)	SE mean	# sites
1 – low	1314	1234-1402	44%	16%	5
2 - medium	1589	1417-1501	7%	1%	4
3 – high	1678	1524-1692	3%	1%	5

Life-history strategies: Growth/survival trade-off

After determining the effect of %RT on age-structure and growth rate we directly assessed the trade-off between these two life-history components. We calculated mean growth (natural logarithm (ln) weight) and survival rates (ln abundance, where abundance was the total count at each) for age-2 to age-4 *O. clarkii lewisi* and hybrids throughout all sites that showed hybridization. Using ANCOVA we tested for significant interaction between genotype and age. A significant result would indicate a genotype-dependent difference in rate of change. The low occurrence of *O. mykiss* throughout the study area precluded the assessment of this genotype during this analysis.

Influence of barriers

All reaches between sites were fully surveyed by foot in order to obtain the coordinates of any migratory barriers. Although each barrier identified appeared to be impassable to upstream fish movement, the validity of barriers is notoriously difficult to confirm given the variability in flow events. In context of this study one must consider hydrological events over the past 100 years (period of *O. mykiss* presence) to truly gauge the validity of each barrier. Side routes for water to bypass a barrier and provide fish with access to upper reaches were considered when assessing the validity of each barrier. No potential

bypasses were reported as falls were typically well confined. Vertical drop of each barrier was obtained with a 30m cloth tape where possible. In other circumstances one crew member photographed with a 2m measuring pole for scale. Vertical drop was then calculated out of the field using Adobe Professional measuring tools.

ANCOVA analysis was used to assess the effect of elevation and site-location (above or below barriers) on the change in %RT alleles across a barrier. Barrier location and overall mean %RT per site were mapped using ArcGIS software. The Alberta base river vector file was overlaid on a digital elevation model (DEM) to display the study area. Sample site and barrier locations were added as points. Hybridization values were estimated between sites when constructing the map.

In 2006, twelve Onset HOBO Water Temp Pro® temperature loggers were installed in well mixed glides throughout the courses of Racehorse (North and South via the mainstem), Vicary and Dutch creeks. Temperatures were recorded every 15 minutes. These streams are neighbouring tributaries of the Oldman River with similar elevational profiles. Temperature profiles were secondarily added to the study after genetic results from 2005 showed pure *O. clarkii lewisi* populations persisting in the headwater reaches of Dutch Creek and North and South Racehorse forks. This presented the situation of investigating the effects of temperature on the spread of hybridization as these streams were similar in genotypic gradient but differed in headwater accessibility (barriers).

Results

Hybridization status

A total of 1168 fish were sampled and genetically identified. Genetic analysis revealed hybridization at 10 of 23 sites, with the degree of hybridization decreasing with elevation as expected (Figure 2-2). It was concluded that F1 hybrids were absent from sampling in both field seasons when no individuals were returned showing heterozygosity at all three nuclear markers. Out of approximately 300 stream km surveyed, 77% were found to contain hybrid fish. The degree of hybridization was found to be significantly related to elevation and not stream order using a multiple regression analysis (Table 2-5). Both hybrid abundance (Figure 2-3) and mean %RT of individual hybrids also decreased with increasing elevation (Figure 2-3). In Figure 2-3 an apparent shift in slope occurs at approximately the Ra1 site. Using a linear regression model of elevation versus mean %RT we sequentially added sites into the model in order of elevation. This was repeated until a decrease in error of the model (MS_{error}) was observed. The last site to be added was then taken back out leaving the model that most accurately predicted the steeper of the two slopes. The last site to be included was in fact Ra1, with an elevation of 1417m and an average hybrid %RT of 10% (Table 2-6).

Table 2-5: Multiple regression results showing significant affect of elevation on site-mean %RT.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.232	.442		5.046	.000
	elevation (m)	-.001	.000	-.833	-5.188	.0001
	stream order	-.043	.034	-.204	-1.272	.219

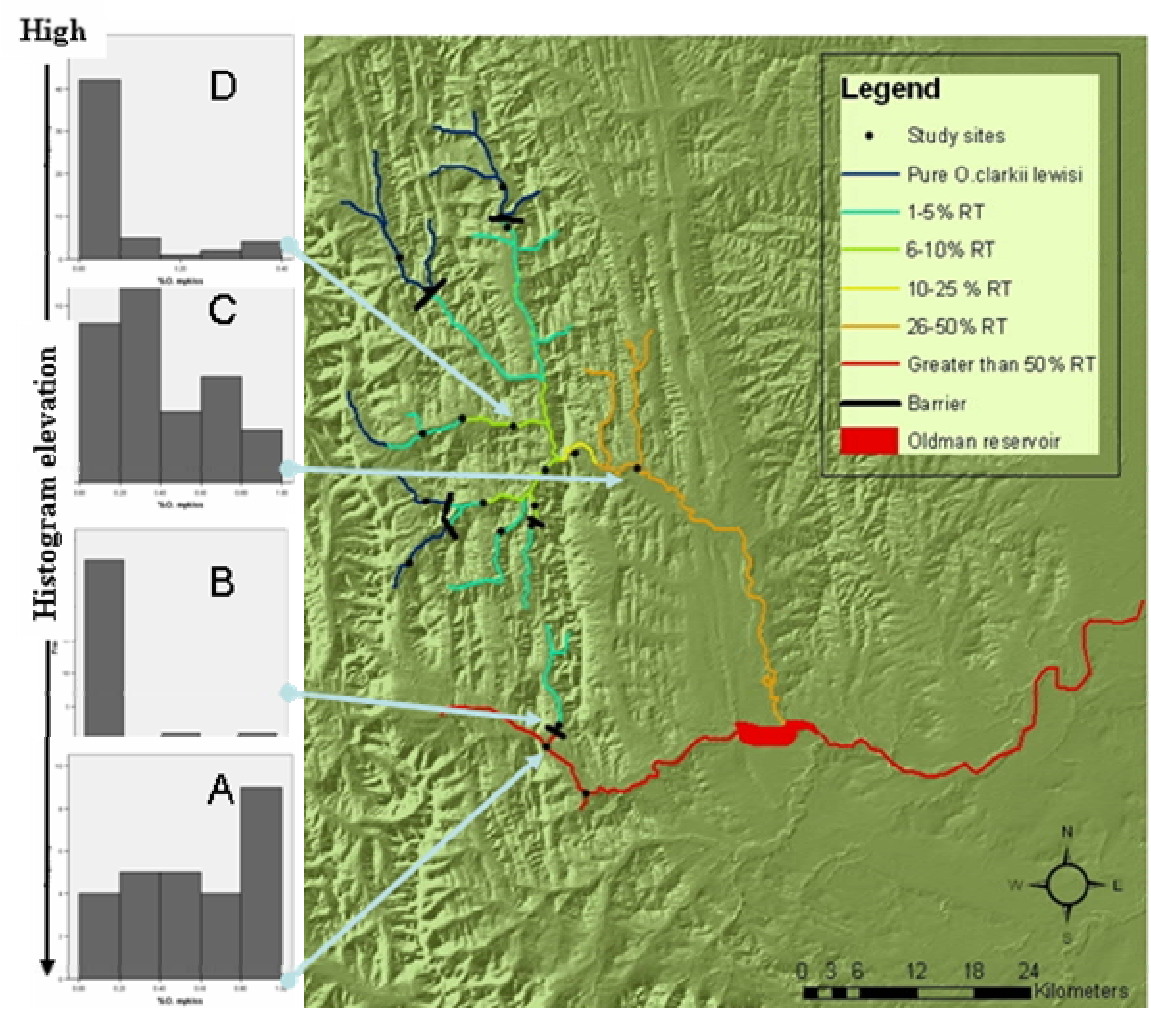

Figure 2-2: Extent of hybridization within the study area. Values represent mean %RT when more then one year of data was collected. Graphs are %RT frequency from four sites along the elevational gradient. Histograms A and B represent below and above barrier sites, respectively.

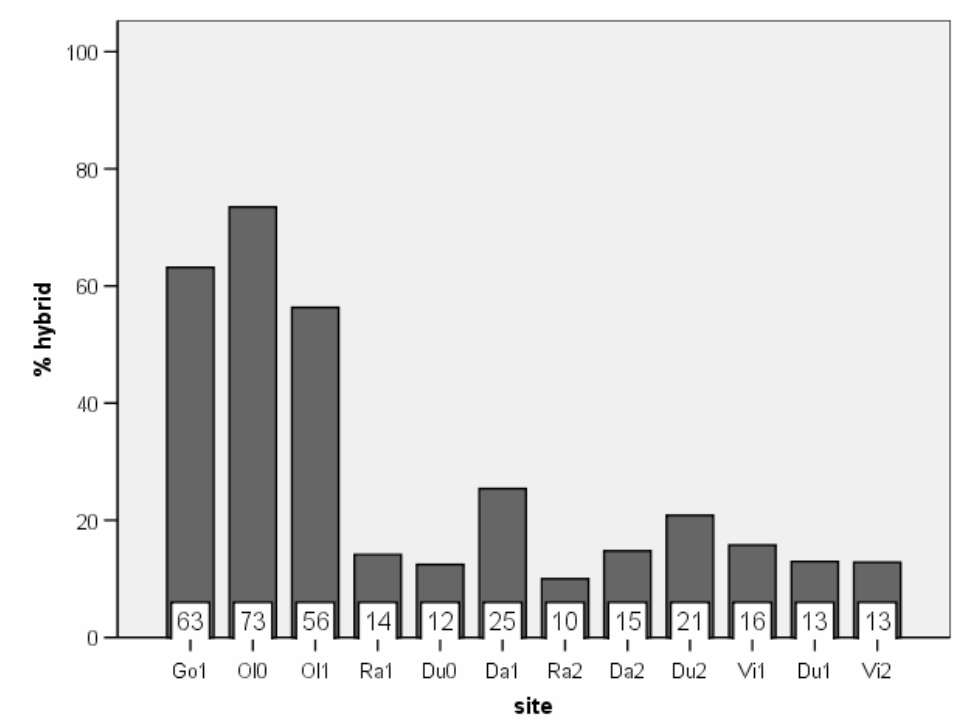

Figure 2-3: Site-specific %hybrid abundance for sites listed by increasing elevation.

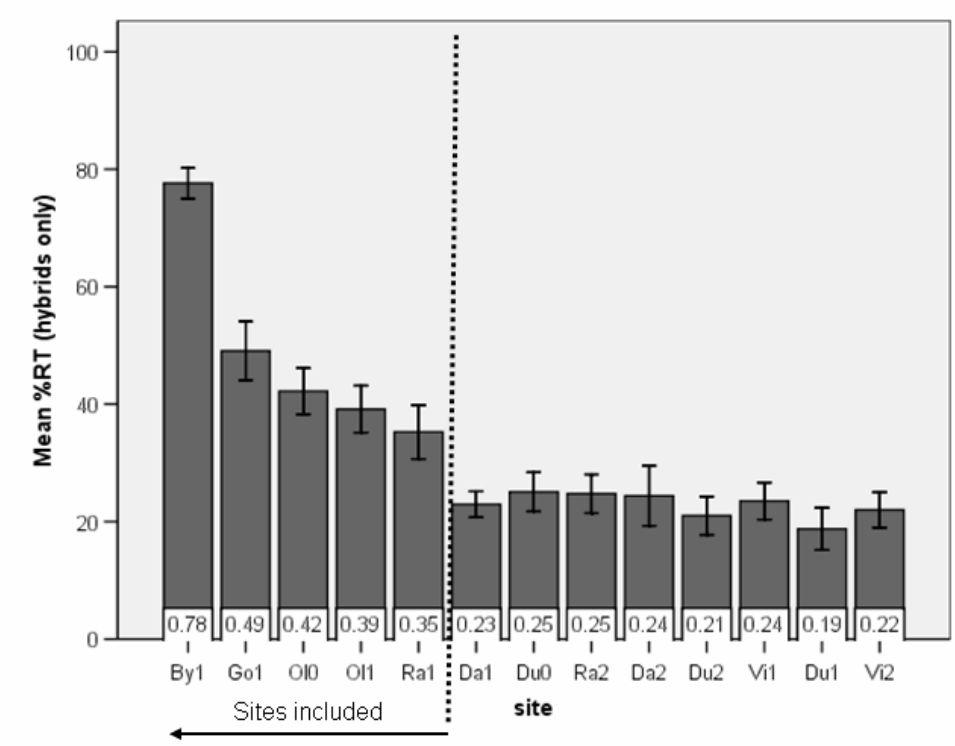

Figure 2-4: Mean %RT of hybrid individuals present in sites arranged with elevational order increasing to the right. Dashed line indicates cut-off point for inclusion in most accurate model.

Table 2-6: Summary of regression results showing sequential addition of next highest site and subsequent MS error value. Also given is the elevation (m) and mean %RT of hybrids only for the highest site included in the model.

Highest site included	Elevation (m) of highest site	Mean %RT of highest site (hybrids only)	MS_{error}
Ol0	1311	38%	0.035
Ol1	1402	24%	0.038
Ra1	1417	10%	0.034
Du0	1471	6%	0.030
Da1	1478	7%	0.029

The genotype frequency histograms were strongly skewed toward *O. mykiss* at low elevations (Figure 2-2a) and *O. clarkii lewisi* at high elevations (Figure 2-2d), with the hybrid mode predominating at medium elevations (Figure 2-2c). Figure 2-2b shows that the genotype frequencies for a low elevation site above an impassible barrier resembles those seen at high elevation sites. This site exhibited a strong skew toward *O. clarkii lewisi*, in contrast to the *O. mykiss* skew (Figure 2-2a) below the barrier.

No significant differences in the degree of hybridization (%RT) were detected among years using a paired t-test (Table 2-7), and thus data for the two field seasons were pooled in all of the analyses (Table 2-7). Six sites showed a mean increase of 2.0%RT, three sites showed a mean decrease of -2.4%RT and seven sites remained unchanged. All of the unchanged sites were pure *O. clarkii lewisi* populations, with four sites above falls that were presumed to be impassable, one above a hung culvert (Be1) and two in streams with unhindered access.

Table 2-7: Table of site-mean %RT for sites sampled in both 2005 and 2006 field seasons.

Site	2005	2006	Mean
Be1	0%	0%	0%
By1	100%	96%	98%
Da1	6%	8%	7%
Du0	3%	8%	6%
Du1	0%	3%	1%
Du2	1%	3%	2%
Du3	0%	0%	0%
Ho1	0%	0%	0%
Li1	0%	0%	0%
Li2	0%	0%	0%
NR	0%	0%	0%
Ol2	0%	0%	0%
Ra1	10%	10%	10%
Ra2	7%	3%	5%
SR	0%	0%	0%
Vi1	6%	5%	6%

Life-history strategies: Age-specific survival rates

As mean age was found to increase with elevation we performed %RT analysis within elevational classes to more accurately address age-structure. When sites were grouped into low, medium and high elevation categories, %RT declined significantly (Table 2-8) with age at all elevation sites (Figure 2-5). As one would expect sample sizes decreased with age-class, and thus the precision for the %RT estimate at higher age class was extremely low. However, when we repeated this same analysis with sequential removal of the oldest age-class from age-10 to age-8, the results remained unchanged. Sample sizes for the means represented in Figure 2-5 are presented in tabular form (Table 2-9).

Table 2-8: Results of linear regression analysis for each elevation class, assessing the affect of age on %RT. Sample sizes are the sum of each elevation class in Table 2-9.

		Unstandardized Coefficients		Standardized Coefficients		
Elevation class		B	Std. error	Beta	t	Sig.
1 - low	(Constant)	.584	.084		6.966	.000
	Age	-.056	.024	-.252	-2.329	.022
2 - medium	(Constant)	.107	.018		5.783	.000
	Age	-.013	.005	-.151	-2.354	.019
3 - high	(Constant)	.062	.013		4.662	.000
	Age	-.008	.003	-.181	-2.524	.012

a. Dependent Variable: %RT

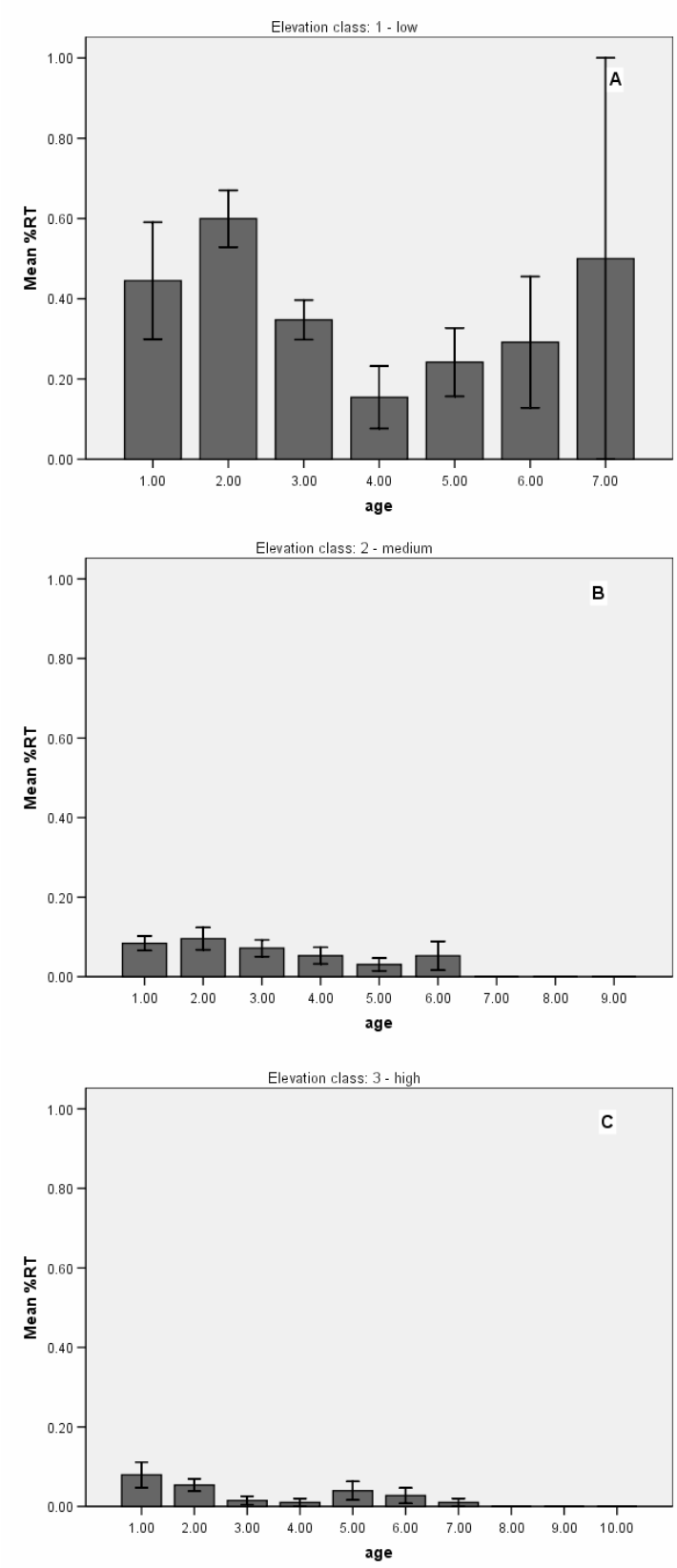

Figure 2-5: Bar graphs depicting mean % *O. mykiss* per age-class for the elevation classes: a) low, b) medium and c) high. Error bars represent ±1 SE. Values are zero where no bars are visible.

Table 2-9: Sample sizes represented by means in Figure 2-5 a-c.

	Elevation class		
	1 - low	2 - medium	3 - high
1.00	6	64	19
2.00	25	57	47
3.00	30	37	42
4.00	7	31	25
5.00	5	27	20
6.00	6	12	14
7.00	2	6	13
8.00	1	3	7
9.00		1	1
10.00			2
Class Total	82	238	190

Proportional hybrid abundance (%hybrid) analyses were also repeated within elevation classes. Overall, hybrid abundance (proportion hybrids) declined significantly (Table 2-10) with age at medium elevation; however, this trend was not observed at high or low elevations (Figure 2-6). Again, results remained unchanged with sequential removal of the oldest age-class from age-10 to age-8. Sample sizes for the means represented in Figure 2-6a-c are presented in tabular form (Table 2-11).

Table 2-10: Linear regression results testing the effect of age on %hybrid with each elevation class. Sample sizes are the sum of each elevation class in Table 2-9.

		Unstandardized Coefficients		Standardized Coefficients		
Elevation code		B	Std. Error	Beta	t	Sig.
1 - low	(Constant)	54.864	16.899		3.247	.004
	age	-1.688	3.918	-.094	-.431	.671
2 - medium	(Constant)	34.494	5.408		6.378	.000
	age	-4.459	1.114	-.610	-4.004	.000
3 - high	(Constant)	25.526	8.147		3.133	.004
	age	-2.292	1.566	-.243	-1.464	.152

a. Dependent Variable: Mean % hybrid

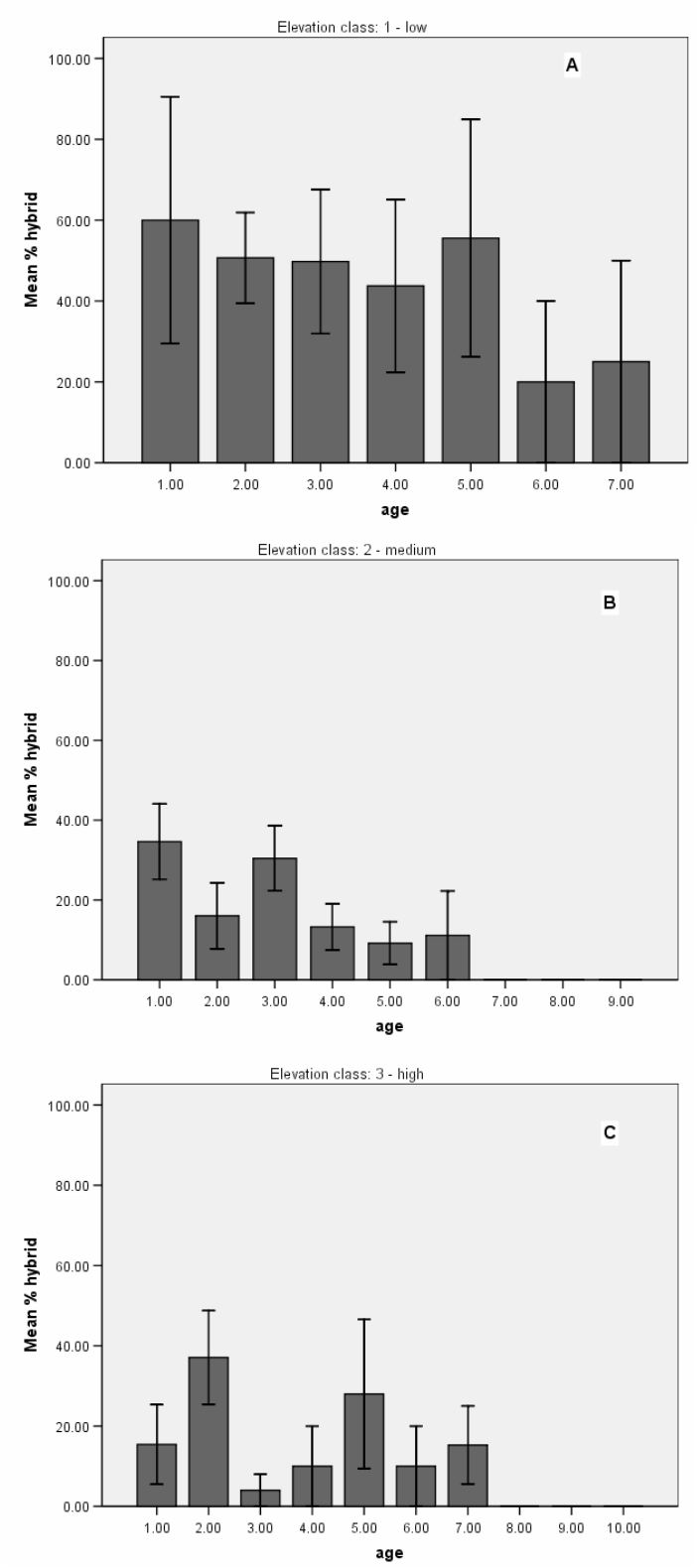

Figure 2-6: Bar graphs depicting mean proportion of hybrids versus age-class for the elevation classes, a) low, b) medium and c) high. Error bars represent ±1 SE.

Table 2-11: Sample sizes represented by means in Figure 2-6 a-c.

	Elevation class		
Age	1 - low	2 - medium	3 - high
1.00	3	4	4
2.00	4	4	5
3.00	4	4	5
4.00	4	4	5
5.00	3	4	5
6.00	2	3	4
7.00	2	3	3
8.00		2	2
9.00		1	1
10.00			2
Class Total	22	29	36

Maximum ages varied with genotype with two *O. clarkii lewisi* found to be age-10, one hybrid of age-8 and the oldest *O. mykiss* observed was age-7. The difference in sample size between these two analyses is a result of the need to average in order to estimate the proportion of hybrids within an age-class, whereas the proportion of *O. mykiss* alleles can be analyzed at the individual level, since multiple markers were used on each individual.

Life-history strategies: Specific growth rate

SGR also showed a very strong pattern of increase with decreasing elevation. Besides elevation, SGR also increased with the degree of hybridization (%RT) and as the average age decreased. Multiple regression showed that the downstream increase in SGR was not a function of elevation alone, but rather, was explained by all three factors, elevation, age and %RT (Table 2-12).

Table 2-12: Results from the SGR multiple regression analysis (n=590).

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.173	.205		15.500	.000
	% O. mykiss	.213	.072	.115	2.934	.003
	elevation (m)	-.001	.000	-.232	-5.912	.000
	age	-.142	.009	-.517	-16.309	.000

a. Dependent Variable: SGR

We analysed SGR within elevation class as with age-specific survival, but furthermore had to restrict comparison to within age classes. Figure 2-7 and Figure 2-8 compare the growth rates of age-2 and age-3 *O. mykiss*, *O. clarkii lewisi*, and hybrids at low, medium and high elevation sites. *O. mykiss* (found only at low elevation) had the highest growth rates, *O. clarkii lewisi* were the lowest, and hybrids were intermediate. Although there was a trend for hybrids to have higher SGR than pure *O. clarkii lewisi*, this difference was not statistically significant. Sample sizes and mean %RT mean SGR values are presented in Table 2-13.

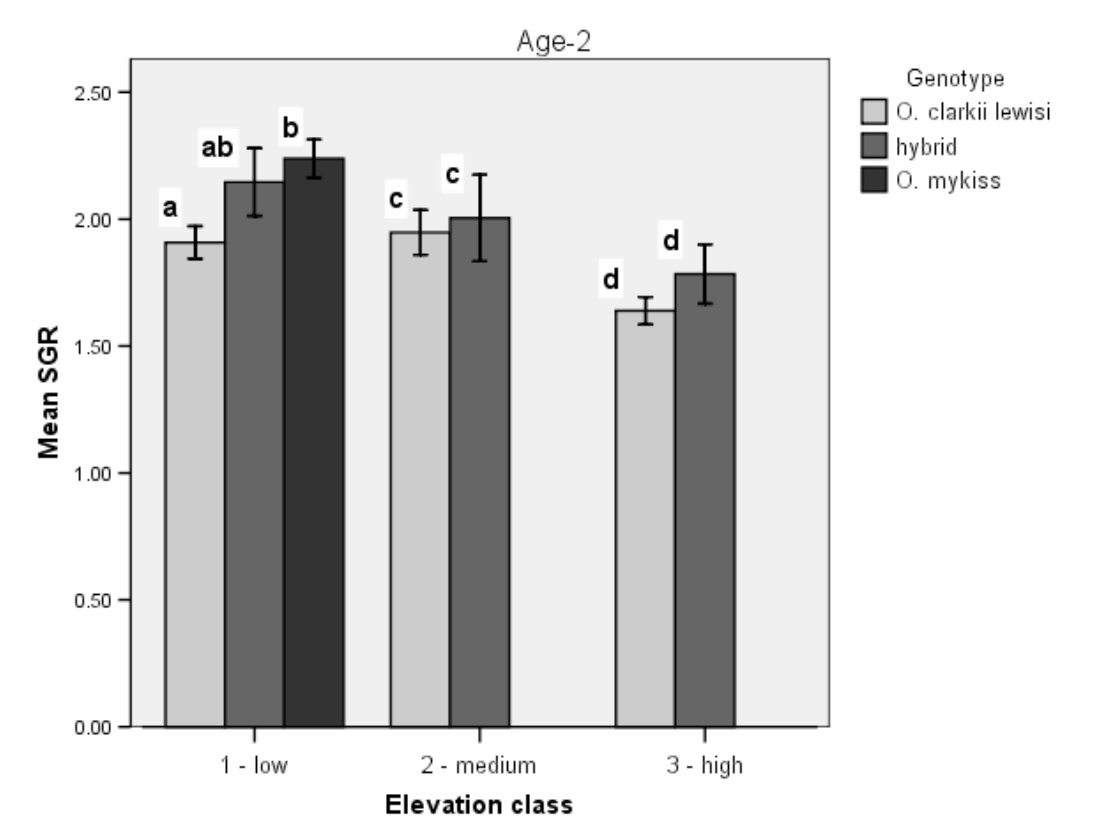

Figure 2-7: Mean SGR for age-2 individuals of genotypes present in each elevational class with ±1 SE error bars. Same letters denote no significant difference within elevational class only as found by LSD multiple comparison analysis.

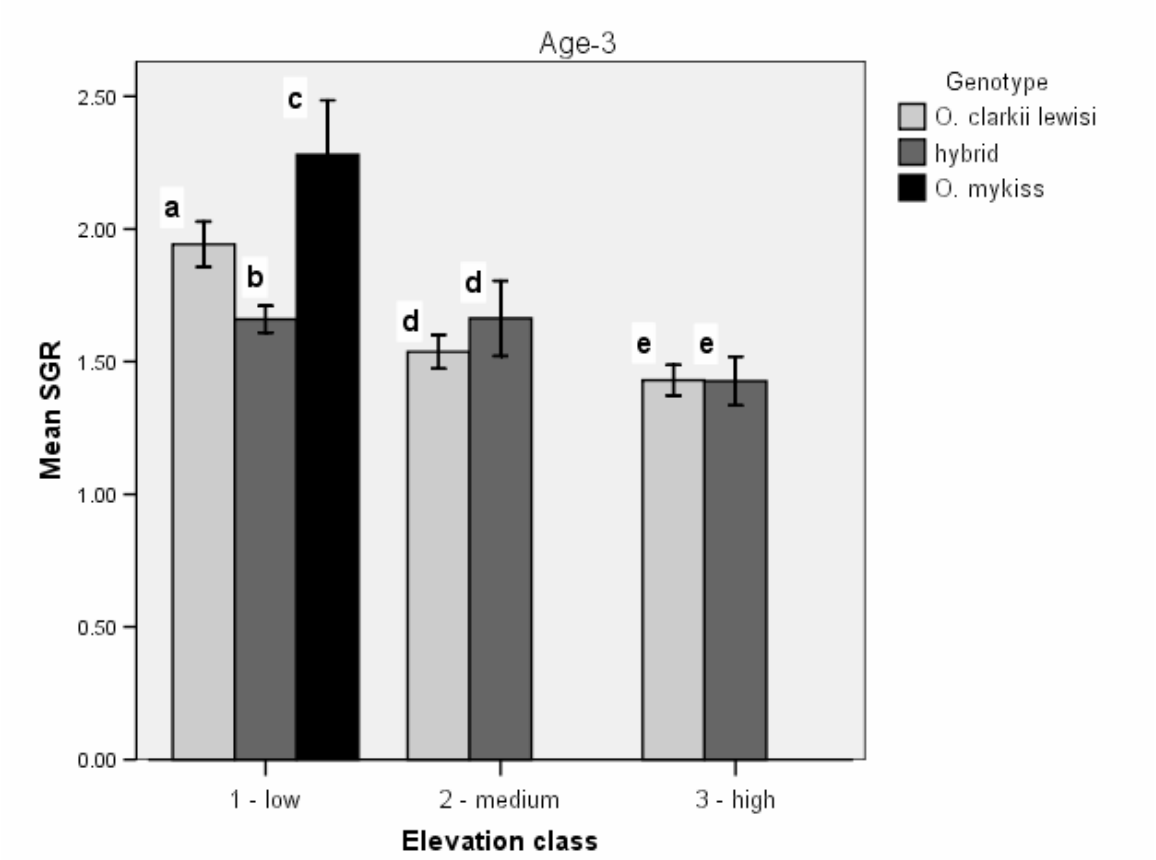

Figure 2-8: Mean SGR for age-3 individuals of genotypes present in each elevational class with ±1 SE error bars. Same letters denote no significant difference within elevational class only as found by LSD multiple comparison analysis.

Table 2-13: Sample sizes and mean %RT of each value presented in Figure 2-7 and Figure 2-8.

	Elevation class													
	1 - low						2 - medium				3 - high			
	O. clarkii lewisi		hybrid		O. mykiss		O. clarkii lewisi		hybrid		O. clarkii lewisi		hybrid	
Age	N	Mean %RT	N	Mean %RT	N	Mean %RT	N	Mean %RT	N	Mean %RT	N	Mean %RT	N	Mean %RT
2	10	0%	17	48%	27	100%	27	0%	12	34%	36	0%	11	23%
3	15	0%	22	43%	4	100%	21	0%	10	23%	32	0%	3	34%

Life-history strategies: Growth rate versus survivorship

When comparing *O. clarkii lewisi* and hybrids, significant genotype-age interactions were detected for growth (Table 2-14) and survival rates (Table 2-15). The average rates at which body weight increased and abundance decreased with age were significantly

different between *O. clarkii lewisi* and hybrids. Hybrids tended to show a higher growth rate (Figure 2-9) and lower survival rate (more negative slope of survival versus age) (Figure 2-10).

Table 2-14: ANCOVA results showing significant effect of genotype*age interaction on ln(weight).

Dependent Variable: ln weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	3.010	1	3.010	475.085	.000
genotype * age	2.176	2	1.088	171.724	.001
Error	.019	3	.006		
Total[a]	138.764	6			

a. R Squared = .991 (Adjusted R Squared = .986)

Table 2-15: ANCOVA results showing significant effect of genotype*age interaction on ln(abundance).

Dependent Variable: ln abundance

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	10.334	1	10.334	105.463	.002
genotype * age	2.670	2	1.335	13.623	.031
Error	.294	3	.098		
Total[a]	82.852	6			

a. R Squared = .901 (Adjusted R Squared = .835)

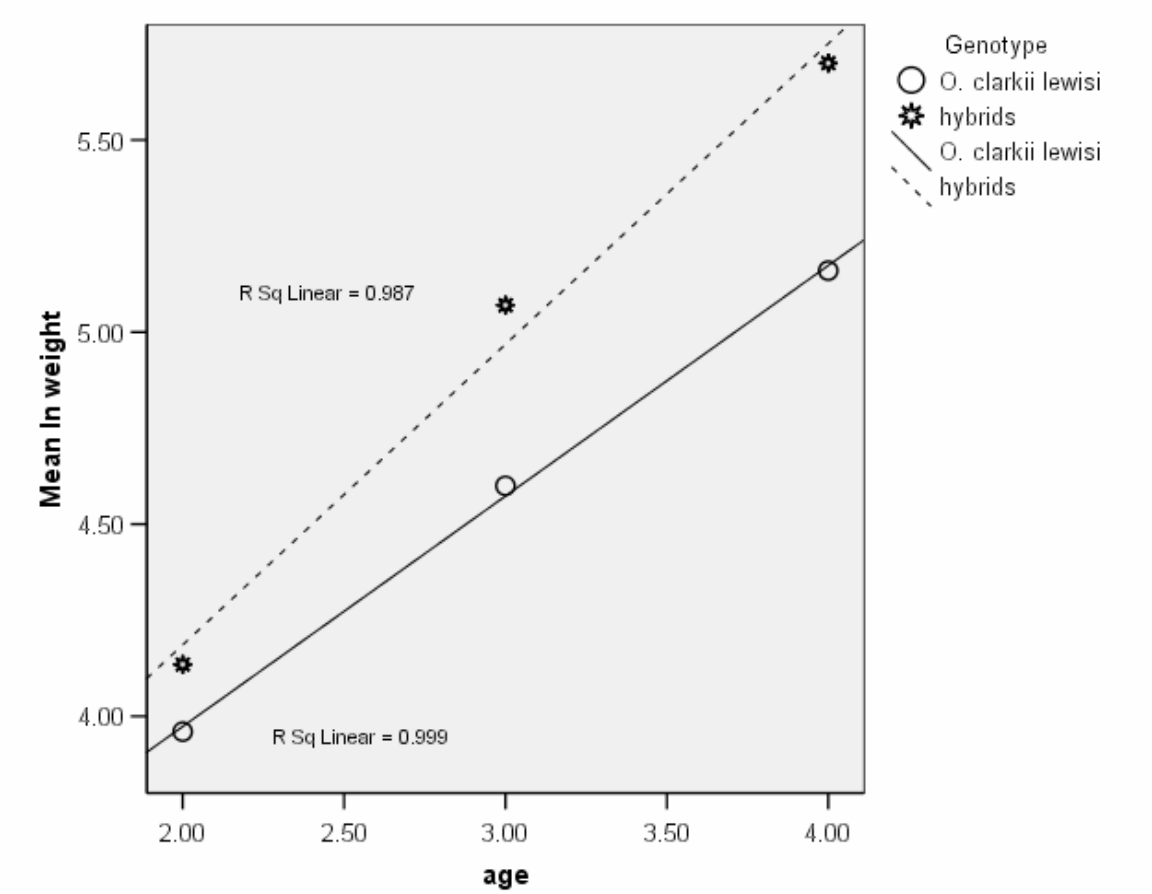

Figure 2-9: Plot showing significantly different mean growth rates (ln weight (g)) for *O. clarkii lewisi* (slope= 0.624) and hybrids (slope=0.759). The overall R^2 from the ANCOVA was 0.991.

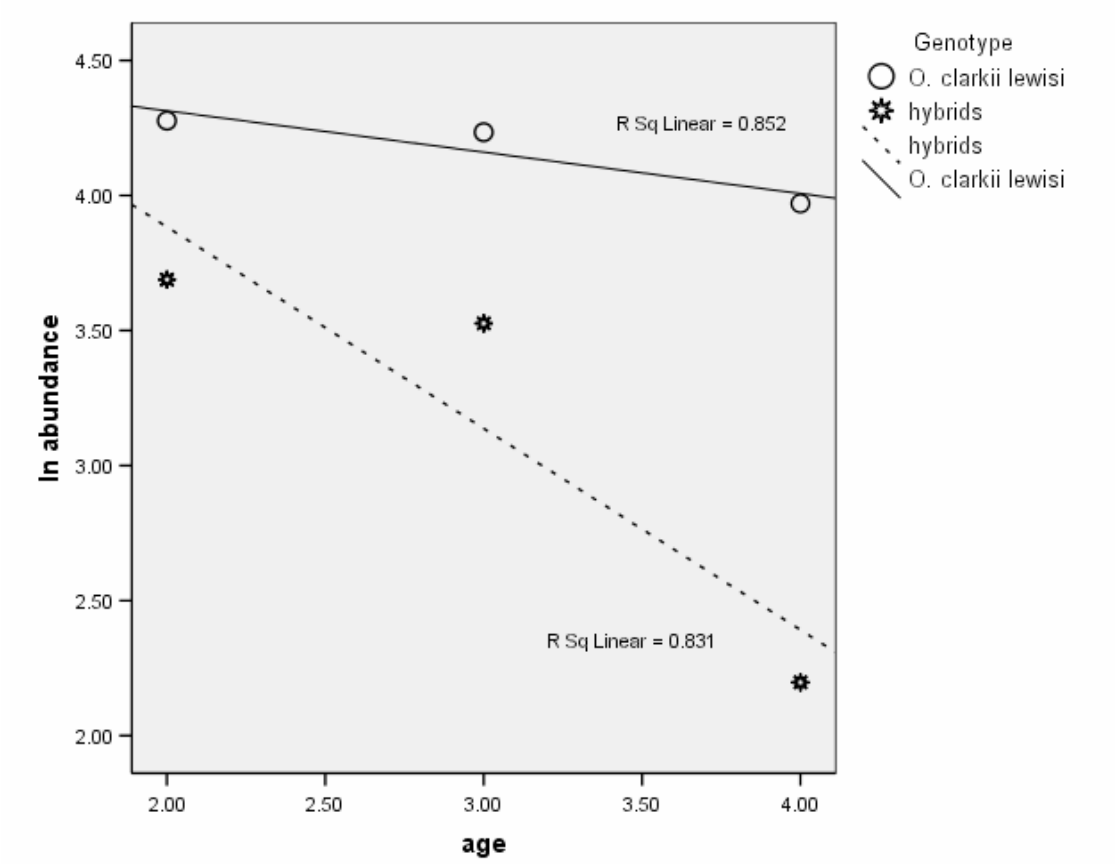

Figure 2-10: Plot showing significantly different rates of mortality (ln abundance) for *O. clarkii lewisi* (slope= -0.270) and hybrids (slope=-0.629). The overall R^2 from the ANCOVA was 0.901.

Influence of barriers and temperature

Six suspected impassable barriers were identified throughout the study area (Table 2-16). All barriers were falls without any stepping. Livingstone Falls are more accurately described as a chute created by an exposed bedding plane. All barriers were well-confined with no potential by-passing side channels. The previously mentioned hung culvert of Beaver Creek (Be1) was not included here as the extent of its existence as a potential barrier is unknown.

Table 2-16: Location and description of potential migratory barriers identified within the study area.

Stream	Barrier type	Estimated drop (m)	Elevation (m)	easting	Northing
Oldman River (upper)	Falls	9.1	1692	675180	5544650
North Racehorse Creek	Falls	3.0	1585	677350	5523400
South Racehorse Creek	Falls	4.6	1615	677350	5520140
Livingstone River	Falls/chute	7.6	1676	683315	5551890
Gold Creek	Falls	12.2	1341	688610	5498640
Daisy Creek	Falls	1.8	1524	686000	5520670

The effect of a barrier in limiting the upstream spread of hybridization was most evident at low elevations, with the falls on Gold Creek (1341m elevation) providing the most striking effect (Figure 2-11). Here %RT was reduced from 56% below the falls to 2% above them. At medium elevations, the falls on Daisy Creek, N. Racehorse Creek and S. Racehorse Creek all had reduced %RT from 5-7% below the falls to 0-2% above. The two high elevation barriers, Oldman River falls and Livingstone River falls all made no difference as %RT was 0% both above and below. Using ANCOVA the main effects of elevation (m) and site location were both found to be significantly related to site-mean %RT (Table 2-17). The interaction between elevation and site location (above or below) was also found to be significant (Table 2-17). Due to the nonlinear trend of the below-barrier sites, %RT was log-transformed enabling the use of ANCOVA analysis. Histograms A and B (Figure 2-2), presented earlier to depict the effectiveness of a barrier in maintaining a genotypic distribution more characteristic of sites at higher elevations, are the pair of sites at 1341m (Figure 2-11).

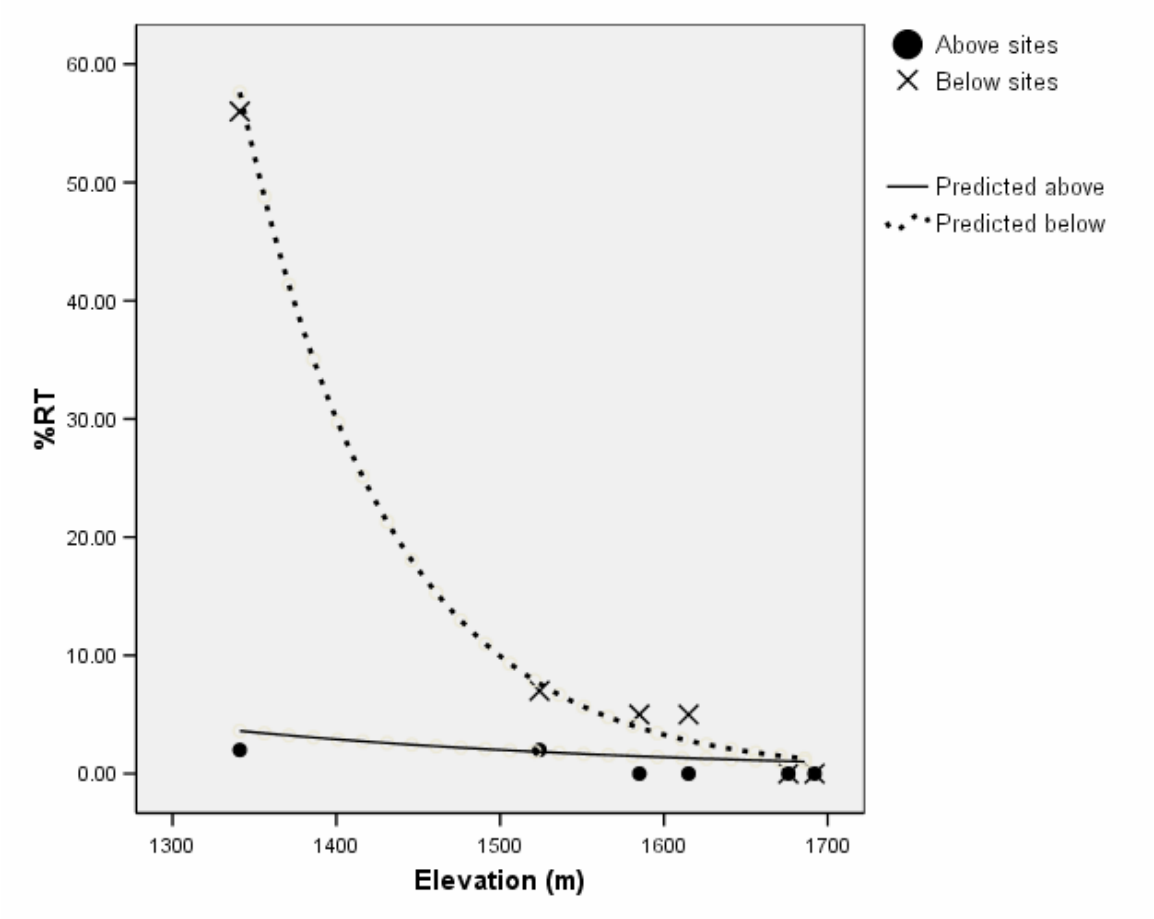

Figure 2-11: Plot of site-mean %RT for sites located above and below barriers. Trend lines calculated from the model of the ANCOVA analysis showing interaction between location and elevation. A significant interaction (B=-0.003) was observed between location and elevation.

Table 2-17: ANCOVA results showing significant effects of the covariate elevation (m) and site location (above or below barriers), as well as a significant interaction (location*elevation= 0.003)

Dependent Variable: log %RT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1.981	1	1.981	57.966	.000
location * elevation	.415	1	.415	12.140	.008
location	.503	1	.503	14.729	.005
elevation	1.697	1	1.697	49.652	.000
Error	.273	8	.034		
Total[a]	5.314	12			

a. R Squared = .914 (Adjusted R Squared = .882)

Data from the temperature loggers showed similar mean June-August ($MDT_{June\text{-}Aug}$) elevational profiles among streams (ΔT = -0.0127°C/m ± 0.002). Although similar in

profiles the streams showed different thermal regimes. Dutch Creek appeared to be colder at any given elevation compared to South Racehorse, North Racehorse (Figure 2-12) and Vicary Creeks, respectively.

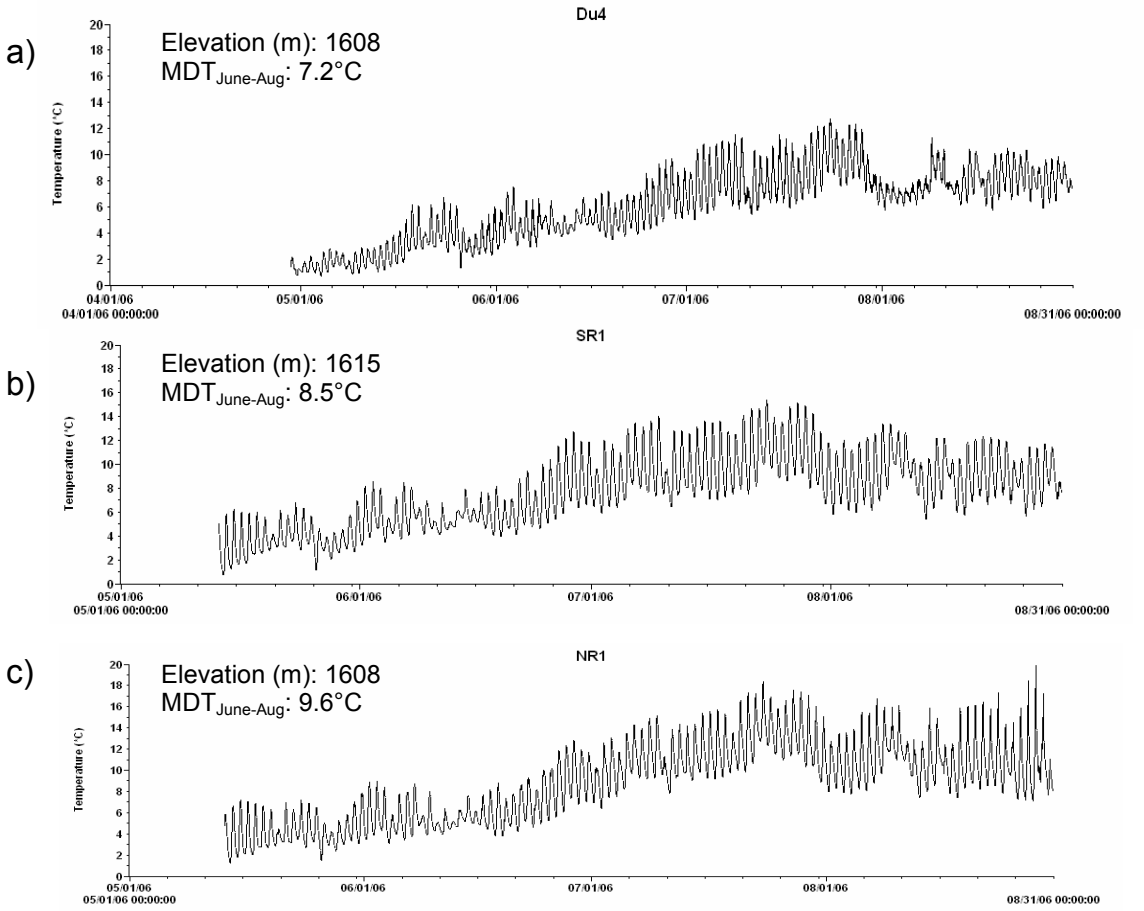

Figure 2-12: Stream temperature profiles of elevationally similar sites on a) Dutch (Du), b) South Racehorse (SR) and c) North Racehorse (NR) Creeks. Average daily June to August values show different thermal regimes of each stream.

Discussion

Hybridization status and genotypic gradient

The hypothesized elevational gradient, with hybridization decreasing upstream, was observed within the study area. We are unaware of any *O. mykiss* x *O. clarkii lewisi* hybridization studies that fail to report this gradient within their study area (Rubidge *et*

al. 2001; Hitt *et al.* 2003; Weigel *et al.* 2003). Hybridization is extensive in both the Oldman River and Crowsnest River drainages. As is commonly reported, no F1 hybrids were identified in either sampling year indicating that the spread of hybridization appears to be facilitated solely by post F1 generations and backcrosses (Hitt *et al.* 2003; Rubidge and Taylor 2004). Highlighting this, Rubidge and Taylor (2004) found only post-F1 hybrids at sites that were reported to be pure *O. clarkii lewisi* approximately three generations before.

The absence of F1 hybrids may be explained by a buffering effect created by the hybrid zone that essentially maintains separation of *O. clarkii lewisi* and *O. mykiss*. The %RT-frequency histograms indicate a broad zone with *O. mykiss* plus hybrids at the lower and hybrids and *O. clarkii lewisi* plus hybrids higher up. This study failed to detect pure *O. mykiss* above an elevation of 1311m and rarely found the two parental species within the same site (n=2). Interestingly, one of the sympatric sites was lower Gold Creek (Go1) were the Gold Creek falls barrier leads to a sharp break in the gradient with no hybrid buffer zone. Thus, like all previous studies, our data suggest that outbreeding depression is either absent or at least too weak to prevent the establishment of an hybrid zone.

The trend observed in the %RT histograms supports the notion that most hybrids exist as back-crosses to either parental taxa. The direction of back-crossing appears to be dependant on elevation with high percentage hybrids trending to *O. mykiss* at low elevations and *O. clarkii lewisi* in higher reaches. Therefore, not only do hybrids decrease in number with elevation, they also decrease in degree of hybridization (%RT) on

average. The majority of sites possessed unimodal distributions skewed towards *O. clarkii lewisi*. This trend was also reported by Rubidge and Taylor (2004). It appears that hybrids are genetically compatible with both parental strains, since both types of backcrosses are common along this gradient.

Life-history strategies: Implications of differing strategies

We hypothesized that preferential movement by *O. mykiss* to downstream reaches is a major contributor to the genetic gradient. Preferential movement is a migratory response to both abiotic (e.g. temperature, channel morphology, land use) and biotic (e.g. competition, metabolism, growth requirements) factors (Paul and Post 2001; Hitt *et al.* 2003). According to this hypothesis, introduced *O. mykiss* preferentially move downstream where they presumably outcompete *O. clarkii lewisi* and become established (Paul and Post 2001).

Habitat preference may ultimately be a reflection of different life-history strategies possessed by *O. clarkii lewisi* and *O. mykiss*; enforced within their respective glacial refugia. The limited distribution of *O. mykiss* throughout the study area precluded large scale comparisons between these two taxa. However, because this hypothesis predicts hybrids to be intermediate in life-history, comparison of *O. clarkii lewisi* to hybrids would in fact seem to be the more instructive comparison. A significant interaction between genotype and age was found when directly comparing *O. clarkii lewisi* and hybrid growth and survival rates. That is, *O. clarkii lewisi* and hybrids have different slopes in both their SGR and survival plots, meaning that hybrids had more rapid growth but a decreased survival. Within elevation class comparisons allowed for the inclusion of

O. mykiss at low elevations, effectively extending this trade-off to the whole genotypic gradient. The same pattern of trade-off between growth and survival along a genotypic/elevational gradient was demonstrated by analyzing the relationship between growth and survival versus age within elevational categories. When comparing SGR within elevation classes we see a significant affect of genotype within the low elevation class at ages 2 and 3. This was explained by a significantly lower *O. clarkii lewisi* SGR compared to *O. mykiss*. Our SGR estimates for hybrids are mixed, but typically show an intermediate rate, although this trend was only statistically significant in the aggregate analysis discussed above.

Individuals that tend to employ an accelerated life-history strategy need areas of high productivity in order to maintain a competitive growth rate (Morinville and Rasmussen 2003). While higher growth rates likely require a higher daily ration the individual would also need to be more competitive to obtain it. This assumes that metabolic costs are similar. However, if RT had higher metabolic costs as well, this expanded metabolic scope, although further increasing food requirements, might allow for a greater investment into interference competition through aggressive behaviour. The results of the metabolic comparison in Chapter 3 will allow for a more complete assessment of this question. The idea of a positive relationship between food requirements and aggressive behaviour is also addressed.

O. clarkii lewisi and *O. mykiss* life-history strategies were also found to differ in survival rate, conforming to a commonly reported trade-off between growth rate and survivorship

(Morinville and Rasmussen 2003). We addressed age-specific survival by analyzing the abundance of *O. mykiss* alleles over age-class. To remove the effect of elevation we analyzed age-specific *O. mykiss* allele abundance (%RT) within elevational classes. In all three classes a negative trend of %RT was significantly predicted by age. This loss of *O. mykiss* alleles with age likely indicates that survivorship is a decreasing function of the number of *O. mykiss* alleles possessed by an individual.

Combining the two life-history aspects assessed we see that, on average, *O. mykiss* employ a faster growing life-history strategy at the cost of reduced survival when compared to *O. clarkii lewisi*. These life-history differences support the hypothesis that introduced *O. mykiss* retain characteristics that reflect their anadromous ancestry and domesticated past, and furthermore, that hybrids represent a broad genetic gradient that are intermediate with regard to both growth and survival. We have previously mentioned the trade-off between growth and survival as one way that reduced survival can be offset. Another mechanism is through sexual selection. In the face of reduced numbers hybrids can achieve adequate productivity by hybrid male being selected by female *O. clarkii lewisi* or hybrid males being more aggressive than *O. clarkii lewisi* males on the spawning grounds. Epafanio and Phillipp (2001) found that even a very slight fitness advantage to hybrids in F2 and further generations may result in the establishment of a hybrid population.

The initial downstream preference of *O. mykiss* reported by Paul and Post (2001) is likely a consequence of their migratory ancestry. Once *O. mykiss* become established, the

gradient may be maintained through a habitat preference/competition mechanism. In order to sustain a competitive growth rate *O. mykiss* must find habitat that can satisfy their higher food requirements. When in a suitable habitat *O. mykiss* must further secure a large enough portion of the available food by being a superior competitor, likely through interference competition. Unlike exploitative competition where a competitor gains advantage by depleting a resource, interference competition further increases energetic demands as the resource is secured through aggressive behaviour. A positive relationship between aggressiveness and growth rate has been observed in other salmonid species (Lahtai *et al.* 2001).

Should the same aggression/growth relationship exist within this study area, the SGR results would suggest that the genotypic gradient is strongly influenced by competitive interactions. We expect that a hybrid would be most successful at the point in the gradient where it has gone far enough up to reduce competition from *O. mykiss*, but can still outperform pure *O. clarkii lewisi*. The exact point would differ depending on its genetic makeup; that is, the more it resembles *O. mykiss*, the further downstream we would expect to find it. The observed distribution of mean %RT of hybrids being negatively related to elevation supports the preferential movement hypothesis. The results of most of our analyses are consistent in that they place hybrids between *O. clarkii lewisi* and *O. mykiss*, but often they were not statistically distinguishable from both parental taxa. This is not surprising considering the broad genetic range among the hybrids (%RT ranged from 0.125 – 0.875 using four markers). In addition, most hybrids have a mean %RT

skewed towards one parental taxon and likely most of their characteristics would be similarly skewed, making statistical resolution difficult.

The role of physical barriers and thermal preferences in maintaining genetically pure *O. clarkii lewisi* populations at high elevation.

Physical barriers doubtlessly have an effect on the spread of hybridization. Many studies report barriers to be the only factor truly limiting the spread of hybridization (Hitt *et al.* 2003; Weigel *et al.* 2003; Rubidge and Taylor 2005). We found the effectiveness of barriers in limiting hybridization to be greater at lower elevation (-0.2% Δ%RT/m), as we had hypothesized. From this it seems likely that a combination of factors may act together to limit the upstream boundary of the hybrid zone. At lower elevations *O. mykiss* and high degree hybrids are within their preferred elevational range, making barriers the only limiting factor. On the other hand, at higher elevations the barriers are less critical since *O. mykiss* and hybrids are effectively outside their preferred elevational range.

The existence of pure *O. clarkii lewisi* populations at high elevations, in absence of any migratory barriers, further suggests that a combination of factors work to maintain the genotypic gradient. The habitat preference discussed above may operate through temperature preferences. In the second year of our study we became increasingly aware of the potential importance of the thermal regime as a probable factor delimiting the hybrid zone, and began to collect detailed temperature records from many of our study sites. The two most upstream sites of Dutch Creek (Du1 and Du3) experienced a %RT gradient from 3%RT (Du1) to a pure *O. clarkii lewisi* population (Du3) without a barrier restricting upstream movement. This gradient was associated with a strong temperature

gradient, with a $MDT_{June\text{-}Aug}$ value of 7.25°C at the lower site (3%RT) and a value of 6.55°C at the upper site (0%RT). If we propose that 7.25°C represents the upper boundary of the hybrid zone (Figure 2-13), presumably because hybrids prefer warmer sites, we see that the migratory barriers of both North and South Racehorse creeks occur at elevations before a $MDT_{June\text{-}Aug}$ value of 7.25°C can be achieved. On the other hand, Vicary Creek (Vi1 and Vi2), where no barriers were present, and the 7.25°C threshold was never reached, was hybridized all the way to its headwaters. Although these limited results do not permit us to make strong conclusions about the exact value of, or the exact role of, such a threshold temperature, they do allow us to propose this as a reasonable hypothesis for further investigation.

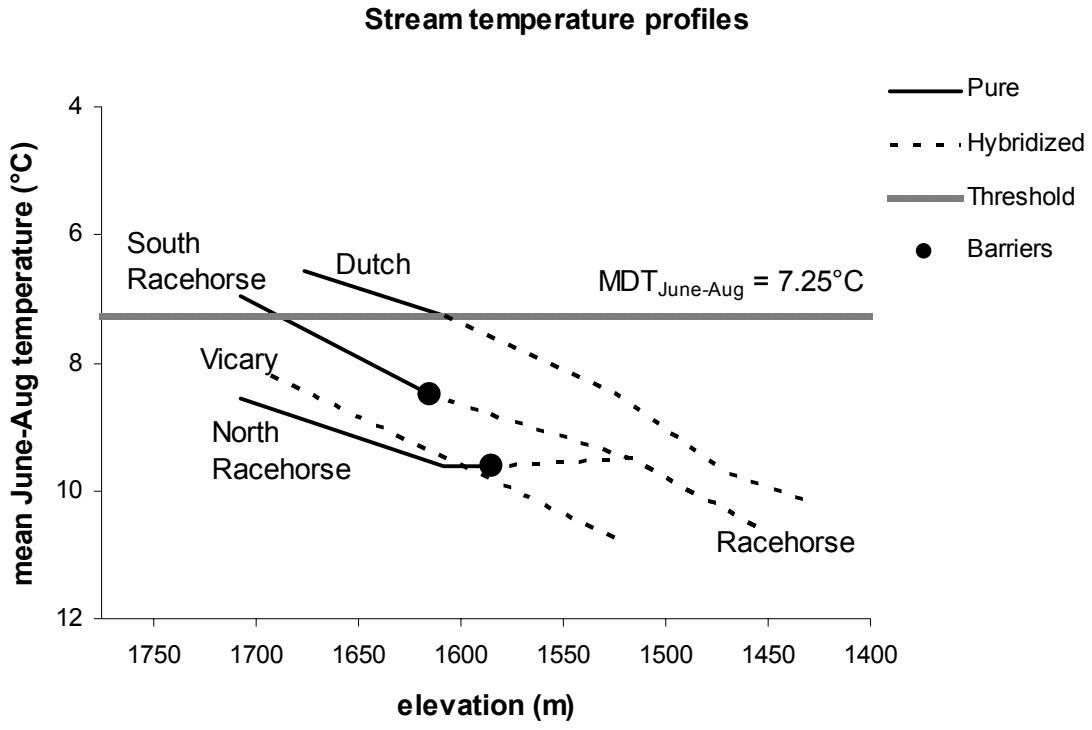

Figure 2-13: Average daily June to August stream temperature profiles of North Racehorse (NR), South Racehorse (SR), Dutch (Du) and Vicary creeks (Vi). Included are barriers of North and South Racehorse creeks and the hypothetical thermal threshold.

In an extensive study of *O. clarkii lewisi*, *O. mykiss* and hybrid distribution in the Madison River Basin of Montana, Sloat (2001) found that reaches occupied by *O. mykiss* were significantly warmer than those occupied by *O. clarkii lewisi*. This study found maximum daily average temperatures (MDAT) of reaches inhabited by *O. mykiss* and *O. clarkii lewisi* to be 11.08°C and 9.84°C, respectively. Similarly, Bozek and Hubert (1992) characterized *O. mykiss* reaches to be the warmest and *O. clarkii lewisi* to be the coldest of the four salmonid temperature regimes they compared. Interesting results are produced when we calculate the MDAT for the location coinciding with the $MDT_{June\text{-}Aug}$ 7.25°C from July to September 15 (same period as Sloat 2001). We estimate the MDAT of this transition point to be 9.71°C. These results, while preliminary, do suggest that we may be able to identify meaningful temperature thresholds that define zones wherein hybrid individuals are most likely to be found. Such thresholds could be very meaningful when considering potential effects of future climate change scenarios on the upstream advance of hybridization.

Sloat (2001) found no significant differences in thermal regimes occupied by hybrids and *O. clarkii lewisi*. This is potentially due to the definition of hybridized sites. Sloat (2001) grouped any sites possessing >10% *O. mykiss* with non-native sites. It is unclear to what degree of hybridization an individual must possess before its behaviour and preference becomes distinguishable from a pure *O. clarkii lewisi*. This method of grouping may have resulted in low %RT sites, whose average preference may not be distinguishable from pure *O. clarkii lewisi* sites, being compared to the former. The effect of environmental

variables (e.g. temperature, discharge, habitat, productivity) on preferential movement has not yet been defined clearly and requires further investigation.

We found the degree of hybridization to be strongly correlated with elevation but not stream order. This was likely due to the minor stream order variation (mean=3.5 ±0.34), with sites typically being located in 3rd or 4th order reaches. Literature on the environmental factors influencing hybridization is mixed. Rubidge and Taylor (2005) found no relationship between the degree of hybridization and stream order, magnitude and elevation. Similarly, Hitt *et al.* (2003) found demographics to be significantly related to degree of hybridization while temperature was found to be significant in only one of four distance classes. Weigel *et al.* (2003), on the other hand, found elevation, and presumably water temperature, to be a significant predictor of hybridization.

The existence of pure *O. clarkii lewisi* populations in headwater reaches supports the elevational refuge hypothesis presented by Paul and Post (2001), but further implies that pure *O. clarkii lewisi* populations in headwater reaches only exist because certain unknown constraints still exist. Further study should be directed at identifying behavioural and physiological factors responsible for the initial preferential movement of *O. mykiss*. Should temperature play an important role in determining the location of a hybrid zone, increases in stream temperature could have devastating effects on remaining *O. clarkii lewisi* populations, specifically with respect to climate change. Keheler and Rahel (1996) predicted an approximate 50% loss of salmonid habitat with a 3°C increase in mean July air temperature. It seems likely that *O. clarkii lewisi* would be the first to be

displaced as species retreat to higher elevations. More detailed studies of competitive interactions between *O. clarkii lewisi*, *O. mykiss* and hybrids would also enhance our understanding of the mechanisms behind the observed gradient, which would likely facilitate management decisions on issues such as continued stocking, possible barrier enhancement and possible *O. clarkii lewisi* introductions above barriers aimed at establishing small residual populations. Since the spread of hybridization in many areas appears to be rapid (Rubidge *et al.* 2001; Hitt *et al.* 2003; Allendorf *et al* 2004), there is an urgent need for an effective management approach to deal with this and other hybridization issues.

Conclusion

This study was successful in describing the genotypic gradient within the study area and presents an elevational pattern that is reasonably similar to that reported by other authors. Furthermore, we have described a gradient in the mean degree of hybridization when considering only hybrid individuals, suggesting that habitat preference is a function of the number of *O. mykiss* alleles an individual possesses. This study found significant differences in both growth rate and survivorship of *O. mykiss* and *O. clarkii lewisi*, showing *O. mykiss* to be a faster growing but shorter lived species on average. This was expected given the differing habitats these two species occupied during the Pleistocene. The contrasting life-history strategies of *O. clarkii lewisi* and *O. mykiss* implies a likelihood that the initial segregation of these two taxa has resulted from an *O. mykiss* preference for highly productive reaches where they can satisfy the higher energetic costs associated with an accelerated life-history. The fact that hybrids were found to be

intermediate in both life-history characteristics and elevational preference supports the hypothesis that habitat preference is a function of genotype.

Our results support the idea that a broad gradient of hybridization exists along the Upper Oldman, and that its position is likely determined by a combination of habitat selection by hybrids (including thermal preferences) and competitive interactions with *O. clarkii lewisi* in the upper reaches and *O. mykiss* lower down. In addition, this study has shown an influence of elevation on the effectiveness of migratory barriers in limiting the upstream spread of hybridization. The fact that some high elevation reaches with no physical barriers still hold genetically pure *O. clarkii lewisi*, despite over 80 years of *O. mykiss* stocking, strongly suggests that temperature preference and/or some as yet unidentified factors become more important in constraining the upstream spread of hybrids with elevation. The importance of demographic factors (Hitt *et al.* 2003; Rubidge and Taylor 2005), stream morphometry, and elevation (Weigel *et al.* 2003) as shown in previous studies, are all consistent with this hypothesis.

These results lead to the final conclusion that hybridization has effectively advanced *O. mykiss* alleles upstream and will likely continue to do so in the same manner that *O. mykiss* invaded inland streams postglacially. It appears from this that there is indeed a very low likelihood *O. mykiss* and *O. clarkii lewisi* will develop reproductive isolation and coexist in sympatry. Both Rubidge *et al.* (2001) and Hitt *et al.* (2003) showed significant upstream advances in hybridization within their respective study areas over roughly 20 years (approximately three generations). The implications of such a scenario

would be that the long-term genetic integrity of *O. clarkii lewisi* is only safe above a true migratory barrier.

3. Physiological differences between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), introduced rainbow trout (*O. mykiss*) and their hybrids, in zone of hybridization along an elevational gradient.

Abstract

This study compared, for the first time, the metabolic capacity of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), rainbow trout (*Oncorhynchus mykiss*) and their hybrids. Individual lactate dehydrogenase (LDH) activity was found to be significantly ($p=0.05$) predicted by degree of hybridization (%RT). Both citrate synthase (CS) assays and a stream-side oxygen consumption rate analysis revealed a significantly higher *O. mykiss* aerobic capacity compared to *O. clarkii lewisi*. Although rarely significantly different from either species, hybrids appear to be metabolically intermediate between the two parental species. A preliminary assessment of five physiological indices (liver glycogen, cortisol, plasma glucose, gill Na^+/K^+ATPase and plasma AchE) not directly related to metabolic capacity found %RT to be a significant predictor of liver glycogen ($p=0.10$) and AchE ($p=0.05$). The higher metabolic rate of *O. mykiss* potentially explains their preference for higher order, more productive reaches where they must outcompete *O. clarkii lewisi* to secure enough of the available food resources. The intermediate metabolic rate of hybrids likely makes them unable to effectively compete with *O. mykiss* forcing hybrids into less productive, upstream reaches where they appear to compete favourably with *O. clarkii lewisi*. Although the lower metabolic scope is likely responsible for this movement it may also allow for hybrids to be successful as their food

requirements are likely also reduced. Under this hypothesis hybridization effectively breaks down the initial species-specific isolating characteristic of metabolism.

Introduction

Hybridization is a common consequence when two naturally allopatric species are made to co-exist as a result of human intervention (e.g. Rubidge *et al.* 2001). The threat of genetic extinction through hybridization appears to be more likely if the involved taxa are closely related (Buss and Wright 1958; see also Chapter 1). Introductions of rainbow trout (*Oncorhynchus mykiss*) have been identified as a main factor in the extinction, extirpation and genetic pollution of many cutthroat trout populations (Behnke 2002; Allendorf *et al.* 2004). Arguably the most widely affected subspecies is the westslope cutthroat trout (*O. clarkii lewisi*). *O. clarkii lewisi* and *O. mykiss* are closely related species that have been identified as sister taxa in several molecular phylogenetic studies (Allendorf and Leary 1988; Crespi and Fulton 2004). When *O. mykiss* are introduced to the native range of *O. clarkii lewisi* they typically move to lower reaches where they appear to outcompete *O. clarkii lewisi* and become established (Paul and Post 2001). Once established these sister taxa readily hybridize producing a hybrid zone spanning an intermediate range along the elevational gradient (Allendorf and Leary 1988; Rubidge *et al.* 2001; Hitt *et al.* 2003; see also Chapter 2). Although the mechanism of this segregation is unclear it is possible that it is linked to the different life-histories of *O. clarkii lewisi* and *O. mykiss*.

O. mykiss have been shown to possess a faster growing, lower survivorship life-history strategy, in keeping with its anadromous ancestry and domestication, while *O. clarkii lewisi*, with its land-locked ancestry, grows more slowly and lives longer (Chapter 2). Furthermore, hybrids have life-histories that are intermediate between the two parental

species, in terms of both growth and survivorship. For a fast growing species to be successful it must find habitat with high enough productivity to satisfy its high energetic demands. Fast growing individuals must also possess a strong competitive ability in order to secure enough of the available resources. Morinville and Rasmussen (2003; 2006) found that in streams open to the sea, the anadromous form of brook trout (*Salvelinus fontinalis*), which have higher energy demands, generally are much more abundant than residents, suggesting that they could outcompete residents from the sections where they co-occur. High energetic requirements can potentially be thought to drive the downstream preference of *O. mykiss* and, as implied from the elevational gradient, place hybrids intermediately in both energetic costs and competitive ability.

Metabolic analysis of fish has been applied to a variety of topics, such as life strategies (Morinville and Rasmussen 2003), trophodynamics (Sherwood *et al.* 2002) and behaviour and social dynamics (Lahtai 2002). Although somewhat plastic (e.g. Guderley *et al.* 1997) the metabolic capacities of a fish are to a large extent predetermined and likely influence individual habitat preference. When selecting suitable habitat, a fish must find an area where food availability is sufficient enough to satisfy the energetic requirements of both its metabolic and growth rates (Moyle and Cech 2004).

In a number of salmonid species migratory life forms have been shown to have elevated metabolism in comparison to residents (Forseth *et al.* 1999; Rikardsen and Elliott 2000; Morinville and Rasmussen 2003). The consequence of this differentiation is that stream habitats suitable for spawning typically do not supply sufficient food to balance the

metabolic/growth budget of a migratory fish throughout its life. In order for migratory individuals to meet their high metabolic costs and still grow rapidly, they must relocate to areas of higher productivity. Although marine environments are commonly associated with higher growth, this contrast can be seen between resident, fluvial and adfluvial freshwater life morphs (Fraley and Shepard 1989) as well.

Since *O. mykiss* are an anadromous species that survived the ice age in coastal refugia and subsequently expanded its range inland, whereas westslope cutthroat trout were residents of inland proglacial lakes (Behnke 2002), we hypothesize that metabolic differences will exist between these two species, and that their hybrids will have intermediate metabolic characteristics. More recent domestication of *O. mykiss* has likely enhanced any differences between these two species. It seems highly probable that differences in habitat selection and competitive ability would imply that *O. mykiss* should have higher aerobic scope and greater power output, because of its recent divergence from a life-style adapted to large-scale migrations. The distribution of *O. clarkii lewisi*, hybrids and *O. mykiss* along the elevational gradient in the upper Oldman River probably reflects the preference of *O. mykiss* for downstream more productive habitats (Paul and Post 2001, also see Chapter 2) needed to support its high metabolic costs. The genotypic gradient also likely reflects their ability to establish themselves in the most productive habitats and to displace native cutthroat from these reaches, which requires a higher metabolic scope and power output. Hybrids, which are distributed within intermediate elevations along the gradient, should thus tend to have intermediate physiological characters and competitive abilities.

In all animals, including fish, there are two basic types of energy-yielding metabolic pathways: aerobic respiration which occurs within the mitochondria and is very efficient, and anaerobic respiration (glycolysis) which takes place within the cytoplasm, and can supply high power output at low efficiency. The differentiation of muscle types reflects the fact that individual fibres are primarily dedicated to one of these pathways (Moyle and Cech 2004). Although energetically less efficient, anaerobic respiration can deliver the quick swimming bursts needed to capture prey, elude predators and defend territory. On the other hand, aerobic respiration is more efficient in ATP synthesis and is therefore capable of providing the constant slow acting muscle contractions required for sustained "normal" activity. Therefore, it would be expected that predominantly insectivorous species, such as *O. clarkii lewisi* and *O. mykiss*, would depend more on aerobic respiration to capture their drifting prey. In fishes, citrate synthase (CS) and lactate dehydrogenase (LDH) are metabolic enzymes commonly used as indicators of maximum aerobic and anaerobic metabolic capacity respectively (Audet and Couture 2003).

There is reason to suspect metabolic difference as a potential factor in the initial preferential movement of *O. mykiss* as it is well understood that productivity increases as one moves downstream (Vannote *et al.* 1980). We have seen in Chapter 2 that specific growth rate (SGR) increases with an increase in %RT. Given Equation 1, *O. mykiss* with a higher SGR and metabolism would need reaches of sufficient productivity in order to satisfy both requirements.

Objective and hypotheses

1.) To assess the maximum metabolic capacities of *O. clarkii lewisi*, *O. mykiss* and their hybrids.

Ha1: *O. mykiss* will possess aerobic and anaerobic metabolic capacities higher than *O. clarkii lewisi* as indicated through enzymatic assays.

2.) To assess oxygen consumption rates in the field using stream-side oxygen consumption tests.

Ha2: *O. mykiss* will consume oxygen at a faster rate than *O. clarkii lewisi* due to a higher aerobic metabolism.

3.) To investigate any relationship between the genotype determined by the mitochondrial marker (nd3) and CS activity.

Ha3: Hybrid individuals that show mitochondria of *O. mykiss* decent will have higher CS activities.

Methods

A sub-sample of the fish collected for the gradient analysis (Chapter 2) was sacrificed in order to obtain muscle samples for enzymatic assays. These fish came from sites ranging in elevation from 1243 to1640m and were obtained during both the 2005 and 2006 field seasons. Fish were killed by cervical dislocation before sampling. White muscle, chosen to be assayed for its dominant proportion of total body muscle mass, was dissected from the caudal peduncle with care taken to ensure all red muscle was removed. Muscle samples were immediately frozen in liquid nitrogen and transferred into a -80°C freezer

until the assays were performed. Fish collection and molecular markers employed were described in Chapter 2, with further description of the genetic techniques to come in Chapter 4.

Sample preparations and enzymatic assays

Samples were prepared following the protocol presented in Sherwood *et al.* (2002). Approximately 0.5g of sample was ground in liquid nitrogen with a mortar and pestle until reduced to a fine powder. The ground sample was then homogenized for 10 seconds in a potassium phosphate buffer solution (0.07 M, pH 7.0 KPO_4 buffer + 1 mM EDTA + 0.2% v/v Triton X-100) at a dilution of 50 volumes using a VWR Power Max drill with a Troemner 7x95mm bit. Approximately 1ml of supernatant (50x) was aliquoted and re-frozen at -80°C for the CS assay. A second dilution (1000x) was performed for LDH and protein analysis and then refrozen as well. Both assays were run in duplicate at 23°C using an Ultrospec 3100pro UV/Visible spectrophotometer with a 1cm path length. Any duplicates that returned greater than 5% standard error were rerun. Activities were analyzed as International Units (IU, amount of enzyme needed to produce 1 μmol of product) standardized to milligrams of protein. Activities calculated from the most significant multiple regression model were also presented graphically in order to assess the intermediary nature of hybrids. Protein was obtained against a BSA standard curve Bradford method and was run the sample day as the enzymatic assays.

LDH (EC 1. 1. 1. 27) was analyzed in a 4.5ml cuvette using the following assay mixture: 2.75 mL potassium phosphate buffer, 0.1ml NADH solution (6 mM) and 0.1ml sodium pyruvate solution (23 mM). The assay was commenced with the addition of 0.05ml of sample. Following a 30-second delay, the reaction was monitored for the reduction of NADH over 90 seconds. This was confirmed to be a linear portion of the kinetic assay for each sample. The change in absorbance (ΔA) per minute was converted to International Units (IU) (Equation 3-1) and standardized to protein content. Samples that ran out of the predetermined optimal range were rerun at an appropriate volume and corrected with a dilution factor (*df*).

$$IU = \Delta A \times 4.84 \times df$$

Equation 3-1: For the LDH assay conversion of absorbance to IU, where 4.84 represents the assay volume (3.0ml) divided by the sample volume (0.1ml) and multiplied by the extinction coefficient of TNB (cm^2/µmol).

CS (EC 4. 1. 3. 7) assays were based on the reaction mixture presented in Pelletier *et al.* (1993). The reaction was performed in a 1.5 mL cuvette by combining 930 uL of CS solution (100 mM Tris/ HCl , 0.1 mM DTNB and 0.2 mM acetyl CoA) and 60 uL of sample. The assay was initiated with the addition of 10 uL of 0.3 mM oxaloacetate solution (omitted from controls). Samples were monitored for 360 seconds to observe the formation of 5-thio-2-nitrobenzoic acid (TNB). This was confirmed to be a linear portion of the kinetic assay for each sample. The change in absorbance (ΔA) per minute was converted to IU (Equation 3-2) and standardized to protein content.

$$IU = \Delta A \times 1.23 \times df$$

Equation 3-2: For the CS assay conversion of absorbance to IU, where 1.23 represents the assay volume (1.0ml) divided by the sample volume (0.060ml) and multiplied by the extinction coefficient of TNB (13.6 cm^2/µmol).

The activities of both enzymes were analyzed using backwards method multiple regression using a criteria of p=0.01 for removal. In both cases predictor variables of %RT, elevation and fork length were included. Comparisons of the effect of %RT on LDH activity were carried out within three fork-length classes with an ANOVA (1: 100-199mm, 2: 200-299mm and 3: >300mm). Comparisons of the effect of %RT on CS activity within the elevational classes described in Chapter 2 were completed with an ANOVA. Due to small sample size, elevational class 3 (high) was excluded. Specifically in regards to hybrid individuals used in CS analysis, the categorical independent variable nd3 (mitochondrial marker) was included in an ANCOVA along with elevation in attempt to identify any relationship between CS activity and this marker.

Oxygen stress tests

A second sub-sample of fish collected was subjected to stream-side oxygen stress tests. Fish were placed in an instream container where they remained in minor current for approximately 30 minutes before testing. Due to size constraints of the container fish tested typically ranged between 30-60 grams and represent 1-3 year age-classes. Tests consisted of an individual fish being placed in a sealed container with a YSI 85 oxygen, conductivity, salinity and temperature meter inserted. The YSI meter was calibrated to water temperature and elevation at the beginning tests for each site. Oxygen consumption (mg O_2/min) was monitored for approximately 30 minutes and was reported as a rate per hour standardized to body mass (kg). Some individuals consumed the available oxygen before 30 minutes resulting in the early termination of the test. After the test was completed fish were immediately revived and monitored while in the instream container

before their release. Mean oxygen consumption rates were analyzed using a multiple regression with % RT, age and elevation as predictor variables.

Physiological comparisons

Secondarily added to the study was the analysis of five physiological parameters not directly indicative of metabolic rate. Thirty-one fish from the individuals used in the metabolic assays were analyzed for acetylcholine esterase (AchE), liver glycogen, blood glucose, plasma cortisol and gill Na^+/K^+ ATPase. AchE was determined using methods described in Chuiko 2000. Methods for determination of liver glycogen and blood glucose levels are described in Levesque *et al.* (2002) while the methods of Levesque *et al.* (2003) were followed to acquire plasma cortisol level and Na^+/K^+ ATPase activity. Fish used in these comparisons came from three sites (By1, Vi1 and Da1) representative of differing elevations and degree of hybridization.

Results

Lactate dehydrogenase (LDH)

Both %RT and fork length were found to be a significant predictors of LDH activity (Table 3-1). The overall mean LDH activity (± SE) was 2.20 ± 0.05 IU/mg protein for an individual mean length of 240 ± 5.4 mm. For literature comparison the mean LDH activity represented per gram of wet muscle mass (±SE) was 1138 ± 0.4 IU/g wet muscle mass. When comparing LDH activities within fork length classes a significant difference was only found between the *O. clarkii lewisi* and hybrid genotypes in the 200-299mm class (Figure 3-1). A trend of *O. clarkii lewisi* possessing lower LDH activity than *O.*

mykiss was observed in all length classes; however, low *O. mykiss* samples sizes likely precluded the reporting of a significant difference (Table 3-2).

Table 3-1: Multiple regression results showing significant effect of %RT and fork length (mm) on LDH activity (U/mg protein) (n=177).

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Sig.
(Constant)	1.229	.189		6.504	.000
%RT	.269	.151	.126	1.784	.076
fork length (mm)	.004	.001	.366	5.191	.000

a. Dependent Variable: LDH (U/ mg protein)

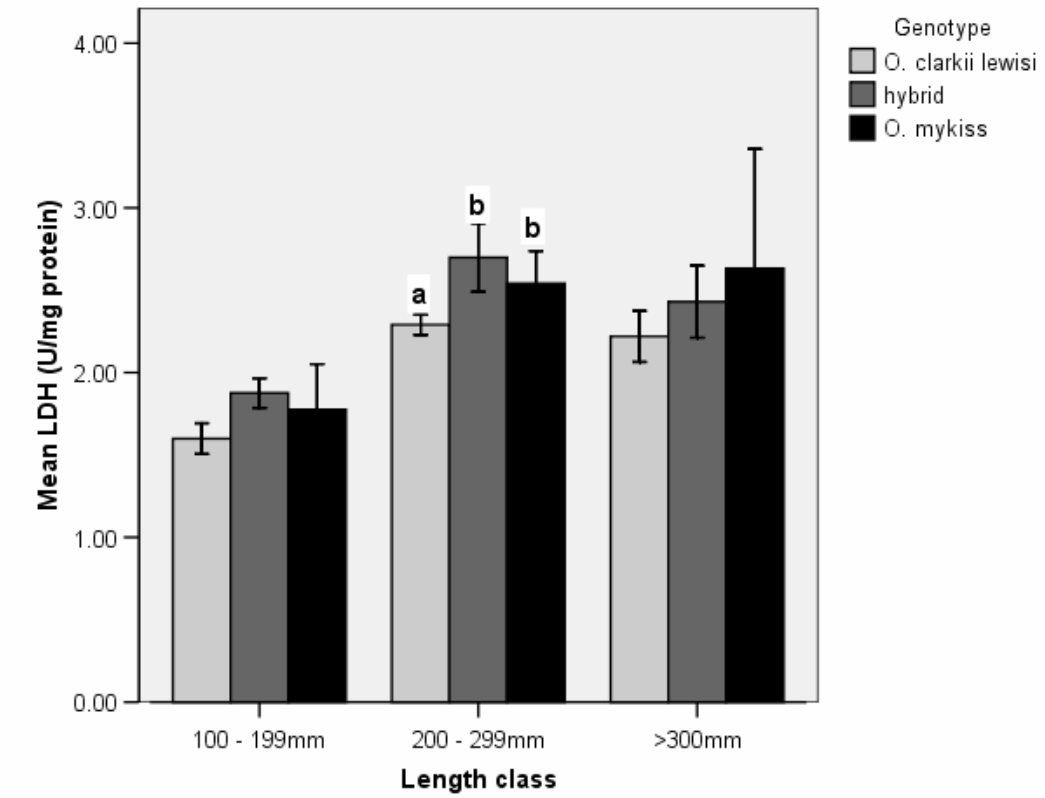

Figure 3-1: Mean white muscle LDH activity for each genotype, grouped by length category. Activities represent mean LDH (IU/mg protein) with 95% C.I. error bars. Same letters denote no significant difference within length class.

Table 3-2: Sample size and mean %RT represented by LDH values in Figure 3-1.

	Length class					
	100 - 199mm		200 - 299mm		>300mm	
Genotype	N	Mean %RT	N	Mean %RT	N	Mean %RT
O. clarkii lewisi	25	0%	73	0%	17	0%
hybrid	11	45%	21	39%	12	33%
O. mykiss	10	100%	5	100%	3	100%

Citrate synthase (CS)

Both % RT and elevation were found to be significant predictors of CS activity (Table 3-3). The mean CS activity represented per gram of wet muscle mass (±SD) was 7.1 ± 2.3 IU/g wet muscle mass. Within hybrids CS was not significantly related to the nd3 marker ($p=0.233$, $n=35$) although low samples size resulted in low power of detection ($\beta<0.50$).

Table 3-3: Table of coefficients of significant predictor variables produced from the CS activity (U/mg protein) multiple regression (n=87).

		Unstandardized Coefficients		Standardized Coefficients		
Model		B	Std. Error	Beta	t	Sig.
1	(Constant)	-.033	.008		-4.085	.000
	%RT	.005	.002	.370	2.971	.004
	elevation	3.16E-005	.000	.726	5.839	.000

a. Dependent Variable: CS activity (U/mg protein)

Comparison of CS activity within elevation classes showed a significant affect of %RT in the low elevation class (Figure 3-2). Low sample sizes did not permit comparison within the high elevation class. *O. mykiss* had significantly higher CS activity than *O. clarkii lewisi* and hybrids at low elevations (Table 3-4).

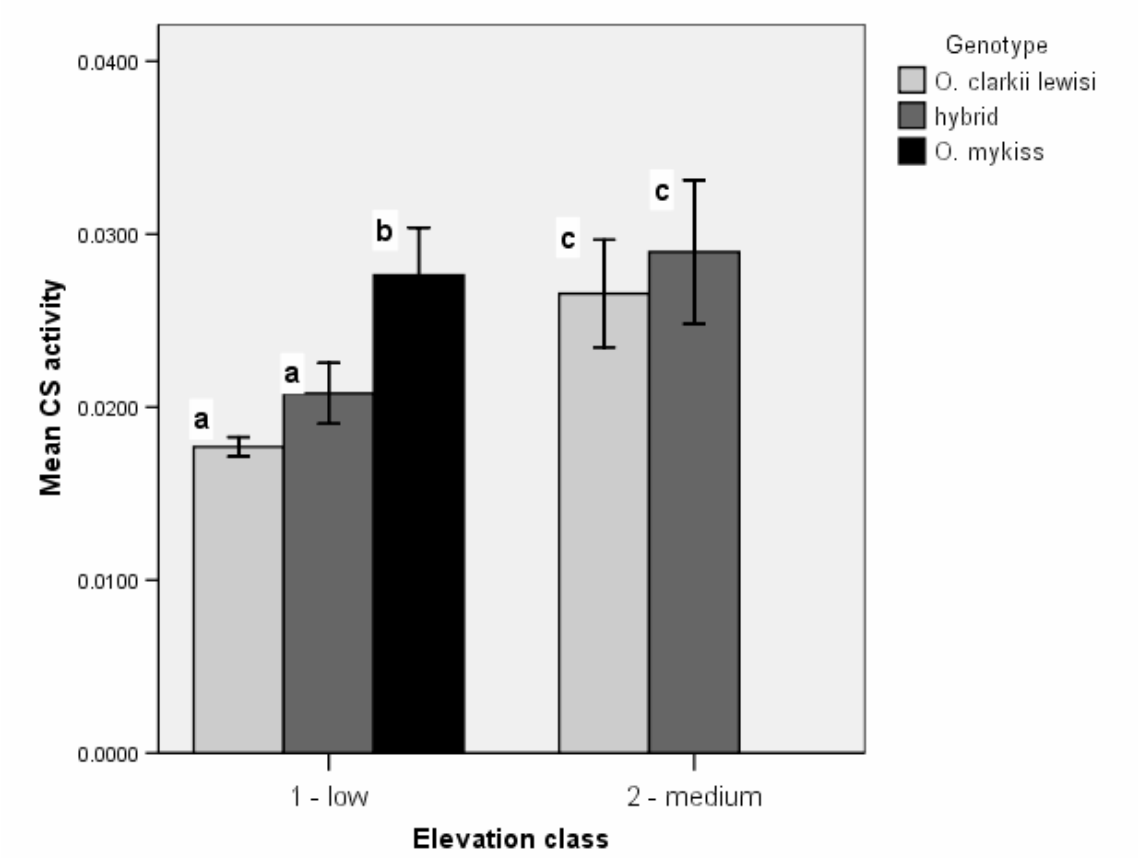

Figure 3-2: Mean CS activities (IU/mg protein) by hybrid classes with ±1 SD error bars. Same letters denote no significant difference within each elevation class only.

Table 3-4: Sample size and mean %RT represented by CS activities in Figure 3-2.

	Elevation class			
	low		medium	
Genotype	N	Mean %RT	N	Mean %RT
O. clarkii lewisi	7	0%	14	0%
hybrid	26	42%	6	29%
O. mykiss	16	100%	---	---

Oxygen stress tests

Oxygen consumption rate (OCR) increased with %RT and decreased with body weight (g) (Table 3-5). To enable comparison among genotypes, five weight classes were created (Table 3-6). Using ANOVA, significant genotype effects were detected in all weight classes (Figure 3-3). Multiple comparison analysis (LSD) could not be used in the <25g

and 76-100g weight classes as the hybrid genotype was represented by only one individual. The multiple comparison tests that could be performed showed that *O. mykiss* had significantly higher rate of oxygen consumption rates than *O. clarkii lewisi* (Table 3-7). Average estimates were higher for hybrids than for *O. clarkii lewisi*, and the difference was statistically significant at the p=0.10 level in the 51-75g class. Table 3-6 and Table 3-8 summarize sample sizes and mean %RT respectively.

Table 3-5: Results of multiple regression analysis showing significant effect of %RT and body weight (g) on oxygen consumption rates (mgO_2/kg hour) (n=97).

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	206.979	11.909		17.380	.000
	%RT	54.527	12.181	.394	4.476	.000
	weight	-.684	.190	-.316	-3.594	.001

a. Dependent Variable: O2hour

Table 3-6: Five weight classes used in oxygen consumption analyses.

	Weight class									
	<25g		26-50g		51-75g		76-100g		101-125g	
	N	mean weight (g)	N	mean weight (g)	N	mean weight (g)	N	mean weight (g)	N	mean weight (g)
O. clarkii lewisi	3	15.77	23	39.35	24	61.40	14	83.69	3	119.17
hybrid	1	18.10	10	38.14	5	58.02	1	83.90	---	---
O. mykiss	---	---	9	42.00	2	57.35	2	95.65	---	---

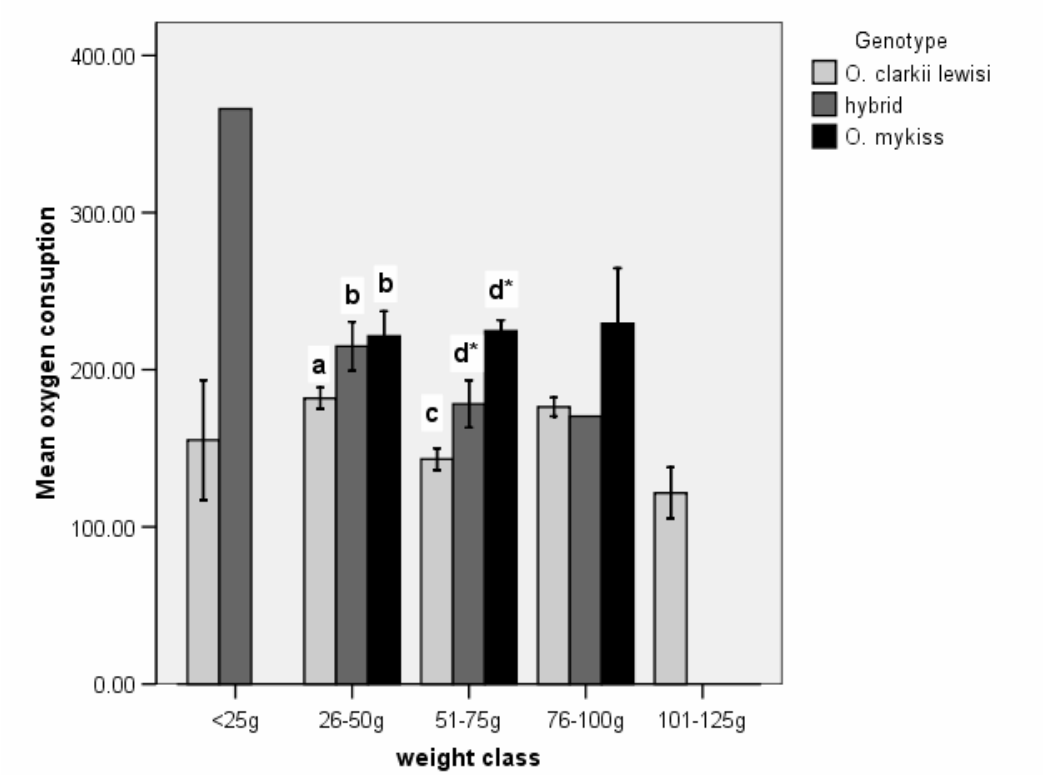

Figure 3-3: Mean oxygen consumption rates (mgO_2/kg hour) grouped by weight class and genotype with ±1 SD. Same letters denotes no significant difference at p=0.05 level, within each weight class as found by multiple comparison analysis (LSD) where possible. * denotes significant difference at the p=0.10 level between same letters.

Table 3-7: Results of multiple comparison analysis for significant differences (p=0.05) noted in Figure 3-3.

Multiple Comparisons

Dependent Variable: oxygen consumption

LSD

weight class	(I) genoCode	(J) genoCode	Mean Difference (I-J)	Std. Error	p-value
26-50g	O. clarkii lewisi	hybrid	-33.06123*	15.17845	.036
		O. mykiss	-39.45963*	15.75521	.017
	hybrid	O. clarkii lewisi	33.06123*	15.17845	.036
		O. mykiss	-6.39839	18.41154	.730
	O. mykiss	O. clarkii lewisi	39.45963*	15.75521	.017
		hybrid	6.39839	18.41154	.730
51-75g	O. clarkii lewisi	hybrid	-35.00000*	16.37668	.041
		O. mykiss	-81.50000*	24.51791	.002
	hybrid	O. clarkii lewisi	35.00000*	16.37668	.041
		O. mykiss	-46.50000	27.87189	.100
	O. mykiss	O. clarkii lewisi	81.50000*	24.51791	.002
		hybrid	46.50000	27.87189	.100

*. The mean difference is significant at the .05 level.

Table 3-8: Mean % RT for which the oxygen consumption rates represent in Figure 3-3.

	weight class				
	<25g	26-50g	51-75g	76-100g	101-125g
Genotype	Mean %RT	Mean %RT	Mean %RT	Mean %RT	Mean %RT
O. clarkii lewisi	0%	0%	0%	0%	0%
hybrid	13%	33%	34%	63%	---
O. mykiss	---	100%	100%	100%	---

Plasma acetylcholine esterase (AchE)

Using multiple regression, %RT and fork length were both significant predictors of plasma AchE activity (Table 3-9). AchE activities (U/ml blood plasma) were negatively related to fork length and positively related to % RT. ANOVA analysis found AchE to be significantly affected by genotype. Multiple comparison analysis (LSD test) showed AchE to be significantly higher in *O. mykiss* than *O. clarkii lewisi* (p=0.003) (Figure 3-4).

ANOVA analysis revealed no significant difference in mean length among the genotypic categories. The mean %RT of the hybrid value was 35%.

Table 3-9: Multiple regression results showing significant effect of %RT and fork length (mm) on AchE activity (U/ml)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	70.450	9.969		7.067	.000
	%RT	19.639	5.950	.454	3.301	.003
	length (mm)	-.155	.047	-.458	-3.325	.002

a. Dependent Variable: AchE

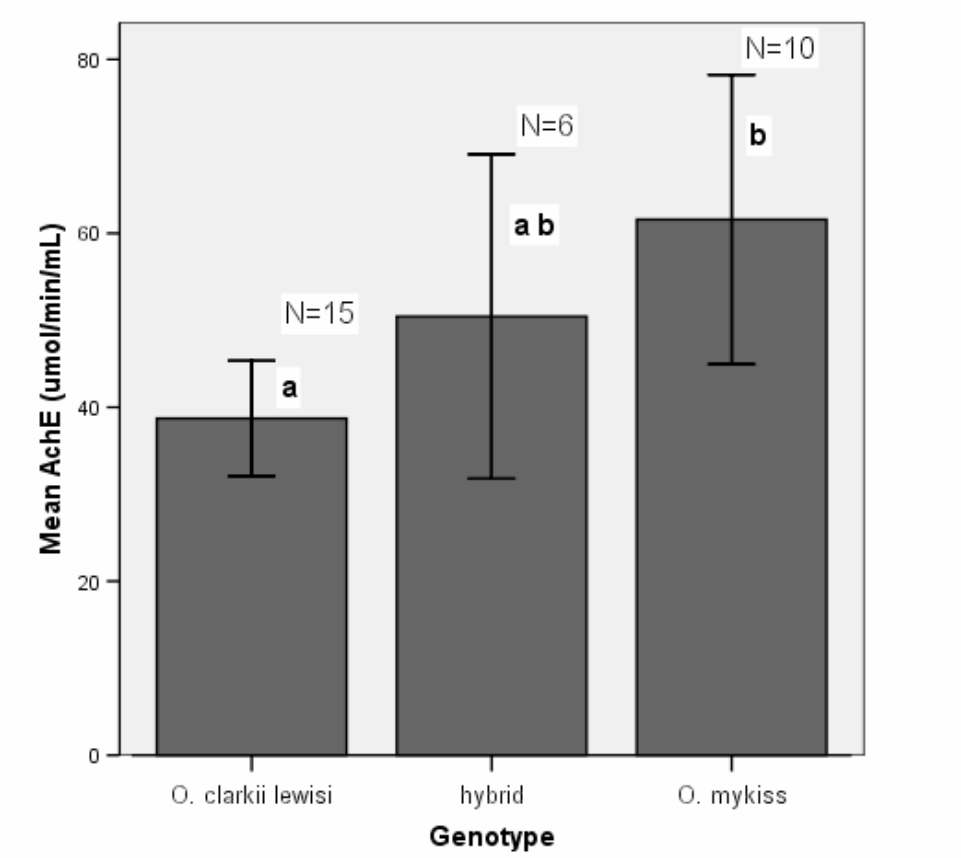

Figure 3-4: Mean AchE activity (µmol/min/ml) by genotype category with ±1 SD error bars. Same letters denotes no significant difference. Mean length between genotype categories showed no significant difference (p=0.505).

Liver glycogen

No significant affect of %RT, elevation (m) or fork length (mm) was found on liver glycogen at the p=0.05 level. However, %RT was significant at the p=0.10 level (p=0.073). Mean liver glycogen levels by genotype show a trend increasing from *O. clarkii lewisi* to *O. mykiss* (Figure 3-5). Low sample sizes, however, provided low power for this comparison (β<0.50). The hybrid genotype represents a mean %RT of 35%.

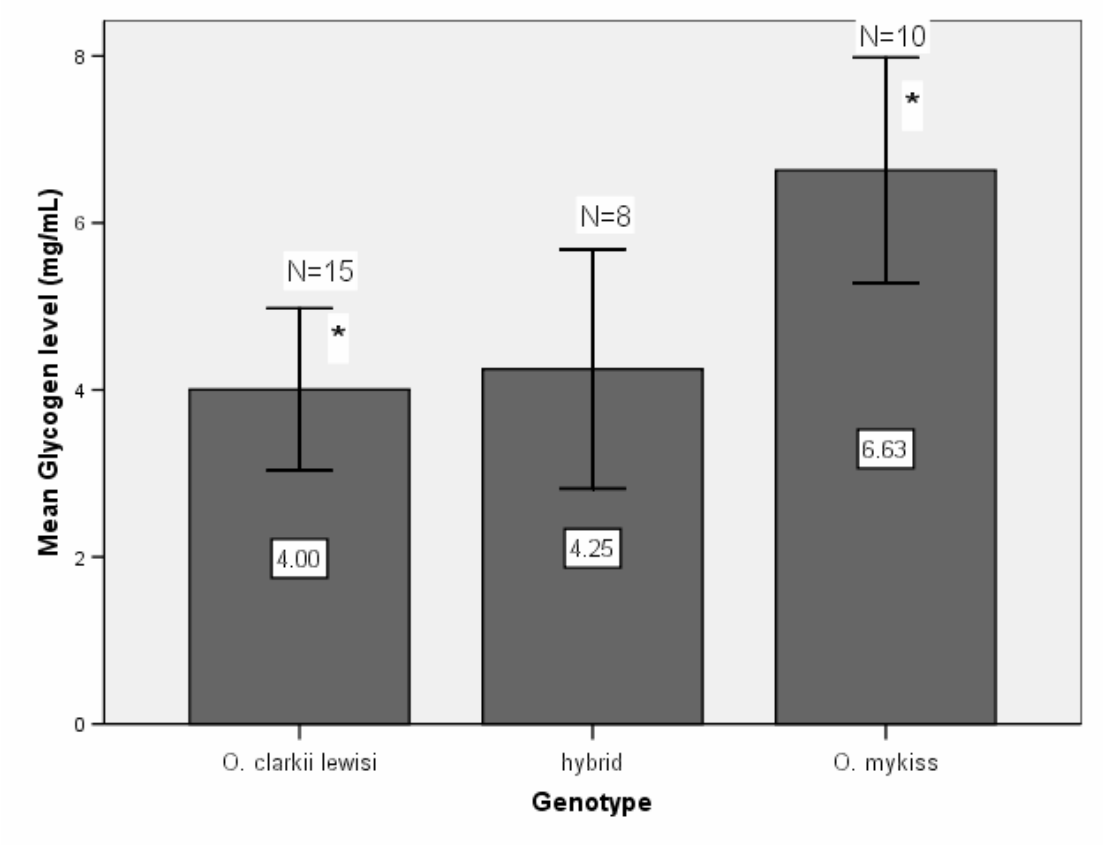

Figure 3-5: Relationship between mean liver glycogen levels (mg/ml) and genotype. Error bars represent ±1 SD. * denotes significant difference at the p=0.10 level.

Glucose

Blood glucose was significantly related to fork length in a curvilinear function, but not %RT or elevation (Figure 3-6).

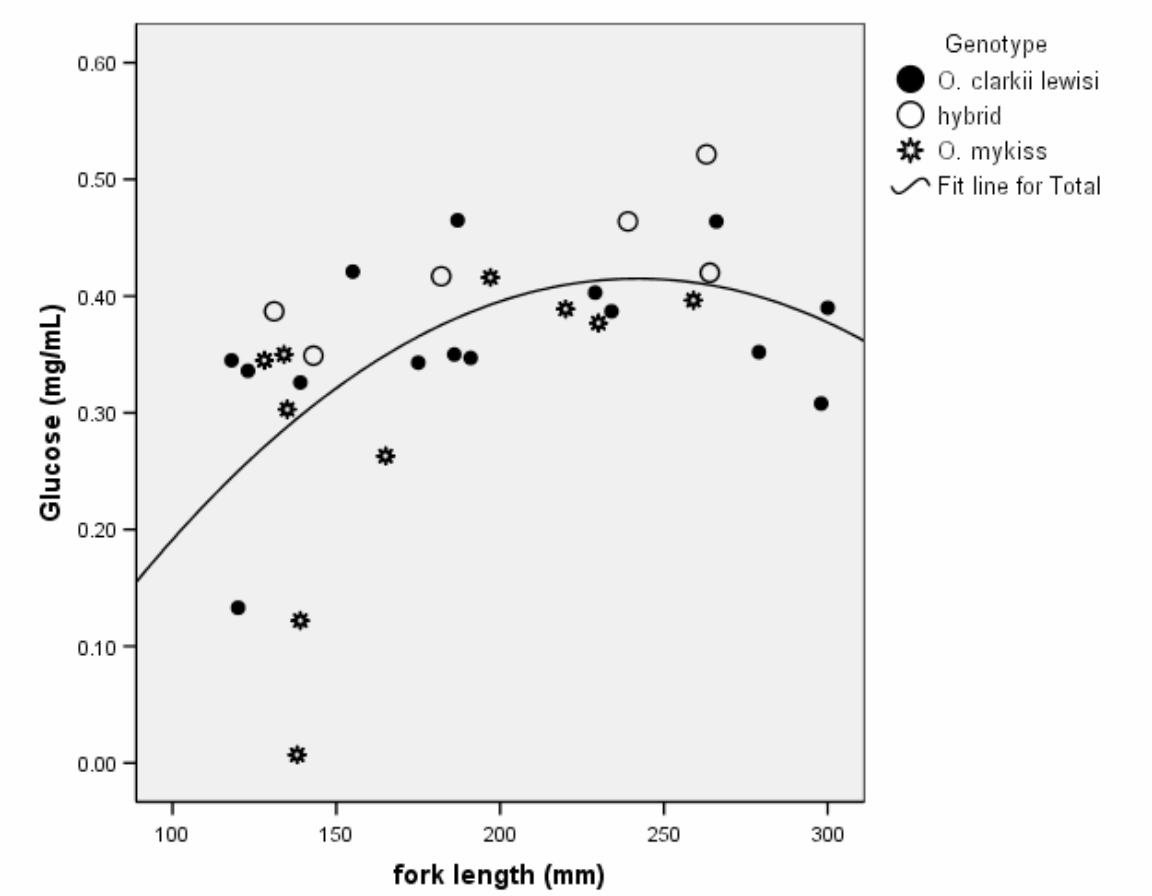

Figure 3-6: Mean blood glucose levels (mg/ml) versus fork length (mm) (p=0.002) (trendline: y= -7E-06x^2 + 0.0038x - 0.0774, r^2=0.27).

Cortisol

Multiple regression analysis showed that cortisol was not significantly predicted by %RT, fork length (mm) or elevation (m) (Table 3-10). Small sample sizes provided low power for this comparison, and consequently a significant difference may exist undetected (β=0.50) (Figure 3-7).

Table 3-10: Results of multiple regression analysis of cortisol levels (ug/ml).

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Sig.
(Constant)	-171.712	294.528		-.583	.565
%RT	59.792	57.236	.696	1.045	.305
fork length (mm)	-1.418	1.252	-.211	-1.132	.267
elevation (m)	.177	.191	.616	.931	.360

a. Dependent Variable: Cortisol (µg/ml)

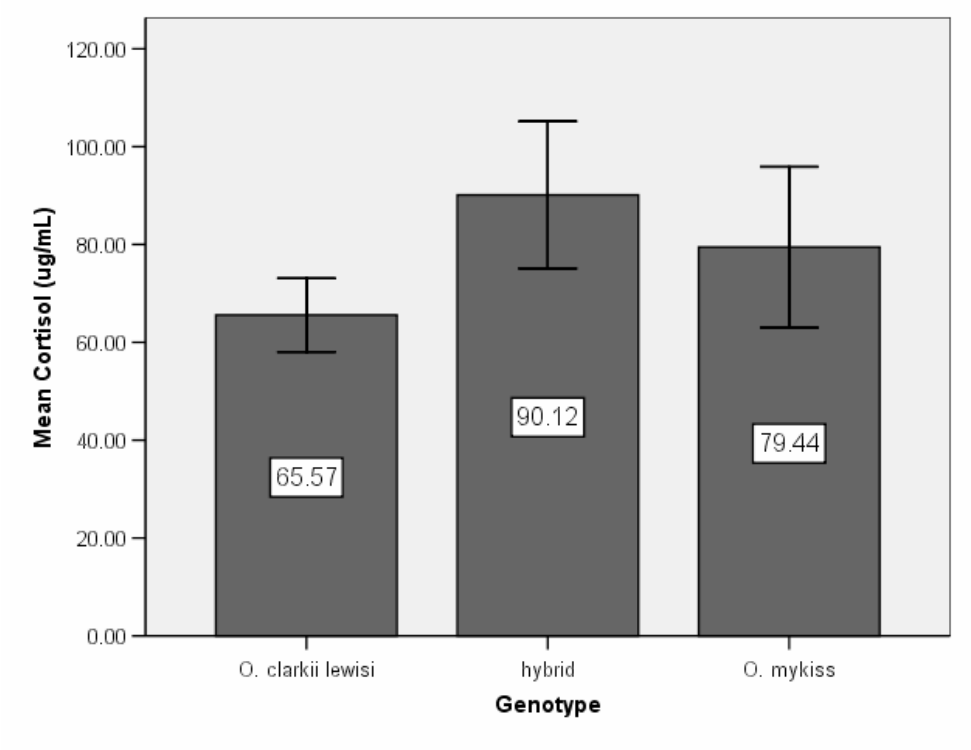

Figure 3-7: Mean cortisol levels for each genotype. Error bars represent ±1 SE.

Gill Na^+/K^+ ATPase

Na^+/K^+ ATPase activity decreased significantly with fork length (mm) but not % RT or elevation (m) (Figure 3-8).

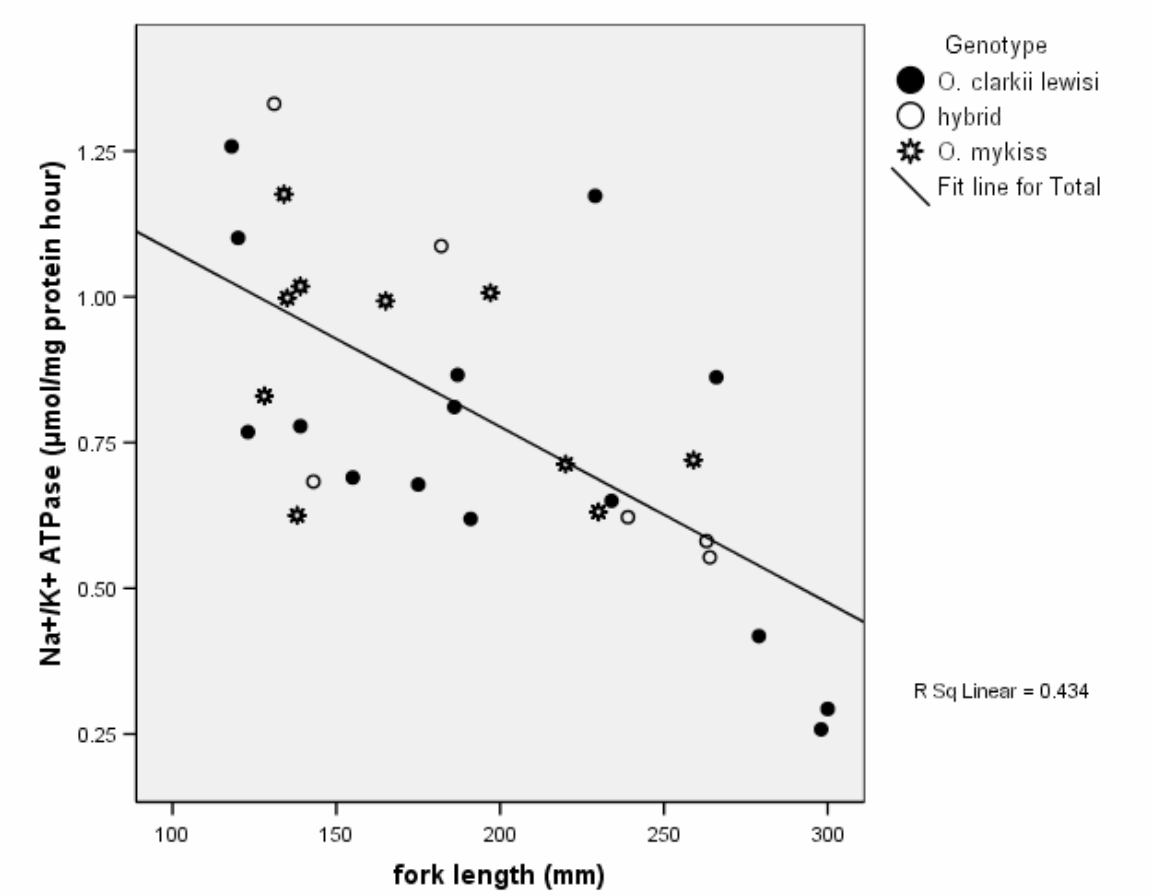

Figure 3-8: Plot showing the negative relationship between gill Na^+/K^+ ATPase levels (ug/mg protein hr) and fork length (mm). (y= -0.0028x + 1.2902, p<0.001)

Discussion

The anadromous life cycle, characteristic of many salmonids, represents an extreme example of an energetic cost vs. benefit trade-off. This life-history strategy requires fish to expend great amounts of energy to support their migratory life-style (Brett 1995). The decision to migrate likely occurs when potential benefits of enhanced growth and fecundity outweigh the increased risk of mortality (Jonsson and Jonsson 1993; Morinville and Rasmussen 2003). Fish with a higher metabolic rate may find a migratory life-cycle profitable since the larger spatial scale upon which such fish live requires a higher output of energy and power. However, the daily rations required to support this metabolic output

will likely require migration to downstream locations with higher productivity. Thus, while a fish with a high metabolic rate is more likely to be a successful migrant, it will be less metabolically efficient and therefore less likely to be a successful resident. Morinville and Rasmussen (2003) showed that anadromous brook trout (*Salvelinus fontinalis*) that spend their first 1 to 2 yr in small nursery streams had higher daily rations but lower growth efficiency (as a result of higher metabolic costs) than similar age residents during this premigratory phase of their life. Estimates of rations that would have been required to support the metabolic rate of these premigrants indicate that such fish would have likely starved to death had they remained in the nursery streams instead of migrating downstream to the estuary where larger food items were present (Morinville and Rasmussen 2006).

Our enzymatic results indicate that the cost of aerobic metabolism increases along a genotypic gradient from *O. clarkii lewisi* to *O. mykiss*, supporting our first hypothesis. The observed gradient of CS activities is expected and is interpreted as a reflection of the anadromous/domesticated ancestry of *O. mykiss*. The significant positive relationship between mean oxygen consumption rate and %RT further supports our prediction of a higher aerobic metabolism in *O. mykiss*. Furthermore, the agreement between CS activity and oxygen consumption rate (OCR) supports the idea that metabolic costs are higher in fish that have more rainbow trout alleles, our third hypothesis. Previous published reports also indicate that *O. mykiss* OCR is higher than that of *O. clarkii* subspecies. In separate assessments of OCR, hatchery raised cutthroat and rainbow trout were found to have daily OCR of 167 and 223 mg/kg body weight per hour, respectively, under identical

temperature and dissolved oxygen conditions (Kindschi *et al.* 1991 Kindschi and Koby 1994). The *O. mykiss* value is an average of one wild and one domestic stock and the cutthroat were from the Snake River, presumably *Oncorhynchus clarkii behnkei*. This difference in oxygen consumption persists across a wide range of temperatures (Dwyer and Kramer 1975; Myrick and Cech 2000) (Figure 3-9). Our data conform well to literature values for both species.

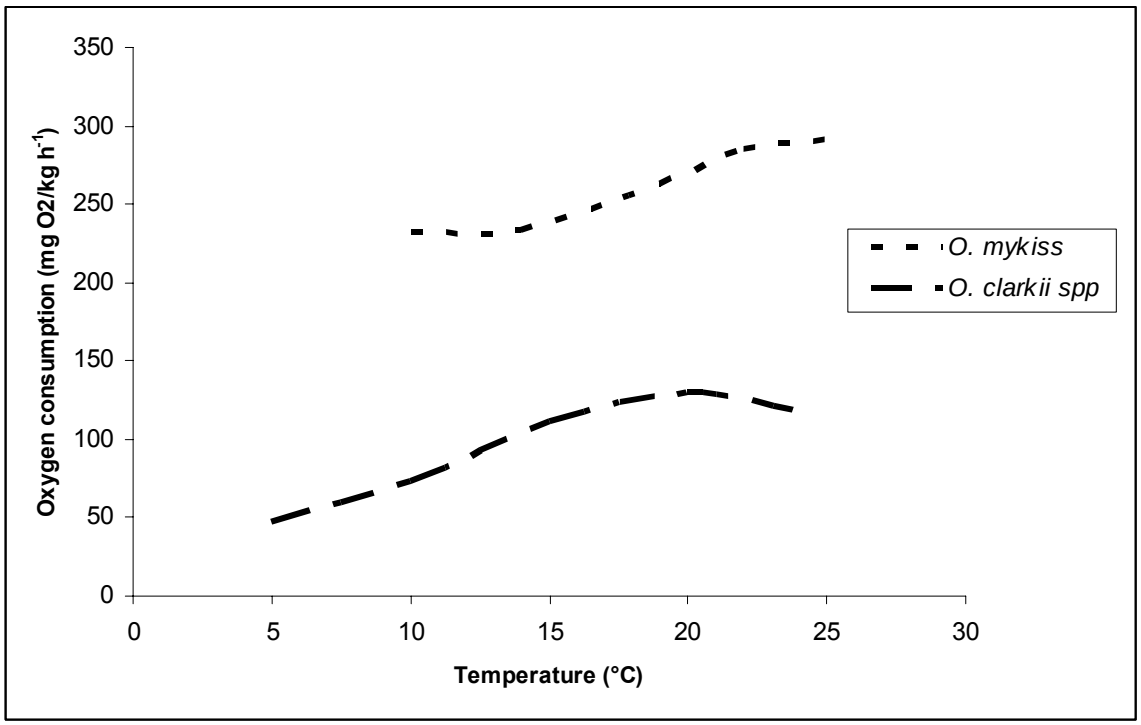

Figure 3-9: Graph showing oxygen consumption of *O. mykiss* and *O. clarkii* subspecies from data presented in Myrick and Cech (2000) and Dwyer and Kramer (1975).

Although the metabolic capabilities of *O. mykiss*, including CS and LDH activities, are well documented, ours are the first interspecific comparisons of CS and LDH activities involving any of the *O. clarkii* subspecies. However, comparisons of swimming velocity have been carried out, and these also indicate that *O. mykiss* have a higher metabolic rate than O. clarkii, since the positive relationship between metabolic rate (OCR) and

swimming velocity is well documented (Webb 1971; Brett 1995; Claireaux et al. 2005). Hawkins and Quinn (1996) tested critical swimming velocities (U_{crit}) of coastal cutthroat trout (*Oncorhynchus clarkii clarki*), steelhead trout (*O. mykiss*) and their reciprocal hybrids. Their results showed not only that *O. mykiss* had the superior U_{crit}, but also that hybrids were intermediate between the parental taxa. Although the authors relate this to morphological differences it could be inferred that OCR, and therefore aerobic metabolic rate would also follow this genetic gradient. Similarly, Taylor and Foote (1991) showed that U_{crit} of sockeye salmon (*Oncorhynchus nerka*) was higher than that of the land-locked *O. nerka* (kokanee). Some indication of an intermediate hybrid U_{crit} was found however, hybrid variability was exceedingly high making statistical comparisons between hybrid and pure forms difficult.

The elevated aerobic metabolic rate of *O. mykiss* is important for two reasons. First, when combined with the SGR results of Chapter 2 it reinforces the idea that *O. mykiss* should have a higher food requirement. Therefore, in order for *O. mykiss* to maintain a competitive growth rate they must seek out habitats of high productivity. It was shown in Chapter 2 that the highly productive Crowsnest River (Rasmussen unpublished data) was more strongly affected by hybridization than the less productive Oldman River, with the lowest site-mean %RT of the former being 58%. This is expected given the higher energetic requirements of *O. mykiss* and implies that the initial preferential downstream movement of *O. mykiss* is, at least in part, due to the need for more productive reaches. As hybrid growth rate and metabolism (Chapters 2 and 3 respectively) have been shown to be intermediate to *O. mykiss* and *O. clarkii lewisi* they should be less dependent on

high productivity, allowing them to be successful farther upstream than *O. mykiss*. The fact that pure *O. clarkii lewisi* populations persist in the Upper Oldman in streams that lack physical barriers (Chapter 2) is likely due to an upstream limit beyond which hybrids are not successful. It is possible that at the upper margins of the hybrid zone any individual possessing even a few *O. mykiss* alleles will tend to prefer downstream reaches productive enough to support them, but not productive enough to bring them into competition with rainbow trout.

Metabolism has been shown to be positively correlated with aggression in several salmonid species (*O. mykiss*, McCarthy 2001, *Salmo salar*, Lahtai *et al.* 2002; Metcalfe *et al.* 2004). These studies relate metabolism to aggression using OCR as a metabolic indicator. Aggressive behaviour requires frequent bursts of high power, and should thus depend on both anaerobic and aerobic capacity. High power comes from the anaerobic pathway, which produces lactate and blood acidosis, whereas the ability to recover and repeat such bursts frequently requires the ability to clear lactate and recoup energy content via the aerobic pathway. Thus, fishes with higher aerobic capacity can recover more quickly after anaerobic exertion (Moyes *et al.* 1992), and therefore make more effective and efficient use of their anaerobic capabilities. The fact that both LDH and CS activity are higher in fish that have more rainbow trout alleles is therefore consistent with the idea that such fish are more aggressive competitors than fish with less rainbow trout alleles. Furthermore, the observed higher metabolic rate of *O. mykiss* conforms to the hypothesis that *O. mykiss* establish downstream where they outcompete *O. clarkii lewisi*.

Although anaerobic respiration is important in many behavioural aspects the predominantly aerobic red muscle of fish is responsible for generating swimming speeds up to 80% U_{crit} (Webb 1971; Hudson 1973). This implies that aerobically derived ATP dominates most movements involved in holding position in the current, general migratory movements and the movements involved in feeding on passively drifting invertebrates, characteristic of stream-dwelling *O. clarkii lewisi* and *O. mykiss*. Within the study area burst swimming will likely only be used when evading predators, feeding in highly competitive environments on large prey, defending territory and overcoming major migratory obstacles.

While our LDH activities compare well to literature values, activities from the CS assay were approximately 50% higher than what has been previously reported for *O. mykiss* (Leary *et al.* 1998; Guderley and Gawlicka 1992; Gamperl *et al.* 2002; Rodnick *et al.* 2004). We suspect that this is due to variation in sample preparation. The method used in this study involved the added steps of grinding with liquid nitrogen as well as two freeze-thaw cycles. The fact that this only affected one of the enzymes assayed comes from the different locations of the enzymes within the cell. LDH is found within the cytoplasm and is therefore liberated as soon as a cell lyses. The additional steps likely had no increased effectiveness on this occurring. However, CS is found within the mitochondria, an organelle that is relatively difficult to lyse. Here our methods would be more likely to increase mitochondrial lysing but induce no further cell lysing; therefore increasing the liberation of CS but not LDH.

Acetylcholinesterase (AchE) activity levels also have implications for the muscle contraction rate. AchE is an important neuro-enzyme that breaks down and removes the neurotransmitter acetylcholine from post-synaptic membranes found at neuromuscular junctions, allowing the neuromuscular junction to fire again with a very short latency period. Randall *et al.* (2002) describe this as "a race" between the diffusion of acetylcholine and its breakdown by AchE. Thus, the significant positive effect of %RT on AchE activity could be seen as facilitating an increased rate of muscular contractions through reducing the latent period of the neuromuscular synapse. No literature was found relating the activity of this enzyme to contraction rate making this inference completely speculative. However, it has been shown that *O. mykiss* possesses AchE isoenzymes that differ in optimal temperature range (Baldwin and Hochachka 1970; Somero and Hochachka 1971). If this is a trait unavailable to *O. clarkii lewisi* then a competitive advantage might exist favouring *O. mykiss* as they would have AchE functioning closer to optimum over a larger thermal range.

Glycogen is an important energy store for fish with the majority of its storage occurring in the liver (Hemre *et al.* 2002). Although glycogen is also found in muscle its metabolism and gluconeogenesis is thought of as a "close-end" system, making liver glycogen an important energy store for the rest of a fish's body (Hemre *et al.* 2002). Depending on food availability fish will tend to metabolize different sources of energy. When well fed, the overall metabolism of *O. mykiss* comes 68% from lipids 20% from carbohydrates and the remainder from protein (Lauff and Wood 1996). However, Lauff and Wood 1996 found that during periods of starvation the contribution of lipids

decreases to 37% while carbohydrate metabolism increases to 37% of total metabolism. This suggests that carbohydrates such as liver glycogen are an important factor under adverse conditions. Although only significant at the p=0.10 level we observed an increase in liver glycogen levels of *O. mykiss* relative to *O. clarkii lewisi*. Higher glycogen stores might be necessary for *O. mykiss* since it might be expected to have a lower threshold for starvation because of its high metabolic rate.

We hypothesize that the upstream limit of the hybrid zone is determined in part by the need for fish with rainbow trout alleles to select more productive habitats because of their high metabolic rate. Stream productivity is known to be enhanced by many factors including: anthropogenic nutrient loading through agricultural (Smith *et al.* 1999; Allen 2004), domestic or industrial sources (Carpenter *et al*. 1998), specifically logging activities (Likens and Bormann 1974). All of these are presently affecting the Upper Oldman watershed to some degree, and could conceivably shift the boundary of the hybrid zone farther upstream, at least temporarily, through stream enrichment. Thus strong consideration should be given to identifying and mitigating sources of nutrient input when considering future management of *O. clarkii lewisi* populations as any increase in upstream nutrient levels could favour fish with rainbow trout alleles.

While we have discussed a great deal the potential advantage that high metabolic rate confers upon fish with rainbow alleles, there is also a potential disadvantage that may make native cutthroat trout more efficient at utilizing the low productivity, small headwater streams. Small streams can be hydrologically unstable, and may turn

ephemeral during dry summer or winter periods, with only a few of the deeper pools remaining. Under these conditions the low metabolic rates of native *O. clarkii lewisi* may allow them to survive in small isolated pools, better than fish with rainbow trout alleles. This could be especially important in small streams of the foothills-prairie transition, where runoff is low and highly seasonal, and cattle ranching frequently exacerbates these hydrological challenges. Small, first order streams, may therefore provide important refuges for native cutthroat trout. It is possible then that if these small streams become invaded by fish with rainbow trout alleles wholesale die-offs might occur during extended dry periods, which pure native cutthroats might have been able to survive. Recent shifts in the climatic regime of the foothills-prairie transition zone in southern Alberta towards warmer, dryer summers may increase the frequency of low runoff periods.

Conclusion

Although the metabolic capabilities of *O. mykiss*, including CS and LDH activities, are well documented, this aspect of *O. clarkii* subspecies has received little study. This study compared, for the first time, the metabolic capacity of *O. clarkii lewisi*, *O. mykiss* and their hybrids. When compared to *O. clarkii lewisi*, *O. mykiss* were found to have both an elevated metabolic aerobic and anaerobic rate. Each of LDH activity, CS activity and oxygen consumption rate were found to be significantly ($p=0.05$) predicted by degree of hybridization (%RT). Although rarely significantly different from either parental species, hybrids appeared to be metabolically intermediate between *O. clarkii lewisi* and *O.*

mykiss. The lack of hybrid significance is not unexpected given that hybrids represent most of the observed genotypic spectrum.

The intermediate metabolic rate of hybrids likely makes them unable to effectively compete with *O. mykiss* forcing hybrids into less productive, upstream reaches where they appear to compete favourably with *O. clarkii lewisi*. Not only is the lower metabolic scope likely responsible for hybrid movement but it also allows for hybrids to be successful as their food requirements are likely also reduced. Under this hypothesis hybridization effectively breaks down the initial species-specific isolating characteristic of metabolism.

The metabolic differences between *O. clarkii lewisi* and *O. mykiss* shown in this study are consistent with their different zoogeographical history and may potentially explain the preference of *O. mykiss* for higher order, more productive reaches. The relatively minor occurrence of postglacial coexistence strongly suggests that *O. mykiss* have outcompeted *O. clarkii lewisi* in most instances likely as a result of *O. mykiss* needing to secure enough of the available food resources. Of the five physiological indices (liver glycogen, cortisol, glucose, Na^+/K^+ ATPase and AchE) assessed, %RT is a significant predictor of liver glycogen and AchE. The notion that *O. mykiss* possess higher AchE levels suggests further competitive advantage. The outcome of historic sympatry between *O. clarkii lewisi* and *O. mykiss* and the apparent superior competitive ability have serious implications for the long-term persistence of pure *O. clarkii lewisi* populations affected by hybridization with introduced *O. mykiss*.

4. Can morphological identification be useful in studies of hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*)?

Abstract

When discussing the identification of hybridized sites, it is commonly believed that genetic-based identification is superior to morphological assessment. Here the potential utilization of morphological approaches as an adjunct to molecular genetics was investigated. This study identified two (out of nine tested) morphological characteristics (jaw slashes and white anal fin tip) found to show a significant statistical relationship to the genotype of westslope cutthroat trout (*O. clarkii lewisi*), rainbow trout (*O. mykiss*) and their hybrids. Morphologically-based identification successfully identified 11/21 (52%) sites that were found through molecular analyses to contained non-pure *O. clarkii lewisi*. Erroneously classified sites were generally areas of low-degree hybridization (mean individual <10% *O. mykiss* alleles) found at intermediate elevations and contained no individuals with greater than 50% *O. mykiss* alleles. Morphology correctly classified 75% of the stream kilometers surveyed and successfully described a front of hybridization, beyond which detailed genetic analysis could be employed to identify low-degree sites. At a drainage-level morphology appears to be an effective, cost-friendly method that limits the number of sites requiring genetic analysis by providing an instant preliminary assessment of target reaches.

Introduction

Hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*) has been identified as the main factor responsible for the decreased range of *O. clarkii lewisi* populations (Allendorf *et al.* 2004). Pure *O. clarkii lewisi* populations are estimated to exist in approximately 15% of their native range in Montana and Idaho (Sheppard *et al.* 2003); similar limited persistence is likely the situation for British Columbian and Albertan populations. Sheppard *et al.* (2003) show the importance of dependable detection of hybridization to a management approach that aims to both limit the spread of hybridization and protect remaining *O. clarkii lewisi* populations.

By exploiting variation in sequence length molecular genetics has become a widely used, effective method of identifying individual genotypes. In hybridization studies target sequences, or markers, are chosen for their observed interspecific differences in fragment length that occur following polymerase chain reaction (PCR) or restriction fragment length polymorphisms (RFLPs). Recently, the use of intron-based markers (IKAROS) or those derived from anonymous DNA sequences (e.g. AFLP, OCC markers) have become more commonly used than microsatellite markers (see Chapter 1) for individual genotype identification. Ideal modern species markers show opposing diagnostic (fixed) expression in both parental genomes and more importantly codominant expression in hybrids. Because genotypic combinations of hybrid zones range from pure individuals to highly backcrossed hybrids, a need exists to have multiple codominant markers. By employing

multiple markers back-crossed hybrids, that show alleles typical of one parental genotype at the majority of loci, have a better chance of being correctly classified as a hybrid.

The notion that genetic-based identification is superior to morphological assessment appears to be well substantiated (e.g. Allendorf *et al.* 2004); however, the potential utility of morphological approaches as an adjunct to molecular genetics has not been considered. One clear benefit of a combined approach would be the acquisition of site-specific hybridization status in the field, while genetic analyses unavoidably require processing time. The purpose of this study is to compare the efficacy with which morphological and genetic assessments can discriminate pure from hybridized *O. clarkii lewisi*. Our goal is not, however, to suggest that morphological techniques can replace molecular techniques, but rather to examine the possibility that they can provide an efficient, cost-effective preliminary screening method to be used in conjunction with genetic techniques.

Allendorf *et al.* (2004) present a thorough contrast of genetic and morphological detection, highlighting the short-comings of the latter approach. While valid, many of their concerns stem from the frequency with which individual fish can be misclassified on the basis of morphology. Many of their concerns are magnified when dealing with individual genotype determination, but do not necessarily produce significant errors at the site or watershed (drainage) levels. Morphology may be used as a preliminary site assessment technique and ultimately aid in drainage-level investigations by reducing the number of sites that require detailed genetic analysis. Because individuals present in a

hybrid zone embody the full range of unique genetic combinations (Rubidge and Taylor 2004) and numerous studies describe a decrease in degree of hybridization with elevation (Allendorf and Leary 1988; Rubidge et al. 2001; Hitt et al. 2003), it would be expected that morphology would be successful up to an elevation where high degree hybrids (high proportion *O. mykiss*) are no longer found. Therefore, morphology should be successful in classifying a site as affected as long as some higher-degree hybrids are present. From this a researcher should be able to identify a front of hybridization, beyond which only low-degree hybrids and pure *O. clarkii lewisi* exist. It is at this point that genetic analyses would be employed to differentiate pure and low-degree hybrid sites.

The prediction that a combined approach (morphology identifying broad-scale hybridization, then focusing with genetics) is dependant on the existence of high-degree hybrids creates concern specific to old hybridization events. One would not expect morphology to be able to detect a limited, historic hybridization event where there have been many generations of backcrossing into one of the parental species leaving most of the hybrids having predominantly those species characteristics.

Generally, concerns about the efficacy of morphology-based identification often center on the high degree of natural variability that morphological characteristics exhibit even among individuals from pure parental lineages (Allendorf *et al.* 2004). Prior to the use of more accurate genetic analyses such as intron-based markers (e.g. IKAROS), the intrinsic variability issue was in fact common to both morphological and genetic approaches. Depending on the microsatelite marker chosen, variability in PCR fragment length can be

enough to create some overlap between taxa if the marker is not fully fixed (Reed *et al.* 1997). To deal with this overlap researchers would support non-diagnostic markers with more diagnostic markers (e.g. Reed *et al.* 1997). As mentioned modern salmonid markers are virtually free of overlap, leaving this concern only valid for morphological methods. As the literature shows that no one morphological criterion is diagnostic between *O. clarkii lewisi* and *O. mykiss*, as exceptions are always present (e.g. Leary *et al.* 1996, Behnke 2002), it seems likely that one could reduce the error of genotype estimation by using multiple characteristics, dominant in either parental taxon. Weigel *et al.* (2003) reported a site-level error of 31% when using a combination of four morphological characteristics suggesting that morphology can be a dependable, cost-effective field technique to be supplemented by genetic analyses.

In this study we compare genetic and morphological descriptions of the extent of hybridization at a drainage level using a categorical dependant variable (*O. clarkii lewisi*, hybrid and *O. mykiss*) to see if preliminary field morphological assessments followed by focused genetic analysis can increase research efficiency. We will further utilize the genetic assessments to develop a continuous dependent variable (percent *O. mykiss* alleles) for each individual fish as well as each site in attempts to identify a threshold degree of hybridization below which morphology is unreliable.

Objectives and hypotheses

The objective of this study is to assess the degree to which morphological assessment can be used to distinguish *O. clarkii lewisi* from hybrids and *O. mykiss* (non-*O. clarkii*

lewisi), by developing a morphological index and comparing it to molecular assessments. In this study we present the following hypotheses:

Ha1: Pure *O. clarkii lewisi* can be reliably distinguished from *O. clarkii lewisi* x *O. mykiss* hybrids in the field using a specific set of morphology characteristics up to a threshold degree of hybridization.

Ha2: Morphological techniques will yield geographic patterns of hybridization similar to those obtained from molecular markers.

Methods

Morphology

A minimum of 30 fish per site were sampled in each year as described in Chapter 2. After reviewing the literature, nine morphological indices were chosen (following in bold) as commonly being indicative of either *O. clarkii lewisi* or *O. mykiss*. Each fish over 100mm in length was scored for each index. *O. clarkii lewisi* get their name from the presence of bright orange slashes present on the underside of the lower jaw (**jaw slashes**) (Figure 4-1). Also typically found in *O. clarkii lewisi* and absent from *O. mykiss* are **basibranchial teeth** (Figure 4-1) (Leary *et al.* 1996). These teeth are relatively inconspicuous and were detected using a blunt probe. Both of these indices were categorical variables recorded as either a presence or absence. *O. clarkii lewisi* are generally thought to be less spotted in two locations: **on top of the head** (Figure 4-1) and

anterior below the lateral line (Figure 4-1) (Behnke 2002). **Spot shape** was found to be a significant determinant between *O. clarkii lewisi* and hybrids in the model of Weigel *et al.* (2003), with *O. clarkii lewisi* typically having round spots and hybrids more irregular. These were the two categorical options used for spot shape in this study. Body spotting was recorded categorically as either absent, light or heavy while number on top of the head was continuous (count). The final categorical predictor variable was presence or absence of a **white anal fin tip** (Figure 4-1). This is found more dominantly on *O. mykiss*. The final three variables are all continuous head measurements: **head length to body length**, **maxillary length relative to body length** and **maxillary length relative to head length** (Figure 4-1). The purpose of two maxillary categories was to maintain independence should head length be found to be a significant predictor of genotype.

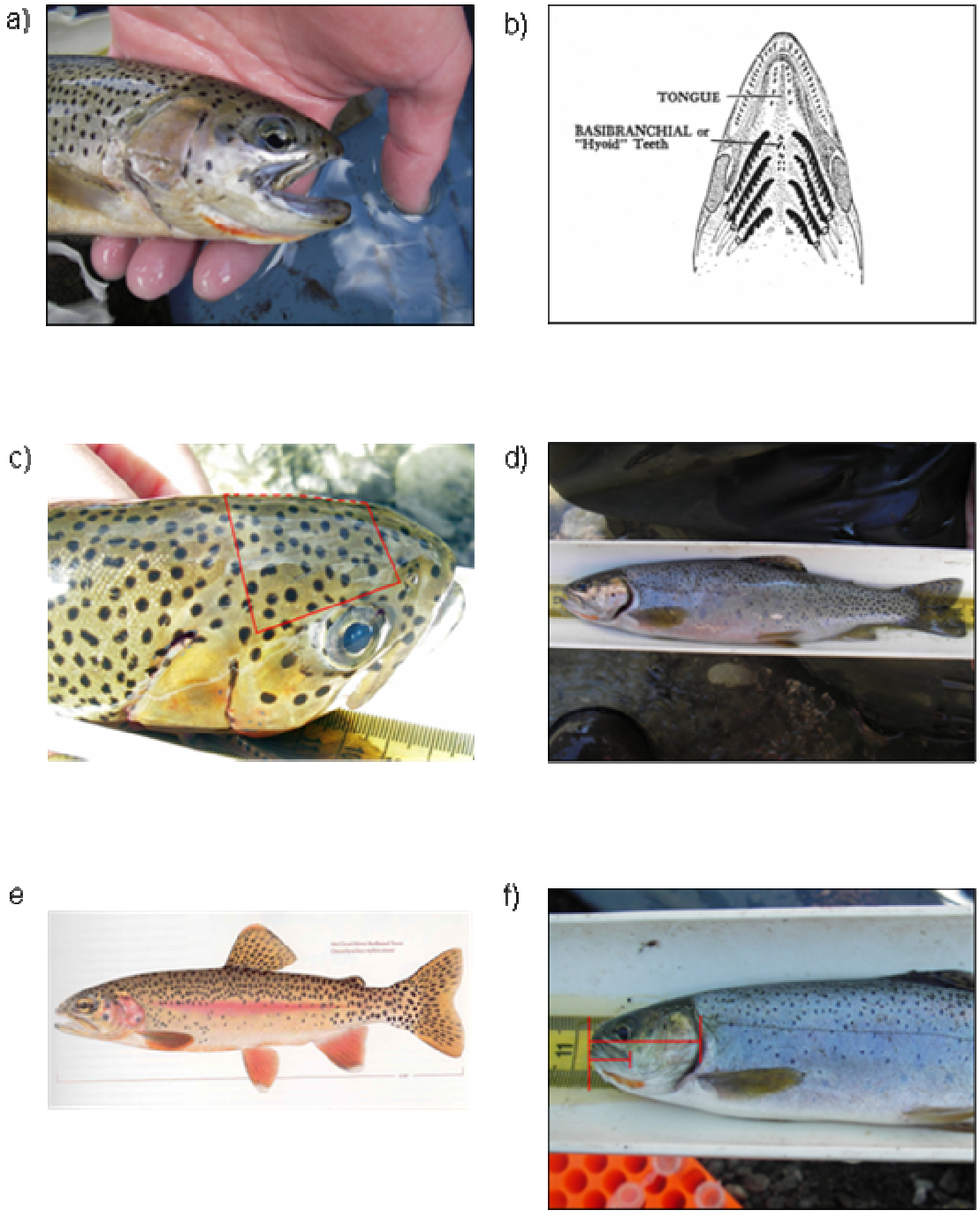

Figure 4-1: Examples of a) jaw slashes typical of *O. clarkii lewisi,* b) basibranchial teeth, c) area used for head spot counts, d) body spotting typical of *O. clarkii lewisi,* e) white anal fin tip of *O. mykiss* (from Behnke 2002) and f) transects used for head measurements.

Genetic analysis

Fin clip samples were digested overnight at 37°C rocking gently in 150μL of lysis buffer (50mM Tris-HCl pH 8.0; 1.0% SDS; 25mM EDTA) with 70 μg proteinase K. DNA was extracted from all samples using a modified column-based technique described in Elphinstone *et al.* (2003). Three diagnostic co-dominant nuclear species markers (OCC16, OCC36 and IKAROS; Table 4-1), and one mitochondrial marker (ND3) were employed in the molecular assessments. All four have been previously reported in the literature (Ostberg et al. 2002; Baker et al. 2002; Docker et al. 2003; Ostberg et al. 2004). Representative samples of *O. clarkii lewisi* and *O. mykiss* were obtained from the Alberta Sustainable Resource Department hatchery stock in order to re-validate each marker.

Polymerase chain reactions (PCR) were performed using standard 25-μL reactions that contained: 10 mM Tris-HCl (pH-8.4) 50 mM KCl, 2.5 mM $MgCl_2$, 200 μM dNTPs, 0.05 μg of each primer, 0.5 units of DNA Taq polymerase, and approximately 100 ηg of genomic DNA template. The optimized thermocycler profile consisted of a 'hot-start' and 2-minute initial denaturation (94^oC), followed by 35-40 cycles of 1-minute denaturation cycle (94^oC), a 1-minute annealing (variable annealing temperatures, see Table 2.2), a 1.5-minute extension (72^oC), and ending with a final 5-minute extension cycle (72^oC). The IKAROS and ND3 species assays included post-PCR restriction enzyme treatment to generate species-specific restriction fragment length polymorphisms (RFLPs): ND3 is cut with *DdeI* while IKAROS is cut with *HinfI* restriction enzymes following manufacture's instructions. PCR products, size polymorphisms and RFLPs were separated by gel electrophoresis at 80-90 V through a 1.8% agarose gel. All fragments were visualized

using ethidium bromide staining and UV transillumination, ambiguous gel images were repeated.

Individual fish were classed into one of three genotypic categories: *O. clarkii lewisi*, *O. mykiss* or hybrid (HYB). Both the *O. clarkii lewisi* and *O. mykiss* categories contained individuals scored as pure-type at all four diagnostic marker loci, while the HYB category represents all individuals exhibiting greater than 0% and less than 100% *O. mykiss* alleles. Although not used in genotype predictions a %RT value (% *O. mykiss* alleles) was calculated to further subcategorize hybridized sites and individuals, allowing for more detailed comparisons. While this study, and others, compare morphologically predicted genotypes to actual genotypes obtained from genetic analysis, it must be noted that the results of genetic analyses are assumed to represent the "true" genotype of an individual. While the classification of a hybrid individual is entirely accurate, using four markers will result in the misclassification of some low degree hybrids as being pure *O. clarkii lewisi*. Therefore, genetic classification is clearly dependant on the number of markers used, as in an area of extensive hybridization each additional marker employed would likely find more suspected pure *O. clarkii lewisi* to actually be low-degree hybrids.

Table 4-1: Four markers employed, PCR fragment lengths and the characteristics of their assessment.

Marker	PCR fragment length (bp)	PCR annealing temperature	Primer sequences (5'→3')
OCC16*	280 *O. mykiss* 380 *O. clarkii spp.*	48°C	GACAGACACATTAAGAGTAGT CAGTAATACAGGTACAGACTG
OCC36**	275–285 *O. mykiss* 325 *O. clarkii spp.*	48°C	ACCGTCTGGTGCGCAACATT CCGGTGTATGGGAGCATTTGA
IKAROS ***	519 *O. mykiss* 813 *O. clarkii spp.*	49°C	TCAGTTTGCACAACCTG CAGAGTAAGTGCCAGCG
ND3****	331 *O. mykiss* 66 & 265 *O. clarkii spp.*	55°C	AAATYTCYCCMGACGCA CTTTTGAGCCGAAATCA

Developer: * Ostberg *et al*. 2002, ** Ostberg *et al*. 2004, *** Baker *et al*. 2002 and ****Docker *et al*. 2003

Statistical analysis

To derive the most accurate morphological model all possible combinations of the nine indices were entered into ordinal logistic regression. The most accurate model was chosen as the combination that produced the lowest number of misclassified individuals from the fewest predictor variables with the highest degree of probability of correct classification. Individuals were classified into one of two groups: *O. clarkii lewisi* or non-*O. clarkii lewisi* hyb/*O. mykiss*. The total number of hybrids predicted was compared to the true status at each site. Sites that possessed any hybrids failed to be classified as pure. The percent error was calculated for each site as from the misclassified individuals. All statistics were run using SPSS 14.0.

Results

Individual-level predictions

Morphological characteristics were recorded from 948 individuals, 141 of which were determined to be hybrids from genetic analysis. It was concluded that no F1 hybrids had been sampled after no individuals were returned heterozygous at all three nuclear markers. Although hybrids of both *O. clarkii lewisi* and *O. mykiss* backcrosses were observed, *O. clarkii lewisi*-backcrosses were more frequent (Figure 4-2).

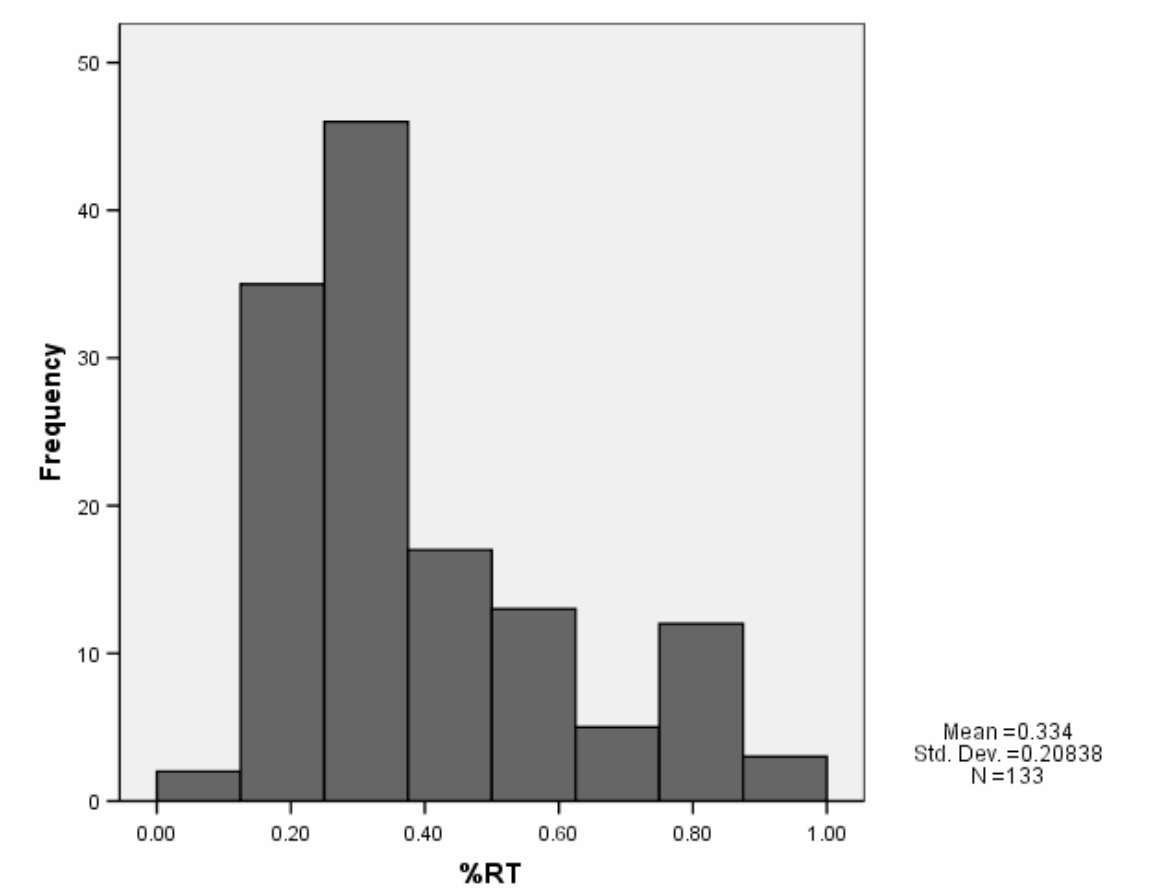

Figure 4-2: Frequency histogram of %RT from all 141 hybrids used in deriving the morphological model.

Of the nine morphological characters only jaw slashes and white anal fin tip were required to successfully classify 740 out of 741 pure *O. clarkii lewisi* individuals (Table 4-2). Typical *O. clarkii lewisi* were found to exhibit jaw slashes and no white anal fin tip, while typical *O. mykiss* showed no jaw slash but had a white anal fin tip. Hybrids were therefore predicted when an individual showed both jaw slashes and white anal fin tip or neither. Non-*O. clarkii lewisi* individuals were incorrectly classified 53% of the time (Table 4-2). This high overall error was largely due to the strong misclassification of <50% hybrids (92%), while accuracies were much higher for >50% hybrids (34% error) and pure *O. mykiss* (0% error) (Table 4-3). The ordinal logistic regression produced high probability of correct classification for both genotype categories (Table 4-4).

Table 4-2: How well do morphology-based categories (*O. clarkii lewisi* vs. hybrid) match genetically-based groupings of *O. clarkii lewisi* or hybrids.

		Actual genotype (genetically-based)	
		O. clarkii lewisi	hyb/O. mykiss
Morphologically-based groups	O. clarkii lewisi	741	109
	hyb/O. mykiss	1	97

Table 4-3: How well do morphology-based categories (*O. clarkii lewisi* vs. hybrid) match genetically-based groupings of pure *O. clarkii lewisi*, <50% hybrids, >50% hybrids and *O. mykiss*.

		Actual genotype (genetically-based)			
		O. clarkii lewisi	<50% RT	>50% RT	O. mykiss
Morphologically-based groups	O. clarkii lewisi	741	97	12	0
	hyb/O. mykiss	1	9	23	65

Table 4-4: Probability of correct classification of an individual into each morphologically-based genotypic grouping.

	Morphologically-based groups			
	O. clarkii lewisi		hyb/O. mykiss	
	Mean	SE mean	Mean	SE mean
Estimated Classification Probability for the morphologically-based group	.87	.00	.99	.00

Site-level predictions

The ordinal logistic regression successfully classified 52% (11/21) of all sites (Table 4-5). In all cases but one (Ho1) the percent error was positive indicating that non-*O. clarkii lewisi* were being misclassified as pure *O. clarkii lewisi*. From the individual results it was shown that almost all observed error came from the misclassification of low degree hybrids. Site Ho1 was an exception in many instances. It was the only site where one pure *O. clarkii lewisi* was classified as a hybrid (i.e. negative % error), and thus this site was the only pure *O. clarkii lewisi* site classified as hybridized. With the exception of Ho1 the observed site-level error was entirely limited to sites that exhibited low-degree (<10 %RT) true hybridization status. All other pure *O. clarkii lewisi* sites and all sites hybridized beyond 10 %RT were classified correctly (Table 4-5). Two sites where no morphological data were collected (Du3 and Oldman Reservoir) are omitted from this table but had true %RT-values of 0% and 97%, respectively.

Table 4-5: True %RT and true and predicted percent of hybrids for each site. Percent error is the number of individuals misclassified at each site. *pure site is defined as no hybrid individual being identified. RT=*O. mykiss* and Hyb=hybridized.

Site	N	True %RT	True % hyb/O. mykiss	Predicted % hyb/*O. mykiss*	% error	True status	Predicted status	Success
Be1	60	0%	0	0	0	pure*	pure	correct
Li1	63	0%	0	0	0	Pure	pure	correct
NR	30	0%	0	0	0	Pure	pure	correct
Ol2	59	0%	0	0	0	Pure	pure	correct
SR	30	0%	0	0	0	Pure	pure	correct
Li2	58	0%	0	0	0	Pure	pure	correct
Ho1	**56**	**0%**	**0**	**2**	**-2**	**Pure**	**Hyb**	**error**
Du1	**62**	**1%**	**6**	**0**	**6**	**Hyb**	**pure**	**error**
Du2	**30**	**2%**	**10**	**0**	**10**	**Hyb**	**pure**	**error**
Go2	**30**	**2%**	**7**	**0**	**7**	**Hyb**	**pure**	**error**
Da2	**30**	**3%**	**17**	**0**	**17**	**Hyb**	**pure**	**error**
Vi2	**30**	**3%**	**13**	**0**	**13**	**Hyb**	**pure**	**error**
Ra2	**28**	**5%**	**14**	**0**	**14**	**Hyb**	**pure**	**error**
Du0	**60**	**6%**	**23**	**0**	**23**	**Hyb**	**pure**	**error**
Vi1	**58**	**6%**	**24**	**0**	**24**	**Hyb**	**pure**	**error**
Da1	**59**	**7%**	**31**	**0**	**31**	**Hyb**	**pure**	**error**
Ra1	68	10%	28	7	21	Hyb	Hyb	correct
Ol1	30	24%	60	37	23	Hyb	Hyb	correct
Ol0	16	48%	94	44	50	Hyb	Hyb	correct
Go1	30	56%	83	50	33	Hyb	Hyb	correct
By1	60	98%	100	98	2	Hyb	Hyb	correct

Site-level error produced a difference in total pure *O. mykiss* stream kilometers. As the purpose of the morphology map was to locate the front of hybridization Ho1 was treated as an outlier and therefore plotted as pure. Morphology predicted *O. mykiss* populations to exist in 42% of stream kilometres (138/327 km) while genetic analysis showed this to more accurately be 77% (252/327 km). The incorrect classification of approximately 35% (114/327 km) occurred in intermediate elevation reaches and above one low elevation barrier (Figure 4-3). All of these reaches contained no hybrids of >50 %RT (Table 4-6)

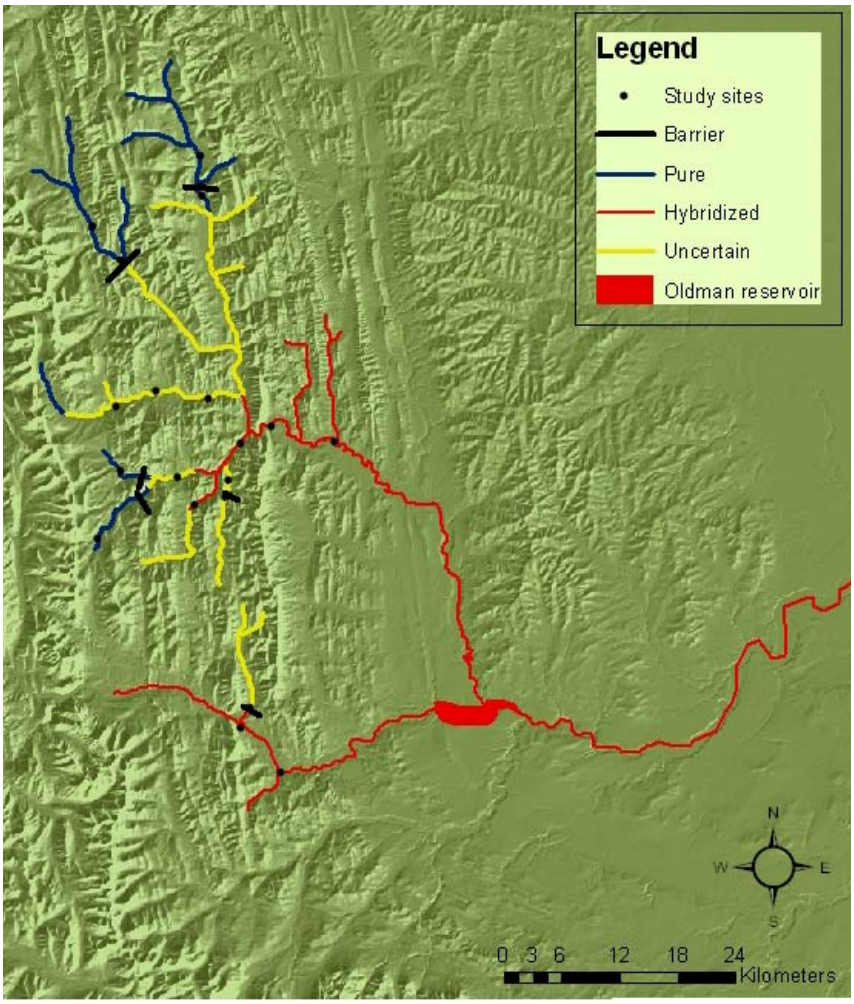

Figure 4-3: Reaches predicted to be hybridized by both morphological and genetic analyses (red), reaches predicted pure by morphology and hybridized by genetics (uncertain = yellow) and reaches predicted pure *O. clarkii lewisi* by both (dark blue).

Table 4-6: Maximum individual %RT for erroneously classified sites.

Site	Maximum %RT
Da1	38%
Da2	38%
Du0	50%
Du1	50%
Du2	33%
Go2	33%
Ho1	0%
Ra2	38%
Vi1	38%

Discussion

The most precise and efficient classification based on morphology was obtained with ordinal logistic regression using jaw slashes and white anal fin tips as the predictors. Because both of these characteristics are nearly fixed in the parental taxa, individuals that exhibited both or neither characteristics were predicted to be hybrids. When addressing individual hybrid success rate we see that only 8% of <50% RT hybrids and 66% of >50% RT hybrids possessed both characteristics, resulting in a much higher individual error rate for low degree hybrids. All pure *O. mykiss* and *O. clarkii lewisi* were correctly classified except one pure *O. clarkii lewisi* (fork length 125mm), which was misclassified as a hybrid at site Ho1 because it lacked both a jaw slash and white anal fin tip.

The misclassification of individual fish resulted in the incorrect classification of nine hybridized sites as pure *O. clarkii lewisi* and one pure *O. clarkii lewisi* site (Ho1) as hybridized. Of the nine hybridized sites, none contained >50% RT hybrids. Excluding site Ho1, incorrectly classified sites contained from 6 – 31% non-*O. clarkii lewisi*. Sites correctly classified as hybridized all contained >60% non-*O. clarkii lewisi*, with the exception of site Ra1, which had only 28% non-*O. clarkii lewisi*. Weigel *et al.* (2003) also found less error when classifying sites with >50% hybridization. Our model correctly classified all pure *O. clarkii lewisi* sites, again with the exception of Ho1. Weigel *et al.* (2003), however, had most difficulty with pure *O. mykiss* sites, which were often misclassified as low degree hybrid sites (<50%). Their model contained a larger number of morphological characters than our model, which tended to error by misclassifying many low degree hybrid sites as pure.

Our results suggest that morphological classification can in fact provide a preliminary picture of the hybridization gradient that is rather similar to that derived from genetic analyses. When comparing the maps of the genetic results to the morphological predictions we see that only 30% of the reaches surveyed were incorrectly classified using morphological characters—all were classified as pure when low level hybrids were actually present.

Although molecular tools unquestionably provide the most precise characterization, studies on the hybridization of cutthroat trout and rainbow trout have often been limited in sample size and spatial scale by the time and expense required to carry out molecular genetic analyses (Sheppard *et al.* 2003). Furthermore, streams are often sampled by government agencies and private consultants during multi-purpose surveys, where the detection of hybridization is not the primary objective. Simple morphological classification models such as the one presented here (see also Weigel *et al.* 2003) can be used in the field during such surveys to broaden the geographical scale of our knowledge of hybridization. Hybridization maps generated using morphological surveys can then be refined by follow-up genetic analyses in the areas targeted as potentially problematic by the morphological surveys. Through the use of morphological classification models, regional fisheries biologists would be able insist the collection of information on hybridization by requesting that this information be included in research license reports. In fact, our morphological models are simple enough that they may even make it possible to manage pure cutthroat trout and >50%RT hybrids with different catch limits, slot

widths and other regulatory devices, which may prove necessary in attempting to conserve pure strains of *O. clarkii lewisi*.

Conclusion

This study has shown that morphological assessments are a feasible preliminary assessment of hybridization on a drainage level. By employing preliminary morphological assessments researchers would be able to limit the extent of genetic analysis required to accurately determine the extent of hybridization by first identifying a zone of uncertain hybridization status. From this genetic techniques could then be used to identify low degree hybridized sites. As the characteristics used in this study are easily determined morphology may even prove to be an important management tool that can be incorporated into regional sport fishing regulations.

5. Conclusion

Hybridization in the upper Oldman River basin of south western Alberta.

Rainbow trout (*Oncorhynchus mykiss*) introduction has been identified as a main factor in the extinction, extirpation and genetic pollution of multiple cutthroat subpopulations, namely the westslope cutthroat trout (*O. clarkii lewisi*) (Behnke 2002; Allendorf *et al.* 2004). Unlike pollution, habitat degradation or over-harvesting, the effects of hybridization can rarely be reverted as this requires complete removal of all introduced individuals. When hybridization is introgressive the complete restoration of the system back to its native state (i.e. removal of introduced alleles) is next to impossible and should be thoroughly examined if being considered as part of a management strategy.

Without question hybridization with introduced *O. mykiss* has had a strong adverse affect on *O. clarkii lewisi* throughout their native range. The study area in question represents a large portion of the entire Alberta population, which together with the Bow River basin population represents the north eastern extent of the historic range of this species. In 2005 the entire Alberta *O. clarkii lewisi* population was recommended by a draft report to be listed as "threatened" by COSEWIC. As is the case throughout the range of *O. clarkii lewisi* (Allendorf *et al.* 2004), hybridization with introduced *O. mykiss* is believed to be a major cause for the decline in abundance of this species in Alberta (COSEWIC 2007).

Hybridization between *O. clarkii lewisi* and *O. mykiss* was found to be extensive throughout the upper Oldman river basin, with evidence in approximately 70% of the

nearly 300 stream kilometres surveyed (1097-1722m elevation). Hybrids were found as high as 1692m (site Vi2), although were much more common at low elevations. Their abundance and geographic extent supports observations of other studies and reports of no apparent selection against hybrid individuals (Rubidge *et al.* 2001; Hitt *et al.* 2003; Weigel *et al.* 2003).

Elevational gradient: The role of life-history strategy and physiology.

During this study genetic analysis confirmed the commonly reported elevational gradient of *O. mykiss* dominant at lower elevations, trending though hybridized sites to pure *O. clarkii lewisi* populations persisting only in headwater reaches (Rubidge *et al.* 2001; Hitt *et al.* 2003; Weigel *et al.* 2003). The preferential downstream movement of *O. mykiss* observed by Paul and Post (2001) was consistent with our results, as *O. mykiss* were not found above an elevation of 1311m despite historical stocking throughout the study area.

We hypothesized that the strong downstream habitat preference of *O. mykiss* is ultimately a result of its recent divergence from an anadromous form. By utilizing the Pacific Ocean as its primary refuge during the Wisconsin (Pleistocene) glaciation, *O. mykiss* would have experienced strong selection for a faster growing, shorter lived species typical of many anadromous species. The enhanced growth rate has then recently been selected for during somestication of *O. mykiss* in hatchery programs. With the exception of Chinook salmon (*O. tshawytscha*), Pacific salmon rarely live past five years of age (Behnke 2002). Also typical of migratory individuals is an elevated metabolic rate relative to resident forms (e.g. Morinville and Rasmussen 2003). A high metabolism is successful only where an

individual has open access to a highly productive environment, usually marine. It seems logical then that species such as *O. clarkii lewisi*, which sought refuge in the less productive inland glacial lakes, would have experienced selective pressures quite different then those that *O. mykiss* were subjected to. In these refugia slower growing, longer lived individuals would likely have been favoured. This "impeded" life-history strategy conforms well to relatively unproductive and dynamic environments where a higher metabolism would not be viable.

As would be expected from their respective Pleistocene refugia and domestication, *O. mykiss* were found to employ a life-history strategy more typical of anadromous individuals, showing significantly faster growth and lower survivorship than *O. clarkii lewisi*. Also in agreement with the glacial refuge hypothesis was the finding of a significantly higher aerobic and anaerobic metabolism possessed by *O. mykiss*. Combining growth and metabolism results strongly suggests that *O. mykiss* will have higher food requirements than *O. clarkii lewisi*, potentially explaining their apparent downstream habitat preference. It was noted that simply moving to areas of higher productivity may not be enough to satisfy higher energetic costs. Once there an individual must ensure that it is able to secure enough of the available resources. A higher metabolism not only allows for more frequent aggressive movements (anaerobic bursts) when defending one's territory, but it would also allow an individual to outright beat its competitors to a prey item when feeding on drifting invertebrates as stream-dwelling *O. clarkii lewisi* and *O. mykiss* do. The significantly higher AchE activity of *O. mykiss*

further suggests superior competitive ability resulting from more frequent muscular contractions.

In addition to the large-scale elevational gradient, we observed a decrease in the mean degree of hybridization (mean %RT) of hybrids with elevation. In other words, not only does the number of hybrids decrease with increased elevation but also does the mean number of *O. mykiss* alleles that they possess. This supports the hypothesis that the geographic distribution is genotype dependent, as high-degree hybrids produced further upstream likely migrate down to more productive reaches and visa versa for low-degree hybrids produced at lower elevations. We have presented the idea that the establishment and maintenance of the overall elevational gradient is likely influenced by life-history strategies and physiology. If so then the primary location of hybrids intermediate along the gradient would suggest that they should also be intermediate in life-history strategies and physiology. In fact, hybrids did tend to show growth, survivorship and metabolic rates intermediate of *O. clarkii lewisi* and *O. mykiss*. However, due to the fact that hybrids represent a broad range of genotypic combinations (1-99% RT) and that these rates likely reflect this variability, the observed trend was rarely found to be significant.

It is unlikely that the establishment and maintenance of the gradient is due to one factor. Physical barriers doubtlessly have an effect on the spread of hybridization. Many studies report barriers to be the only factor truly limiting the spread of hybridization (Hitt *et al.* 2003; Weigel *et al.* 2003; Rubidge and Taylor 2005). We found the effectiveness of barriers limiting hybridization to be greater at lower elevation (-0.2% Δ%RT/m). This

supports the notion that a combination of factors may act together to limit the upstream boundary of the hybrid zone. At lower elevations *O. mykiss* and high-degree hybrids are within their preferred elevational range, making barriers the only limiting factor. On the other hand, at higher elevations the barriers are less critical since *O. mykiss* and hybrids are effectively outside their preferred elevational range, allowing for pure *O. clarkii lewisi* populations to exist in headwater reaches in absence any migratory barriers.

The existence of pure *O. clarkii lewisi* populations at high elevations, in absence of any barriers, further suggests that a combination of factors work to maintain the genotypic gradient. Although this can be explained by habitat preference only our results indicate that temperature may influence the distribution of *O. clarkii lewisi* and *O. mykiss*. A hypothetical temperature threshold (7.25°C) to hybridization was presented in an attempt to explain why some high elevation reaches with no physical barriers were found to still hold genetically pure *O. clarkii lewisi*, despite over 80 years of *O. mykiss* stocking. Overall, it is suspected that differences in life-history strategy, and potentially temperature preference, play an integral role in habitat selection and competitive ability of these two species, potentially explaining the observed gradient.

Identification and Management

Fisheries managers must have an accurate account of the extent of hybridization within their regions if they wish to effectively manage remaining pure *O. clarkii lewisi* populations. Often financial constraints limit the extent to which genetic analyses can be utilized. This study has presented a combined approach of a preliminary morphological

assessment to determine areas where genetic analysis would then be most beneficial. By performing a morphological-based assessment a researcher is able to identify a front of hybridization, above which (elevationally) all hybrids are of low enough %RT to be erroneously classified as pure *O. clarkii lewisi*. It is at this point that genetic analysis can be carried out to differentiate between low degree hybridized sites and truly pure sites.

The issue of how *O. clarkii lewisi* populations should be managed is quite contentious. While arguments and qualifications (e.g. threshold for acceptable degree of hybridization) for the protection of pure *O. clarkii lewisi* populations have been presented (Allendorf *et al.* 2004) there is some reluctance with the implications of the broad definitions involved in listing this species as endangered (Campton and Kaeding 2005). The root of most of this debate comes from what approach should be taken.

If the management approach is the termination of further *O. mykiss* introductions followed by the targeted removal of naturalized *O. mykiss* and hybrid populations then the definition of a hybrid must be developed. This prompts the question, "at what degree of hybridization does an individual possess enough *O. mykiss* alleles to be worth removing?" Is more harm than good done by removing an individual that returns one *O. mykiss* allele out of a possible eight (the lowest potential result using four nuclear markers) then good? As was previously mentioned the degree of hybridization within an affected stream network typically decreases with elevation. It could then be argued that the chances of an *O. clarkii lewisi* hybridizing would increase with its migratory tendencies. If true then a targeted removal of all fish possessing any *O. mykiss* alleles

would likely remove the migratory *O. clarkii lewisi* genotype and effectively fragment resident, headwater populations. It would seem that this would result in a situation not unlike that created by hybridization itself. Fragmented populations are undesirable not only as they have an increased risk of extinction (Rieman and Dunham 2000) but as population size decreased the chance of hybridization increases (Rhymer and Simberloff 1996).

Regardless of the approach fisheries managers must act promptly if any action is to be taken. Both Rubidge *et al.* (2001) and Hitt *et al.* (2003) showed significant upstream advances in hybridization within their respective study areas over roughly 20 years (approximately three generations). These advances imply that the long-term genetic integrity of *O. clarkii lewisi* is only safe above a true migratory barrier.

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