The comparative growth and survival of a naturalized and aquaculture strain of rainbow trout (*Oncorhynchus mykiss*) in laboratory and whole-ecosystem experiments

by

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ABSTRACT

This thesis investigates the comparative growth and mortality of a naturalized (wild) and domestic (aquaculture) strain of rainbow trout (*Oncorhynchus mykiss*) common to Lake Huron. I first conducted a laboratory-based experiment, comparing the growth rates of the two strains. Under optimal and competition treatments, the domestic strain achieved a body weight ~2x that of wild conspecifics. Next, I conducted a replicated, whole-ecosystem study comparing the same strains. Both strains experienced equally low survival and the domestic strain segregated into a fast-growing group, (~3x growth relative to the wild strain), and a slow-growing group that had a lower growth rate than wild trout. A high growth rate for fast-growing domestic trout was achieved by a reliance on high energy prey as well as through low metabolic costs relative to wild strains. Together, these results demonstrate that aquaculture strains of rainbow trout have greater growth potential relative to wild conspecifics and may outcompete them in nature.

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Chapter 1. Introduction

1.1 Aquaculture

Wild catch fisheries are considered one of the most prevalent and ancient practices in the world for harvesting food. However, the manner in which fish are obtained has changed considerably in the past century. As the human population continues to grow, demand for commercially important species of fishes will continue to increase (FAO 2012). Many experts believe that wild catch fisheries alone will not be enough to support the future need for food fish (Anderson and Fong 1997). To accommodate this demand, commercial aquaculture production of fish and seafood is on the rise. For example, the contribution of aquaculture to total fisheries supply has increased from <1 million tonnes annually in 1950 to >55 million tonnes in 2009 (FAO 2012). This is equivalent to a 10-fold annual per capita increase in fish production globally from aquaculture, compared to a relatively stable trend in capture fisheries over the past six decades (FAO 2012).

The term 'aquaculture' is loosely defined as all forms of culture of aquatic animals and plants in fresh, brackish and marine environments. Organisms are commonly reared in pond, raceway, cage, pen or raft culture (Pillay 1990). The earliest evidence of fish first being cultured is from China around 1500 BC where farmers caged and reared the common carp (*Cyprinus carpio*) (FAO 2012). Over time, aquaculture has evolved to accommodate the needs of a growing human population. Today, aquaculture has transformed into a multinational industry with major operations in both freshwater and marine systems.

One of the requirements of aquaculture operations is an adequate supply of quality water for efficient production (Pillay 1990). Cage culture is one of the most popular forms of aquaculture because of its ability to make use of existing water bodies for this purpose. Pens or cages may be installed directly into freshwater or marine systems to access natural water for culturing operations. Cage types consist of fixed, floating, submersible and submerged configurations (Beveridge 1987). Of these types, fixed cages are one of the most commonly used in Canadian aquaculture (Patterson 2010) and typically consist of a floating frame with a net bag attached by posts and anchored to the substrate of a water body (Beveridge 1987). Historically, fixed cages have been used to rear salmonids (salmon, charr and trout) in both marine and freshwater. Salmonid aquaculture remains one of the most highly demanded and well established branches of the fish farming industry, ranking 3rd in total production globally and comprising two-thirds of total aquaculture production in Canada (Masser and Bridger 2007; FAO 2010).

Presently, salmonid production occurs in a wide variety of geographical locations. Norway and Chile are the leaders in salmonid production worldwide, accounting for 36% and 19% of total production respectively, but a significant proportion is still contributed by North American countries at 8% (FAO 2012). Among salmonid aquaculture species, Atlantic salmon (*Salmo salar*) has quickly become one of the most profitable and extensively farmed species in marine systems. In fact, although commercial farming of Atlantic salmon has only occurred for about 40 years, its biomass in aquaculture had surpassed that in nature by the end of the

20th century (94% of total Atlantic salmon biomass is in culture) (Boghen 1995; Gross 1998). In addition to Atlantic salmon, freshwater salmonids such as Arctic charr (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) are continuing to increase in annual production in the Canadian aquaculture sector (Figure 1.1). Rainbow trout is the most commonly farmed freshwater species in Canada, with aquaculture operations in almost every province (DFO2007a; DFO 2007b). Although these freshwater operations are spread throughout Canada, the majority of rainbow trout production occurs at <10 facilities located in northern Lake Huron (Masser and Bridger 2007; Moccia and Bevan 2007). Considering the rapid rate of expansion the aquaculture industry has experienced in the recent past, a common concern of this industry for both marine and freshwaters is the potential negative impacts on the surrounding natural environment, especially related to water quality, disease and escaped farmed fish (reviewed in: Gross 1998; Naylor et al. 2005; Leggatt et al. 2010).

Despite diligence by fish farmers, occurrences of escape events from holding facilities are common (McGinnity et al. 2003; Naylor et al. 2005; Abrantes et al. 2011). Most well-known are episodic escape events that release large number of domesticated salmonids into the wild. These large scale events are typically the result of damage to holding pens from storms, vandalism and predators (Morris et al. 2008). Large scale events are required to be reported by farm operators in most regions with substantial net-pen aquaculture production (Leggat et al. 2010). However, these reports are likely an underestimate of escapes as they do not

consider chronic, low rates of escapes due to tears in pen meshing or improper farming practices that are a regular occurrence in the industry (Carr et al. 1997).

Because of its broad geographic scope of production, Atlantic salmon have become the model for understanding escapees of farmed salmonids. Studies detailing accounts of escaped Atlantic salmon detections in the Atlantic and Pacific Oceans are common. Hansen et al. (1993) observed feeding grounds in the northeast Atlantic Ocean where 25-48% of all adult Atlantic salmon were escapees from farms. Similar findings have been reported in Norwegian rivers with 20-30% of all adults originating as escapees from sea pens and >80% in some rivers (Gross 1998). Although the data from Canada on Atlantic salmon escapes is incomplete, similar findings to the northeastern Atlantic Ocean have been documented. Known numbers of farmed Atlantic salmon escapes in Canada's Atlantic Ocean can be >160 000 per year (Morris et al. 2008) and >80 000 escapes per year in Canada's Pacific Ocean (BCMAL 2009). Due to incomplete reporting and neglecting to note escapes due to leakage, these numbers are likely an underestimate. For example, Morton and Volpe (2001) show that escaped Atlantic salmon numbers can reach >10 000 caught in only 17 days of netting off the coast of British Columbia. The proportion of farmed Atlantic salmon found in the wild are not surprising statistics considering the immense level of production of this species.

Occurrences of salmonid escapes in freshwater are similar to proportions in marine systems, although this has been less intensively studied. Monitoring of escaped farmed rainbow trout confirms that escapees can account for 1-6% of

total aquaculture production in a variety of geographical areas (Penczak et al. 1982; Phillips et al. 1985; Carss 1990; Thorstad et al. 2008; Consuegra et al. 2011). Although some research exists, there is presently a knowledge gap with our understanding of the prevalence of farmed salmonid escapes in freshwater, and especially in North America.

1.2. Phenotypic characteristics of farmed and wild salmonids

As a consequence of culture rearing, these farmed or "domestic" salmonids possess a divergence in genotype and phenotype from wild conspecifics that can be directly attributed to selective breeding practices. Selective breeding refers to the process of propagating stocks of animals that possess traits desirable for commercial market. By selectively breeding a particular stock of fish with favourable genetics for aquaculture production, the characteristics of future populations can be radically different from that of their parents. The effect that selective breeding has had on salmonids is evidenced by the genetic divergence seen among domestic, hatchery and wild strains (Einum and Fleming 1997; Fleming and Einum 1997; Overturf et al. 2003, 2010; Skaala et al. 2005; Tymchuk et al. 2009a, 2009b; Wringe et al. 2010). Genotype deviations from wild conspecifics have quantitatively been shown to affect phenotypic expression in physiology, behaviour, survival and growth (reviewed in Table 1.1). However, expression of phenotypic characteristics among strains is ultimately dependent on genotype-by-environment interactions, so the context of rearing environment is an important factor to consider (Devlin et al. 2004; Sundström et al. 2007).

The primary concern of escape events from aquaculture is that due to these physiological and behavioural differences in domestic salmonids, interactions of escaped fish with ecosystem components will occur in ways not experienced by indigenous fish stocks. Understanding how selective pressures can impact the success of domestic salmonids in the wild can be the key to fully comprehending the interactions that these strains would have on a natural system. Because genetics have been shown to be divergent in domestic and wild strains, it follows that phenotypic expression of certain characteristics will result in certain costs and benefits specific to a strain. Herein I outline the most important differences in the characteristics of wild and domesticated salmonids to better depict the potential outcomes of competition and sympatry in the wild.

1.2.1 Reproductive characteristics

The long-term, chronic risk of establishment of a domestic genotype of salmonid will ultimately depend on the population's ability for reproductive success and propagation of that specific gene pool. Genetic heritability of reproductive characteristics in salmonids, such as spawn timing and sexual maturation is highly variable (Table 1.1), suggesting that selection may have a large influence on reproductive success within a species (Quinn et al. 2000). For example, several studies have shown that domesticated Atlantic salmon may establish populations faster than wild conspecifics due to their accelerated rate of spawning and earlier development (Lura et al. 1993; Sægrov et al. 1997; Fleming et al. 2000). These data suggest that domestic salmonids have the ability to take advantage of productive spawning beds, emerge from eggs and establish competitive

dominance prior to later season spawners, which would presumably be wild conspecifics. Alternatively, earlier spawning has not always been found to be the case in domestic salmonids. Some studies have documented domestic Atlantic salmon spawning days to weeks after wild salmon, potentially destroying wild egg beds or depositing overtop of wild nests (Gudjonsson 1991; Gausen and Moen 1991; Webb et al. 1991, 1993a, 1993b; Carr et al. 1997). Regardless of spawn timing, reproductive success is generally thought to be reduced in domestic compared to wild fish. Domestic salmonid breeding success can be as low as 1-3% of wild fish due to failure to spawn, poorly constructed redds and low egg numbers (Fleming et al. 1996; Volpe et al. 2001). Irrespective of the apparent reduced ability to effectively spawn in the wild, escapee numbers are high in the natural environment and risk of disruption of wild fish stock reproductive success remains high.

Another area of phenotypic divergence between wild and domestic strains of salmonids is aggressive behaviour. One of the artifacts of aquaculture conditioning is a greater degree of agonistic behaviour in domestic strains of salmonids compared to wild conspecifics. Aggressive behaviour in salmonids has been shown to affect population density (Grant and Kramer 1990), distribution (Hoar 1951; Chapman 1962), and access to food supply (Kalleberg 1958), however, the majority of domestic salmonid literature relates to aggression involved with territorial defense of breeding grounds (van den Berghe and Gross 1989; Einum and Fleming 1997; Fleming and Einum 1997; McGinnity 2003). Often during the breeding season, the number of fish exceeds the number of

territories, and as a result, competition for space occurs. Growth-enhanced salmonids may have an advantage in territorial disputes because of their increased aggression and size compared to wild fish (Jönsson et al. 1998; Jonsson and Jonsson 2006). Conversely, some studies have described decreased or variable aggressive behaviour in domestic salmonids compared to wild conspecifics (Doyle and Talbot 1986; Fleming and Einum 1997; Mork et al. 1999; Weir et al. 2004). For example, in mixed groups of wild and farmed Atlantic salmon in aquaria, Mork et al. (1999) observed wild strains showing greater aggressive activity directed towards domestic strains than the opposite. Ultimately it seems that the genotype and the type of environment could both be factors in determining overall aggressiveness of a particular strain.

Long-term establishment of the domestic genome in the wild would occur as a result of interbreeding between farmed and naturalized strains of salmonid. Evidence of genetic introgression of domestic alleles into wild populations of salmonid is common among various species including: Atlantic salmon (Crozier 1993; Webb et al. 1993a, 1993b; Clifford et al. 1998), brown trout (*Salmo trutta*) (Fritzner et al. 2001, Hansen et al. 2001) and Pacific anadromous rainbow trout (Campton and Johnston 1985; Currens et al. 1997). Hybridization of domestic salmonids with wild populations can lead to outbreeding depression of the F2 generation (Gharrett et al. 1999; Gilk et al. 2004). As a result, future populations of salmonids are expected to have reduced fitness compared to parent generations (Gharrett and Smoker 1991). A scenario where hybridization may lead to a population crash in nature has been termed an “extinction vortex," described as

the decline of vulnerable populations of wild salmonids due to the additive genetic effects of domestic salmonids (McGinnity et al. 2003).

1.2.2 Growth

The major phenotypic difference between farmed salmonids and their wild counterparts is growth (Table 1.1). As previously mentioned, growth enhancement of farmed fish is achieved by selective breeding of fast-growing individuals and families. Generally, domestic fish will have superior growth rates compared to wild fish; however, this is not always the case. Growth of salmonids is greatly dependent on the degree of domestication in parent generations (Tymchuk and Deviln 2005), but is an additive component to the environment in which they are reared (genotype x environment interactions). For example, fast-growing coho salmon (*Oncorhynchus kisutch*) grew almost 3 times longer than wild conspecifics under hatchery conditions, but only 20% longer under naturalized stream conditions when strains were allowed to forage on natural prey-items (Sundström et al. 2007). Additionally, aquaculture strains of salmonids are known to have considerably high heritability for growth enhancement (h^2 = >0.5 for rainbow trout) (Gjerde and Schaeffer 1989; Martyniuk et al. 2003), meaning great variability of growth rates among individuals and populations are possible (Gjedrem 2000). In some experiments, domestic rainbow trout growth rate in culture has been shown to be as high as ~8-fold (Tymchuk and Devlin 2005) relative to wild rainbow trout. Domestic rainbow trout growth advantage in culture is also generally maintained in natural freshwater systems. In Canadian prairie lakes, domestic strains of rainbow trout originating from a variety of

regions show ~1.5-fold greater growth than wild conspecifics at time of harvesting (Ayles and Baker 1983). Studies in BC interior lakes have shown growth in domestic rainbow trout to be twice that of size-matched wild fish (Biro et al. 2004a, 2006).

Resource competition in the wild is an additional component to comparative growth between salmonid strains. The success of a particular strain is dependent on its ability to compete for a limiting resource (Tilman 1982). If two strains are competing for a single resource, there is potential for the competitively inferior strain to be excluded. Domestic salmonid characteristics such as increased aggression, larger body size, greater appetite or ability to exploit productive prey-items earlier than wild conspecifics may lead to a competitive advantage. Additionally a potential switch to selection of larger prey items may also decrease risk of predation through reduced foraging time (>high energy food items), increased growth rate to surpass vulnerable sizes and increased reproductive fitness (Ivlev 1961; Weatherly 1974; Tyler and Dunn 1976; Werner 1979; Grossman 1980). In conclusion, many of the phenotypic characteristics in domestic salmonids likely lead to enhanced growth and competitive advantage relative to wild conspecifics.

1.2.3 Survival

Domestication of aquaculture strains has allowed them to attain near maximum growth rates in culture that are not seen in wild fish. Although natural selection and competitive interactions among various taxa are driven by differences in

body-size (Wilson 1975; Mittelbach 1981; Smith and Brown 1986; Nagel and Schluter 1998; Kingslover and Pfennig 2004), organisms rarely grow to their genetic potential for size in the wild (Arendt 1997). Therefore, it can be surmised that there are certain costs associated with high growth rate in the wild (Werner and Anholt 1993; Gottard 1994; Mangel and Stamps 2001; Stoks et al. 2005). Theory would suggest that animals increase their activity, foraging rates and the use of risky habitat when food is scarce or to achieve a maximum growth rate (Houston et al. 1993). This behaviour can be important in two ways as a component of understanding the differences in survival of domestic and wild strains of salmonids. First, domestic salmonids have been conditioned to intake more calories than wild conspecifics as a result of culture conditioning (Suboski and Templeton 1989), genetics (Fleming et al. 2002; Tymchuk et al. 2006a), or a combination of both. Second, domestic salmonids have been shown to have a reduced anti-predator response relative to wild conspecifics (Biro et al. 2004a, 2006; Tymchuk et al. 2007; Houde et al. 2009). Whereas wild salmonids have ingrained instincts to avoid predators by using cover and reducing foraging effort in risky areas spatially and temporally, domestic strains have no predisposition to such selective pressures and would presumably retain behaviour acquired during aquaculture rearing. Therefore, in natural settings, domestic strains have the potential to incur mortality due to natural selection that would not be experienced by wild conspecifics from greater foraging effort in predator risky habitats.

Several field studies have attempted to examine survival of domestic salmonids; although, the majority focus on Atlantic salmon in marine environments

(McGinnity et al. 1997, 2003; Fleming et al. 2000; Saloniemi et al. 2004). Furthermore, most studies of salmonid survival are either mensurative or do not incorporate manipulative experimentation and controls into their design. Of the literature on freshwater species, a number of studies focus on hatchery genotypes of brown trout, a relatively unimportant commercial species of salmonids to the Canadian freshwater industry (Flick and Webster 1964, 1976; Mason et al. 1967; Fraser 1989; Lachance and Magnan 1990; Aerestrup et al. 2000). Several studies have examined domestic genotype survivorship of rainbow trout in whole-lake and aquaculture release experiments, whereby, domestic rainbow trout experienced high annual mortality (~%50) (Blanchfield et al. 2009; Patterson 2010), which has been found to be about twice that of wild conspecifics in other studies (Biro et al. 2004a, 2006). Low survival in domestic rainbow trout has been corroborated in semi-natural enclosures, with greater growth rates being correlated with increased mortality when predation was high (Tymchuk et al. 2007). In summary, depending on environmental variables, including food availability and predation risk, fast-growing salmonids have the potential to incur greater levels of mortality compared to wild conspecifics.

1.3 Freshwater salmonid aquaculture in Canada

Commercial production of rainbow trout in the North Channel region of Lake Huron constitutes ~60% of Canada's freshwater aquaculture industry (DFO 2007a). Annual production is ~4 500 tonnes (Hunter 2009), but poised to rapidly expand in the next decade. Given the inevitable release of domestic salmonids into freshwater environments from aquaculture, and the radically different

characteristics that they possess relative to wild conspecifics, it is important to understand the potential implications such occurrences can have on a given ecosystem. The fate of escaped domestic fish in freshwater are poorly described, and even less well understood is the interaction between wild and domestic strains that inhabit Lake Huron.

Examples of influxes of aquaculture fish into Canadian waters include 600 000 - 700 000 rainbow trout from a commercial operation in Lake Diefenbaker, Saskatchewan since 1992, ~360 000 rainbow trout from one event at a fish culture station in eastern Lake Ontario in 1997 (Hoyle et al. 1999) and ~200 000 from one event in Lake Huron in 2012 (McCutcheon 2012). Although these occurrences are considered to be of ecological concern, basic data on characteristics of escapees in freshwater such as survival, growth and competition have received limited scientific consideration. Only a few aforementioned studies attempt to take an experimental approach to determining the ecosystem impacts of domesticated salmonids in freshwater (Biro et al. 2004a, 2006; Blanchfield et al. 2009; Patterson 2010). Blanchfield et al. (2009) acoustically-tagged domestic rainbow trout and annually released them from an experimental aquaculture operation in a 23 ha lake at the Experimental Lakes Area (ELA) in northwestern Ontario. This study's goals were to define site fidelity, survival and overall movement after release from aquaculture. The authors were able to determine that released rainbow trout experienced high annual mortality (~50%) and spent a significant portion of time around the cage site, but the authors did not compare them explicitly to wild conspecifics. Similarly, simulated rainbow trout escapes

from aquaculture sites on Lake Huron resulted in low survival (~50%), low site fidelity (~15%), but maintained high growth rates after 3 months (Patterson 2010). A series of whole-ecosystem stocking studies in BC lakes have examined differences in growth and mortality between a wild and domestic strain of rainbow trout (Biro et al. 2004a, 2006). These studies conclude that domestic rainbow trout generally have superior growth compared to wild conspecifics, but have greater mortality when predation pressure is high. One advantage of a study of this design is that it is able to accurately quantify growth and mortality of different strains of fish concurrently in the same environment.

Due to the lack of knowledge on the performance and fate of escaped salmonids from aquaculture into the wild, there is a need for further research to better predict the interactions between escapees and wild conspecifics in natural systems, as well as to understand the potential impacts of escapees to native fish communities and food webs (Podemski and Blanchfield 2006). Previous research comparing wild and domestic rainbow trout interactions may not be directly applicable to the Laurentian Great Lakes (LGL), the site of most freshwater aquaculture in Canada. First, past experiments have evaluated growth and mortality of hatchery and wild strains of rainbow trout in lakes with a limited food web, which may not elicit the full range of potential results related to growth and survival. A possible improvement to this design would be to introduce a domestic and wild strain of rainbow trout to a system with a more complex food web, including forage fish species, potentially relieving the need for fish to focus exclusively on aquatic invertebrate species for food sources. Second, many studies that compare wild

and domestic salmonids in nature have done so with strains that would have no potential to interact and are not regularly in competition. Furthermore, these studies use hatchery strains that are not explicitly intended for commercial applications and may have lower growth rates than those intended for aquaculture. The advantage of the present study is the availability of a complex food web and strains of wild and domestic rainbow trout that would potentially be in direct competition in Lake Huron. In addition, results from my study may better support evidence that domestic escapees may cause deleterious effects on other LGL fish species, specifically in terms of reduction of resources (food, habitat and mates). In this case, results from my experiments are directly applicable to understanding the ecological consequences of escape occurrences in Canadian freshwater aquaculture.

The research conducted through this thesis is important in strengthening the database of research that has been performed looking at the differences and interactions between wild and domestic salmonids to date. By producing meaningful results as it relates to this issue, more informed and accurate management decisions can be made in consideration of regulations on Canadian aquaculture facilities.

1.4 Objectives

In response to the knowledge gaps of our understanding of potential impacts of escaped farmed salmonids in Canadian freshwater systems, I directly compared the growth and survival of a wild and domestic genotype of rainbow trout. The

strains being compared in this study are both common to the same water body, and have the potential for direct competition. The two strains originate from a naturalized population (wild) and a farmed aquaculture operation (domestic) in Lake Huron.

I conducted a controlled laboratory experiment coupled with a whole-ecosystem stocking study to achieve the following objectives:

1. Quantify the growth of a domestic and wild genotype of rainbow trout under optimal and competitive conditions in laboratory (Chapter 2).

2. Quantify the growth and mortality of a domestic and wild genotype of rainbow trout in a whole-lake study (Chapter 3).

3. Define the variables that account for growth differences between strains, using modeling approaches (Chapters 2 and 3).

4. Provide a better understanding to aquaculture operators, fisheries management and the scientific community in general to the potential impacts of domestic salmonids on native populations of fish, especially in the Canadian freshwater context.

1.5 Rainbow trout (Oncorhynchus mykiss)

Because rainbow trout are stocked in high numbers in Ontario and have a naturally reproductive population in the LGL, they are of economic, recreational and biological importance in Canada. In addition, rainbow trout show high plasticity and phenotypic variability among genotype, so they are an ideal

candidate for growth rate and behaviour manipulation studies (Gjedrem 2000; Hutchings 2004).

The rainbow trout's native range is exclusive to eastern Pacific Ocean coastal watersheds and extends from Baja California in the south up to Kamchatka Peninsula and Alaska in the north (Thorgaard 1983; Stewart and Watkinson 2004). Though they are not native east of the Rocky Mountains, rainbow trout have been planted in northeastern North America since 1874, with the establishment of the Caledonia Hatchery in New York State (MacCrimmon and Gots 1972). The first watershed on Lake Huron to be stocked with rainbow trout was the Au Sable River in northeast Michigan in 1876 from the Northville hatchery, supplied with the same trout as the Caledonia Hatchery (MacCrimmon and Gots 1972; Behnke 2007). Today, rainbow trout are one of the most widespread species globally, having been introduced in every continent except for Antarctica (MacCrimmon 1971; Casal 2006).

Spawning in rainbow trout will vary temporally depending on geography and genealogy (Bromage et al. 1992). However, North American subspecies are most commonly known to spawn in proximity to inlets or outlets of lakes and streams in the spring, between April and June (Lindsey et al. 1959; Hartman et al. 1962). Some Great Lakes populations have even been observed to spawn as early as late December to late April (Dodge and MacCrimmon 1970). Spawning sites of rainbow trout will consist of beds of fine gravel where the female will dig a redd and deposit her eggs, which are then fertilized by males. This typically occurs in water temperatures between 10.0 – 15.5^{o} C (Scott and Crossman 1973). Eggs will

hatch and alevins will emerge from the nest approximately 4-7 weeks later (Shapovalov and Taft 1954; Scott and Crossman 1973). Fry will then spend 1-3 years in streams before migrating to the lake or ocean in anadromous subspecies known as steelhead trout. At sexual maturity (3-6 years of age) rainbow trout will return to tributaries to spawn (Scott and Crossman 1973). Unlike some other salmonids, rainbow trout are iteroparous and may return to spawn as many as 5 successive years (Scott and Crossman 1973; Holm et al. 2009). The life expectancy of rainbow trout is variable depending on region. LGL populations appear to live between 6-11 years (Scott and Crossman 1973; Holm et al. 2009).

Rainbow trout will typically experience optimal growth at water temperatures between 16 - 18º C (Javaid and Anderson 1967; Dickson and Kramer 1971; McCauley and Pond 1971; Hokanson et al. 1977; Wurtsbaugh and Davies 1977; Papoutsoglou and Papaparaskeva-Papoutsoglou 1978; Austreng et al. 1987) and lethal upper temperature limits between 25 – 26.5º C (Alabaster and Welcomme 1962; Bidgood and Berst 1969; Charlon et al. 1970; Cherry et al. 1975; Hokanson et al. 1977). Rainbow trout are known to prefer habitats with dissolved oxygen content >6mg • L^{-1} and maintain a lower limit of approximately 3 mg • L^{-1} (Gutsell 1929; Matthews and Berg 1997; Barrow and Peters 2001). Adult rainbow trout prefer shallow water depths, and velocities >20 cm • s^{-1} in streams characterized by riffles and pools and a low tolerance to turbidity (Johnson and Kucera 1985; Baltz et al. 1991; Stewart and Watkinson 2004). In summer months, rainbow trout select areas of gravel and cobble and begin to move to areas of

cobble, boulders and macrophyte beds as water temperature begins to cool (Johnson and Kucera 1985; Riehle and Griffith 1993; Meyer and Griffith 1997).

Younger age-classes of rainbow trout will typically feed on various invertebrates including crustaceans, aquatic insects, snails, leeches as well as fish eggs (Scott and Crossman 1973; Nilsson and Northcote 1981; Beauchamp 1990; Warner and Quinn 1995; Rikardsen and Sandring 2006; Godby et al. 2007). As rainbow trout mature, an ontogenetic shift in diet occurs attributing to their accelerated growth rate after the juvenile stage and having surpassed gape size limitations (Stewart and Watkinson 2004). Rainbow trout will typically forage most actively during crepuscular periods (Contor and Griffith 1993; Riehle and Griffith 1993). Adults are mostly piscivorous and are known to feed on small forage fishes such as alewife (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*) and cyprinid species in the LGL (Jude et al. 1987; Holm et al. 2009).

1.5.1 Strains

Since the modernization of salmonid domestication approximately a century ago, several genetic lines of rainbow trout have developed due to artificial selection or geographical barriers, whether intentional or not. Generally, the long-term effects of interactions between domesticated and wild stocks of salmonids will arise primarily from genetic effects (Tymchuk et al. 2006a); however, environment and culture conditioning will also contribute to overall phenotypic expression of certain traits within a strain. This so-called "continuum" of phenotypic traits is created by the synergistic effect of genetics and environment. The spectrum of

phenotype ranges from highly selected aquaculture strains, to hatchery fish, to purely wild fish. The range of phenotypes observed among different wild populations are anticipated to overlap considerably with each other and with hatchery strains derived from them. Domesticated strains that have undergone directed and unintentional selection are expected to possess phenotypes different from wild and hatchery populations and are often in a common direction away from the wild phenotype (Tymchuk et al. 2006b) (Figure 1.2).

Both the laboratory and the whole-lake components of my thesis compare a wild and a domesticated strain of rainbow trout. A major advantage of this study is that the two strains used have the potential to interact with one another in real-world scenarios. The domestic strain is cultured in a water body that also contains naturalized strains of rainbow trout (Lake Huron). Escape events from commercial aquaculture operations on Lake Huron, would result in domesticated strains of rainbow trout interacting with wild strains of the same species. By quantifying the growth and survival of both of these strains in the laboratory and experimental lakes, I am then able to extrapolate the results on a larger scale.

Both the wild and domestic strains have a different lineage and culture history that will allow them to express very different characteristics as part of the phenotypic continuum. Although much of the knowledge of sub-speciation and origin of rainbow trout strains is varied and somewhat anecdotal, some evidence and records are available to determine lineages. The species, *Oncorhynchus mykiss*, Behnke (1992) includes three major groups: (1) the redband trout of the Columbia River basin and in the upper Fraser River basin, *O. mykiss gairdneri*;

(2) the redband trout of the Sacramento and McCloud river basins, *O. m. gilbert, O. m. aguabonita, O.m. shasta, O. m. stonei*; and (3) coastal rainbow trout or steelhead, *O. m. irideus, O. m. mykiss*. Based on records from the U.S. Fish Commission's fish culture operations, it seems that hatchery rainbow trout originated from the McCloud River hatchery strain of non-migratory rainbow trout – *Oncorhynchus mykiss shasta* (Needham and Behnke 1962). This sub-species of rainbow trout has the characteristics of bright colouration, large, sparse spots and elliptical parr markings (Behnke 1992). It has been documented, however, that coastal steelhead and inland sub-species of eggs were not differentiated when collected for propagation in the McCloud River hatchery, so the resulting hatchery strain originating from the 1870s was likely a hybrid of the two sub-species (*O. mykiss shasta* x *O. mykiss irideus*) (Needham and Behnke 1962; Dollar and Katz 1964). The hatchery strain of rainbow trout is considered to retain the morphological characteristics and genetic background more closely associated with sea-run steelhead trout (Behnke 1992).

The McCloud River hatchery rainbow trout were first brought to eastern North America sometime in the late 1870s when eggs were incubated at the Caledonia hatchery in New York State (MacCrimmon and Gots 1972). The two strains that were obtained for the purposes of my experiments originated initially from this brood stock. The two strains are:

1. Wild genotype – Although rainbow trout are not native to the LGL, there now exists a well-established introduced population. They are considered "naturalized" because they are not historically indigenous to the region but

have sufficient reproduction to sustain their population. Plantings of rainbow trout in the LGL first occurred in 1884 from the Caledonia hatchery into the Genesee water shed (MacCrimmon and Gots 1972). Subsequent stocking on the Canadian side of Lake Ontario occurred later in Bronte creek in 1927 and 1930 (MacCrimmon and Gots 1972). These initial stocking events led to the establishment of a naturalized population that could then be found in most watersheds of Lake Ontario, including Ganaraska. Since 1984, the Ontario Ministry of Natural Resources (OMNR) has exclusively stocked the Ganaraska River population of rainbow trout into Lake Ontario. This strain continues to be used in many areas of Ontario including Lake Huron and is common in a number of watersheds (OMNR 2005). The "Ganaraska" strain that was used for my experiments was reared from the OMNR's Dorian Fish Hatchery (east of Thunder Bay) in Ontario, Canada. It was used as the "wild" strain in both the laboratory and whole-lake experiments. Eggs of Ganaraska trout are obtained from the wild and are reared in a raceway culture until reaching appropriate stocking size at this facility.

2. Domestic genotype – This strain of rainbow trout has a history of selective breeding for the purposes of commercial aquaculture. The domestic strain used in these experiments was reared from a brood stock at Cedar Crest Fish Farms in Hanover, Ontario, Canada. The purpose of this fish farm is to mainly supply ~100-150 mm length fish to open-pen cage aquaculture operations on Manitoulin Island, Ontario, where they are subsequently

grown to an appropriate size for market. Cedar Crest's stock is routinely mixed with brood stocks from Kamloops, Spring Valley and other California-based farms every 3-5 years to counter inbreeding (Cedar Crest Fish Farms, personal communication). This strain is historically from McCloud River parent generations but is ultimately an amalgamation of various genetic lineages. Cedar Crest rainbow trout were used in the tank growth trials and in the whole-lake studies. Carryover effects stemming from differences in previous rearing environments and not specifically a result of genotype should be noted, but not explicated controlled for in these experiments.

1.6 Project summary and design

To address the objectives of my research, I undertook a laboratory-based and a replicated whole-lake experiment. In both cases, I use a substitutive design scheme to directly compare the two strains of rainbow trout. Weber and Faush (2003) explain that very few studies determine interactions comparing hatchery-bred and wild salmonids using substitutive design. Purely mensurative and additive experiments may have the benefit of being more realistic to situations in nature, but do not effectively control for possible effects of equal density, stock rate and acclimation biases. By using a substitutive design measure (equal stocking densities of wild and domestic fish) I was able to directly compare both strains under identical conditions. Application of this design to both approaches allowed me to account for growth with and without the selective pressures of a natural system. Because phenotypic expression of most characteristics in

salmonids is plastic and consists of a genetic and environment component, by controlling for environmental interactions in the laboratory trails, I am able to accurately quantify growth purely based on genetic effects of a particular strain.

1.6.1 Growth trials (Chapter 2)

The controlled growth trials quantified the growth rate of domestic and wild genotype rainbow trout over 102 d in a flow-through tank system. The purpose of this experiment was to determine growth rate differences between the wild and domestic strain under optimal conditions to compare to growth achieved in the whole-ecosystem experiment. My hypothesis is that under these culture conditions, domestic strains will have superior growth compared to wild conspecifics. The divergence in growth trajectories between domestic and wild strains under competition are expected to be greater owing to the increased aggression, feed intake and appetite in domestic salmonids (Table 1.1).

Rainbow trout were subjected to two separate treatments: (1) Strains reared in intra-strain isolation and fed commercial feed to satiation daily. This treatment was designed to determine the maximum potential for growth in the absence of inter-strain competition when food availability was high. (2) Strains reared together in the same tank, fed a reduced ration to drive competitive interactions between strains (1.2% of bodyweight daily). This treatment was designed to determine if a strain's growth is significantly different from treatment 1, due to inter-strain competition. Fish were sampled approximately every 25 d to test for differences in growth trajectories between strains and treatments.

1.6.2 Whole-ecosystem experiment (Chapter 3)

Subsequent to quantifying the difference in growth rate of both strains under optimal conditions, a whole-ecosystem experiment was designed to determine the growth and mortality of each strain under natural conditions. Theoretically, outcomes of the whole-ecosystem experiment should differ from the controlled study because of the radically different environment, range of habitats, risk of predation and variety of prey-items. By analyzing the results of most research, my hypothesis for this experiment is that domestic rainbow trout are likely to grow at a greater rate, but incur costs due to their increased growth, specifically greater mortality.

The whole-lake experiment consisted of stocking replicate lakes (Lake 303 and Lake 304) at the Experimental Lakes Area in northwestern Ontario with equal densities of size-matched domestic and wild genotype rainbow trout. The lakes chosen are both head-water lakes, with similar biotic communities consisting of cyprinids, benthic invertebrates, zooplankton, amphibians and turtles. In addition, each lake is frequented by piscivorous birds: common merganser (*Mergus merganser americanus*), common loon (*Gavia immer*) and other species to a lesser extent. Both lakes have been monitored and sampled 2 years prior to the rainbow trout introduction. Monitoring included sampling of biota (minnow, invertebrate and primary producer), water chemistry and physical parameters. Sampling protocol of these parameters was continued throughout the experiment year. A “fish fence” was constructed at the outflow of each lake to ensure that no fish could escape each lake during the duration of the experiment.

Table 1.1 List of studies examining differences in phenotype between artificially selected salmonids (domestic; D) compared to wild (W) conspecifics.

Trait	Result	Environment	Species	Source
Genetics	GH expression elevated in D relative to W	-	Coho salmon	Devlin et al. 2009
	Genetic divergence in several fitness-related traits	-	Atlantic salmon	Fleming and Einum 1997
	D are similar to one another and have reduced genetic variability relative to W	-	Coho salmon	Overturf et al. 2003, 2010
	D differed on most loci relative to W	-	Atlantic salmon	Skaala et al. 2005
	Differences in IGF-1 expression between D and W	-	Rainbow trout/Coho salmon	Tymchuk et al. 2009a
	3 - 9% of genes differed between D and W	-	Rainbow trout	Tymchuk et al. 2009b
	Reduced genetic variation in D relative to W	-	Rainbow trout	Wringe et al. 2010
Morphology	D showed more robust bodies and smaller rayed fins relative to W	Culture	Atlantic salmon	Fleming and Einum 1997
Growth	D ~1.5x growth of W	Wild	Rainbow trout	Ayles and Baker 1983
	D ~1.5x growth of W	Wild	Rainbow trout	Ayles et al. 1976
	D ~2x growth of W	Wild	Rainbow trout	Biro et al. 2004a, 2006
	D ~3x growth of W	Culture	Rainbow trout	Devlin et al. 2001
	D ~1.3x growth of W	Culture	Atlantic salmon	Einum and Fleming 1997
	D ~1.6x growth of W	Wild	Atlantic salmon	Einum and Fleming 1997

Table 1.1 (continued) List of studies examining differences in phenotype between artificially selected salmonids (domestic; D) compared to wild (W) conspecifics.

Growth	D ~1.1x growth of W	Culture	Atlantic salmon	Fleming and Einum 1997
	W ~1.1x growth of D	Semi-natural	Atlantic salmon	Fleming and Einum 1997
	D ~1.1x growth of W	Wild	Atlantic salmon	Fleming et al. 2000
	D achieved 3x size of W at end of experiment	Culture	Atlantic salmon	Fleming et al. 2002
	D ~1.2x growth of W	Culture	Atlantic salmon	Handeland et al. 2003
	D ~1.4x growth of W	Culture	Coho salmon	Hershberger et al. 1990
	D ~1.2x growth of W	Culture	Coho salmon	McClelland et al. 2005
	D > growth relative to W	Wild	Atlantic salmon	McGinnity et al. 1997
	D ~1.1x growth of W	Culture	Coho salmon	Overturf et al. 2003
	D ~8x growth of W	Culture	Rainbow trout	Tymchuk and Devlin 2005
	D ~1.3x growth of W	Culture	Rainbow trout	Tymchuk et al. 2006a
	D ~1.5x growth of W	Culture	Coho salmon	Tymchuk et al. 2007, 2009a
	D 1 - 1.2x growth of W	Culture	Rainbow trout	Valente et al. 2001
Feed efficiency	D feed efficiency greater relative to W	Culture	Atlantic salmon	Grisdale-Helland and Helland 1998
	D 1-1.3x greater feed efficiency relative to W	Culture	Atlantic salmon	Handeland et al. 2003
	D only more efficient when fed $\geq$ usual daily ration	Culture	Brown trout	Mambrini et al. 2004
	No Difference between D and W	Culture	Brown trout	Sanchez et al. 2001
	D ~1.25x greater feed efficiency relative to W	Culture	Atlantic salmon	Thodesen et al. 1999

Table 1.1 (continued) List of studies examining differences in phenotype between artificially selected salmonids (domestic; D) compared to wild (W) conspecifics.

	No difference between D and W	Culture	Rainbow trout	Valente et al. 2001
Anti-predator response	D spent more time in 'risky' areas of lake relative to W	Wild	Rainbow trout	Biro et al. 2004a, 2006
	D left cover sooner relative to W after simulated predator presence	Culture	Atlantic salmon	Einum and Fleming 1997
	D reappeared sooner relative to W after simulated predator attack	Culture	Atlantic salmon	Fleming and Einum 1997
	D exhibited decreased anti-predator response in presence of simulated predator relative to W	Semi-natural	Atlantic salmon	Houde et al. 2009
	D were spent more time foraging in presence of predator relative to W	Culture	Rainbow trout	Johnsson and Abrahams 1991
	D spent less time feeding after predator exposure relative to W	Semi-natural	Rainbow trout	Tymchuk et al. 2007
Survival	Similar survival between D (18-37%) and W (14-32%)	Wild	Rainbow trout	Ayles and Baker 1983
	D survival ~60% relative to W with high predator pressure	Wild	Rainbow trout	Biro et al. 2004a, 2006
	Early life mortality greater in D relative to W	Wild	Atlantic salmon	Fleming et al. 2000

Table 1.1 (continued) List of studies examining differences in phenotype between artificially selected salmonids (domestic; D) compared to wild (W) conspecifics.

	D had 2% lifetime survival relative to W	Wild	Atlantic salmon	McGinnity et al. 2003
	D had 51-77% recapture rate relative to W	Wild	Atlantic salmon	McGinnity et al. 1997
	D are modeled to have decreased survival relative to W when predation is high	Semi-natural	Rainbow trout	Tymchuk et al. 2007
Aggression	D dominated W in pairwise contests	Culture	Atlantic salmon	Einum and Fleming 1997
	D more aggressive relative to W	Culture	Atlantic salmon	Fleming and Einum 1997
	W dominated D in pairwise contents	Semi-natural	Atlantic salmon	Fleming and Einum 1997
	D initiated more aggressive patterns in divided substrate, W initiated more in fibreglass substrate	Culture	Atlantic salmon	Mork et al. 1999
	No difference between D and W	Semi-natural	Atlantic salmon	Fleming et al. 1996
	W use dominance hierarchies more effectively to spawn relative to D	Semi-natural	Atlantic salmon	Weir et al. 2004
Spawn timing	D females migrate to spawning areas 1-2 weeks earlier relative to W	Wild	Atlantic salmon	Fleming et al. 2000
	D ascend spawning sites ~2 months later relative to W	Wild	Atlantic salmon	Gausen and Moen 1991

Table 1.1 (continued) List of studies examining differences in phenotype between artificially selected salmonids (domestic; D) compared to wild (W) conspecifics.

	D ascend spawning sites later relative to W	Wild	Atlantic salmon	Gudjonsson 1991
	D spawned 21-26 days earlier relative to W	Wild	Atlantic salmon	Lura et al. 1993
	D peak spawning occurred 32 days earlier relative to W	Wild	Atlantic salmon	Sægrov et al. 1997
	D spawned 1-2 weeks later relative to W	Wild	Atlantic salmon	Webb et al. 1991, 1993a, 1993b
Sexual maturation	D attained sexual maturity faster relative to W	Wild	Atlantic salmon	Kallio-Nyberg and Koljonen 1997
Reproductive success	D redds account for 35-55% of total	Wild	Atlantic salmon	Carr et al. 1997
	High frequency of D biomarker in some sites relative to W	Wild	Atlantic salmon	Clifford et al. 1998
	D reproductive success ~30% relative to W	Semi-natural	Atlantic salmon	Fleming et al. 1996
	16% lifetime reproductive success of D relative to W	Wild	Atlantic salmon	Fleming et al. 2000
	D spawning success thought to be lower relative to W	Wild	Atlantic salmon	Lura et al. 1993
	D redds account for at least 45% of total	Wild	Atlantic salmon	Sægrov et al. 1997
	D are expected to have reduced spawning success relative to W	Semi-natural	Atlantic salmon	Weir et al. 2004

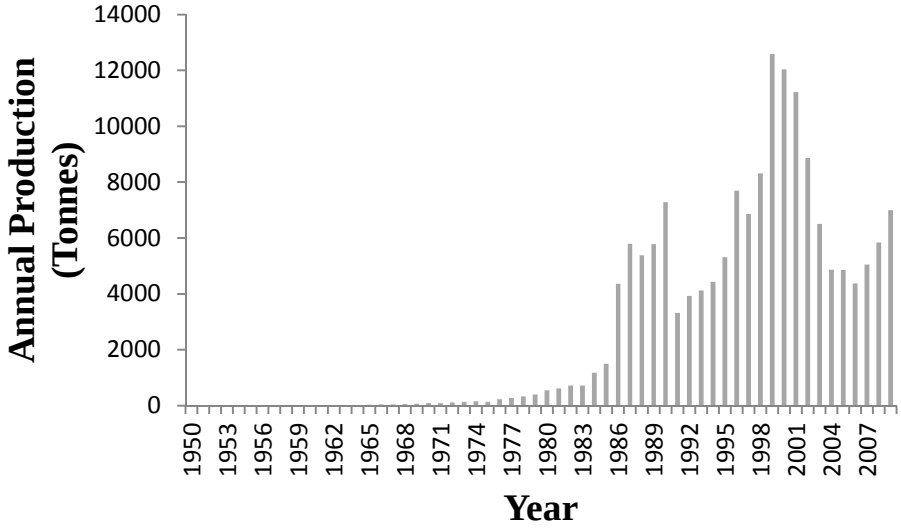

Figure 1.1 Annual Canadian aquaculture production of salmonids in freshwater systems. Calculated as summed reported production from all Canadian Aquaculture facilities per year (FAO - FishStatJ 2010).

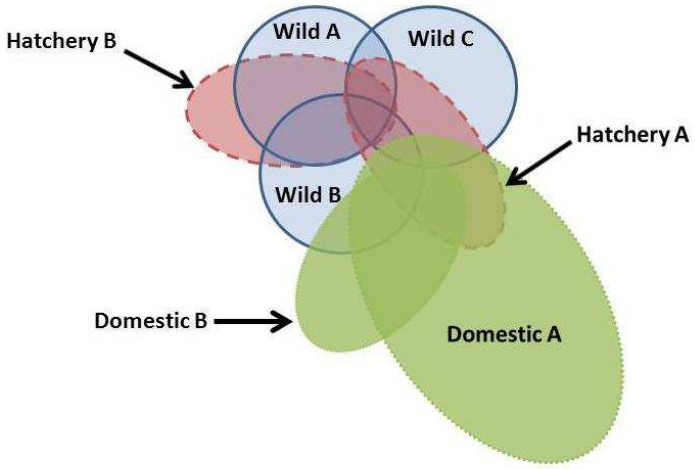

Figure 1.2 Hypothetical relationships among phenotypic states of wild, hatchery and farmed domestic strains of salmonids. Ranges of phenotype observed are anticipated to overlap, dependent on origin and genotype of strain. Selected strains (domestic) are anticipated to possess characteristics in a common direction away from wild phenotypes (adapted from Tymchuk et al. 2006b).

Chapter 2. Growth of aquaculture and wild strains of rainbow trout in a controlled laboratory-setting

2.1 Introduction

2.1.1 Salmonid genotypes

Aquaculture and naturalized strains of salmonids are known to occur sympatrically in both freshwater (Penzack et al. 1982; Phillips et al. 1985) and marine environments (Hansen et al. 1993; Carr et al. 1997; Naylor et al. 2005). These strains are thought to compete with one another for food, habitat and mates (Fleming et al. 2000, 2002; Jonsson et al. 2006). The driver for the competitive interactions between aquaculture and naturalized strains of salmonids is the result of the fundamental behavioural and physiological differences between cultured and wild fish (Table 1.1). This divergence in phenotype can be directly attributed to generations of selective breeding and culture conditioning in domesticated strains of fish used in commercial aquaculture. Selective breeding is essential to the efficiency of fish production for commercial market. Growth rates up to, and exceeding twice that of wild conspecifics are not uncommon (see Table 1.1) and are achieved as a result of phenotypic differences occurring from selective breeding. Unlike most other domesticated animals such as poultry and cattle, genetic selection of fish has occurred comparatively recently (Overturf et al. 2003). This allows a unique opportunity to directly quantify differences in phenotypic expression between farmed and wild strains of a single species of fish.

2.1.2 Growth rates

Growth rate differences between farmed and wild conspecific salmonids in culture are well documented in scientific literature (Fleming and Einum 1997; Valente et al. 2001; Fleming et al. 2002; McClelland et al. 2005; Tymchuk et al. 2006a, 2007, 2009a; Wringe et al. 2010) and primarily focus on Atlantic salmon (*Salmo salar*). Einum and Fleming (1997) compared growth among wild, domestic and hybrid Atlantic salmon in a hatchery setting. Domestic treatment groups had statistically greater growth rates than both wild and hybrid groups. These same researchers have also shown that the advantage of greater growth rate is exaggerated at higher temperatures for domestic Atlantic salmon (Fleming and Einum 1997). In another study, domestic Atlantic salmon maintained a significant size advantage over wild conspecifics when reared in outdoor flow-through tanks (Neregard et al. 2008). Body mass, length, condition factor and specific growth rate (SGR) were all greater in domestic Atlantic salmon. Thodesen et al. (1999) also found selected lines of Atlantic salmon have better growth performance than wild conspecifics under identical conditions. The domesticated strain of Atlantic salmon in these experiments had a significantly greater feeding efficiency and temperature independent growth rate (thermal growth coefficient; TGC) than wild strains. Similar studies confirm that farmed Atlantic salmon have superior growth rates than their wild counterparts in a variety of life stages, water qualities, and temperatures (Fleming et al. 2002; Handeland et al. 2003).

2.1.3 Rainbow trout

The majority of research has focused on growth and the interactions between domestic and wild strains of salmonid species in marine culture, while

comparatively less is understood and documented for freshwater aquaculture species. Of the freshwater comparative studies that have been conducted, most focus on the rainbow trout (*Oncorhynchus mykiss*) model. By quantifying percentage of domestic alleles in a spectrum of domestic x wild hybrids of rainbow trout, researchers have concluded that additive effects due to domestic genotype result in greater growth (Tymchuk et al. 2005, 2007). These studies took what they considered to be fully domesticated rainbow trout and reared a series of hybrids of pure-wild conspecifics so that varying degrees of domestic alleles were retained in the offspring generations (i.e., 100% domestic, 75% domestic, etc.). Succinctly, the degree of selection in rainbow trout is positively correlated with maximum growth potential that can be selected for in a given strain (Figure 2.1). This result agrees with other laboratory and field experiments comparing domestic and wild rainbow trout (Valente et al. 2001; Biro et al. 2004a, 2006). Much of the past research on growth divergence between wild and domestic strains of rainbow trout has been conducted on west coast strains in Canada (i.e. native strains; Devlin et al. 2001; Biro et al. 2004a, 2006; Tymchuk et al. 2007, 2009a, 2009b), although the majority of freshwater aquaculture occurs in the Laurentian Great Lakes (LGL; Moccia and Bevan 2007). This is an important distinction for two reasons. First, the domesticated rainbow trout used in experiments on the west coast are bred at farms and hatcheries mostly for the purposes of stocking ponds and lakes and for recreational purposes. These strains are not specifically intended for the high growth rate associated with large-scale, commercial, open-pen aquaculture production. Therefore, it is possible that

previous research comparing relative growth rates of wild and domestic strains may not encompass the full range of divergence expected under a commercial application. Second, while rainbow trout are the most commercially produced freshwater species in Canada, they are not native to the locales where the majority of commercial production occurs (i.e. Lake Huron/Georgian Bay, ON and Lake Diefenbaker, SK). Thus, there is not a fundamental understanding of potential growth divergence between "naturalized" strains of rainbow trout (stocked into the LGL in the late 1800's) and the domestication of this naturalized strain for commercial production that has progressively been occurring over the past 100 years.

Most commercial open-pen farming of rainbow trout occurs in the LGL and produces 1000-5000 t annually, or 1-5 million fish reared to 1 kg (FAO 2010). The regular escape of farmed fish to the wild associated with open-pen production (McGinnity et al. 2003; Naylor et al. 2005) allows these strains of rainbow trout to directly compete with wild (naturalized) strains. Yet, we are not able to predict the potential impact of escapees on wild fish as these strains have not been examined in the past for a direct comparison of growth rates. Given the absence of studies on these strains, additional comparative growth experiments are needed to understand maximum growth rate potential as a benchmark to how they may relate to competition in the wild. By controlling for stochastic variation that would be apparent in a natural system, we may quantify growth rate while eliminating random effects, so that differences between strains can be said to be genetically driven.

2.1.4 Objectives

I conducted replicated, controlled lab experiments to quantify growth differences between a wild strain of rainbow trout from Lake Huron relative to the strain used in commercial aquaculture (domestic) in Lake Huron.

The objectives of these experiments were to:

1. Quantify the maximum growth divergence between a wild and domestic strain of rainbow trout
2. Quantify the difference in growth of a wild and domestic strain of rainbow trout under inter-strain competition.

My hypothesis is that under optimal culture conditions in which fish are fed to satiation, domestic strains are predicted to have superior growth compared to wild conspecifics. The divergence in growth trajectories between domestic and wild strains under competition are expected to be greater than those grown without inter-strain competition, due to the increased aggression, feed intake and inherent growth rate in domestic salmonids.

2.2 Methods

2.2.1 Experimental design

Growth trials were conducted at the Aquatic Biotechnology and Ecotoxicology Lab (ABEL) at Lakehead University in Thunder Bay, Ontario, Canada. A total of 9, 160 L tanks made of insulated fibre glass were installed in the lab area. Each

tank was 1.24 m x 0.60 m in area and 0.52 m high (Figure 2.2). Tanks were configured in a flow-through system and received dechlorinated water at rate of 1 L • min^{-1} from a municipal source (Lake Superior). Water was allowed to drain through a stand-pipe covered with mesh in the centre of each tank to maintain flow. The initial water temperature ($\bar{x}$ ± 1 S.D.) before the addition of fish was 12.67 ± 0.15 ºC, dissolved oxygen (DO) = 10.66 ± 0.14 mg • L^{-1}, and pH = 7.5 among 9 tanks.

Ganaraska strain (referred to as "wild" here forth) were obtained from the fish hatchery in Dorion, Ontario, Canada (see section 1.51 for strain details) on October 6, 2010. At this date, initial weight (0.01 g) and fork length (1.0 mm) were recorded. Fish were not fed until October 13, 2010 and then fed a maintenance diet of 1% average body weight • day^{-1}. On October 19, 2010, the wild strain were weighed and measured again to obtain t_0 values. On October 21, 2010, the domestic strain arrived from Cedar Crest Fish Farms (Hanover, Ontario, Canada). Equal numbers of fish from each strain (n = 30 for treatment 1; n = 15 for treatment 2) were distributed among tanks so that mean fish mass between strains was similar among all tanks in both treatments (treatment 1 = 6.62 ± 1.98 g; ANOVA, $F_{(5,174)} = 0.125$, $P = 0.997$; treatment 2 = 6.66 ± 1.85 g; ANOVA, $F_{(5,84)} = 0.074$, $P = 0.996$). Treatments were randomly assigned to each tank to eliminate bias in tank arrangement. Both strains of rainbow trout were subjected to two separate treatments.

2.2.1.1 Treatment 1: growth potential

Each strain was reared in isolation and fed to apparent satiation 3 times daily. This treatment was designed to determine the maximum potential for growth in the absence of inter-strain competition when food availability was high. A total of 6 tanks were used, each held an equal number (n = 30) of either wild or domestic fish. Fish were fed 1.5 mm fish feed (Corey Nutrition Company, Fredericton, NB, Canada; 52% crude protein, 18% crude fat). Satiation was defined as continuous feeding until 3 consecutive pellets reached the bottom of the tank (i.e. fell) without being eaten. Feed was administered manually to the tanks, small amounts at a time, to ensure that determination of satiation was accurate. Excess feed was siphoned from the tank subsequent to feeding. The total amount of feed for each tank for each day was calculated by weighing a bulk amount prior to feeding and then again afterward to calculate total difference (0.01 g).

2.2.1.2 Treatment 2: growth under inter-strain competition

Strains were reared together in the same tank (n=15 wild; n=15 domestic; n = 3 tanks) and fed a reduced ration to enhance competitive interactions between strains. This treatment was designed to test divergence in growth trajectories between strains relative to maximum growth (treatment 1) as an indicator of inter-strain competition. A feed weight of 1.2% of average individual fish mass per tank was distributed daily. A ration of 1.2% daily body weight • day^{-1} was chosen to elicit a competitive response and was selected based on a reduction from the 2% daily body weight • day^{-1} used to promote growth at the Dorion Fish Hatchery (OMNR, personal communication). Feeding procedure was similar to treatment 1, but instead of being fed to satiation, each tank was fed a pre-calculated ration

divided between 3 feeding events. This 1.2% of body weight was calculated based on the average weight of all fish within each tank. Feed amount was determined by calculating the mean weight of both wild and domestic strains, taking 1.2% of that average, and then multiplying by the total number of fish per tank. For example, if average fish weight was 6 g, then 1.2% of this amount (0.072 g) would be allotted to each fish (n = 30) for a total daily tank feed weight of 2.16 g, which would be divided into thirds and delivered at every feeding period per day. When growth was measured (every 25 d) or mortality occurred, the total amount of feed was recalculated. If any feed would remain after feeding had ceased, it was siphoned as in treatment 1 tanks.

To differentiate between strains, domestic rainbow trout received a visible implant elastomer (VIE) dye injection (Northwest Marine Technology, Shaw Island, WA, USA). The dye was injected into the adipose tissue posterior to the eye. The dye acts as a colour marker that has a high retention rate and fluoresces under black light.

2.2.2 Daily condition monitoring

Water temperature and dissolved oxygen concentration (DO) were measured and recorded daily in each tank with a hand-held YSI 550a temperature (0.1ºC) and oxygen (0.01 mg • L^{-1}) probe (YSI Inc., Yellow Springs, OH, USA). In addition, the flow though system was monitored to ensure fresh water was being continuously circulated and the flow rate was appropriate (measured at 1 L • min^{-1}). The lights in the lab were active for 24 h a day. In order to achieve a 12:12 h

light and dark cycle, coroplast covers (painted black) were removed and replaced for each tank, every day of the experiment. To consistently maintain diel cycles, this was done at the first and last feed time each day (~8:00 and 20:00). If a mortality occurred during the duration of the experiment, date, strain, tank, weight and fork length of the fish were recorded and the sample was subsequently placed in a WhirlPak® bag and immediately frozen (-20ºC) .

2.2.3 Data collection

The total duration of the experiment was 102 days (October 21, 2010 to January 31, 2011). At the start of the trials and approximately every 25 days, body size was assessed for each strain. All fish were taken off feed one day prior to measuring in order to reduce activity. Each fish was anaesthetized in tricanine methane sulphonate (TMS™ **(**MS222); Argent Chemical Laboratories, Inc., Redmond, WA, USA) prior to handling in order to sedate fish and reduce handling stress. A bath solution was prepared with a concentration of 40 mg • L^{-1} TMS buffered with 80 mg • L^{-1} $NaHCO_3$ to neutralize the solution pH. Fish were introduced to the anaesthesia bath and once sufficiently sedated, were measured for fork length (1.0 mm) and mass (0.01 g). Afterwards, fish were moved to a recovery bath, which consisted of a cooler of water receiving oxygen.

Each tank was processed separately at each weighing and measuring event. This took place on three occasions after the initiation of the experiment: (1) November 16, 2010 (2) December 13, 2010 and (3) January 3, 2011. The experiment concluded on January 31, 2011 when a final measurement of each rainbow trout

was taken. All rainbow trout were euthanized in a bath of 200 mg • L^{-1} TMS buffered with 400 mg • L^{-1} $NaHCO_3$. Fish were administered to the solution and were monitored until opercular movements had ceased. Five minutes after the cessation of gill movements, fish were placed in WhirlPak® bags with date, strain, fork length, weight and ID number recorded on them and in a log book. Samples were immediately frozen (-20ºC) for future total mercury analysis as part of a bioenergetics model (section 2.2.6).

2.2.4. Data analysis

To assess whether there was a random effect of “tank” on the outcomes of the experiments in treatments 1 and 2, a linear mixed effects model with all terms was compared to a model without the random effect of “tank” on the response variable. This comparison was performed to determine if the inclusion of "tank" contributed significantly to the overall model and explained significantly more variance than a linear model that included only the fixed variables: "time" and "strain".

Comparison of the simpler model with the general linear model (GLM) was carried out using a log-likelihood ratio test similar to the methods of Rennie and Evans (2012). This follows the null hypothesis that the model that includes the random effect of tank on mass (eq. 2.1) does not explain significantly more of the variation than the simpler model (eq. 2.2).

(eq. 2.1) $y = \text{Strain} + \text{Time} + \text{Time(Tank)} + \text{Strain*Time(Tank)} + \varepsilon$

(eq. 2.2) $y = \text{Strain} + \text{Time} + \text{Strain*Time} + \varepsilon$

The distribution of this test statistic is derived from a chi-squared distribution and attempts to express how many times more likely each model predicts "mass" (Huelsenbeck et al. 1996). Both models were subjected to the log-likelihood ratio test because of its high statistical power, making it ideal for distinguishing between nested models (one simple model and one with random constraints) (Neyman and Pearson 1933; Huelsenbeck and Crandall 1997). I conducted this analysis in the statistical program R on the two linear models being compared (eq. 2.1 and eq. 2.2) with the *lme4* package for fitting linear and generalized linear mixed-effects models. The random effect of "tank" on average fish mass was non-significant for treatment 1 ($P = 0.146$) and treatment 2 ($P = 0.070$), indicating that and the simpler model (eq. 2.2) for growth can be used.

The absence of any tank affect allowed for linear models using repeated measures to determine significant differences in the response variables: mass, fork length, condition factor and feed conversion ratio between strains over time. This approach was also used to compare differences between growth trajectories of the same strains subjected to different treatments.

2.2.5 Calculations

Growth was expressed using two measures: the thermal growth coefficient (TGC) and specific growth rate (SGR). The TGC measure is routinely used as a model for comparison of salmonid performance because it provides a standardized measure of growth that is unaffected by live weight, time interval and temperature (Dumas et al. 2007; 2010). The TGC is in the form,

(eq. 2.3) $$TGC = (BW_1^{1/3} - BW_0^{1/3}) \bullet (\Sigma T)^{-1}$$

where, BW_0 and BW_1 are initial and final mean tank body weights (g) respectively and ΣT is the sum day – degrees C°. ΣT was calculated by multiplying the average temperature among experimental tanks for that time period by the number of days in that time period (Iwama and Tautz 1981; Cho 1992; Dumas et al. 2007).

Specific growth rate (SGR) was calculated in addition to TGC as a growth measure for comparison to literature values:

(eq. 2.4) $$SGR = [(\ln BW_1 - \ln BW_0) \bullet (\text{time in days})^{-1}] \bullet 100$$

where, BW_0 and BW_1 are initial and final mean fish body weights (g) per tank, respectively. Interpretation of the SGR should be taken with caution considering the equation assumes that fish growth is approximately exponential and is relatively unaffected by temperature (Dumas et. al 2010).

Feed conversion ratio (FCR) is used a measure of efficiency by which food is transformed into body mass (Bureau et al. 2000; Valente et al. 2001; Overturf et al. 2003). Fish with lower FCR values gain a greater mass per unit of feed, and therefore are considered to be more efficient users of feed.

(eq. 2.5) $$FCR = [\text{total feed consumed (g)}] \bullet [\text{average weight gain (g)}]^{-1}$$

FCR was calculated for each tank and averaged among replicate tanks for wild and domestic strains in treatment 1. FCR could only accurately be calculated in

treatment 1 because partitioning of feed consumed between strains held in the same tank was not possible for treatment 2.

Condition factor (K) is a ratio used to quantify the relationship between fish length and weight between strains of fish (Fulton 1904):

(eq. 2.6) $$K = (BW \bullet L^{-3}) \bullet 10^{5}$$

where, BW is body weight (g) and L is the fork length (mm) of the fish.

All statistical analyses were performed using the statistical package R (Version 2.13.1, The R Foundation for Statistical Computing, 2011).

2.2.6 Bioenergetics modeling to estimate consumption

A modeling approach was used to fit the measured growth and feed consumption values to a commonly used predictive bioenergetics model (Kitchell and Stewart 1977). Bioenergetics models (BM) typically use validated standard equations and coefficients that are common to the species studied. These models rely on accurate user inputs to produce results that are precise and robust. By fitting the BM to known measurements of consumption, initial and final average fish mass, feed energy and temperature, one may solve for coefficients that are specific to each strain of fish that can be subsequently used in future field studies. For this model, I was able to solve for the intercept of the allometric growth function (*RA*; a component of the respiration equation) by fitting the BM to these measured parameters from these growth trials. Following similar methods for adjusting respiration in whitefish (*Coregonus clupeaformis*) bioenergetics modeling

(Madenjian et al. 2006), the *RA* was adjusted in the model because: (1) respiration in fish BMs are highly sensitive compared to estimates of elimination (Bartell et al. 1986); and (2) to simplify the calibration process by only altering one term in the respiration equation.

2.3 Results

2.3.1 Physical parameters

Water temperature decreased over the course of the study (October to late January) as a consequence of using a natural source (Lake Superior). Temperature of each tank ($\bar{x}$ ± 1 S.D.) steadily decreased from 12.67 ± 0.15 ºC at t_0 to 5.83 ± 0.22 ºC at t_{102} among tanks. Due to the decrease in water temperature over time, the solubility of dissolved oxygen (DO) steadily increased over the duration of the experiment (from 10.66 ± 0.14 mg • L^{-1} at t_0 to 12.53 ± 0.33 mg • L^{-1} at t_{102} among all tanks) (Figure 2.3).

2.3.2 *Mortality*

Survival rates were high throughout the experiment. In total, 10 rainbow trout died during the 102-day experiment, 2 domestic strains from treatment 1 and 5 domestic and 3 wild among tanks from treatment 2.

2.3.3 Growth

2.3.3.1 Treatment 1

Wild and domestic strains of rainbow trout fed to satiation showed steady growth throughout the study. On average, domestic strains increased their mass ~3x that of wild strains and achieved ~2x the total final mass (Figure 2.4). Domestic and wild strains increased their initial mean mass (6.58 g) by 26.28 and 8.52 g, respectively. The response variable “mass” differed significantly between strains (ANOVA, $F_{(1,4)} = 239.73$, $P < 0.001$). The interaction term indicates that there is a significant time effect, meaning that both strains changed over time at different rates (ANOVA, $F_{(1,4)} = 74.70$, $P < 0.001$). The coefficient of variation (CV) for body mass also increased over time and was greater in wild tanks than domestics (wild = 48% ± 8; domestic = 35% ± 4 at 102 days) (Figure 2.6). Therefore, even though domestic fish achieved a larger size and had a greater range in size distribution relative to wild fish, the variation in body mass compared to the sample mean was consistently greater in wild rainbow trout (Figure 2.7). Domestic fish size distribution was approximately normal, while wild mass distribution was skewed to the right (a greater proportion of individuals were greater than the median value of 13.8 g). In addition to size, morphological differences were observed between strains (Figure 2.5). The domestic strains possess a deeper profile with a smaller head compared to body size. Wild rainbow trout had a 'streamlined' profile with the presence of parr markings that were not as evident in the domestic strain. Significant differences between strains over time were also observed for fork length (mm) (ANOVA, $F_{(1,4)} = 152.07$, $P < 0.001$) and condition factor ($g \cdot mm^{-3} \cdot 100000$) (ANOVA, $F_{(1,4)} = 477.59$, $P < 0.001$) (Table 2.1). Standardized growth metrics based on mean tank mass were greater

for domestic strain fish than wild. Total TGC was greater in domestic fish (1.48) than wild (0.67) and SGR was also greater in domestic fish than wild (3.47 and 2.70, respectively) (Table 2.2).

It is important to recall that temperature steadily declined during the experiment (Fig 2.3). As a result of cold water temperatures, I was not able to fully assess differences in growth potential between rainbow trout strains in this study because the thermal environment in the experimental tanks was not ideal for rainbow trout growth. To overcome this limitation, I used the calculated TGC coefficients from the study to assess growth of wild and domestic fish based on a scenario in which temperature remained constant throughout the duration of the experiment. Maintaining a static 12.7°C (average t_0 temperature) throughout the experiment resulted in an additional 18.07 g and 4.62 g increase in average mass for domestic and wild strains, respectively (Figure 2.8). Thus, had this growth trial been conducted under a constant water temperature of 12.7°C instead of the observed declining water temperature, domestic rainbow trout would have achieved a final size of 49.77 g, roughly 2.8-fold greater than wild fish (21.12 g). Therefore, the growth divergence between the domestic strain relative to the wild is not maximized to its full potential, as a result of sub-optimal water temperatures in this experiment.

By fitting the BM to the measured values for the daily consumption of feed, initial and final weight and daily temperature, allometric growth coefficients (*RA*) for each strain were calculated (Table 2.3). The *RA* for domestic (0.004916) and wild strains (0.0039811) were found to be different from each other and from the

default measure used in the BM (0.00264). The optimized RA values more accurately predicted observed final mass of domestic and wild rainbow trout by 18.5% and 15.4%, respectively. Using these calculated *RA* values based on known input parameters from the satiation growth trial, I compared observed versus modeled consumption rates. Although total modelled consumption is equal to observed, the model tends to greatly overestimate initial consumption and consistently underestimate consumption for the latter two-thirds of the experiment (from day 30 onwards; Figure 2.9). In addition, inconsistencies between modeled and observed estimates of consumption were greater for domestic relative to wild rainbow trout. Using these optimized parameters for each strain, calculated coefficients can then be applied to estimate relative strain consumption in a field study.

2.3.3.2 Treatment 2

Similar to the treatment 1, where fish were fed to satiation, rainbow trout fed a reduced ration (1.2% of body weight) grew steadily throughout the 102 day trail. Under the competitive environment of treatment 2, domestic strains achieved 2.16 times the mass of wild strain fish at the end of the experiment. On average, domestic and wild strains increased their mass by 22.38 and 6.85 g, respectively (Figure 2.10). Domestic fish retained size advantages in mass (g) (ANOVA, $F_{(1,4)} = 463.91$, $P < 0.001$), fork length (mm) (ANOVA, $F_{(1,4)} = 376.55$, $P < 0.001$) and condition factor ($g \bullet mm^{-3} \bullet 100000$) (ANOVA, $F_{(1,4)} = 327.53$, $P < 0.001$) relative to wild conspecifics (Table 2.4). The CV for mass increased over the duration of the experiment, and was consistently greater in wild strains. At the end of the

experiment, CV in wild strains was roughly one-third greater (45% ± 11) than for domestic strains (33% ± 9) (Figure 2.11). Domestic fish in treatment 2 also had a greater range in size distribution relative to wild fish (Figure 2.7). The distribution of sizes for domestic fish was approximately normally distributed where wild mass was right-skewed Standardized growth metrics were also greater in domestic than wild rainbow trout. Total TGC was greater for domestic fish than wild (1.29 and 0.54, respectively) and SGR was greater in domestic tanks than wild tanks (3.35 and 2.58, respectively) (Table 2.5).

2.3.4 Comparison of growth between treatments

Growth trajectories of domestic and wild rainbow trout strains in treatment 2 (Figure 2.10) followed a similar pattern to that in treatment 1 (Figure 2.4). Domestic fish fed to satiation in treatment 1 only achieved an average final mass 3.75 g greater than those in treatment 2, and was significant over time between treatments (ANOVA, $F_{(1,4)} = 19.44$, $P < 0.001$). Wild strain fish in treatment 1 also achieved a significantly greater mass (1.64 g) than fish in competition (ANOVA, $F_{(1,4)} = 9.42$, $P < 0.01$). However, the interaction term between treatments was not significant over time (ANOVA $F_{(1,4)} = 0.70$, $P = 0.60$). The greatest change in growth between treatments occurs at day 25 and was maintained throughout the experiment (Figure 2.12). This suggests that after the initial acclimation to each treatment, growth trajectories maintained similar slopes until the final measurement event.

2.3.5 Feed consumption

Daily feed intake as a percentage of body weight was greatest in treatment 1 at the beginning of the experiment for both strains (Figure 2.13). Percentage feed intake per body weight decreased gradually throughout the duration of the experiment, but domestic fish consistently had a higher percentage of feed intake than wild fish. At approximately day 75 in domestic tanks and day 50 in wild tanks, treatment 1 fish began to have equal or less feed intake than treatment 2, which was set at a fixed rate of 1.2% per day.

Feed conversion ratio (FCR) was consistently lower in domestic strains of rainbow trout than wild strains throughout the experiment (ANOVA, $F_{(1,3)} = 12.95$, $P < 0.001$), meaning that domestic strains gained more mass per unit of feed than wild fish (Figure 2.14). Total feed conversion ratio was 0.97 ± 0.02 g • g^{-1} in domestic tanks and 1.24 ± 0.24 g • g^{-1} in wild tanks, with wild strains having much higher variability compared to domestics. Time effect was insignificant, which is likely attributed to domestic FCR remaining constant over time. Wild strain fish in this study required 22% more feed to obtain the same increase in mass over time as a domestic fish. The divergence in FCR between strains was its greatest at days 50-75. At this time interval, wild rainbow trout would need ~40% more feed to achieve the same mass as domestics under these conditions. Additionally, domestic fish groups consistently consumed a greater mass of feed in total than wild tanks during the entire experiment in treatment 1 (Figure 2.15).

2.4 Discussion

Salmonids selectively bred for marine aquaculture are generally known to have greater growth rates than wild conspecifics. Comparatively little work has been done to assess strains that are commercially important to freshwater aquaculture. Here, I conducted a set of laboratory experiments to specifically quantify the divergence in growth between a domestic and wild strain of rainbow trout that are commonly found sympatrically in the LGL. The "Ganaraska" strain has been naturalized in Lake Huron since the late 1800's, while the aquaculture strain is reared in open-pen farms on the same lake. Escape events from these aquaculture farms make the interactions between these two genotypes inevitable and their comparative growth under controlled conditions relevant to my study. I intended to quantify the growth rate of each strain, fed to satiation, reared in strain-specific isolation and compare these results to a treatment involving an inter-strain competitive environment.

2.4.1 Growth

2.4.1.1 Optimal conditions

I found that under optimal conditions, the domestic strain of rainbow trout used in commercial aquaculture in the LGL (i.e., Lake Huron) had 3-fold greater growth than wild fish and was able to achieve a final mass of over twice that of the wild strain at the conclusion of the experiment. Superior growth in domesticated salmonids compared to wild conspecifics is generally in agreement with other lab-based growth-trials (see Table 1.1). Recalling that the phenotypic states of salmonids are plastic and scale on a continuum, the growth results in these

experiments have the potential to be more divergent than experiments that do not incorporate strains that are specifically bred for maximal size. Many studies compare strains that may be considered, "domestic" but are intended for hatchery and recreation rather than commercial industry. The variability in growth between these LGL strains is not exempt from this phenotypic continuum. Although the domestic strain has superior growth compared to the wild conspecifics in this experiment, they also retain less variability. This difference in mean mass deviation is reflected in the CV of mass over time between the strains. Variation in mass among wild fish steadily increases over time while the domestic strain remains relatively stable (Figure 2.6). This result seems to be intuitive. Domestic strains, selectively bred for aquaculture, are shown to have reduced heterogeneity in culture due to generations of selective breeding (Clifford et al. 1998; Overturf et al. 2003; Skaala et al. 2005). Domestic strains should have a parallel phenotypic response to culture rearing as a consequence of enhancing the genotype for this purpose. Wild strains conversely, have a more varied phenotypic response to a culture environment. Because selection of a specific trait is not perpetuated within the wild genotype, we tend to see greater divergence in growth rates relative to domesticated strains selected for growth. Inter-population genetic variation can be explained by the increased heterogeneity of wild genotypes of salmonids to increase growth rates.

Rainbow trout typically experience optimal growth at 16 - 18º C (Javaid and Anderson 1967; Dickson and Kramer 1971; McCauley and Pond 1971; Hokanson et al. 1977; Wurtsbaugh and Davies 1977; Papoutsoglou and Papaparaskeva-

Papoutsoglou 1978; Austreng et al. 1987). Temperature highly influences growth rates in salmonids and teleosts in general through metabolism (Cho 1992; Jobling 1994; Azevedo et al. 1998; Bureau et al. 2000). In Treatment 1 of my experiment, domestic rainbow trout achieved twice the mass of wild strains within a period of ~3 months. However, by the end of the experiment, water temperatures had decreased to ~6°C following a pattern of ambient water temperature in Lake Superior, well below optimal for growth. I used the thermal growth coefficients (TGC) calculated from the satiation growth trials for each strain and applied them to a model in which initial temperature (12.7°C) was maintained throughout the entire 102 days. Under this modeled temperature scenario, domestic and wild strains have the potential to increase their growth in mass by an additional 36% and 22%, respectively, compared to observed values (Figure 2.8). It would seem that based on this model, the domestic strain of rainbow trout used in these experiments would continue to experience exponential growth increases at incremental temperature rises. In contrast, the wild strain appears to be closer to its maximum growth potential under both conditions. Most comparative studies examining wild and domestic salmonid growth demonstrate that divergence in growth trajectories are ~1-2-fold (see Table 1.1). Data from the TGC model suggest that the potential for growth divergence between the two strains from my experiments can be up to 3-fold at a temperature of 13°C. The divergence in growth would potentially be maximized at optimal temperatures for growth (16-18°). Therefore, higher quality food sources and warmer temperature regimes in a

whole-lake experiment are expected to be exaggerated from results elicited from this growth trial.

Another consideration to the impedance of growth may be the time of year this experiment was conducted (October - January). Typically, temperate species of fish will begin to shift energy allocation towards fat storage and away from somatic growth, reduce activity and optimize metabolism expenditure in the fall of each year in preparation for winter (Post and Parkinson 2001; Biro et al. 2004b, 2005). It is possible that rainbow trout in this experiment may have experienced behaviour similar to energy sequestering observed in a natural environment, especially the wild strain. It has been suggested that colder water temperatures induce a strong suppression of the growth hormone – insulin-like-growth factor (GH-IGF-I) system (McCormick et al. 2000; Agustsson et al. 2001), which has limited growth in other salmonid growth trials (Neregard et al. 2008). Coupled with temperature patterns, photoperiod is an additional environmental factor that may be confounding observed patterns of fish growth. Because photoperiod was kept at a consistent 12:12 h throughout the experiment, it is possible that the fish were unable to establish realistic diel cycles that would be common in the wild. Establishment of natural cycles of light to dark that is consistent with those that coincide with seasonal changes are important in determining how fish hormone levels react. For example, secretion of growth hormone (GH) and feeding demand is strongly correlated with duration and timing of photoperiod (Boujard and Leatherland 1992; Björnsson et al. 1995; Boeuf and Bail 1999). Controlling for a

more realistic day to night ratio in the future may help to elicit results closer to that which would be observed in nature.

In addition to greater growth and size through the experiment, the domestic rainbow trout also maintained consistently greater condition (K) at every interval of the experiment (Table 2.1). This is not a surprising result considering that the domestic strain has been bred to accumulate mass in a short period of time to be successful in aquaculture. Furthermore, statistical differences between strains in terms of length and mass were seen after the first 25 days of the experiment (Student's one-tailed t-test, t-stat = 2.13, $P < 0.005$). Condition factor also provides a good indication of body morphology between the two strains. A greater value of K denotes enhanced body depth (i.e. greater mass) compared to fork length, common to the domestic strain. Conversely, the wild strain is characterized by a more "streamlined" profile (Figure 2.5). Interestingly, increased condition seems to be solely from the processes of domestication and not through anthropogenically enhanced growth hormone. Several studies demonstrate that an increase in IGF-I as a result of either GH injection or genetic engineering in salmonids stimulates a response decreasing condition factor (McLean et al. 1997; Devlin et al. 2001), as opposed to what is observed in the domestic strain from my experiments.

2.4.1.2 Competitive environment/comparison to treatment 1

Comparatively fewer studies have been conducted on quantifying the growth of domestic salmonids under inter-strain competitive rearing environments, as

opposed to those in which one strain of fish is grown under optimal conditions. The original hypothesis that growth divergence would be greater under competition (reduced feed) than optimal conditions did not hold true for these experiments. Instead, I observed that domestic strains maintained a roughly 3-fold growth advantage over the wild strains, as in treatment 1, and achieved a final mass about twice that of wild fish. Growth trajectories of domestic and wild strains were approximately similar in both treatments and varied at most by 15% (day 75) and 13% (day 50), respectively (Figure 2.12). The lower growth observed in treatment 2 was presumably driven by reduced feed and was not greatly influenced by competition between strains. By the first measuring period (day 25) mass of wild rainbow trout was ~12% greater in the satiation trial and was consistent for the entire experiment. Additionally, the domestic strain had a greater mass difference between the wild strain at every interval. Findings from this inter-strain competition treatment seem to be in agreement with similar studies. Tymchuk et al. (2007) found that domestic rainbow trout were able to maintain their growth advantage over wild conspecifics reared under both optimal and competitive conditions. Furthermore, SGR ratios between domestic and wild strains were approximately the same for the two environments, similar to the findings of this study. Likewise, no evidence of inter-population competition affecting growth rates in farmed versus wild Atlantic salmon in mixed groups was detected in one experiment (Fleming et al. 2002). It seems that it may be difficult to truly elicit competitive interactions between two conspecific strains under hatchery conditions. However, some studies have reported that particular strains

of domestic Atlantic salmon do exhibit a statistically significant depression in growth performance under wild competition in hatchery compared to controls (Einum and Fleming 1997; Fleming and Einum 1997). Results show that size advantage was maintained for domestic strains in hatchery but not in semi-natural conditions. This finding seems to support the hypothesis that varying genotypes of salmonids will respond plastically to changes in environmental conditions (i.e. wild strains may display greater growth under natural conditions; see Sundtröm et al. 2007).

2.4.2 Feed consumption

Comparing feeding efficiency among groups of fish is important in determining the mechanisms in which growth rates depend upon. All else being equal, the quantity of feed consumed in controlled laboratory experiments should be a good predictor of fish growth. In addition, quantifying food consumption in the present study allowed me to assess feeding efficiency as a feed conversion ratio (FCR). A possible reason for the lack of difference in growth trajectories between treatments is that feeding rate in the competition trial (treatment 2) was too great to induce competitive dominance of one strain over the other. Initially, the 1.2% of average body weight daily ration seemed to be an adequate reduction in feed, as amount of feed as a percentage of body weight was 1.5 – 3% lower in treatment 2 compared to treatment 1 (Figure 2.13). However, towards the end of the experiment, treatment 2 tanks were actually receiving a greater amount of feed than the satiation treatment. As noted earlier, steady declines in water temperature as the study progressed, reduced consumption rates by fish to the point where

satiation feeding fell below 1.2%. Perhaps food was not a limiting resource in this scenario, reducing the occurrence of inter-strain competitive interactions between strains. This outcome has been noted in other salmonid growth trial studies. In experiments involving mixed transgenic/wild coho salmon (*Oncorhynchus kisutch*), the absence of competitive interactions has been attributed to food being supplied at a high rate and all fish receiving equal access to feed (Tymchuk et al. 2005). Future studies would benefit from quantifying differences in mixed tanks with varying amounts of feed, to see at what level, feed amount produces a significant competitive response to growth. In my experiment, it is noteworthy that rainbow trout in treatment 1 exhibited greater variation in growth compared to treatment 2 (Figure 2.6, 2.11). This relates back to inter-strain populations having the ability to show a greater growth expression and spread among individuals with the opportunity to consume more food in the satiation treatment.

The domestic strain was more efficient at converting feed into mass gain than the wild strain (Figure 2.14). The higher FCR in domestic versus wild fish can be explained by farmed rainbow trout being artificially selected for this trait. Based on these results, it requires ~25% more feed for wild rainbow trout to grow the same size as domestics. Increased feed efficiency has also been found to be greater in domestic Atlantic salmon (Thodesen et al. 1999; Grisdale-Helland and Helland; Handeland et al. 2003), but not in brown trout (*Salmo trutta*) (Sanchez et al. 2001; Mambrini et al. 2004). This is likely due to Atlantic salmon having a more intensive history of genetic selection relative to brown trout. The lowest FCR values for both strains in my experiments occurred at an average temperature

of 10.4°C among tanks for the first 56 days, yet were highly variable throughout the experiment (Figure 2.14). Although metabolism and feeding rate peak at temperatures around 15-18°C for rainbow trout, feed efficiency does not seem to correlate with temperature (Alanara 1994; Azevedo et al. 1998; Bureau et al. 2000; Valente et al. 2001). This finding is in contrast to Atlantic salmon, for which 10°C has been reported as the optimal temperature for feed conversion efficiency (Thodesen et al. 1999; Handeland et al. 2003). Much research attributes faster growth in selected over the wild strains simply to the mass of feed consumed and not feed efficiency. In contrast, the domestic strain used in my experiments would have a two-fold advantage over wild conspecifics: they have greater feed efficiency and consume a greater mass of feed pellets (Figure 2.15).

2.4.3 Modeling predictions

Modeling energy budget data can be a useful approach to differentiating bioenergetic strategies between conspecfic strains of fish. Data collected from these growth trials allowed for calculation of coefficients that are specific to each strain of rainbow trout. The intercept of the allometric mass function (*RA*), may be adjusted to fit observed consumption in the experiment. The *RA* coefficient is important in determining the degree of energy that is retained for growth versus that which is eliminated from an organism, through respiration. The *RA* values for domestic and wild (0.0049 and 0.0038, respectively) were greater than the original calculated literature value of 0.0026 for rainbow trout (Rand et al. 1993). By adjusting the value for respiration, the model more accurately reflects the observed consumption in my experiment. Other researchers have also adjusted

respiration rates within bioenergetics equations to achieve more reliable results for rainbow trout (Railsback and Rose 1999; van Poorten and Walters 2010). Parameters of respiration in the Wisconsin model based on laboratory trials may be considered incomplete and were only calibrated using tank temperatures of 5°C and 15°C (Rao 1968, 1971). In revising the BM in these experiments, the parameters are optimized to predict total consumption and final weight at a given temperature and are more sensitive to individual physiological strain differences, which will be critical when estimating consumption in the field component of this thesis (Chapter 3). This is achieved by using the individual *RA* functions that are theoretically environment independent so that bioenergetics modeling of each strain for the field component will more precisely predict energy elimination and retention through this respiration term.

2.4.4 Conclusions and recommendations

I have demonstrated that wild and domesticated rainbow trout common to the same water body (Lake Huron) possess the potential to have extremely different growth rates under identical culture conditions. When separated into treatments for optimal growth and competitive environments, much of the same growth rates are maintained between treatments for both strains. Domestic rainbow trout grew 3-fold faster than wild conspecifics and achieved a final mass ~2 times that of wild conspecifics, both when fed to satiation and under competition in mixed tanks that involved a reduction of feed. Several main conclusions can be drawn from this experiment: (1) domestic strains of rainbow trout are able to attain over twice the size of wild strains under optimal conditions; (2) based on model

predictions, growth rates appears to increase with temperature for domestic, but not as much for wild strains and the divergence has the potential to be greater at optimized temperatures (16-18°C) and; (3) domestic strains of rainbow trout achieve greater size through the combined effect of increased consumption and higher conversion efficiency of food to mass, relative to wild strains.

A recommendation for future studies would be to increase the statistical power of the experiment. Because the rainbow trout being measured in culture for this experiment are the sampling units, and each tank is considered to be the experimental unit, each treatment has only n = 3 to draw conclusions from. By increasing the number of tanks, the robustness of the statistical analysis will be enhanced. Another consideration would be to increase the number of treatments, if logistically possible. Observations from this experiment indicate that temperature and amount of feed are important variables in determining the overall outcome of the tests. It would be interesting to quantify the differences in growth if trials were conducted at varying temperature and feeding regimes. Finally, results from growth trials of this nature are very dependent on the strains used. This study had the advantage of using strains that are commonly found in sympatry in nature. It would be of value to observe if strains of rainbow trout from various aquaculture suppliers, regions or even different species of salmonids would respond similarly to the treatments applied in these rainbow trout.

Table 2.1 Size, condition and variation in mass of wild and domestic strains of rainbow trout fed to satiation (treatment 1). Data represent $\bar{x}$ ± 1 S.D. from three replicate tanks (n = 3) each containing 30 fish.

Strain	Time (d)	Mass (g)	Fork Length (mm)	Condition Factor ($g \cdot mm^{-3} \cdot 100000$)	Coefficient of Variation (mass)
Wild	0	6.6 ± 0.17	84.7 ± 0.73	1.05 ± 0.01	28.3 ± 2.5
	25	8.9 ± 0.77	91.9 ± 2.24	1.11 ± 0.03	35.1 ± 6.1
	50	11.5 ± 1.57	99.9 ± 4.05	1.08 ± 0.03	40.8 ± 6.3
	75	13.4 ± 2.23	103.6 ± 4.92	1.13 ± 0.02	43.6 ± 7.2
	100	15.1 ± 2.57	108.4 ± 6.02	1.09 ± 0.01	48.2 ± 8.1
Domestic	0	6.6 ± 0.08	80.0 ± 0.42	1.27 ± 0.01	28.3 ± 2.0
	25	11.6 ± 0.22	95.7 ± 0.95	1.29 ± 0.02	32.4 ± 4.3
	50	18.8 ± 0.68	114.0 ± 1.20	1.23 ± 0.02	33.7 ± 4.5
	75	25.0 ± 1.22	125.5 ± 1.23	1.23 ± 0.02	34.9 ± 3.1
	100	32.9 ± 1.52	138.2 ± 1.58	1.21 ± 0.03	34.7 ± 4.2

Table 2.2 Standardized growth and feed efficiency measurements of wild and domestic strains of rainbow trout fed to satiation (treatment 1); TGC = thermal growth coefficient (multiplied by 1000 to bring to unity); SGR = specific growth rate; FCR = feed conversion ratio. FCR data represent the $\bar{x}$ ± 1 S.D. All data is calculated from three replicate tanks (n = 3) each containing 30 fish. Total values are calculated from measurements taken on day 0 and day 102 of the experiment.

Strain	Time (d)	TGC x 1000	SGR	FCR
Wild	0 - 25	0.64	2.12	1.15 ± 0.21
	25 - 50	0.66	2.36	1.16 ± 0.31
	50 - 75	0.77	2.48	1.17 ± 0.23
	75 - 100	0.51	2.62	1.63 ± 0.18
	Total ($t_0 - t_{102}$)	0.67	2.70	1.24 ± 0.24
Domestic	0 - 25	1.26	2.38	0.96 ± 0.08
	25 - 50	1.47	2.84	0.94 ± 0.05
	50 - 75	1.62	3.08	0.99 ± 0.03
	75 - 100	1.54	3.38	1.04 ± 0.04
	Total ($t_0 - t_{102}$)	1.48	3.47	0.97 ± 0.02

Table 2.3 Input parameters for bioenergetics model.

Parameter	Description	Value	Units
Consumption Equation 3 [1]			
CA	Intercept of allometric mass function	0.628	$g \cdot g^{-1} \cdot d^{-1}$
CB	Slope of the allometric mass function	-0.3	
CQ	Temperature for CK_1	5	°C
CTO	Temperature for 0.98 of C_{max} on increasing curve	20	°C
CTM	Temperature for 0.98 of C_{max} on decreasing curve	20	°C
CTL	Temperature at which dependence is CK_4	24	°C
CK_1	Small fraction of maximum rate	0.33	
CK_4	Reduced fraction of maximum rate	0.20	
E_p	Prey energy density	19800	$J \cdot g^{-1}$
Respiration Equation 1 [2]			
RA	Intercept of the allometric mass function	0.004916* 0.003811†	$g \cdot O^2 \cdot g^{-1} \cdot d^{-1}$
RB	Slope of the allometric mass function	-0.217	
RQ	Approximates the Q_{10} (rate of increase in low temperatures)	0.06818	$°C^{-1}$
RTO	Coefficient for swimming speed dependence on metabolism	0.0234	$s \cdot cm^{-1}$
RTM	Lethal water temperature	0	°C
RTL	Cut-off temperature at which activity relationship changes	25	°C
RK_1	Intercept for swimming speed above RTL	1	$cm \cdot s^{-1}$
RK_4	Coefficient of mass-dependence on all swimming speeds	0.13	
ACT	Intercept for swimming speed - water temperature function < RTL	9.7	$cm \cdot s^{-1}$
BACT	Coefficient of swimming speed on water temperature dependence < RTL	0.0405	$°C^{-1}$

Table 2.3 (continued) Input parameters for bioenergetics model.

SDA	Assimilated energy lost to specific dynamic action	0.172
Egestion/Excretion Equation 3 [2]		
FA	Intercept of the proportion of consumed energy egested vs. water temperature	0.212
FB	Coefficient of water temperature dependence on egestion	-0.222
FG	Coefficient of feeding level dependence of egestion	0.631
UA	Intercept of the proportion of consumed energy excreted vs. water temperature	0.0314
UB	Coefficient of water temperature dependence on excretion	0.58
UG	Coefficient of feeding level dependence of excretion	-0.299

All equations were taken from Hanson et al. 1997. * RA used for domestic tanks; † RA used for wild tanks. [1] Thornton and Lessem 1978 [2] Stewart et al. 1983.

Table 2.4 Size, condition and variation in mass of wild and domestic strains of rainbow trout fed a reduced ration and reared in same tanks (treatment 2). Data represent the $\bar{x}$ ± 1 S.D. from three replicate tanks (n = 3) each containing 15 fish.

Strain	Time (d)	Mass (g)	Fork Length (mm)	Condition Factor ($g \cdot mm^{-3} \cdot 100000$)	Coefficient of Variation (mass)
Wild	0	6.6 ± 0.11	84.8 ± 0.41	1.06 ± 0.03	29.3 ± 3.2
	25	7.9 ± 0.38	88.9 ± 0.43	1.08 ± 0.05	32.5 ± 5.8
	50	10.0 ± 0.64	95.2 ± 1.10	1.11 ± 0.01	36.5 ± 9.1
	75	11.7 ± 1.27	99.8 ± 2.49	1.12 ± 0.01	39.4 ± 10.9
	100	13.5 ± 1.84	105.0 ± 3.10	1.08 ± 0.02	45.3 ± 11.2
Domestic	0	6.7 ± 0.16	80.5 ± 0.18	1.26 ± 0.03	27.6 ± 4.2
	25	10.2 ± 0.32	92.2 ± 0.59	1.27 ± 0.04	31.5 ± 5.4
	50	16.0 ± 0.59	108.6 ± 1.62	1.22 ± 0.02	30.7 ± 5.8
	75	21.2 ± 0.60	119.3 ± 1.18	1.21 ± 0.03	31.1 ± 7.1
	100	29.1 ± 0.71	133.7 ± 1.86	1.17 ± 0.03	33.1 ± 9.0

Table 2.5 Standardized growth and feed efficiency measurements of wild and domestic strains of rainbow trout fed to satiation (treatment 1); TGC = thermal growth coefficient (multiplied by 1000 to bring to unity); SGR = specific growth rate. Data is calculated from three replicate tanks (n = 3) each containing 30 fish. Total values are calculated from measurements taken on day 0 and day 102 of the experiment.

Strain	Time (d)	TGC x 1000	SGR
Wild	0 - 25	0.35	1.99
	25 - 50	0.34	2.22
	50 - 75	0.24	2.35
	75 - 100	0.17	2.51
	Total ($t_0 - t_{102}$)	0.54	2.58
Domestic	0 - 25	0.90	2.25
	25 - 50	0.73	2.68
	50 - 75	0.50	2.92
	75 - 100	0.50	3.26
	Total ($t_0 - t_{102}$)	1.29	3.35

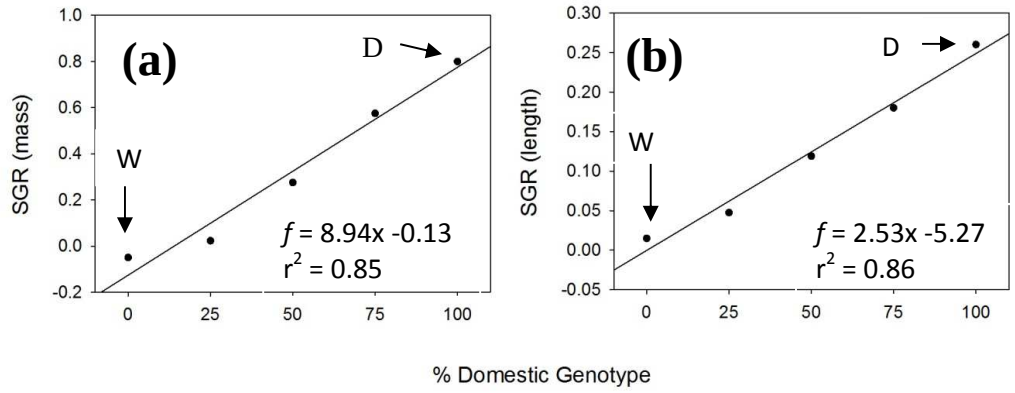

Figure 2.1 Specific growth rate (SGR) of pure wild (W), pure domestic (D) and hybrid rainbow trout as measured by mass (a) and length (b) as a function domestic alleles (%) possessed by the strain. Modified from Tymchuk and Devlin (2005).

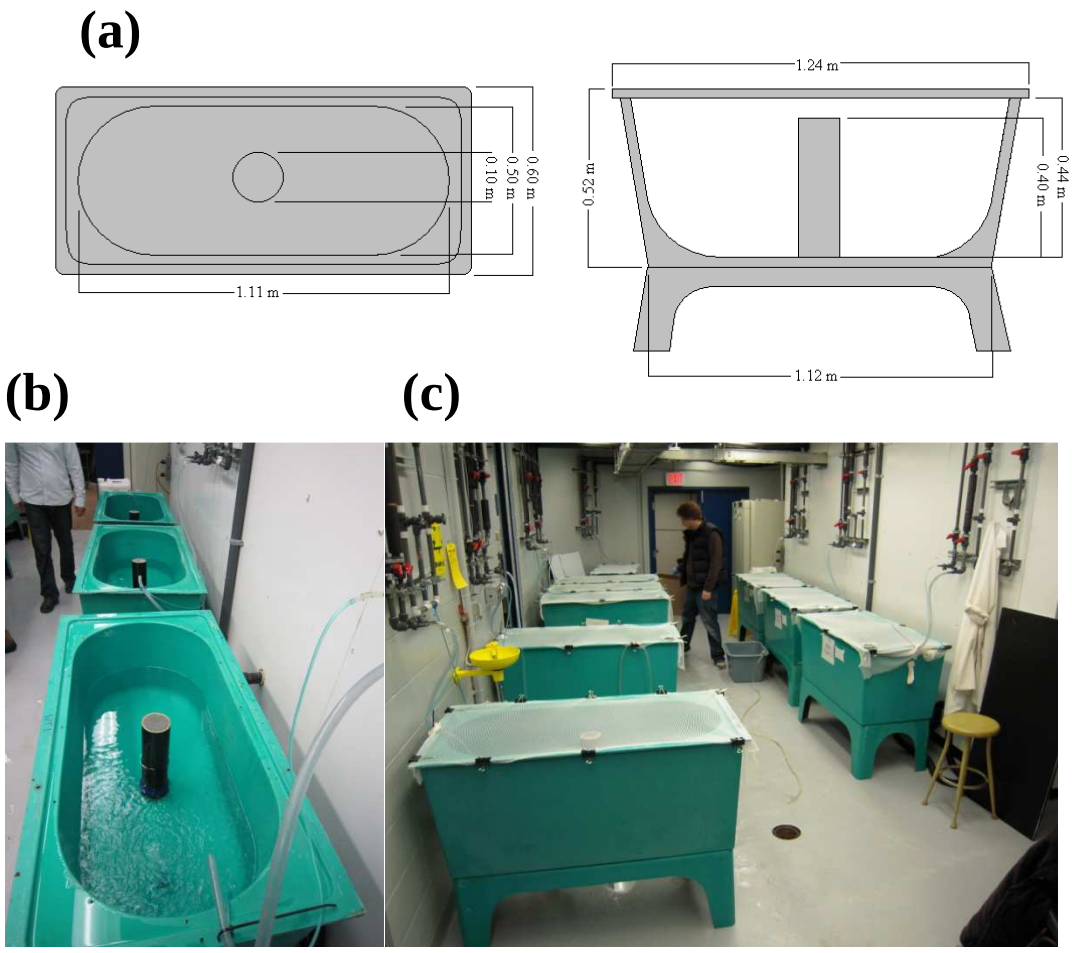

Figure 2.2 (a) Schematic and measurements of fiberglass, 160 L flow-through tank used in growth trial experiments, (b) close-up of flow-through system and (c) arrangement of tanks in experimental area.

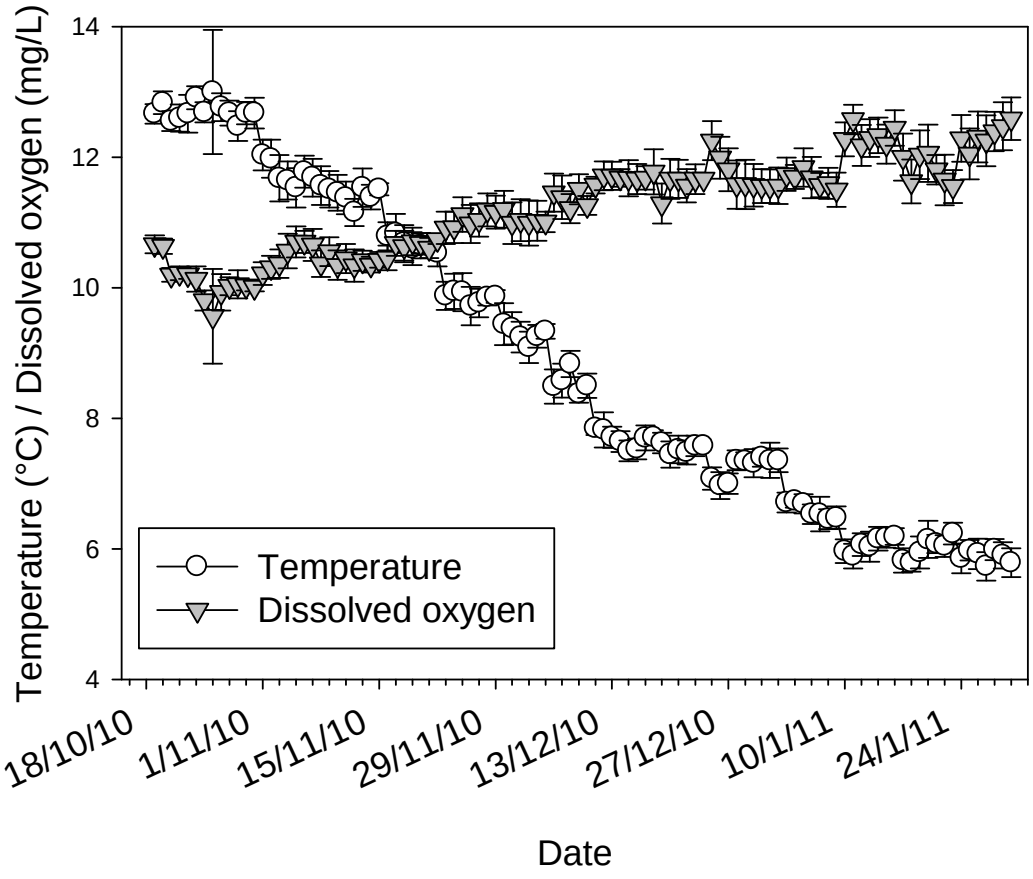

Figure 2.3 Tank values ($\bar{x}$ ± 1 S.D.) of daily water temperature and dissolved oxygen. Averages include all tanks in both treatments (n = 9).

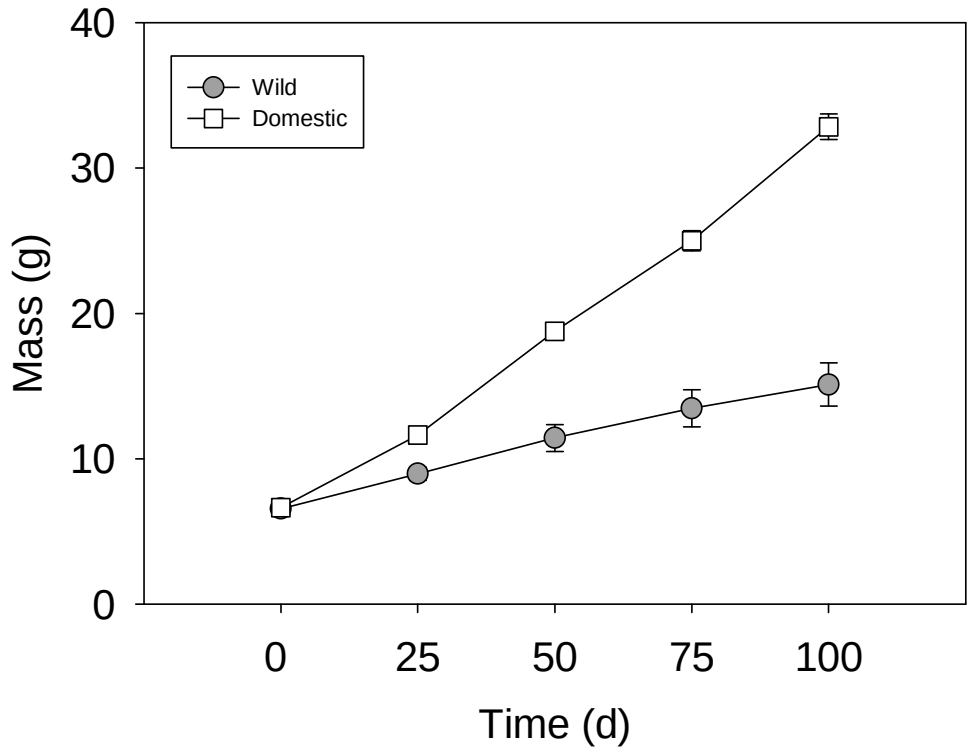

Figure 2.4 Mean body mass (g) of wild and domestic rainbow trout from three replicate tanks (n=3) fed to satiation daily over 102 days (treatment 1); $\bar{x}$ ± 1 S.E.

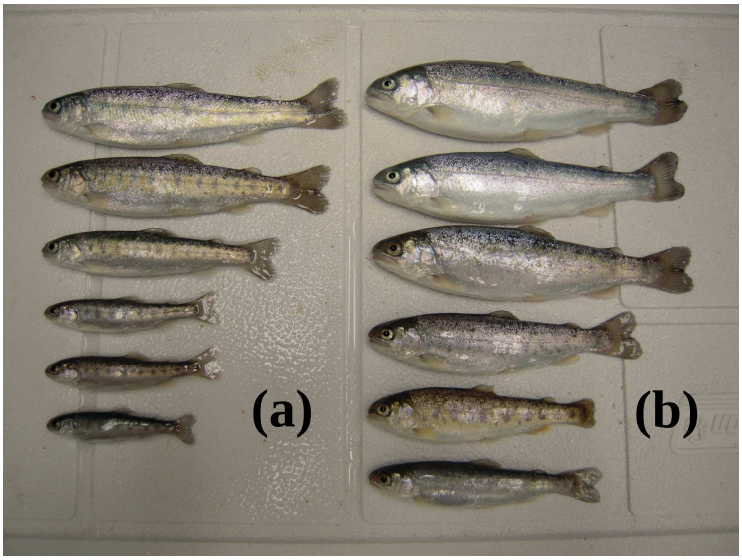

Figure 2.5 Size-range from biggest to smallest wild (a) and domestic (b) rainbow trout fed to satiation daily (treatment 1) at the end of experiment (102 days). All individuals shown were obtained from same tank for that strain.

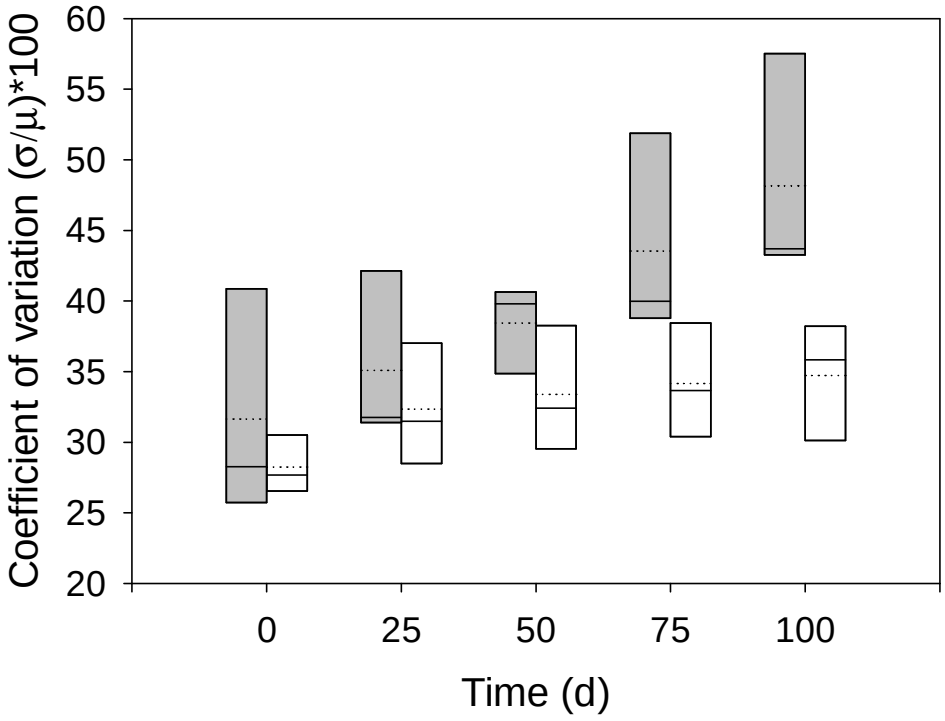

Figure 2.6 Coefficient of variation (CV) of individual mass (g) of wild (grey) and domestic (white) strains of rainbow trout fed to satiation (treatment 1). The lower and upper boundary of the box represents the 25th and 75th percentiles, respectively. The solid line within the box marks the median value and the dotted line represents the mean of three replicate tanks (n = 3).

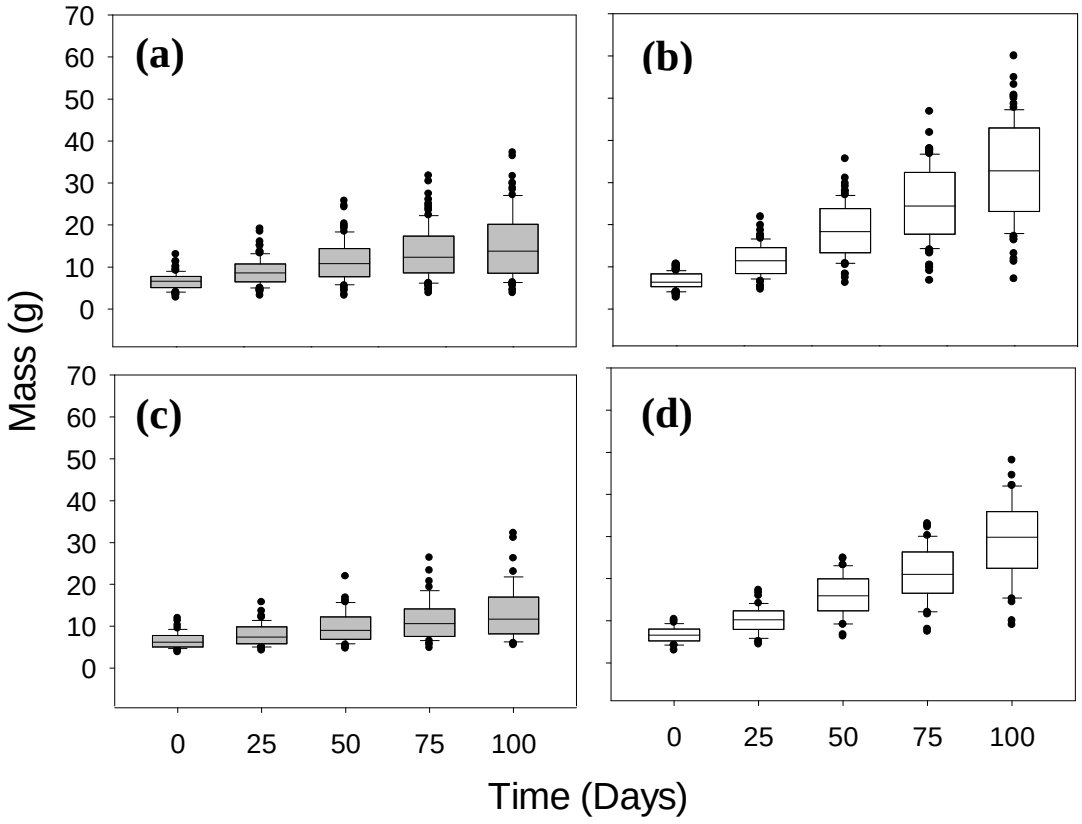

Figure 2.7 Range in mass of wild (a) and domestic (b) strains of rainbow trout fed to satiation (treatment 1) and wild (c) and domestic (d) strains of rainbow trout fed a reduced ration (treatment 2). The lower and upper boundary of the box represents the 25th and 75th percentiles, respectively. The line within the box marks the median value. The error bars above and below the box represent the 90th and 10th percentiles, respectively. The dots represent outliers greater or lesser than the error bars. Individual fish mass are used as data points (treatment 1: 90; treatment 2: 45).

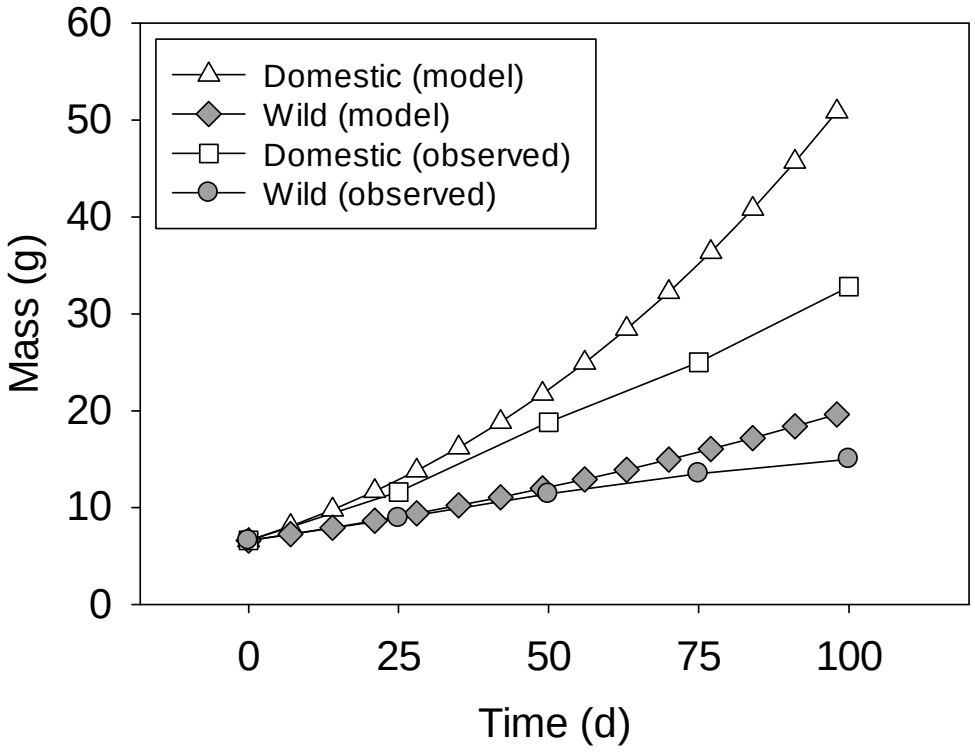

Figure 2.8 Comparison of observed versus modeled growth based on calculated thermal growth coefficient (TGC) values from wild and domestic strains of rainbow trout fed to satiation (treatment 1). Modeled growth curve was based on a constant temperature of 12.7°C (average t_0 temperature).

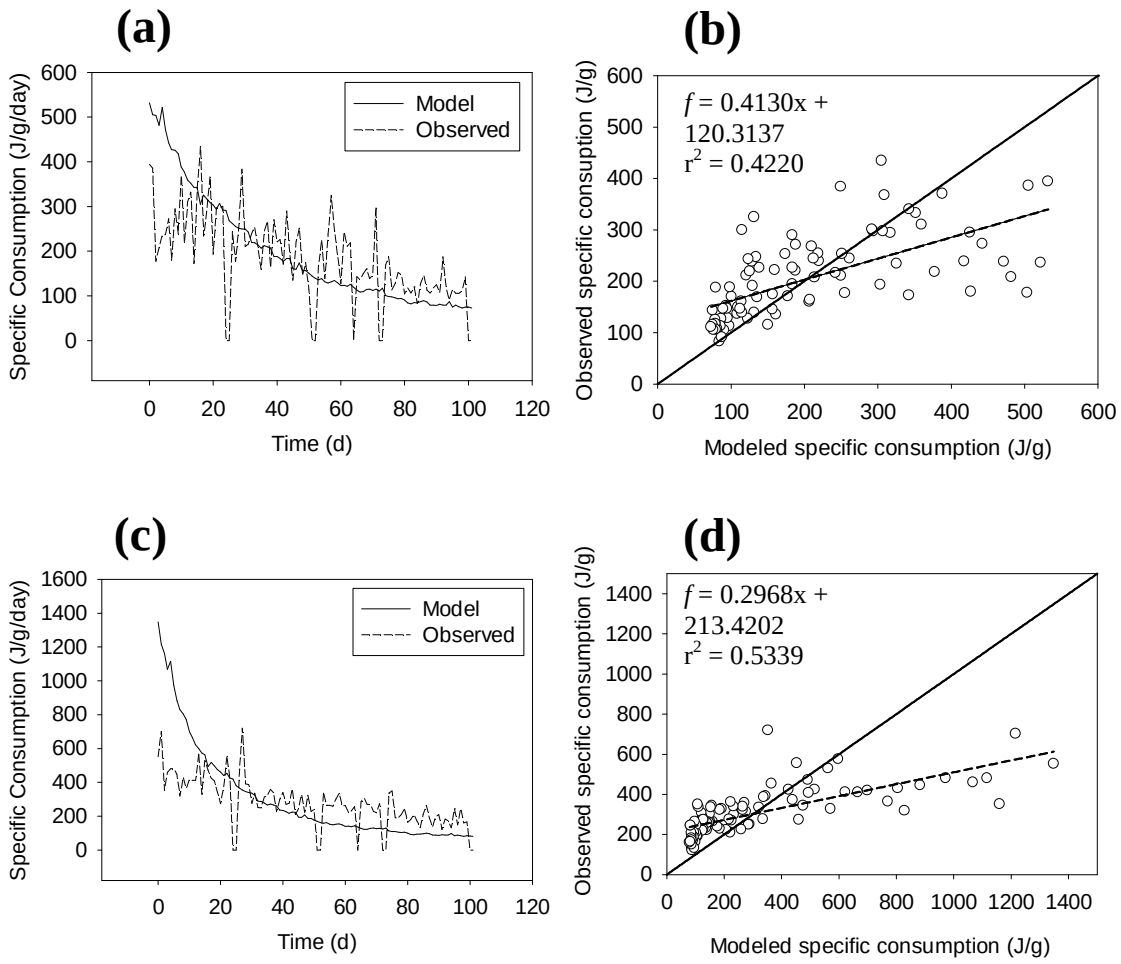

Figure 2.9 Modeled consumption from a bioenergetics model (BM) with optimized strain specific allometic mass functions (*RA*) comparing observed consumption of wild (a) and domestic (b) rainbow trout and strains fed to satiation (treatment 1) over time and wild (c) and domestic (d) observed versus modeled consumption compared to a 1:1 line.

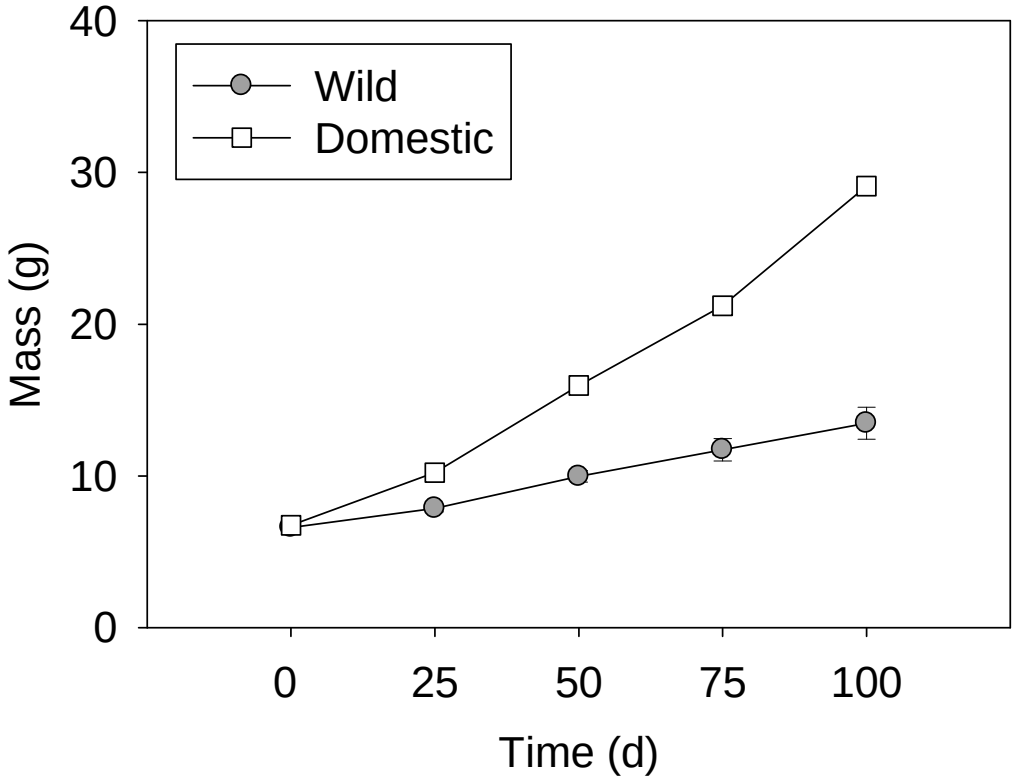

Figure 2.10 Mean body mass (g) of wild and domestic strain rainbow trout fed a reduced ration of feed (1.2% body weight) over 102 days (treatment 2); $\bar{x}$ ± 1 S.E.

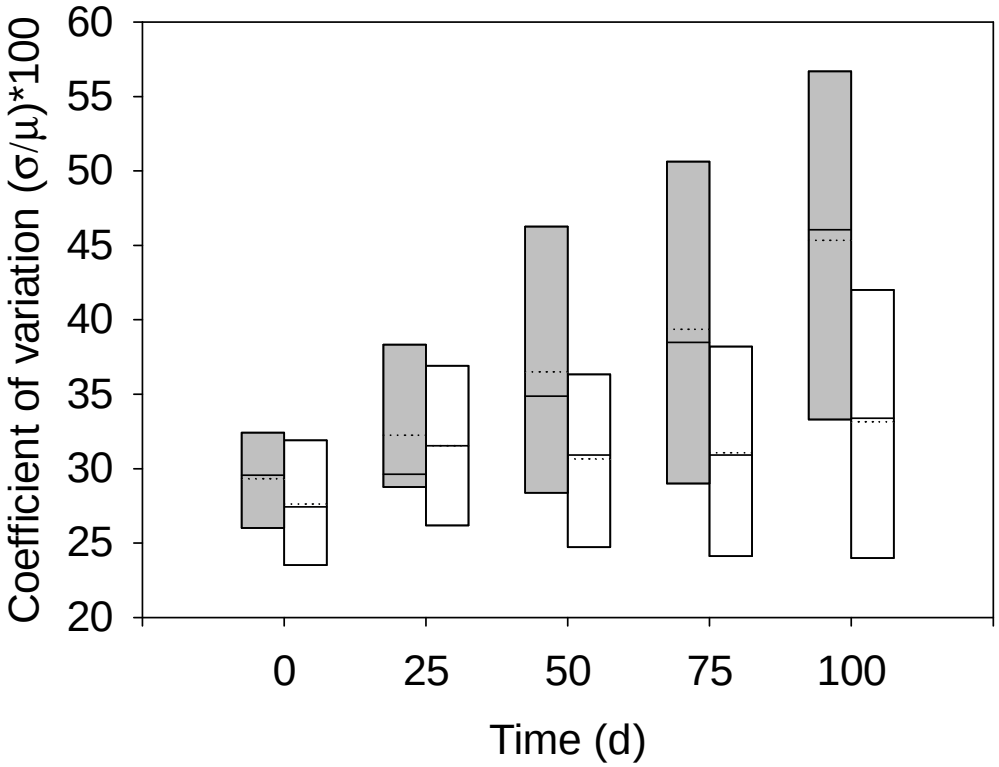

Figure 2.11 Coefficient of variation (CV) of individual mass (g) of wild (grey) and domestic (white) strain rainbow trout fed a reduced ration of feed (1.2% body weight; treatment 2). The lower and upper boundary of the box represents the 25th and 75th percentiles, respectively. The solid line within the box marks the median value and the dotted line represents the mean of three replicate tanks (n = 3).

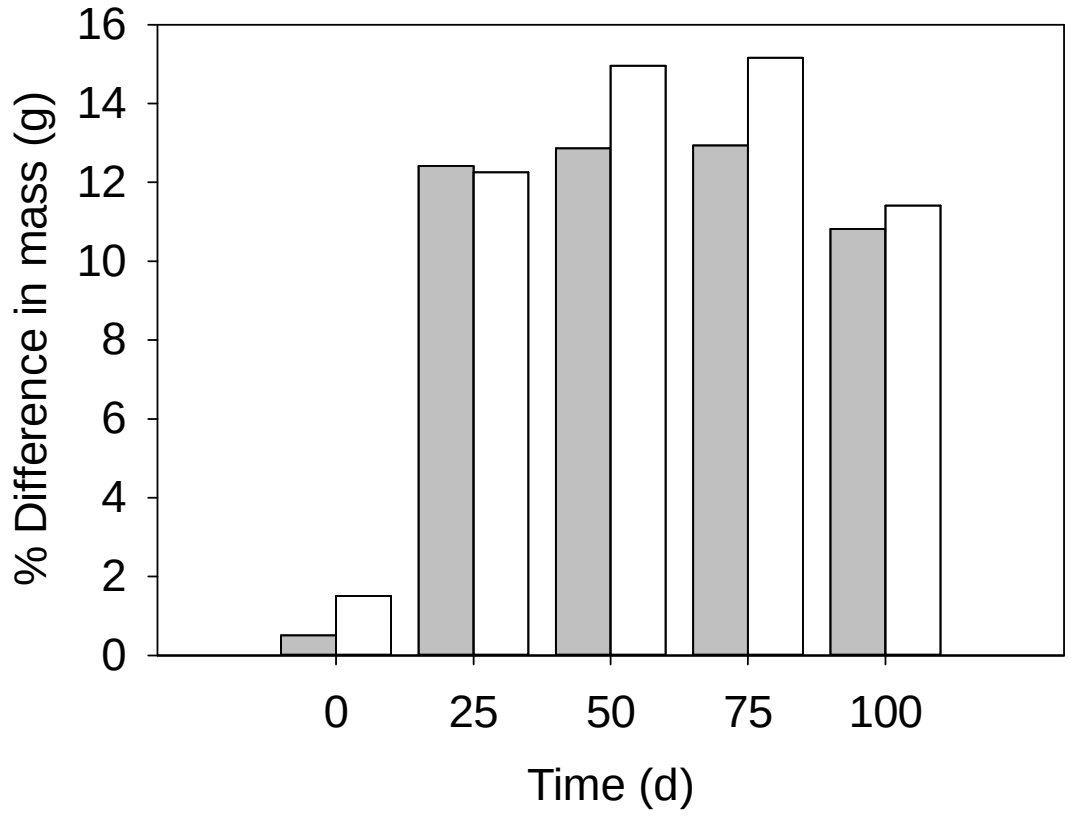

Figure 2.12 Percent difference in mass between wild (white) and domestic (white) strains of rainbow trout fed to satiation (treatment 1) and fed a reduced ration (1.2% body weight; treatment 2). Percentage difference was calculated as: [(mean mass treatment 1 - mean mass treatment 2) • (mean mass treatment 1)$^{-1}$] • 100%.

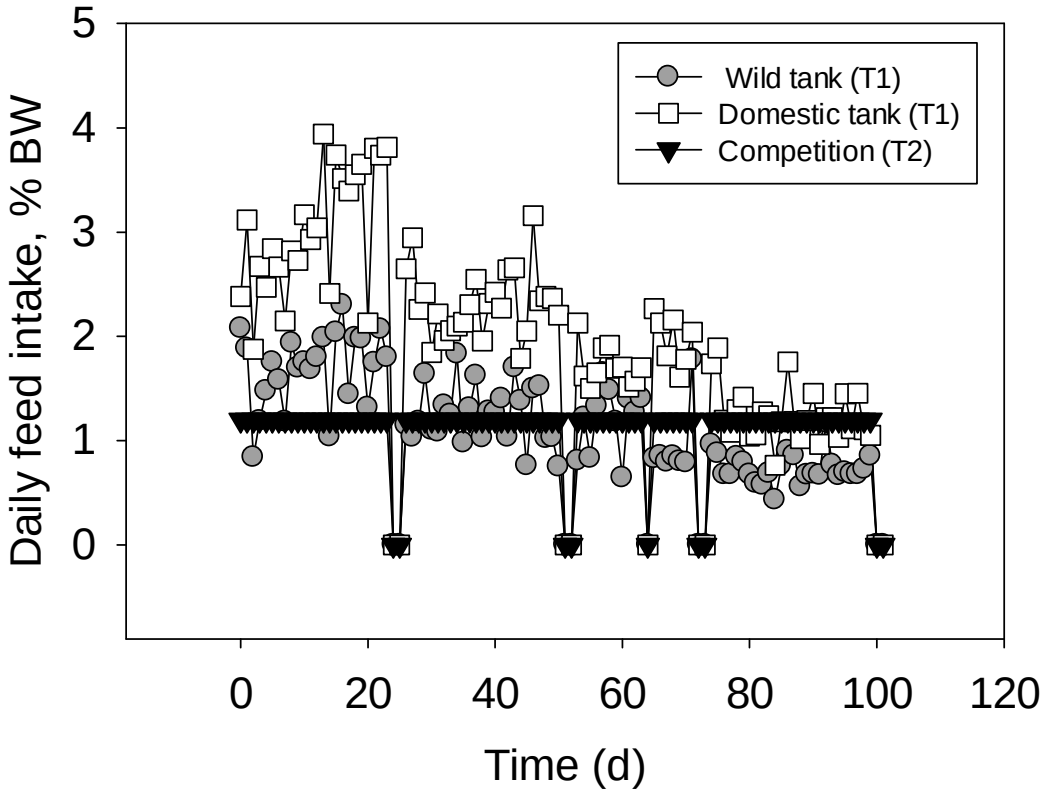

Figure 2.13 Mean feed intake as a percentage of bodyweight in wild and domestic strain rainbow trout fed to satiation (treatment 1) over time compared to rainbow trout strains fed a reduced, constant ration (1.2% body weight daily) in treatment 2.

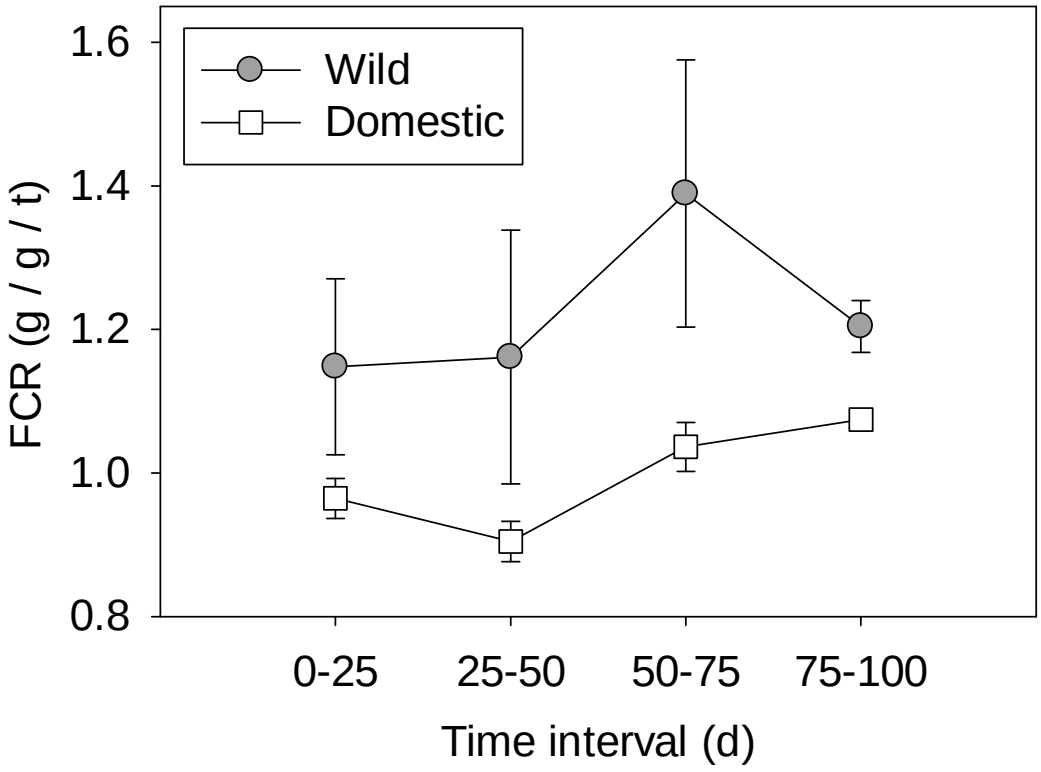

Figure 2.14 Feed conversion ratio (FCR) of wild and domestic strains of rainbow trout fed to satiation (treatment 1), over 25 day time periods. Each data point represents the mean of three replicate tanks (n = 3; $\bar{x}$ ± 1 S.E.).

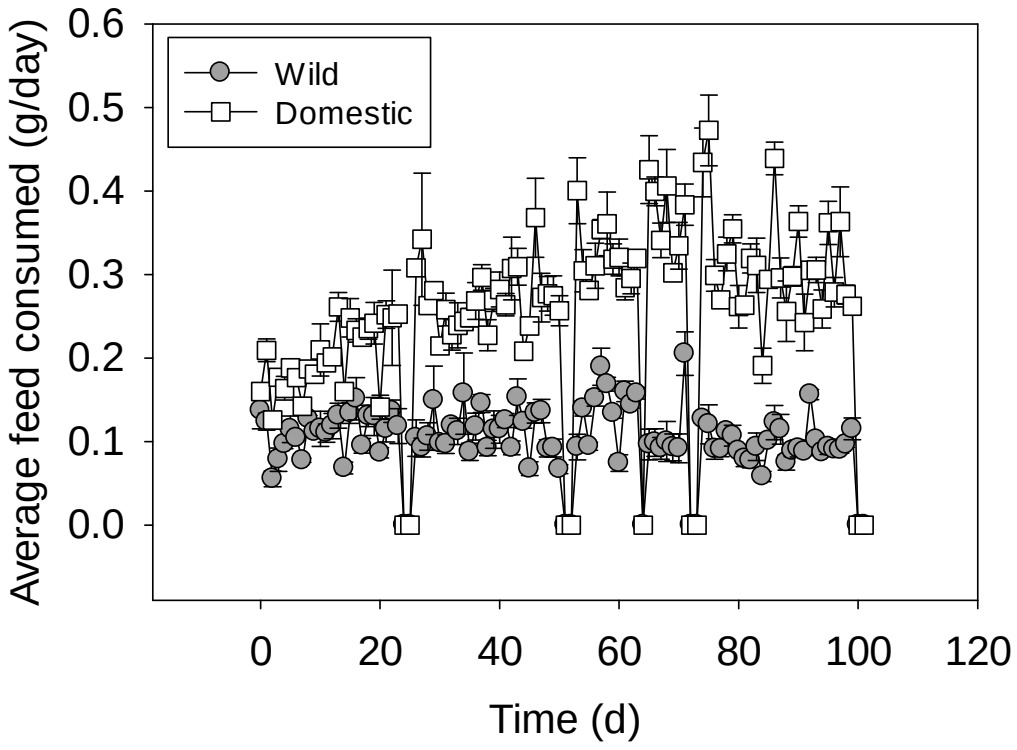

Figure 2.15 Mean feed consumed per day for wild and domestic strains of rainbow trout fed to satiation (treatment 1). Each data point represents the mean of three replicate tanks (n = 3; $\bar{x}$ ± 1 S.E.).

Chapter 3. Relative growth and mortality of domestic and wild strains of rainbow trout in a whole-ecosystem experiment

3.1 Introduction

3.1.1 Domestic salmonid escapes into natural systems

The freshwater aquaculture industry has increased production by >15 million tonnes globally in the past 10 years (FAO 2010). One reason behind this industry growth is the ability of aquaculture operators to culture fish in naturally occurring water bodies by the use of open net pens. Farmed (domestic) strains of fish are commonly held at high densities and are selectively bred for rapid growth. These farmed strains of fish have radically divergent phenotypic characteristics as compared to their wild conspecifics. The divergence in phenotypic and behavioural characteristics between salmonid strains selectively bred and used in aquaculture versus their wild counterparts underpins a major concern for this industry in terms of competition, interbreeding and transfer of pathogens (Naylor et al. 2005; Weir and Grant 2005; Podemski and Blanchfield 2006).

As shown in Chapter 2 of this thesis, domestic and wild strains of rainbow trout (*Oncorhynchus mykiss*) from the Laurentian Great Lakes (LGL) have significantly different growth trajectories and feeding efficiencies in a laboratory setting. Although much research has directly compared the growth and mortality between domestic and wild strains of rainbow trout for risk assessment, the majority of these studies have been performed in culture (see Table 1.1). A

primary concern of commercial aquaculture is the potential for domestic fish to escape from cages, enter an aquatic system and compete with established fish populations, including wild conspecifics. This concern is realistic, considering the number of escapes that are routinely reported in marine environments. McGinnity et al. (2003) estimates >2 million farmed Atlantic salmon annually escape into the north Atlantic Ocean. Although less data exists in freshwater systems, many reports cite that escapes of fish in the magnitude of 100's of thousands are possible from single events (Hoyle et al. 1999; McCutcheon 2012). Therefore, it is pertinent that studies be conducted in a natural setting, closer to real-world applications, and using strains of fish expected to be in direct competition should an escape event occur from commercial freshwater aquaculture operations. Because phenotypic expression in salmonids is strongly influenced by environmental variables, the context in which we choose to compare domestic and wild strains is critical.

Survival is a component of fitness that can vary greatly among salmonid species and strains (Tymchuk et al. 2006b and references therein). The degree of predator pressure, environmental conditions, strain tolerance and behaviour are all important variables that influence relative survival in the wild. Laboratory and semi-natural experiments do not fully encompass the suite of variables affecting survival that would be present in natural environments. In addition to survival, differences in growth rate can lead to a number of competitive advantages including earlier maturation, access to gape-limited food sources, dominance and reduced susceptibility to size-specific predation. By comparing inter-strain

differences in growth and survival only in culture, studies do not fully evaluate the degree of expression and magnitude of various attributes under natural conditions. Tymchuk et al. (2006b) suggest that more rigorous assessments of the plasticity of cultured and wild strains are necessary to determine whether domestic genotypes have a response to environmental variables that differ from wild genotypes in non-parallel ways. By enhancing our understanding of domestic and wild salmonid interactions in nature, we are better able to determine which environmental variables likely contribute to the success of a particular strain. This type of information is critical to better inform risk assessments as it relates to escapes of domestic salmonids from aquaculture.

3.1.2 Salmonid survival and growth studies

The majority of studies to date on the survival and growth of aquaculture-reared salmonids in the wild have examined Atlantic salmon (*Salmo salar*) and rainbow trout. McGinnity et al. (1997) found domestic genotype Atlantic salmon were caught less frequently each subsequent year after release compared to wild conspecifics (51 – 77% of wild), which the authors attributed to lower survival. Survivorship of domestic Atlantic salmon has been found to be lower compared to wild genotypes at all life-stages, in both fresh and marine waters (Fleming et al. 2000; McGinnity et al. 2003). Generally, reduced survival is also observed in domestic strains of rainbow trout. Some of the earliest work with rainbow trout involved quantifying survival of domestic, hybrid and wild strains in Canadian prairie freshwater lakes (Ayles et al. 1976; Ayles and Baker 1983). These studies found considerable variance in survivorship among strains, but most of the

domestic strains of rainbow trout studied had slightly greater survival (18-37%) compared to wild fish (14-32%). Additionally, the authors go on to suggest that hybrid vigour may be a primary mechanism for increased domestic strain success in the wild. More recently, a series of stocking experiments has examined the survival of rainbow trout in a number of small interior freshwater lakes in British Columbia, Canada. Domestic strains of rainbow trout incurred significantly greater mortality in lakes with high bird predation, which was attributed to increased foraging activity in more productive, yet riskier, offshore areas of the lakes relative to the wild strain (Biro et al. 2006).

Growth rates of escaped salmonids in the wild are typically greater than that of wild conspecifics. Domestic genotypes relative to naturalized Atlantic salmon show superior growth in the wild (Einum and Fleming 1997; McGinnity et al. 1997; Fleming et al. 2000). Similarly, freshwater rainbow trout have been shown to achieve growth twice that of wild conspecifcs subsequent to size-matched stocking in Canadian prairie (Ayles et al. 1976; Ayles and Baker 1983) and BC interior lakes (Biro et al. 2004a, 2006). However, no studies to date have specifically compared growth and survival of wild and domestic strains of rainbow trout from a region where the majority of Canadian freshwater aquaculture production occurs.

3.1.3 Aquaculture in Canadian Shield lakes

The concentration of freshwater aquaculture production in Canada is around Manitoulin Island in Lake Huron where rainbow trout are primarily cultured

(Moccia and Bevan 2007). The Canadian freshwater industry is poised to increase production several-fold in the next decade with new operations proposed for the LGL as well as expansion of existing operations in Lake Diefenbaker. To date, there have been no field based studies comparing the relative growth and survival of wild and domestic strains of rainbow trout from the LGL, and their potential for interaction in the wild. Past field studies examining relative growth and survival of wild and hatchery strains of rainbow trout may not be directly applicable to the LGL situation because the strains were not selectively bred for commercial aquaculture and the study lakes contained a limited food web in which zooplankton was the primary prey for fish (Biro et al. 2004a, 2006).

Initial research on escapes of the LGL aquaculture strain of rainbow trout has shown the potential for high growth, extensive movement and habitat overlap with naturalized strains (Blanchfield et al. 2009; Patterson 2010). An experimental release of rainbow trout to simulate an escape event in Lake Huron demonstrated that aquaculture strains have the potential to widely disperse from their cage site (Patterson 2010). The extensive movements and elevated growth rates of domestic strains in the LGL is corroborated by a study at the Experimental Lakes Area (ELA) tracking the same strains of rainbow trout after an intentional release from an experimental aquaculture operation (Blanchfield et al. 2009). Data from these experiments suggest the potential for significant habitat overlap and the likely possibility of competition with naturalized strains, especially in the littoral regions of lakes, although each study did not explicitly examine domestic strain interactions with wild rainbow trout.

The ELA is an ideal setting to evaluate the relative growth and survival of domestic and wild LGL strains of rainbow trout. Situated in the boreal shield, ELA lakes possess many of the same characteristics as the LGL, but on a smaller scale. ELA lakes are pristine and free from human disturbance than can confound experimental studies. In addition, the presence of minnows in the diet of escaped farmed trout in the ELA study (Blanchfield et al. 2009), suggests that this component of the food web may shape habitat use in boreal lakes in a manner different than past studies, such as in the BC lakes where the greatest biomass of food was zooplankton in the pelagic areas of lakes (Biro et al. 2004a, 2006). I specifically chose ELA lakes that contained natural populations of forage fish (cyprinids) in which to introduce both strains of rainbow trout.

3.1.4 Chapter objectives

There is a need for relevant data on the potential growth and survival differences of natural and selected strains of salmonids in freshwater systems. I conducted a replicated, whole-ecosystem stocking study to directly compare the growth and mortality of a domestic strain of rainbow trout used in commercial freshwater aquaculture relative to that of a wild (naturalized) strain from the LGL. I conducted this study in a pair of isolated lakes at the ELA in northwestern Ontario. Specifically, my objectives were to:

1) Quantify the incremental growth of both strains over a growing season.
2) Document the comparative and total survival of each strain over a growing season.

In order to address these objectives, I have taken several approaches to best describe growth and mortality.

3.1.5 Hypothesis

Results from Chapter 2 indicate that under controlled conditions, domestic strains of rainbow trout are able to achieve growth rates greater than twice that of wild conspecifics. Considering that elevated growth rates from aquaculture rearing typically lead to reduced survival in the wild, I predict from this field experiment:

1) The domestic strain of rainbow trout will achieve a greater growth rate than size-matched wild rainbow trout.

2) If avian predation pressure is high, domestic strains will incur higher mortality rates than wild rainbow trout.

3.2 Methods

3.2.1 Study lakes

This experiment look place at Lake 303 (49°39' 51.20" N, 93°44'30.42"W) and Lake 304 (49°39'33.10"N, 93°44'54.47"W) at the ELA in northwestern Ontario, Canada (Figure 3.1). Both lakes are headwater systems with outflows that ultimately drain into Lake 240. These lakes are ideal for a whole-ecosystem manipulation experiment because they are small enough to be easily managed, are in close proximity to the ELA base camp and are adjacent to one another so sampling is efficient. Both lakes support a diverse ecological community of

zooplankton, benthic and surface invertebrates, forage fish, amphibians, reptiles and bird species. Although both lakes share similar biotic characteristics, they are different morphometrically (Figure 3.2). Lake 303 is a 10 ha cold polymictic lake with a 2.5 m maximum depth. Lake 304 is a dimictic lake with a greater maximum depth (6.5 m) and is comparatively smaller in area (4 ha) than Lake 303. Water volume is similar between lakes. In Lake 304, a zone of oxygen-depleted water occurs at depths >4 m in the summer months. Lake 303 has an approximately uniform, shallow depth and structure throughout, while Lake 304 possesses well-defined offshore and littoral areas (Figure 3.2). This difference in morphometry between lakes potentially influences variables such as foraging behaviour, refuge, and available habitat for the stocked rainbow trout. To prevent any escape of stocked rainbow trout, fish barrier fences were constructed at the outlets of each lake (Figure 3.2). Barrier fences consisted of 1 cm^2 steel mesh attached to wood frames and sandbagged to the lake bottom. These fences were constructed in the winter of 2011 and installed after ice-off, prior to the rainbow trout introduction.

3.2.2 Limnological conditions

The physical conditions of a lake have the ability to affect fish survival and success in the wild. For instance, light penetration and turbidity may affect a rainbow trout's ability to forage (Ware 1973). Water temperature and dissolved oxygen content may define the zones of the lake that are preferable and inhabitable to rainbow trout. For optimal growth, rainbow trout prefer water temperatures of 16-18ºC (Javaid and Anderson 1967; Dickson and Kramer 1971;

McCauley and Pond 1971; Hokanson et al. 1977; Wurtsbaugh and Davies 1977; Papoutsoglou and Papaparaskeva-Papoutsoglou 1978; Austreng et al. 1987) and >6 mg • L^{-1} dissolved oxygen (Gutsell 1929; Matthews and Berg 1997; Barrow and Peters 2001). Reduced access to cover in the littoral and benthic zones due to high temperatures or low oxygen can force fish to spend more time in less ideal micro-habitats. Temperature and dissolved oxygen were recorded weekly by profiling the water column at the centre buoy (CB) of each lake (Figure 3.2) at 0.5 m intervals with a hand held probe (model 550A, YSI Inc./ Xylem Inc., Yellow Springs, OH, USA). In addition, data loggers (StowAway TidbiT Temp Logger, Onset Computer Corporation, Cape Cod, MA, USA) programmed to record water temperature every 30 min were set on a weighted string at the CB. Lake 304 data loggers were set at depths of 0, 1, 2, 3, 4, and 6 m. Lake 303 data loggers were set at 0 and 3 m depths. Standard sampling occurred every 4 weeks in both lakes following common methods used at the ELA (Shearer 1978; Cruikshank 1994; Xenopoulos et al. 2009; Sandilands, personal communications). This consisted of photo-penetration, integrated water sampling, Secchi depth, cloud cover, wave height, wind direction and speed, air temperature and water colour.

Integrated epilimnion water sampling occurred 0.5 m above the metalimnion determined as the depth where water temperature increased by a rate of 1ºC • m^{-1}. Integrated metalimnion water sampling only occurred in Lake 304 and not Lake 303 (complete thermal mixing throughout water column). The sampling zone was determined as where temperature change was >0.25ºC for a 0.25 m change in depth to where light intensity was 1% of surface depth. Water samples at 1 m

intervals on L304 and epilimnion grab samples on both lakes were taken every 4 weeks throughout the field season. Light penetration was calculated every 0.5 m in the water column using a LI-1400 data logger and light meter coupled with a LI-192 underwater quantum sensor (LI-COR Environmental, Lincoln, NB, USA). Cloud cover and wave height were recorded every 4 weeks. Cloud cover was recorded based on a scale of 0-10 and wave height was estimated by approximating maximum amplitude as 0, 1-5 or 5-10 cm.

3.2.3 Rainbow trout stocking procedure

Wild and domestic strains of rainbow trout used in these experiments are of the same origins as those used in the growth trials (Chapter 2) and are fully described in Chapter 1 (Section 1.5.1).

The wild Ganaraska strain was obtained from the Dorian Fish Hatchery in, Ontario, Canada. On May 25, 2011, I selected ~2000 wild rainbow trout from the hatchery at an approximate size of 10 g. The selected fish were transferred to a separate tank where the adipose fin was removed with clippers to differentiate them from the domestic strain once stocked at the ELA. On June 24, 2011, the wild strain was netted from the hatchery and transported to the ELA in oxygenated plastic bags.

Domestic strain rainbow trout were reared at Cedar Crest Fish Farms in Hanover, Ontario, Canada, and transported to a commercial aquaculture operation on Manitoulin Island. Cedar Crest had separated the fish into the desirable weight of approximately 10 g. On June 24, 2011, ~2000 domestic fish were transferred to an

oxygenated recirculation tank secured to the back of a truck and transported from Manitoulin Island to the ELA. Once both strains had arrived at the ELA on June 25, 2011, they were immediately transferred to one of 6, 160 L flow-through fiberglass tanks (Figure 2.1), which were supplied with oxygen via aeration stones. Due to the stress of the transportation, some mortality had occurred, mostly in the wild strain (~5%).

Prior to stocking, 500 rainbow trout (half domestic, half wild) destined for each lake were weighed (0.1 g) and measured for fork length (1 mm) to obtain t_0 values. Fish were sedated prior to handling following the same procedures outlined in section 2.2.3. Measured fish also received a decimal coded wire tag (DCWT; Northwest Marine Technology (NMT), Shaw Island, WA, USA) so that individual growth rate could be calculated upon recapture. Each DCWT was 1.1 mm of stainless steel wire marked with rows of numbers denoting individual codes and injected 2-3 mm into the snout of the fish. The snout site of the injection was selected by recommendation from NMT and has a retention rate of 95-100% in salmonids over 24-30 d (Hale and Gray 1998). Each DCWT had a reference tag associated with the initial mass and fork length recorded so that the fish could be accurately identified upon recapture.

After processing, fish were immediately transferred to oxygenated bags, secured in coolers, and transported to Lake 303 and Lake 304 via ATVs (June 25, 2011, approximately 2 – 6 h after arrival at field camp). Prior to release, bags containing the rainbow trout were allowed to acclimate slowly to lake water. At the approximate time of release, surface water temperature in Lake 303 and Lake 304

were 20.82 and 21.75, respectively (Figure 3.4). A total of 2294 rainbow trout were released in Lake 303 and 1000 in Lake 304, consisting of half wild and domestic fish for each lake. Stocking density was based on Ontario Ministry of Natural Resources (OMNR) protocol for "put-grow-take" purposes, of 4.5 kg • ha^{-1} for small lakes (OMNR 2000). To promote fish growth, I selected a lower stocking density of about 3.5 kg • ha^{-1} (3.72 and 3.03 kg • ha^{-1} in Lake 304 and Lake 303, respectively).

3.2.4 Survival

3.2.4.1 Rainbow trout sampling

I divided each lake into a grid of 50 m^2 quadrats for sampling stocked rainbow trout periodically throughout the summer and fall of 2011. During each sampling period, equal numbers of offshore (>2 m depth) and inshore (<2 m depth) quadrats were randomly selected using a random number generator. In general, sampling consisted of gillnetting a specified quadrat for a pre-determined interval of time (details of nets used are provided below). Inshore quadrats were sampled by attaching one end of a net to shore and setting the net perpendicular, letting the lead line sink to the lake bottom. Sampling of offshore quadrats consisted of setting nets in 0 – 4 m depth using a series of floats while anchoring the net at each end. I sampled in this way for two reasons: (1) the entire available habitat in the water column was being sampled and (2) the gillnet was sampling above the anoxic zone of the lake (>4 m in Lake 304).

I chose a series of mesh sizes of gillnet to sample inshore and offshore habitats such that all possible sizes of fish could be recruited to the sampling gear. These included:

1. Fall Walleye Index Nets (FWIN) – monofilament gillnet gang 60.8 m long comprised of eight panels 1.8 m deep x 7.6 m long, sewn together, each consisting of one of the following sequential stretched mesh sizes; 25, 38, 51, 64, 76, 102, 127 and 143 mm.
2. Nordic Nets – monofilament single mesh size nets 25 m long and 1.8 m in depth. A variety of mesh sizes were used including; 13, 25, 32, 38, 44, 51, 64 and 76 mm. Occasionally two nets would be stitched together to form a multi-panel net.

In order to create a comparison to the laboratory-based study, each lake was sampled at a similar time interval (~every 30 d). At each sampling interval, a lake was sampled for 5 d. If <25 fish were captured, an additional week of sampling was performed. Initial set-time was 30 min at each sampling interval, if no samples were recovered, set-time gradually increased, reaching a maximum duration of 12 h, which usually occurred overnight to take advantage of activity during the crepuscular period. When a rainbow trout was caught in a net, time and location in the net and region of the lake was recorded. As the field study progressed, additional nets were employed in the inshore regions on the lakes where the majority of fish were being recaptured.

In addition to gillnet effort, I incorporated a number of other methods to capture fish, including, cast netting, trap netting, fyke netting, beach seining and angling.

Of these additional methods only trap netting was effective, yielding 3 domestic rainbow trout in Lake 304 near the northwestern shore of the lake (Figure 3.7). Each trap net consisted of a 1.2 m x 1.2 m house with 6.35 mm mesh netting. The set-up involved attaching one wing to shore and one wing anchored offshore (see Beamish 1973). Two nets were set for a total of two weeks in August and were checked every two days.

3.2.4.2 Avian predation

As a means to better describe the selective pressures that rainbow trout experienced throughout the field season, avian predation was monitored in both lakes. Piscivorous birds have been cited as one of the greatest sources to fish mortality in freshwater lakes (Steinmetz et al. 2003; Beckmann et al. 2006). To monitor the presence of fish-eating birds throughout the duration of the experiment, a time-lapse camera (Moultrie Plot Stalker MFH-DGS-PS 8MP EBSCO Inc., Birmingham, AL, USA) was strategically positioned on Lakes 303 and 304 to take photographs of the majority of open water for each lake (Figure 3.2). Each camera took a photograph every 30 min from dawn until dusk each day of the experiment. Memory cards were removed from cameras and uploaded into a database, weekly. Photos were later examined for known piscivorous bird species presence throughout the field season including common mergansers (*Mergus merganser americanus*), common loons (*Gavia immer*), bald eagles (*Haliaeetus leucocephalus*), buffleheads (*Bucephala albeola*), gulls (*Laridae* spp.), belted kingfishers (*Megaceryle alcyon*) and other ducks (*Anatidae* spp.). Daily presence/absence data was tabulated along with the number of a particular

bird species observed. In addition to time-lapse photography, bird species presence was recorded with each site visit. Diving time was also recorded for mergansers and loons as an approximate measure of foraging time of each species. When a predatory bird was observed on either lake, time spent under water was recorded for 30 min intervals. Foraging success was difficult to determine as feeding was rarely observed above water and loons and mergansers are known to consume the majority of their prey while submerged (Barr 1996; Beckmann et al. 2006; Wiese et al. 2008).

I calculated risk of predation from presence/absence data coupled with foraging activity for mergansers and loons. I used a term for predation risk (PR) that I modified from equations incorporated by Beckmann et al. (2006):

(eq. 3.1) $$PR_s = \frac{\sum_{d=1}^{n} Ns}{Vs} \bullet D_{avg} \bullet P_s$$

where, V_s is the total number of days that a bird species was observed focally and by camera, N_s is the number of individuals of a particular bird species observed on a particular day d, D_{avg} is the average daylength and P_s is the estimated proportion of time spent foraging. The term *PR* should be considered a relative metric and not absolute. As there is no control for comparison, the PR's purpose is to provide a contrast of predator pressure among lakes, months and bird species as well as a measure of total predation pressure for both species.

3.2.4.3 Population estimation

Population abundance was estimated at each gillnet sampling event. An estimation of population size was based on a model used to estimate mortality of similar sized rainbow trout (50-328 mm) as those retrieved in this study (Biro et al. 2003). The model attempts to predict size-selective capture of rainbow trout based on the probability of a particular size being captured over 5 d of netting. The calculation relies on the assumption that the smallest fish are caught with a much lower probability than larger fish (Post et al. 1999). Using rainbow trout mark recapture data, the proportion of a particular fork length of fish was used as input into the model. The most likely formula that best describes the data as determined by the comparison of Akaike Information Criterion (AIC) is a logistic function in the form:

(eq. 3.2) $$P_{capture} = 0.431 \bullet 1 / [1 + (e^{1.36-1.18FL})^{0.02}]$$

where, $P_{capture}$ is the proportion of fish of a given fork length (FL) in mm captured over a time interval. To estimate remaining population, the number of fish caught at a 10 mm size bin was divided by the probability of capture of that size of fish and summed over total catch.

3.2.5 Growth

When a rainbow trout was caught in a net, it was removed from the gear and placed in a bucket of water in the boat until the entire net was checked. Fish were transported onshore where they were sacrificed in an overdose of TMS (see section 2.2.3 for detailed procedure), transferred to a labelled Whirlpak® bag, placed on ice in a cooler and transported back to the laboratory at the ELA base camp. Each fish was identified for strain based on presence/absence of adipose

fin, weighed (0.1 g) and measured for fork length (1 mm). The gastrointestinal tract and liver of each fish was then removed and placed in a Whirlpak® bag. Organ removal involved making an incision on the ventral side of each fish from the central margin of the lower lip to the anus. The organs were then severed at the esophagus and at the lower intestine and removed using forceps. The remaining carcass of each sample along with the separated organs were immediately frozen at -20ºC.

Individual rainbow trout were checked for the presence of a DCWT at the Freshwater Institute (Winnipeg, MB) in winter 2011-2012, using a handheld wand detector (Northwest Marine Technology). Detection required repeatedly passing the wand over the head of each fish at the approximate site of tag injection. When a tag was detected the wand would beep and an LED light would turn on. The tag could then be located and dissected from the head of a fish. Each DCWT was identified using a device specifically made to magnify and view these tags (MangniViewer, Northwest Marine Technology). The device consists of a high intensity light source, a magnetic reading pencil, and a 25x microscope. Once each tag was read, it was catalogued with its respective fish ID number so it could be associated with original weight and fork length at time of stocking. At this time, rainbow trout internal organs were then transferred to a 95% ethanol solution, in preparation for dissection.

3.2.5.1 Stomach contents analysis

I dissected individual stomachs from the gastrointestinal tract and removed its contents into a petrie dish. Several drops of water were added to the stomach

contents, which were then viewed using Leica Wild M10 and MZ8 binocular dissecting microscopes (Leica Microsystems, Inc., Richmond Hill, ON). Stomach contents were identified, enumerated and separated according to: class (*Insecta* (miscellaneous), *Arachnida*, *Copepod*), subclass (*Oligochaeta*), order (*Amphipoda*, *Odonata*, *Ephemeroptera, Cladocera, Coleoptera, Diptera*) and family (*Corixidae, Cyprinidae*). Indigestible items, detritus and plant material were also found in several stomachs but were discarded and not included in the analysis. Stomach contents for each fish stomach were separated for prey type and subsequently transferred to pre-weighed 44 mm aluminum weigh boats. To obtain dry mass, samples were dried in an oven at 60ºC until mass difference between drying was no greater than 5%. Total prey type mass per dish was then assessed on a digital scale and recorded (0.0001 g).

The domestic strain of rainbow trout was separated into two groups for all analyses based on growth trajectory, defined as either fast- or slow-growing domestic, and compared to the wild strain (see Results). Relative Importance (RI) of a prey item was calculated using frequency of occurrence, % total numbers and % total weight of a food item (George and Hadley 1979) (eq. 3.3). I chose to use RI to describe rainbow trout diet because it incorporates all three measures of prey importance, opposed to a single measure. RI is derived from the Absolute Importance Index (AI). The AI for item *i* is:

(eq. 3.3) AI_i = % frequency of occurrence + % of total numbers + % total weight for prey item *i*

To calculate the RI for item *i*:

(eq. 3.4) $RI_i = 100\ AI_i / \sum_{i=1}^{n} AI_i$

where, *n* is the number of different prey items.

A commonly used method to determine diet overlap between groups of animals can be calculated using the Schoener Index (SI) (Schoener 1968, 1970). This measure uses individual stomach prey weight of each category to determine diet overlap between groups. A value of 100 would represent full overlap and 0, no overlap, and values > 0.60 are considered to be biologically significant (Zaret and Rand 1971). The SI is calculated as:

(eq. 3.5) $SI_{x,y} = 100\ (1\text{-}0.5\ \Sigma\ |p_{x,i} - p_{y,i}|)$

where, p_{xi} and p_{yi} are the $mass_i$ of group *x* and *y* respectively in prey item category *i*.

3.2.5.2 Stable isotope analysis (SIA)

I quantified the two most commonly used stable isotopes in food web ecology, $\delta^{13}C$ and $\delta^{15}N$, to describe the relative trophic interactions among species in Lake 304. $\delta^{13}C$ is associated with sequestered CO^2 during photosynthesis by primary producers and is elevated in aquatic organisms that are more closely associated with littoral vs. offshore zones and benthic vs. pelagic zones of ecosystems (Fry 2006). $\delta^{13}C$ fractionates minimally between trophic transfers (<1 ‰) (DeNiro and Epstein 1978; Michener and Schell 1994). In contrast, $\delta^{15}N$ fractionates significantly and is used as a measure of trophic position in aquatic food webs (Post 2002). Organisms that are at higher trophic positions have an enriched value of $\delta^{15}N$ relative to their food sources (Minigawa and Wada 1984). Samples were collected of t_0 rainbow trout specimens, t_x rainbow trout captured throughout the

experiment, forage fish species, benthic, surface and terrestrial invertebrates, bulk zooplankton, aquatic primary producers and a piscivorous bird (list of samples and replicates can be found in Appendix 1).

I examined stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ from both white muscle and liver tissues from recaptured rainbow trout. Examining tissues with varying turnover rates elicits trends based on scaling temporal attributes due to growth and metabolic activity (Peterson and Fry 1987; Pinnegar and Polumin 1999; Hesslein et al. 1993; Kurle and Worthy 2002). Liver, being the more metabolically active tissue compared to white muscle in rainbow trout, would therefore reflect diet over a shorter period of time than muscle, with diet composition being reflected in isotope signatures in the magnitude of weeks and months for liver and muscle tissue, respectively (Hobson and Clark 1992; Perga and Gerdeaux 2005).

Approximately 1 g of white dorsal muscle was removed from the caudal peduncle. The entire liver was dissected from the preserved internal organs. The merganser muscle and liver samples were obtained by dissecting a 1 g segment of the breast and liver tissue, respectively. Benthic organisms were collected both in the littoral and profundal zone of the lake. Profundal sampling consisted of taking an Ekman grab in the offshore organic substrate and separating the contents by sieving with a 250 µm mesh to isolate invertebrates (profundal chironomids). The remainder of the benthic invertebrates were collected from the littoral zone of the lake. A D-frame dip net (Wildco, Yulee, FL, USA) was used to collect the suspended benthic invertebrates from the water column as a result of "kick-sampling". Invertebrates were then isolated and separated afterwards using a

coarse-sized sieve (420 µm). Surface invertebrates (*Gyrinidae* spp.) were collected using a dip net on the water surface. Benthic and surface invertebrates were collected in Lake 303 and Lake 304 on September 15 and 19, respectively. Zooplankton samples were obtained monthly (May-October 2011) by hauling a 0.5 m, 150µm mesh-size net vertically through the water column close to centre buoy of the lake. When enough zooplankton was obtained for mercury and stable isotope analysis (~1 g for each), it was transferred to a Whirlpak® bag and frozen at -20°C.

Rainbow trout muscle and liver tissue was dried in an oven at 60˚C for 48 hours in 10 mL glass scintillation vials. Invertebrate and primary producer samples were freeze dried at -50˚C (FreeZone 18 L, 7755042, Labconco, Kansas City, MO, USA). After moisture had been removed from all samples, they were homogenized and ground using a porcelain motor and pestle. Homogenized samples were then transferred to 0.5 mg tin capsules (350-400 µg), packaged and analyzed at the Stable Isotopes in Nature Laboratory (SINLAB) at the University of New Brunswick, Canada. Samples were analyzed for carbon and nitrogen stable isotopes using continuous flow isotope mass spectrometry (CFIRMS; Finnigan Delta^{Plus}, Thermo Finnigan, San Jose, CA, USA) coupled with a Costech 4010 elemental analyzer (Costech Instruments, Valencia, CA, USA). Standard reference material for carbon was Pee Dee Belemnite carbonate for CO_2 and atmospheric nitrogen for N_2. In addition, 10 different laboratory reference standards were analyzed along with sample replicates every ten runs to ensure instrument precision. Stable isotope values are expressed in parts per thousand

(‰) also referred to as parts per mil. Stable isotope ratio values are expressed using the notation, "δ" and are in the form:

(eq. 3.5) $$\delta X = [(R_{sample}/R_{standard})-1] \bullet 1000$$

where, X is the is ^{13}C or ^{15}N and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Stable isotope data were pooled over the entire summer for wild, fast-growing and slow-growing domestic rainbow trout. In order to simultaneously compare the $\delta^{13}C$ and the $\delta^{15}N$ means among groups, a MANOVA paired with an ANOVA on the $\delta^{13}C$ and $\delta^{15}N$ values was performed. Isotopic niche area and overlap was also calculated between groups. Niche space describes the degree of specialization versus generalized diet of a population of fish (Layman and Allgeier 2012). The overlap of niche spaces represents the degree of shared diet between populations (or strains) of fish being analyzed. Calculation of niche area was carried out using the SIAR package in the statistical program R. SIAR generates 95% confidence standard ellipses (SEA_B), which are bivariate equivalents to standard deviations representing the calculated dietary 'niche' that a particular group encompasses based on isotopic values (Jackson et al. 2011). I calculated isotopic niche overlap applying a correction factor for small sample size (SEA_c) to compare diets of wild, fast-growing domestic and slow-growing domestic rainbow trout. I also compare overlap of niche areas using the total area (TA) of the convex hull. TA represents the niche width calculated as the total area encompassed by the smallest convex polygon containing all of the group individuals in $\delta^{13}C$ by $\delta^{15}N$ niche space (Layman et al. 2007). This metric of diet composition of a group of

fish is less integrative than the standard ellipse method, yet accounts for all individuals in the population, and as a result, encompasses a greater degree of variability among individual fish (Layman et al. 2011).

3.2.5.3 Bioenergetics and mercury mass balance modeling

Bioenergetics models (BM) have been used as a tool by biologists to compare and quantify the processes in which aquatic organisms utilize and expend energy. Here, I use BMs to estimate the energy budgets of wild, fast-growing and slow-growing domestic rainbow trout recaptured from the field experiment. The model uses a combination of a mercury (Hg) mass balance model (MMBM, Trudel and Rasmussen 2001); with a traditional BM (Kitchell and Stewart 1977). The combination model was the same used by Rennie et al. (2005) for modeling activity costs of yellow perch (*Perca flavescens*). The model uses the mass-balancing of mercury to predict consumption rates by calculating the concentration of mercury at t_0 and t_x. Since the primary source of mercury uptake in uncontaminated water bodies is through food (Cabana et al. 1994; Hall et al. 1997; Leaner and Mason 2002; Hrenchuk et al. 2012), one can assume that the total increase in mercury over a certain time period is directly proportional to the rate of consumption of food sources. The predominant species of mercury bioaccumulated in fish is in the form monomethyl-mercury (MeHg) (Bloom 1992; Becker and Bigham 1995; Mason et al. 1995; Scheuhammer et al. 2007). MeHg is considered to be fat-soluble and stored in muscle tissue. Assuming instantaneous loss rate of MeHg to the gonads is negligible in sexually immature rainbow trout and that egestion rates can be considered constants over small time steps (i.e., 1

d), though integration, the MMBM can be rearranged to solve for consumption rate:

(eq. 3.6) $$C_t = [Hg_t - Hg_0 e^{-(E+G)t} / \alpha Cd(1-e^{-(E+G)t})] \bullet (E+G)$$

where, C_t is the mass-specific food consumption rate (d^{-1}) at time *t*; Hg is the MeHg concentration of the fish (μg Hg • g^{-1}); E is a function describing the instantaneous elimination rate of Hg (d^{-1}); G is the mass-specific growth rate (d^{-1}); α is the assimilation efficiency of MeHg from food and C_d is the concentration of MeHg in food (μg Hg • g^{-1}). The MMBM (eq. 3.6) is connected to the BM through the common term C*t*.

By linking both models, one has the ability to use contaminant mass-balancing and traditional bioenergetics equations to accurately predict fish energy budget. The BM is expressed as:

(eq. 3.7) $$W_t = W_0 + (C_t \bullet ED_{prey}) - (F + U + R_T) / ED_{fish}$$

where, W_t is the fish weight (g) at time *t*; W_0 is the initial fish weight (g), C*t* is the ingestion rate (J • g^{-1}), ED_{prey} is the energy density of prey (J • g^{-1}), ED_{fish} is the energy density of fish (J • g^{-1}), F is losses due to egestion (J • g^{-1}); U is losses due to excretion (J • g^{-1}); and R_T is losses due to metabolism (J • d^{-1}). Metabolism can be further decomposed into:

(eq. 3.8) $$R_T = ACT \bullet R_s + R_d$$

where, R_d is specific dynamic action (J • d^{-1}), or the heat increment required to processes food for energy and storage, R_s represents losses due to standard

metabolism ($J \bullet d^{-1}$), and ACT represents an activity multiplier to standard metabolism that can be estimated iteratively (Winberg 1956). Model inputs and a description of how these are calculated can be found in Appendix 1.

Consumption and growth rates tend to scale with fish size, making a comparison between strains problematic (Hewett and Kraft 1993; Trudel et al. 2001; Rennie et al. 2010). However, rates may be compared among fish of different sizes if mass is raised to an appropriate scaling exponent (Jobling 1994). To test whether literature scaling exponents were appropriate to apply to my data set, I regressed absolute growth and consumption rates by mass to produce a scaling factor. The mean (± 1 S.E.) observed value of the absolute growth scaling exponent (0.61 ± 0.12) and mean absolute consumption (0.74 ± 0.03) were comparable to those reported by the literature, so I chose to scale mass by the exponent 0.63 (Jobling 1994) and 0.82 (Cho 1992) for growth and consumption, respectively.

3.2.5.4 Prey energy density

BMs require estimates of caloric content of prey items, which I calculated using calorimetry. Samples for this analysis were obtained and freeze dried at -50˚C following the same procedure as for SIA. Dried samples were crushed and homogenized using a mortar and pestle. The powdered sample was then pressed into concentrated pellets using a punch and die of a pellet press (Parr Instrument Company, Moline, IL, USA). The pellet was weighed (0.0001 g) and placed on the firing cap in an oxygen bomb. The sample was then combusted and measured for energy content using a 6725 semi-micro oxygen bomb calorimeter coupled with a 6772 precision thermometer (Parr Instrument Company). The instrument

was calibrated using standards of benzoic acid. All standard values were <2% of each other.

3.2.5.5 Methyl-mercury (MeHg) determination

Rainbow trout and minnows were processed by removing approximately 0.2-0.3 g of white dorsal muscle tissue and frozen at -20°C in 22 mL amber glass vials with Teflon-lined lids (National Scientific Company, Rockwood, TN). Samples were carefully handled using stainless-steel or Teflon tools that had been soaked in 10% HCl baths. Tools were cleaned between samples using 95% ethanol and milli-Q water. Lake 304 bulk zooplankton samples (see section 3.2.4.2) were freeze dried prior to MeHg analysis.

Samples were analyzed at the Canadian Centre for Inland Waters (CCIW) in Burlington, Ontario, Canada. Fish samples were analyzed for wet weight total mercury (THg) concentrations. THg can be considered to be approximately equivalent to MeHg in fish muscle tissue, so one may assume THg = MeHg (Turner and Rudd 1983; Bloom 1992). Concentrations of THg were measured with a direct mercury analyzer (DMA-80), determined using atomic absorption spectrophotometry at 254 nm (Milestone, S.r.l., Fatebenefratelli, Italy). A duplicate measurement occurred after every 10^{th} sample processed. National Research Council of Canada (NRCC) certified biological reference standards DOLT-2, DORM-3 and TORT-2 were analyzed along with National Institute of Standards and Technology (NIST) standards 1566 and 2976 to ensure quality assurance and control. All but three measured values fell within 10% of the reported values (still within %15) of the NRCC and NIST (Appendix 1).

Direct MeHg concentrations were measured for Lake 304 bulk zooplankton samples because THg cannot be assumed to be equal to MeHg in invertebrate organisms (Tremblay and Lucotte 1997; Bowles et al. 2001). Digestion of zooplankton samples followed Hintelmann and Nguyen (2005). This involved weighing approximately 20 mg of sample into Teflon digestion vials. Each sample was then spiked with $CH_3^{201}Hg+$ and 5 mL of 40% Nitric acid was added. The vials were then tightly capped and heated for 16 h. Analysis was by aqueous phase ethylation using $NaBEt_4$, pre-concentration onto Tenax traps, followed by gas chromatography (GC) separation, pyrolysis, and detection via cold vapour atomic fluorescence spectrophotometry (CVAFS) using an automated methyl-mercury analyzer (Brooks Rand Labs, Seattle, WA, USA). This method resulted in a 0.1 ng • g^{-1} method detection limit (MDL). Duplicates accounted for >25% of total runs. Analytical average spike recovery equaled 90.54% and all NRCC and NIST reference standards were within 15% of reported values. A description of the calculation of model inputs can be found in the Appendix 1.

For a direct comparison to growth trial data (Chapter 2), the model was run for 100 d. A final mean rainbow trout size could not be calculated because fish were collected throughout the field study, so I fitted growth-curve equations to mass (g) data over 100 d to produce an estimate of t_{100} for each strain being examined. For the growth trajectory of each group, a dynamic fit was applied to the data points to maximally reduce residuals. Similarly, MeHg values were plotted against the measured mass values for rainbow trout in each size group, using the same fitting method as for growth. Curves were calculated and the estimated mass for each

strain at t_{100} was matched with the estimated MeHg value as inputs into the model.

3.3 Results

3.3.1 Limnological conditions

Lake 303, which mixes throughout the year, was iso-thermal from surface to sediment within the water column. As a result of continuous mixing, constant inputs of atmospheric oxygen were introduced into the surface water of Lake 303 and dissolved oxygen remained approximately constant at about 8 – 9 mg • L^{-1} throughout the field season and was never <5 mg • L^{-1}. Conversely, Lake 304, being about twice as deep as Lake 303, formed strongly stratified thermal layers during the summer. This resulted in an oxygen-depleted zone found below 4 m depth (~4 mg • L^{-1}). Water temperatures reached 26ºC, a temperature considered lethal for rainbow trout (Alabaster and Welcomme 1962; Bidgood and Berst 1969; Charlon et al. 1970; Cherry et al. 1975; Hokanson et al. 1977), at some depths in Lakes 303 and 304 towards the end of July 2011 (Figure 3.3).

Light attenuation in Lake 304 was stable throughout the field season (~1.0; Figure 3.4) and consistent with patterns from the previous two years. Secchi depth as a measure of light penetration was more variable than light attenuation from month to month, with values that ranged from 1.5 to 3.75 m. In 2010 and 2011 Secchi depth was greatest (~3.75 m) for the months of July and August; a pattern, not evident in light attenuation data (Figure 3.4). Similarly, percentage light

penetration reached deeper depths in 2011, compared to the previous two years (Figure 3.5).

3.3.2 Rainbow trout recaptures

Few rainbow trout were caught during the 2011 field season. Although effort was high (Table 3.1), a total of 20 domestic and 7 wild rainbow trout were recaptured throughout the season, and only in Lake 304 (an additional wild rainbow trout was caught in Lake 304 the following April). Standardized catch rates were greatest in July and declined significantly over the field season (Figure 3.6). Because no rainbow trout were recaptured in Lake 303, data analysis will focus primarily on Lake 304.

Wild and domestic rainbow trout were recaptured in similar areas of Lake 304 (Figure 3.7). The proportion of fish captured nearshore (<2 m depth) was 82% and 86% for domestic and wild fish, respectively. There was no significant spatial segregation of the two strains, which shared similar littoral habitat. Of those locations around the lake, rainbow trout were caught in the eastern shore of the lake more often. A general habitat feature that characterized nearshore site catches was proximity to a relatively steep drop off. Catch locations began to trend towards inshore zones for both strains as the season progressed (Figure 3.8). Total catches offshore ranged from 4 in July to only 1 in October. Conversely, nearshore catches increased slightly from 3 catches in July to 4 in October. Domestic rainbow trout were readily caught at the beginning of the season, while the wild strain had no recaptures until August of 2011.

3.3.3 Avian predation

Predation pressure by piscivorous birds was evident from their regular presence on both Lake 303 and Lake 304. From April – October 2011, sightings were recorded of common mergansers and common loons with gulls, belted kingfishers, bald eagles, and other ducks species to a lesser extent. Mergansers and loons were present in the study lakes throughout the field season, whereas all other bird species were sighted <5% of the time and were not observed to make any foraging attempts. Presence of mergansers and loons were highest from late spring to early summer in both lakes (40-60% of days present; May – July), and began to taper off as the season progressed into fall (5-15% presence) (Figure 3.9). Foraging behaviour was only observed and recorded for mergansers and loons. The pattern of foraging differed between bird species, with mergansers making shorter, frequent dives while loons dive times were 3-4 times longer. Monthly diving ($\bar{x}$ ± 1 S.D.) time varied from 11.4 ± 4.9 sec (n = 29) to 15.2 ± 5.3 sec (n=52) for mergansers and 44.3 ± 16.5 sec (n = 27) to 64.0 ± 34.7 sec (n=19) for loons (Figure 3.10).

Rainbow trout predation risk (PR) from piscivorous birds was greatest in July and August of each year in both study lakes (Figure 3.11). Loon PR is the greatest in August in Lake 304 (12.6) and May and in Lake 303 (13.3). Merganser PR is approximately uniform throughout the field season in Lake 304 (0.4 – 2.4) and is only present in Lake 303 from July – October (1.5 – 4.2) (Figure 3.11). Total summed PR for both species follows a similar pattern for both lakes with maximum PR in July for Lake 304 and Lake 303 (14.8 and 13.6, respectively),

followed by an eventual decrease to 2.4 and 1.5, respectively by the end of the season in October (Figure 3.11).

3.3.4 Survival

Upon stocking, some acute initial mortality occurred in both lakes. Estimation from snorkel survey the following day post-stocking revealed that 10-20% of the fish stocked incurred mortality upon best estimate, presumably due to the stress and shock of transportation and the stocking event. Survival was estimated using a predictive model based on the probability of a particular recapture length of rainbow trout (eq. 3.2). Using this equation, estimated monthly abundance of each strain was low and approximately similar (Table 3.2). The model predicted extremely low October survival rates of domestic and wild strains of rainbow trout in Lake 304 (5 and 7 fish • lake^{-1}, respectively). The sample sizes required to optimize the results from this model were not achieved (n=17 domestic; n=7 wild) and therefore, the model has a low statistical power to estimate abundance.

3.3.5 Growth

Effort was taken to ensure the initial size of rainbow trout at time of stocking was as similar as possible. Average mass (±1 S.D.) at t_0 for wild and domestic rainbow trout in Lake 304 was 14.2 ± 5.0 g and 12.7 ± 6.6 g, respectively. Although I considered this difference in mass to be adequate for t_0 size matching, there was a statistical difference between groups (Student's t-test for equal variance, $P <$ 0.005; Table 3.3).

Growth rates of stocked rainbow trout varied significantly, not only between strains but also within the domestic strain. Growth rates of domestic rainbow trout

showed two trajectories and were subdivided into fast and slow-growing groups (Figure 3.12). The fast-growing domestic group exhibited a 34-fold increase in mass, fitting an exponential growth equation over time. Wild strain rainbow trout showed a 6-fold increase in mass that was also best fit to an exponential growth curve. The slow-growing domestic group grew the least. For some individuals, final mass was less than the mean t_0 values, as fit by linear regression. Using these curves to fit the data, at t_{100} the mass for fast-growing domestic, wild, and slow-growing domestic rainbow trout are 438.0 g, 83.8 g and 28.7 g, respectively.

3.3.5.1 Individual growth

Of the 28 rainbow trout that were recovered in Lake 304, 11 retained decimal coded wire tags that were successfully removed from the fish (7 domestic and 4 wild - one fish was half eaten when recovered), identified and matched to t_0 values for fork length and mass. This allowed for individual growth rates to be used in calculation of specific growth rate (SGR) and thermal growth coefficient (TGC) to compare between groups. Results show that fast-growing domestic rainbow trout achieved greater than twice the growth rate (SGR=3.12) of both wild (SGR=1.43) and slow-growing (SGR=1.31) domestic groups (Table 3.4). Likewise, the TGC of fast-growing domestic rainbow trout was >3-fold that of both wild and slow-growing domestic groups of rainbow trout. An additional observation is that the individual rainbow trout that were able to achieve elevated growth rates compared to others, is at least partially due to them having a size advantage at t_0. Regressing initial mass (g) over total SGR results in a linear, positive relationship among individual fish (Figure 3.13; $P = 0.06$, $r^2 = 0.38$).

Based on the n-vale for each recaptured group, it is recognised that the statistical strength of the analysis is weak and therefore comparison between groups is largely hypothetical.

3.3.5.2 Stomach contents analysis

A total of 26 rainbow trout stomachs were analyzed for prey items (2 slow-growing domestic stomachs were empty). Prey selection was variable among wild, fast-growing and slow-growing domestic rainbow trout as revealed by the relative importance (RI) index (Figure 3.14). Fast-growing domestic rainbow trout had the greatest reliance on forage fish (cyprinids = 36%) followed by wild (26%) and slow-growing domestic (4%) groups. The most dominant item in fast-growing domestic rainbow trout was benthic and terrestrial invertebrates at 38% including *Odonata*, *Ephemeroptera*, *Diptera* (larvae), *Amphipoda*, *Insecta* (misc.) and *Oligochaeta*. Slow-growing domestic and wild rainbow trout had benthic/terrestrial RI values of 8% and 11%, respectively. Slow-growing domestic rainbow trout had the greatest preference for zooplankton (56%) followed by fast-growing domestic (7%) and wild (0%). Fast-growing domestic, slow-growing domestic and wild rainbow trout all had a significant dietary contribution from emergent *Diptera* (20%, 32%, and 63%, respectively). Diet contribution by prey weight resulted in fast and slow-growing domestics being great for cyprinids (77% and 96%, respectively). Wild rainbow trout had a lower comparative contribution of cyprinds to prey weight (28%) and relied most heavily on *Diptera* (71%) (Figure 3.15). Based on the Schoener's index (SI), the diets of fast and

slow-growing domestic rainbow trout overlapped significantly with each other, but not with the wild strain (Table 3.5).

3.3.5.3 Stable isotope analysis (SIA)

There was a difference in muscle (MANOVA, $F_{(2,16)} = 12.6$, $P < 0.05$) and liver (MANOVA, $F_{(2,17)} = 32.3$, $P < 0.05$) stable isotope values at t_0 of the field experiment between domestic and wild rainbow trout. However, isotope values were within ~1 ‰ of each other for muscle (domestic: $\delta^{13}C = -18.24 \pm 0.49$, $\delta^{15}N = 10.99 \pm 0.90$; wild: $\delta^{13}C = -17.35 \pm .25$, $\delta^{15}N = 11.41 \pm 0.29$) and liver (domestic: $\delta^{13}C = -18.81 \pm 0.59$, $\delta^{15}N = 10.75 \pm 0.51$; wild: $\delta^{13}C = -18.33 \pm 0.26$, $\delta^{15}N = 9.77 \pm 0.29$). Wild, fast-growing domestic and slow-growing domestic rainbow trout captured from Lake 304 did not differ (slightly non-significant) in muscle stable isotope signatures (MANOVA, $F_{(2, 24)} = 2.5$, $P = 0.057$), but were significantly different in liver stable isotope signatures (MANOVA, $F_{(2, 23)} = 3.4$, $P = 0.015$) (Table 3.6). This finding indicates that there was a statistical difference in diet pathways, analyzed from fast turnover tissue for stable isotopes among wild, fast-growing and slow-growing domestic rainbow trout. Most notably, there was little difference in trophic position among wild, fast and slow-growing rainbow trout ($\delta^{15}N < 1‰$). Instead, differences among groups in source of prey ($\delta^{13}C$ ~3‰) were most obvious with slow-growing domestic trout reflecting a more littoral diet than wild or fast-growing domestic fish (Figure 3.16b). Native cyprinids, rainbow trout and the top predator, merganser showed trophic enrichment in $\delta^{15}N$ relative to prey items while carbon signatures reflected variable reliance on inshore and offshore food sources. Wild rainbow trout were

most similar to cyprinids in isotopic signatures (Figure 3.16b). Both domestic groups were trophically elevated in mean $\delta^{15}N$ relative to cyprinids and wild rainbow trout, although this difference was minor (slow-growing domestic = 9.30, fast-growing domestic = 9.50, wild = 8.76). This result confirms that dietary analysis whereby fast-growing domestic fish showed greater reliance on forage fish compared to slow-growing domestic rainbow trout. Despite the high relative importance (RI) of zooplankton (Figure 3.14) or mass of cyprinids (Figure 3.15) in the diet of slow-growing domestic fish, they exhibited a more littoral $\delta^{13}C$ signature than either fast-growing domestics or wild fish. The merganser was elevated ~1-2‰ $\delta^{15}N$ greater than both rainbow trout and cyprinids, indicating that they probably consumed a combination of fish species. Application of the stable isotope data allowed for calculation of functional ecological niches. Slow-growing domestic rainbow trout had the largest niche area, which is indicative of a variable diet. Wild and fast-growing domestic rainbow trout had comparatively smaller niches, reflective of a more specialized diet than slow-growing domestic rainbow trout. Significant niche overlap among the three groups of rainbow trout occurred when using muscle isotope data (Figure 3.17a); however, groups begin to reflect dietary specialization when liver tissue, with a faster turnover rate, was examined (Figure 3.17b). Slow-growing domestic and wild rainbow trout showed a high degree of niche overlap based on liver isotope signatures, whereas the niche of fast-growing domestics had little overlap with slow-growing domestic rainbow trout and almost zero with wild (Table 3.7).

3.3.5.4 Bioenergetics

An estimation of the energy budget by the BM/MMBM, revealed that each group of rainbow trout has radically different metabolic requirements for growth. The model predicted positive growth at different rates for wild and fast-growing domestics and negative growth for slow-growing domestics (the model did not converge). For this reason, comparison of energetic characteristics will focus mainly between wild and fast-growing domestic groups.

Size corrected food consumption rates ($\bar{x} \pm 1$ S.E.) were 1539.13 ± 14.78 J • g^{-1} in wild and 1268.60 ± 14.89 J • g^{-1} in fast-growing domestic rainbow trout (Figure 3.18a). Despite the 5-fold lower growth rate in wild rainbow trout, they consumed ~18% more food per g of body mass than fast-growing domestics. Absolute consumption increased linearly with growth rates of both strains; however, the slope of the relationship between growth and consumption was greater for fast-growing domestic rainbow trout (0.99), indicating that they allocated a greater ratio of food consumed to growth compared to wild fish (slope = 0.59) (Figure 3.18). Similarly, food conversion efficiency was 36% greater in fast-growing domestic rainbow trout compared to wild (Figure 3.18b). Model output estimated that on average (wild rainbow trout allocate approximately half (48.9 ± 0.29%) of total consumption to metabolic costs, roughly 3-fold more than fast-growing domestic rainbow trout (15.5 ± 0.03%) (Figure 3.18c).

3.4 Discussion

The occurrence of aquaculture strains of salmonids escaping into freshwater is common and poses a risk to the stability of native fish species. By quantifying the

comparative expression of growth and mortality of domestic and naturalized Laurentian Great Lake (LGL) genotypes of rainbow trout in the wild, this study aims to characterize the degree of competition between these conspecifics. I previously established that the domesticated strain of rainbow trout achieved a ~3-fold greater mass increase relative to the wild strain under controlled conditions and survival was universally high (Chapter 2). Here, my objectives were to quantify the comparative growth and mortality of these same strains under the selective pressures of a natural system. Overall, survival was low for both domestic and wild strains of LGL rainbow trout stocked into small boreal lakes at the Experimental Lakes Area (ELA). A major finding of this study was that the domestic strain of rainbow trout segregated into two distinct groups with different growth trajectories: a fast-growing group that had a ~34-fold increase in mass and a slow-growing group that doubled in size over the 100 d field study. The wild strain of rainbow trout increased its mass on average by 6-fold. Differences in growth among rainbow trout groups appeared to not be related to habitat choices, but instead to dietary and metabolic differences. Stomach content analysis revealed that superior growth of fast-growing domestic rainbow trout compared to slow-growing domestic and wild was a result of selection for high energy food sources (forage fish) in frequency and prey-weight. Utilization of high energy food sources by fast-growing domestic rainbow trout allowed for a greater energy allocation to somatic growth compared to slow-growing domestic and wild rainbow trout.

3.4.1 Survival

Rainbow trout survival is a major component to this study and reveals how selective pressures affect naturalized and domestic strains of salmonids in the wild. The initial shock of stocking can be a major contributor to mortality. Studies examining fish survival rates post-stocking have reported the greatest incidence of mortality in the first few days of the acclimation phase (Berg and Jorgensen 1991; Pitman and Gutreuter 1993; Aarestrup et al. 2005). Stress induced plasma cortisol levels in rainbow trout have been observed to rise dramatically during stocking events and can remain elevated up to 8 days post-stocking (Barton et al. 1980). During the process of transport from hatchery to lake, wild and domestic rainbow trout were under a high level of stress from oxygen depletion, handling agitation, sedations, and temperature and pH fluctuations. As a result, some rainbow trout died prior to stocking while others did not acclimate successfully to the lakes and were found dead upon snorkel survey the day after stocking (10-20% by best estimate). Although difficult to quantify directly, initial stocking mortality contributed to the overall low survival of stocked rainbow trout in Lakes 303 and 304.

An absolute estimate of survival was difficult to ascertain for both strains of rainbow trout because of the low number of recaptures during the field experiment (20 domestic and 8 wild in L304 and zero catches in L303). Domestic rainbow trout were caught at a greater rate than wild, especially in the first month after stocking (Figure 3.8); however, this can be a result of some domestic salmonids having greater activity and therefore being more susceptible to passive sampling (Biro et al. 2004a, 2007; Jackson and Brown 2011). Previous

experiments stocking similar sized domestic and wild strains of rainbow trout have also resulted in high estimates of mortality over a field season (Post et al. 1999; Biro et al. 2003). Lake 304 mortality calculations from month to month ranged from 96.5 - 99.5% in domestics to 98.6 - 99.5% in wild rainbow trout (Table 3.2). Based on these calculations, both strains experienced equally high mortality in the ELA experiment. Perhaps a better way to assess survival in an experiment such as this would be to follow similar procedures as Biro et al. (2003). In this study, a number of tagged rainbow trout were released prior to intensive fall gillnetting so that the probability of recapture rates could be calculated. This method would eliminate the need to use pre-defined probability curves that may be strain and environment specific.

Predation by avian piscivorous birds is likely the primary mechanism of rainbow trout mortality in Lakes 303 and 304 in the field season of 2011, post stocking. Presence of the most abundant avian predators, loons and mergansers accounted for ~50% of total days observed from May-July on Lakes 303 and 304 and then declined through August-October. Observing behaviour patterns of loons and mergansers on both lakes revealed that foraging by these birds can account for approximately 54% and 13% of the total time present on a lake, respectively. Total bird predation risk (PR) can be calculated by a combination of the terms for presence and foraging activity in both lakes with values starting high in May, peaking in July and steadily declining through the fall. Comparing piscivorous bird activity on Lake 303 and Lake 304 to other studies, it is reasonable to estimate that loons and mergansers potentially contributed 80-90% of total fish

mortality from May-October, 2011 (not including initial stocking mortality). Beckmann et al. (2006) estimates loon consumption rates while foraging on age-1 rainbow trout in BC interior lakes to be 46% of total fish stocked over a 3-month summer field season. However, this is likely an underestimation of realized loon predation. The PR in this study was ~1.5 total for the experiment, whereas PR in Lakes 303 and 304 ranged from ~2-15. PR is greater in the ELA study lakes because proportion of days present was much higher compared to the BC lakes. The present study had the advantage of monitoring every half hour using a combination of focal observations and time-lapse photography, where the BC study was limited to infrequent visits. Age-0 rainbow trout in the BC lakes experienced even greater mortality evidenced by extremely low catch rates (< 1 fish • h^{-1} • ha^{-1} • lake $area^{-1}$) in lakes with high loon presence, accounting for up to 81% of total age-0 rainbow trout mortality after one field season (Beckmann et al. 2006; Biro et al. 2006). Post et al. (1999) also estimated that rainbow trout survival correlates with PR, whereby age-0 fish were predicted to have 1-4% population mortality per day. Population mortality of 1-4% in Lake 303 and Lake 304 would therefore account for upwards of 75-100% of total mortality from May to the end of October.

Limnological conditions may have exacerbated mortality through avian predation in this study. At the time that avian presence on each lake was at its peak in the summer months (May – August), water temperature was reaching up to 22 - 26ºC in some parts of both lakes. This thermal regime has the ability to spatially constrain rainbow trout to the cooler regions of the lake between 2-4 m depth,

where cover is limited and PR would be at its greatest. In partial support of this idea, rainbow trout were captured in the offshore zone of Lake 304 in mid-summer, but captured inshore as water temperatures cooled in fall. In addition to temperature stressors, light attenuation, penetration and Secchi depth are strongest in the mid-summer months (Figure 3.4, 3.5). As higher water temperatures constrain habitable zones in the lakes, vulnerability to visual predators such as loons and mergansers may be amplified from decreasing turbidity in the water column (McIntyre and Barr 1983; McIntyre 1994). Water clarity has been listed as a primary requirement for feeding success in piscivorous diving birds (Barr 1973, 1986) and as a consequence, indirectly contributes to mortality. Besides avian predation, there is also the potential risk of predation from turtles, which was observed during night gillnetting and trap netting.

As a direct mechanism for the mortality, temperature must be considered as the greatest abiotic facator on teleosts (Brett et al. 1969; Fry 1971; Wildhaber and Crowder 1983; Michalsen et al. 1998). High water temperatures throughout the summer in Lake 304 likely had a major influence on survival the upper maximum for rainbow trout was reached in the experimental lakes (~25˚C; Alabaster and Welcomme 1962; Bidgood and Berst 1969; Charlon et al. 1970; Cherry et al. 1975; Hokanson et al. 1977). At this threshold, a few physical, chemical and physiological phenomena occur which are detrimental to fish health: (1) water oxygen content has less ability to dissolve into the water column at higher temperatures which negatively effects respiration for fish; (2) Food consumption is normally distributed over ambient water temperature for teleosts (Ojanguren et

al. 2001; Hofmann and Fischer 2003; Yong-Pu Zhang et al. 2009). At a certain temperature threshold, fish will reduce and eventually stop feeding because the cost of metabolising (specific dynamic action) energy is too great, resulting in reduced growth and even death. For example, in brown trout (*Salmo trutta*), standardized growth rate is a function of ambient temperature. Brown trout in one experiment were observed to increase standardized growth with increasing temperature to a maximum of 17ºC and were observed to have a sharp decline in growth in excess of that temperature (Ojanguren et al. 2001). Metabolic processes past this tipping point are too energetically costly and fish will drastically decrease their energy intake to offset these costs. (3) Temperatures too high to be preferable for rainbow trout will constrain habitat and relegate residence in areas of the lake that may leave them susceptible to predators. Lake 304's physical optimal habitat was from 2-4 m depth where fish were able to obtain sufficient oxygen and preferable water temperatures. Lake 303 provided no refugia from temperature spatially and with depth, creating a potential scenario for summer fish-kill. High water temperatures coupled with predation risk is a likely reason that no rainbow trout were recaptured in Lake 303.

3.4.2 Growth

Two major findings from the whole-lake experiment were the greater than expected differences in growth trajectories between wild and domestic strains of rainbow trout, and extreme divergence in growth within the domestic strain. Fast-growing domestic fish achieved >5 times the mass of wild conspecifics after 100 d (Figure 3.13). My study resulted in rainbow trout growth trajectories in the wild

that were similar to previous Canadian studies (Ayles and Baker 1983) or greater (Biro et al. 2004a, 2006). Ayles and Baker (1983) describe their experimental study lakes as typical shallow Canadian prairie lakes that experience winterkill and are highly eutrophic, productive and unstratified. It is unclear the structure of the ecological community and what species were present as a source of forage for stocked rainbow trout, but most of the experimental lakes were thought to contain no native fishes (Sunde and Barica 1975). Likewise, in experimental studies on fishless BC lakes, domestic rainbow trout achieved double the mass of wild fish with zooplankton as the primary prey (Biro et al. 2006). To achieve accelerated growth rates, salmonids will typically shift their preference to high energy food sources (i.e., minnows) after surpassing gape-size limitations (Gretchen et al. 2004; Keeley and Grant 2001; Mittelbach and Persson 1998; Trippel and Beamish 1993). Growth potential of freshwater salmonids is therefore strongly dependent on the energy sources available in a given ecosystem. The great dichotomy between slow-growing and fast-growing domestic rainbow trout in this experiment is an example of differences in energy acquisition through diet. The high energy food (forage fish) in the diets of fast-growing domestic trout was almost 10-fold greater than for their slow-growing counterparts.

Stable isotope analysis provided additional insight into selection of prey items and relative position in the food web for the stocked strains of rainbow trout. In this study, isotopic signatures from liver and muscle tissue confirmed stomach content analysis. Fast-growing domestic rainbow trout had a consistently greater $\delta^{15}N$ value than other groups, indicating their reliance on higher trophic level food

sources (Minagawa and Wada 1984). Slow-growing domestic and wild rainbow trout had similar $\delta^{15}N$ signatures (9.30 and 8.76, respectively), but wild fish showed a lower $\delta^{13}C$ value, which was very similar to Lake 304's minnow species, suggesting that they foraged on much of the same prey sources. Slow-growing domestic rainbow trout also had a lower signature in $\delta^{13}C$ than fast-growing rainbow trout. This finding may reflect that slow-growing rainbow trout selected for more littoral/benthic invertebrates compared to fast-growing domestic trout, but could also be an artifact of low tissue turnover and retention of hatchery food $\delta^{13}C$ signatures. Calculation by Bayesian statistics confirmed that fast-growing domestics have a narrow and specialized diet that differs from slow-growing domestic and wild rainbow trout. The most variable niche width and largest area is that of slow-growing domestics, which was largely driven by variable $\delta^{13}C$ values, indicating that a variety of prey sources for this group that included basal food web sources such as benthic invertebrates (Fry 2006). Wild rainbow trout overlapped with slow-growing domestics, which is consistent with each group's selection for a variety of invertebrate species. Increased separation among groups in isotopic space is evident in liver versus muscle tissue (Figure 3.17). Though much of the same patterns emerge in both tissues, the enhanced turnover in the more metabolically active liver tissue allows greater resolution in isotopic signatures and is better at reflecting prey item choices at a smaller time scale compared to muscle. One confounding factor in this data set however, is that $\delta^{15}N$ values for slow-growing domestics did not scale well with body size. Slow-growing domestics in some cases had even greater $\delta^{15}N$ values than both larger

body domestic and wild rainbow trout. There are two possible reasons for this finding: (1) growth was low in this group and as a result, low tissue turnover. Slow-growing domestics may have retained isotopic signatures from hatchery food or (2) slow-growing domestic rainbow trout were potentially in a state of severe food limitation (i.e., starvation) and were forced to oxidize muscle protein as a substrate for energy relative to carbon rich glycogen and lipid stores. In absence of another significant energy source, structural protein can be catabolized to provide a metabolic fuel to teleosts resulting in decreased growth (Maddock and Burton 1994; Martin et al. 2002), causing $\delta^{15}N$ values to be elevated even if growth rate is stagnant or decreasing. Evidence of elevated stable nitrogen isotope values as a result of amino acid cycling/wasting has been observed in a number of nutrient deficient taxa including: mammals (Polischuk et al. 2000), invertebrates (Scrimgeour et al. 1995; Webb et al. 1998), reptiles (McCue and Pollock 2008) and fish (Doucett et al. 1999; Gaye-Siessegger et al. 2004).

An interesting finding in my study is the divergence in stomach contents among wild, fast-growing and slow-growing domestic rainbow trout, although all groups were not segregated spatially. Catch locations suggest that after the first month subsequent to stocking, both wild and domestic strains of rainbow trout were found mostly in the littoral region of Lake 304. This is corroborated with stomach contents of each group being characteristic of littoral prey-items (i.e., *Leptodora* species of Cladocera found near shore). The selection of mostly near shore prey items among all three groups is consistent with greater catch rates inshore versus offshore and observations of near shore habitat use by escaped farmed fish in

other studies (Blanchfield et al. 2009; Patterson 2010). Much of the diet associated with slow-growing domestic and wild rainbow trout is the same that has been observed in smaller size classes (Scott and Crossman 1973; Nilsson and Northcote 1981; Beauchamp 1990; Warner and Quinn 1995). In Lake Michigan tributaries, age-0 rainbow trout fed on similar taxa as those observed in wild and some slow-growing domestics in Lake 304 with the majority of stomach contents consisting of benthic invertebrates (Godby et al. 2007). Conversely, younger age classes of farmed rainbow trout in marine Norwegian waters showed an increasing preference for forage fish as time from release from aquaculture increased similar to the pattern observed in fast-growing domestic rainbow trout in Lake 304 (Rikardsen and Sandring 2006). Divergence in prey item selection between LGL strains of domestic and wild rainbow trout is a novel finding in this study and has not been demonstrated previously. This finding suggests that both wild and domestic rainbow trout broadly select similar habitat types, but their diets reflect differences in micro-habitat or prey selection.

3.4.2.1 Bioenergetics modeling

Using standard bioenergetics equations to solve for metabolic processes allowed for detailed insight into energy acquisition and expenditure by different strains of rainbow trout in this field study. For example, wild rainbow trout had the greatest relative size-corrected consumption rates, but relative metabolic costs accounted for a greater proportion of their energy budget than fast-growing domestics (Figure 3.18c). Additionally, conversion efficiency was lower in wild compared to fast-growing domestics (Figure 3.18b), so the slower growth in wild rainbow

trout can be attributed to greater metabolic costs in this strain and not a low rate of food consumption. Conversely, fast-growing domestics invested comparably less energy into metabolic costs, presumably because they consumed high energy food items and spent less time forging for zooplankton and benthic invertebrates. This finding is corroborated by the diet analysis in which fast-growing domestics fed predominantly of forage fish (Figure 3.16). Superior growth in the fast-growing LGL aquaculture strain is supported by increased feed intake, conversion efficiency and consumption rate in laboratory growth trials (Chapter 2). Fast-growing domestic attributes of metabolism relate well to a fish farm scenario: an individual fish may feed *Ad libitum* with little consequence of predators or the need to find food. However, this energetic tactic may not be ideal in every environment. Water bodies that do not provide food of great abundance or energy may force domestic rainbow trout to forage for pelagic prey items. This is the case in studies by Biro et al. (2004a, 2006). Pelagic foraging for zooplankton by domestic rainbow trout in these experiments led to greater mortality costs, especially in low food and high predator presence lakes. Since fast-growing rainbow trout were not provided an option for high energy food, they needed to expend more energy to acquire food items to offset metabolic costs. Furthermore, domestic strains greater energetic allocation to stoma at a given consumption rate (Figure 3.19), can be disadvantageous in temperate climates. For example, in Canadian lakes, wild populations typically allocate greater energy to lipid reserves over stoma when they pass size-dependent risk of predation and in anticipation of winter (Biro et al. 2005).

3.4.3 Conclusions

The stocking of LGL strains of naturalized and domestic rainbow trout into a common system resulted in outcomes that were unexpected and specific to each group. Survival rates were very low in Lakes 303 and 304 throughout the field season, likely a consequence of a number of mechanisms. The water temperatures at ELA rose steadily throughout the summer, which constrained habitat thermally and spatially, presumably forcing rainbow trout to the littoral zone of the lakes. Both strains sharing similar habitat led to equal levels of predation and mortality throughout the experiment. Using a common section of the lake also allowed for each strain to use the same inshore habitat to select prey-items. A dichotomy in growth trajectories occurred as a result of differentiation in prey-items. The fast-growing domestics fed predominantly on high energy food (minnows), while the slow-growing domestics and wild shared a greater reliance on aquatic invertebrates. The move to the inshore zone of Lake 304 is a possible reason that the slow-growing domestic rainbow trout were not able to achieve high growth rates. As evidenced from the individual growth analysis, slow-growing domestics were likely the smaller fish to begin with, and did not have the opportunity to surpass the gape-size limitations before being forced inshore from high temperatures. Overall, the potential for greater growth in domestics is evident as they are able to take advantage of high energy food items and employ more efficient energetic strategies compared to wild conspecifics.

Table 3.1 Total gillnet hours (h • # of nets) fished for Lake 303 and Lake 304 at the Experimental Lakes Area in 2011. Gillnet hours is a product the total number of hours a gillnet was fishing in a lake by the number of nets set in the lake.

Lake	July	August	September	October	Total
303					
Inshore	422	3912	11111	15539	30984
Offshore	391	3414	0	2204	6009
304					
Inshore	971	4317	34727	37492	77507
Offshore	1024	4412	14125	21722	41283

Table 3.2 Estimated survival (total number of fish remaining) in Lake 304 at the Experimental Lakes Area in 2011 based on a formula for probability of recapture (Biro et al. 2003) of a specific fork length of a rainbow trout. Monthly estimations are based on recapture and size values from the respective months.

	Strain	
Month	Wild	Domestic
June	500	500
July	no data	17.49
August	2.36	2.52
September	7.11	14.49
October	7.16	4.83

Table 3.3 Initial mass (g) and fork length (mm) ± 1 S.D. of wild and domestic strains of rainbow trout stocked into Lake 303 and Lake 304 at the Experimental Lakes Area on June 25, 2011. Differences in size were determined by a Student's t test for equal variance.

	Wild	Domestic	p-value
Lake 304			
Mass	14.2 ± 5.0	12.7 ± 6.6	<0.005*
Fork length	110 ± 12	98 ± 12	<0.0001*
n	249	248	-
Lake 303			
Mass	14.0 ± 4.4	12.2 ± 4.8	<0.0001*
Fork length	110 ± 12	97 ± 10	<0.0001*
n	250	249	-

Table 3.4 Initial, final mass (g), fork length (m), specific growth rate (SGR) and thermal growth coefficient (TGC) of wild, fast-growing domestic and slow-growing domestic groups of rainbow trout recaptured from Lake 304 at the Experimental Lakes Area in 2011 (± 1 S.D.). SGR and TGC are calculated by t_0 and t_x mass of decimal coded wire tags (DCWT). TGC was multiplied by 1000 to bring to unity.

Group	Mass (g)		Fork Length (mm)		
	t_0	t_x	t_0	t_x	Time (d)
Wild (n =3)	26.0	73.4	138	183	90
	12.4	33.0	107	146	64
	23.6	66.5	133	180	64
SGR	1.43 ± 0.25				
TGC	0.94 ± 0.17				
Fast-growing	39.2	104.1	146	199	26
domestic (n = 2)	36.2	227.7	143	243	74
SGR	3.12 ± 0.90				
TGC	2.96 ± 0.98				
Slow-growing	31.0	48.1	131	158	26
domestic (n = 5)	9.5	14.9	91	106	64
	16.0	34.6	106	141	26
	15.8	15.7	105	111	28
	9.0	27.6	90	134	94
SGR	1.31 ± 1.12				
TGC	0.88 ± 0.84				

Table 3.5 Schoener's index (SI) based on prey weight (g) among wild, fast-growing and slow-growing domestic rainbow trout stomach contents recaptured from Lake 304 at the Experimental Lakes Area in 2011. SI values > 0.60 (*) represent biologically significant dietary overlap in prey items.

Groups compared	Schoener's Index
Wild vs. Domestic (fast growth)	0.30
Wild vs. Domestic (slow growth)	0.30
Domestic (fast growth) vs. Domestic (slow growth)	0.78*

Table 3.6 MANOVA and ANOVA of the δ13C and $\delta^{15}N$ stable isotopes among wild, fast-growing and slow-growing domestic rainbow trout recaptured from Lake 304 at the Experimental Lakes Area in 2011. * indicates statistical significance ($P<0.05$).

MANOVA ($\delta^{13}C$-$\delta^{15}N$)	DF	Pillai Stat	F	Num. DF	*P*
Liver					
Strain	2	0.459	3.425	4	0.015*****
Residuals	23				
ANOVA ($\delta^{13}C$)	DF	Sum Sq.	Mean Sq.	F-value	
Strain	2	46.49	23.24	3.28	0.056
Residuals	23	162.83	7.08		
ANOVA ($\delta^{15}N$)	DF	Sum Sq.	Mean Sq.	F-value	
Strain	2	2.00	1.00	2.5053	0.104
Residuals	23	9.18	0.40		
Muscle					
Strain	2	0.341	2.471	4	0.057
Residuals	24				
ANOVA ($\delta^{13}C$)	DF	Sum Sq.	Mean Sq.	F-value	
Strain	2	58.22	29.11	2.73	0.085
Residuals	24	255.94	10.66		
ANOVA ($\delta^{15}N$)	DF	Sum Sq.	Mean Sq.	F-value	
Strain	2	0.53	0.26	0.53	0.593
Residuals	24	11.83	0.49		

Table 3.7 Standard ellipse area corrected for small sample size (SEA_c), total area of the convex hull (TA), and % niche overlap as calculated by Bayesian estimates of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values from liver and muscle tissues removed from wild and fast-growing and slow-growing domestic rainbow trout recaptured from Lake 304 at the Experimental Lakes Area in 2011.

			Overlap (% total area)		
Comparison	SEA_c	TA	vs. Wild	vs. Domestic (fast-growing)	vs. Domestic (slow-growing)
Liver					
Wild	2.67	3.19	0.00	<0.01	17.63
Domestic (fast-growing)	0.84	0.90	<0.01	0.00	2.38
Domestic (slow-growing)	7.14	13.90	17.63	2.38	0.00
Muscle					
Wild	1.56	1.79	0.00	20.13	15.14
Domestic (fast-growing)	1.63	1.33	20.13	0.00	5.26
Domestic (slow-growing)	7.20	16.75	15.14	5.26	0.00

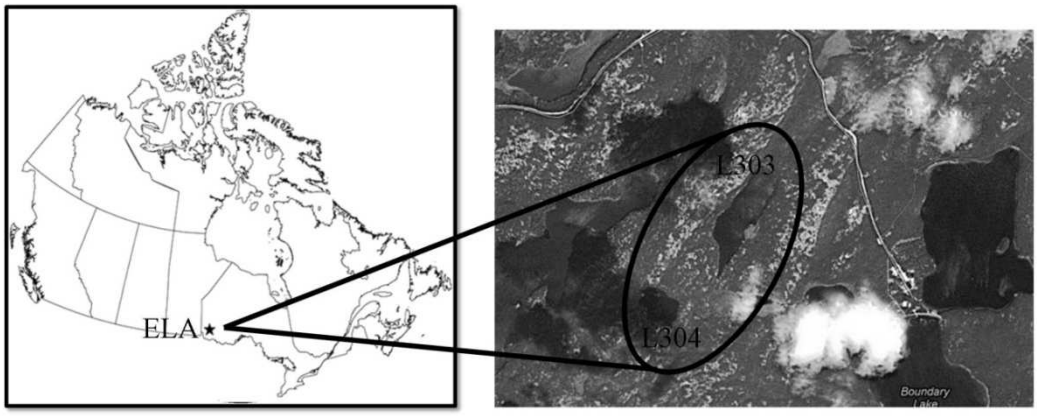

Figure 3.1 Lake 303 and Lake 304 at the Experimental Lakes Area in northwestern Ontario, Canada. The map on the left shows the location of the ELA in relation to the rest of Canada. The map on the right is an aerial representation produced from Google earth™ (2012).

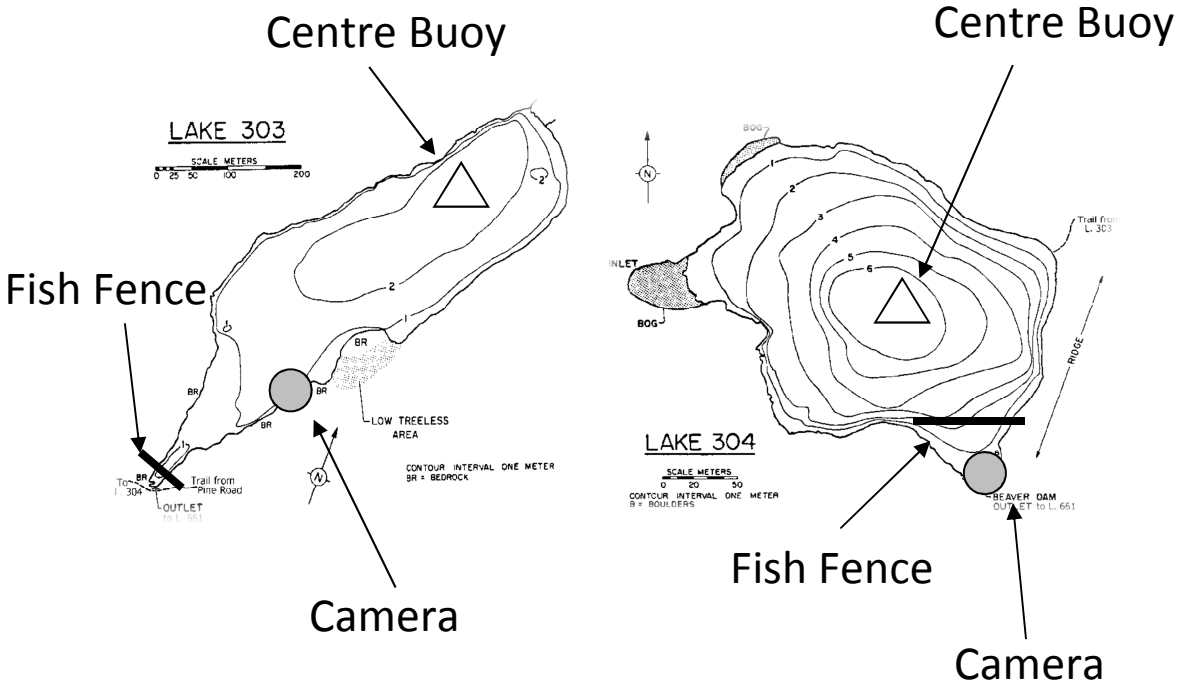

Figure 3.2 Lake 303 and Lake 304 at the Experimental Lakes Area in northwestern Ontario, Canada. Lake 303: Max Depth (m): 2.5; Volume (m^3): 150023; Area (ha): 9.93. A barrier fish fence is located at the southwestern outflow of the lake. A time-lapse camera is attached to a tree on the southwestern portion of the lake on the east shore facing northeast. The centre buoy marker for the lake is located at the deepest section of the lake in the northeast section within the 2 m isobath. Lake 304: Max Depth (m): 6.7; Volume (m^3): 114826; Area (ha): 3.62. A barrier fish fence is located at the southern outflow of the lake, north of a beaver dam. A time-lapse camera is attached to a tree behind the barrier fence on the south shore of the lake facing north. The centre buoy marker for the lake is located at the deepest section of the lake at the approximate centre within the 6 m isobath.

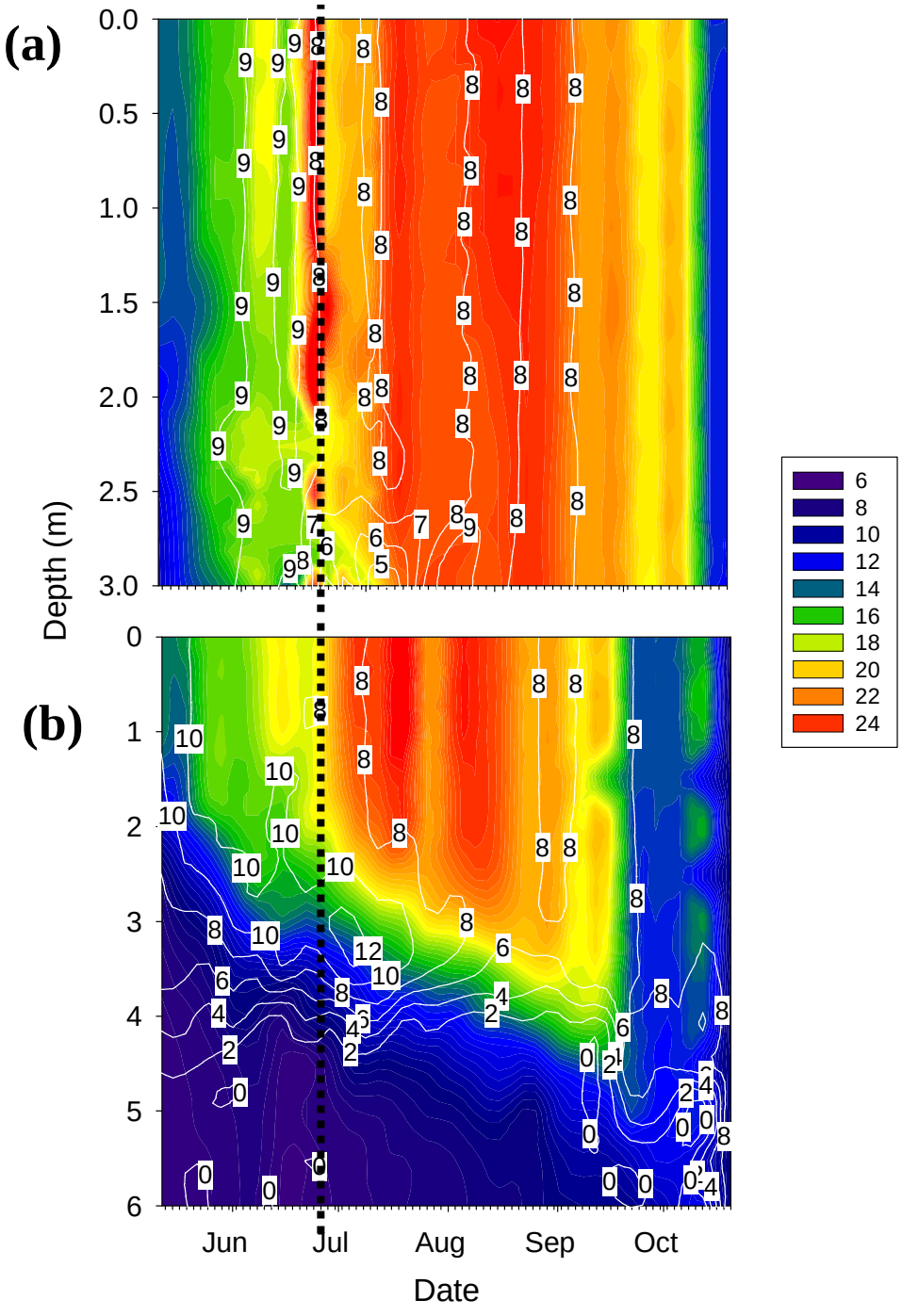

Figure 3.3 Lake 303 (a) and Lake 304 (b) temperature and dissolved oxygen (isobaths) depth plots from April to October 2011 at the Experimental Lakes Area. Temperature is from data obtained by TidbiT temperature logger at specified depths at ~ 12:00 daily. Dissolved oxygen data was obtained from weekly profiling with a YSI device. The dashed black line indicates the stocking date of wild and domestic strains of rainbow trout.

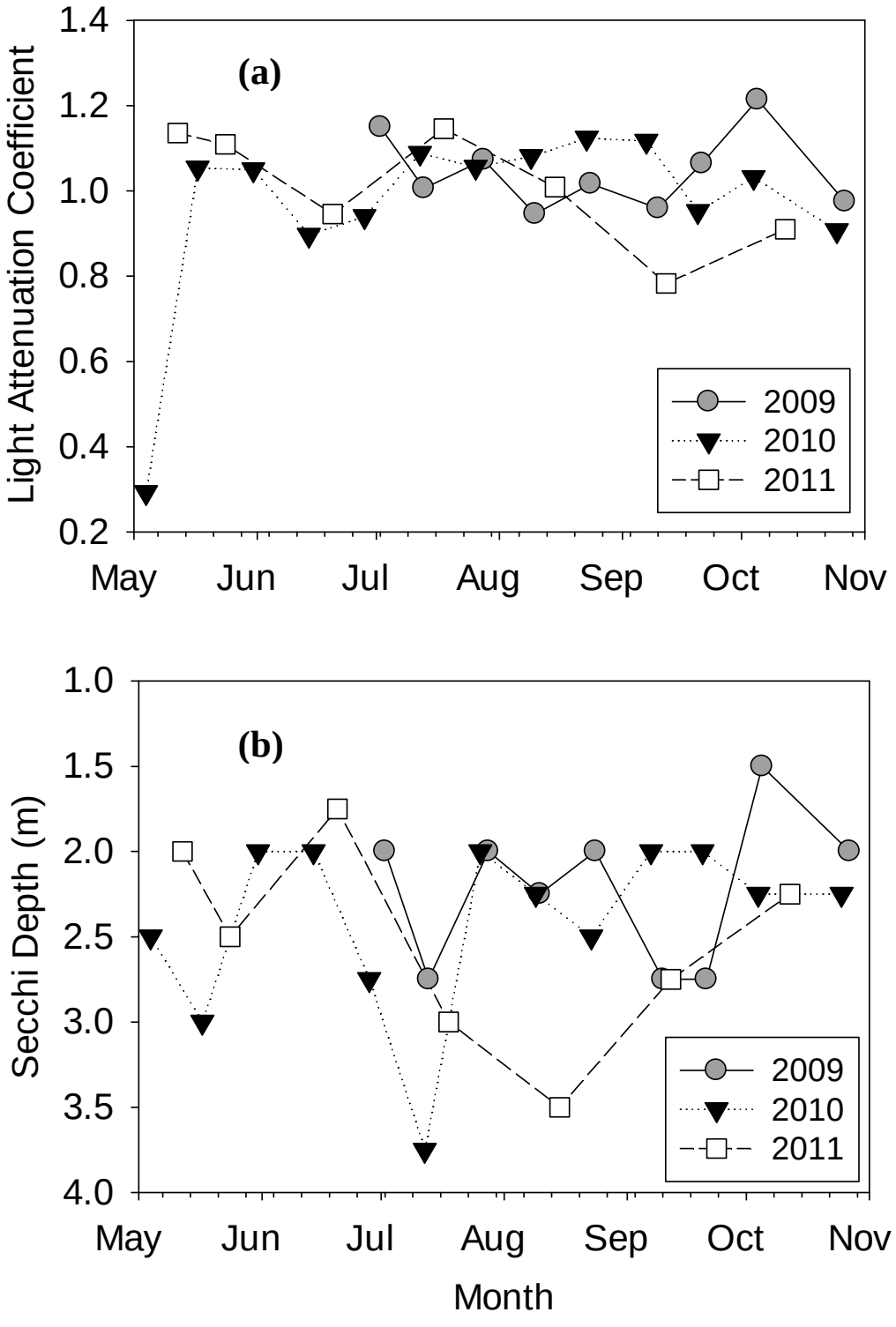

Figure 3.4 Light attenuation coefficient (a) and Secchi depth (b) measured in Lake 304 at the Experimental Lakes Area from 2009 – 2011.

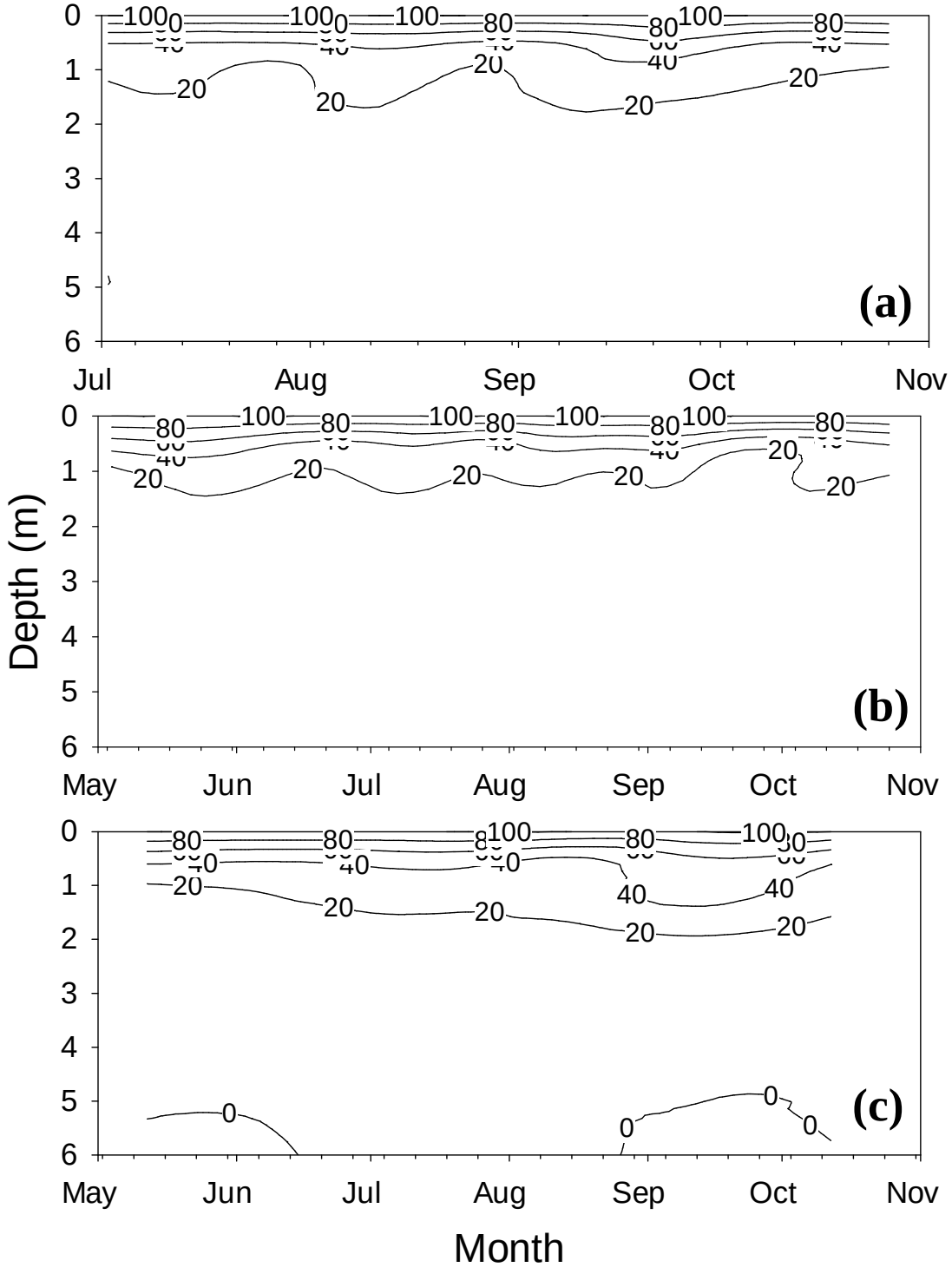

Figure 3.5 Light penetration (%) in 2009 (a), 2010 (b) and 2011 (c) in Lake 304 at the Experimental Lakes Area.

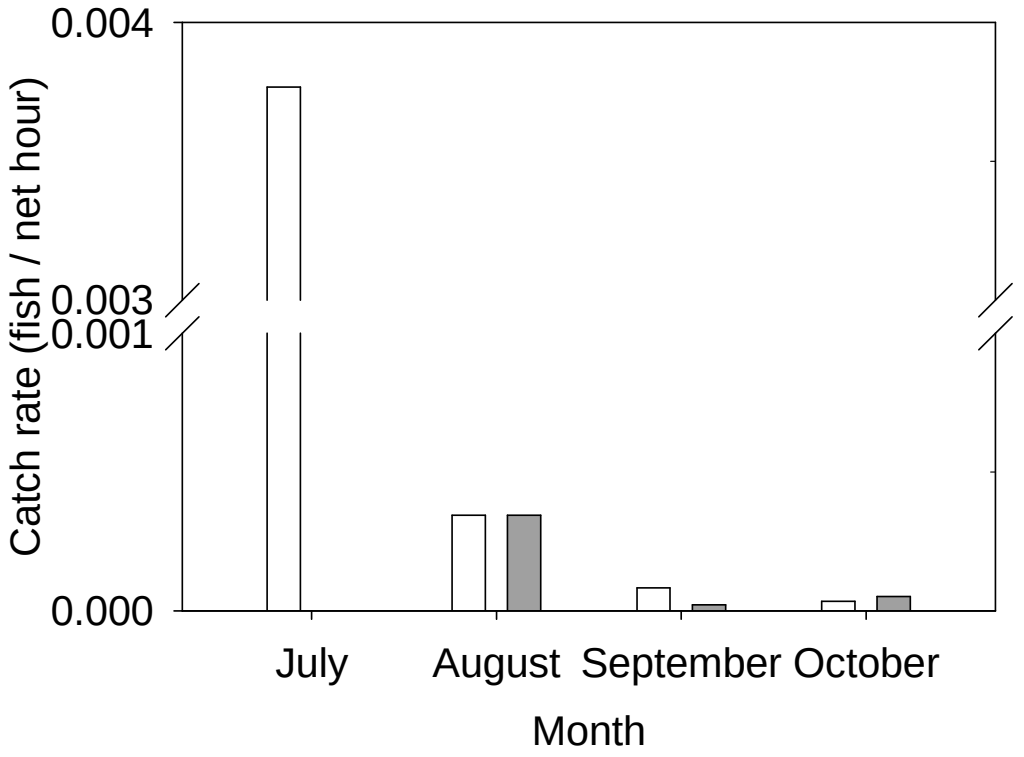

Figure 3.6 Monthly catch rates of wild (grey bars) and domestic (white bars) rainbow trout in Lake 304 at the Experimental Lakes Area in 2011. Net hours are a product of the total amount of hours a gillnet was fished by the number of nets set in the lake.

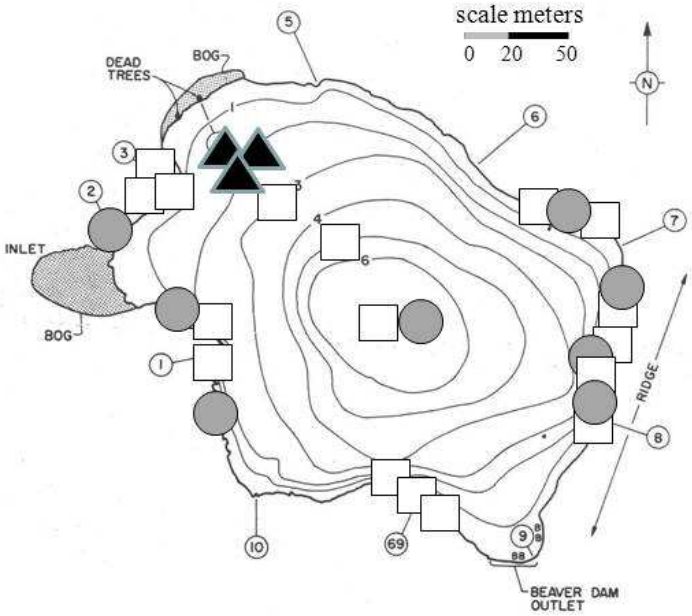

Figure 3.7 Locations of gillnet-caught wild (grey circle), gillnet-caught domestic (open square) and trap net-caught domestic (black triangle) rainbow trout in Lake 304 at the Experimental Lakes Area in 2011. Contour intervals are one meter. B = boulder; circled numbers = station numbers.

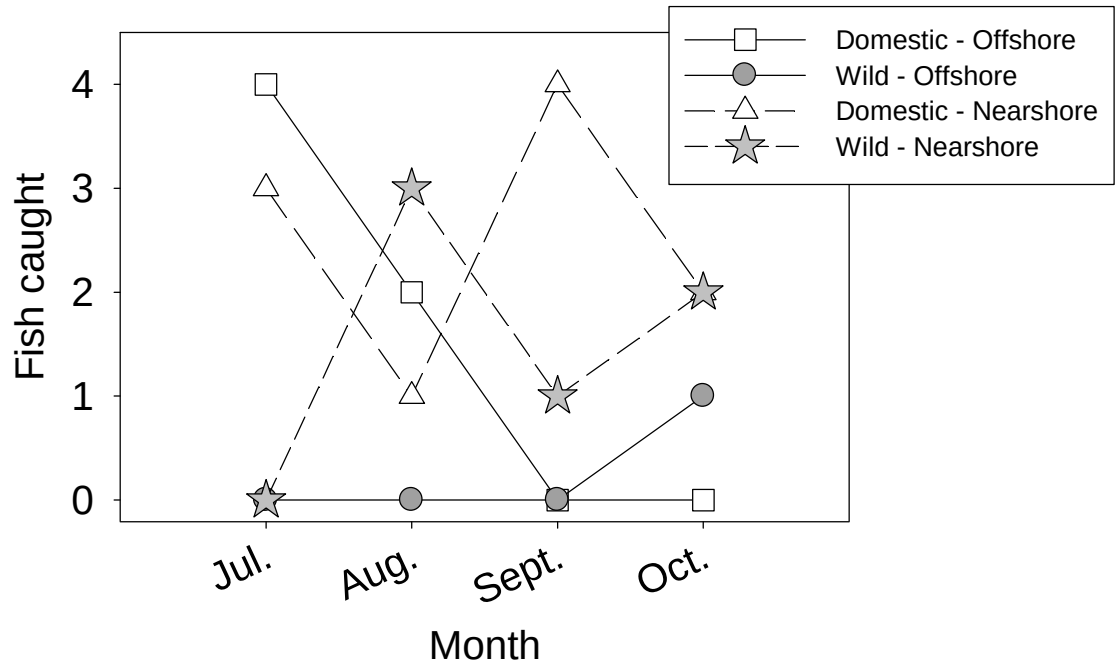

Figure 3.8 Monthly gillnet catch locations of stocked wild and domestic rainbow trout in Lake 304 at the Experimental Lakes Area in 2011.

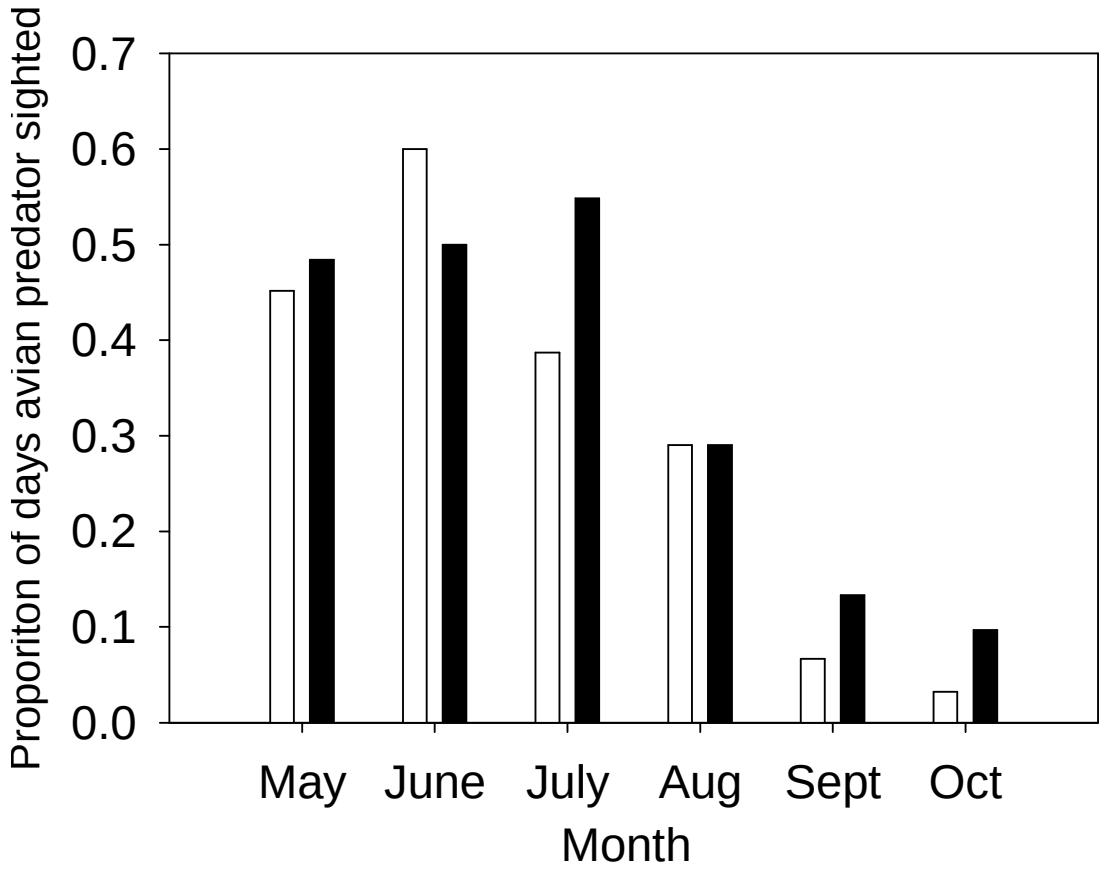

Figure 3.9 Days an avian predator was sighted on Lake 303 (black bars) and Lake 304 (white bars) at the Experimental Lakes Area in 2011 as proportion of the total number of days of observation. Observations were based on a combination of time-lapse photography and personal focal observations.

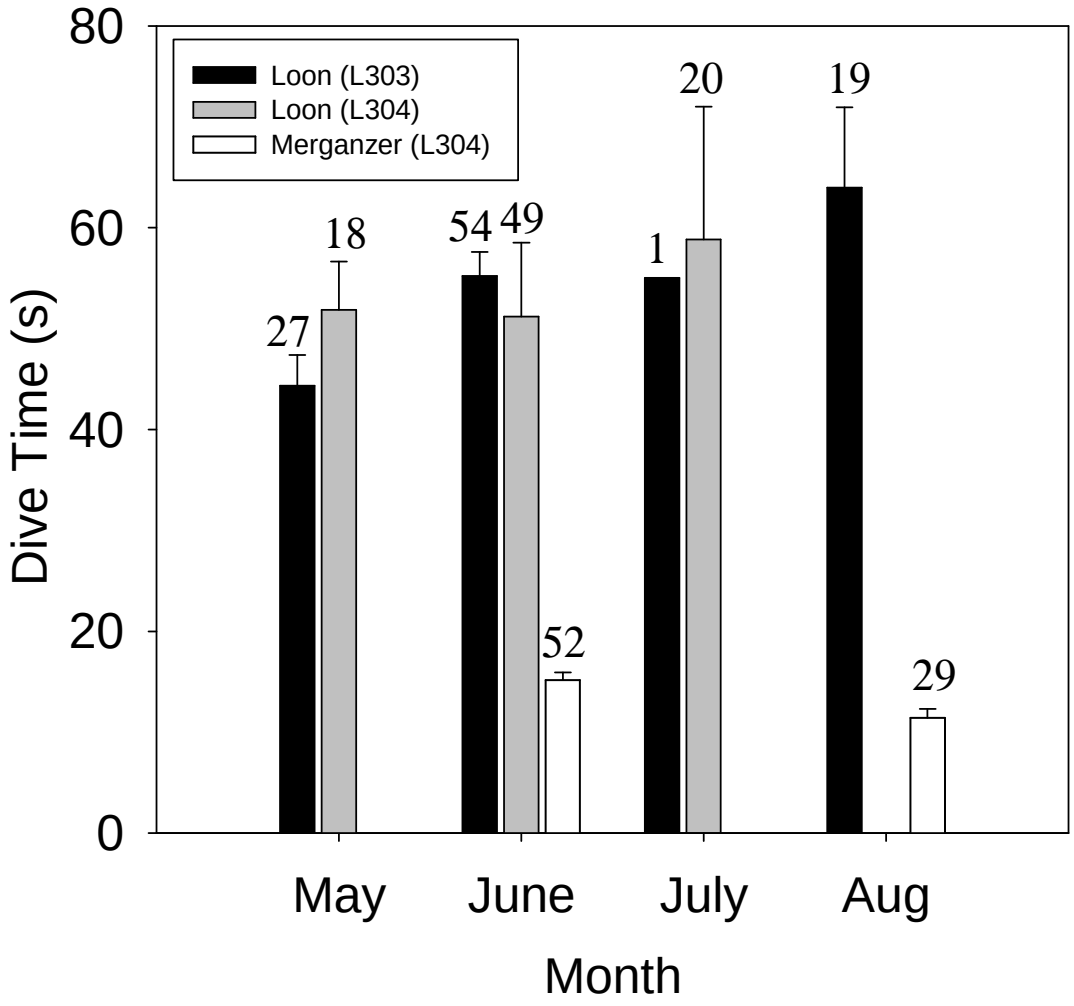

Figure 3.10 Mean dive time for loons and mergansers in Lake 303 and Lake 304 at the Experimental Lakes Area in 2011. The number above each bar represents the number of dive times recorded. Error bars are ±1 S.E.

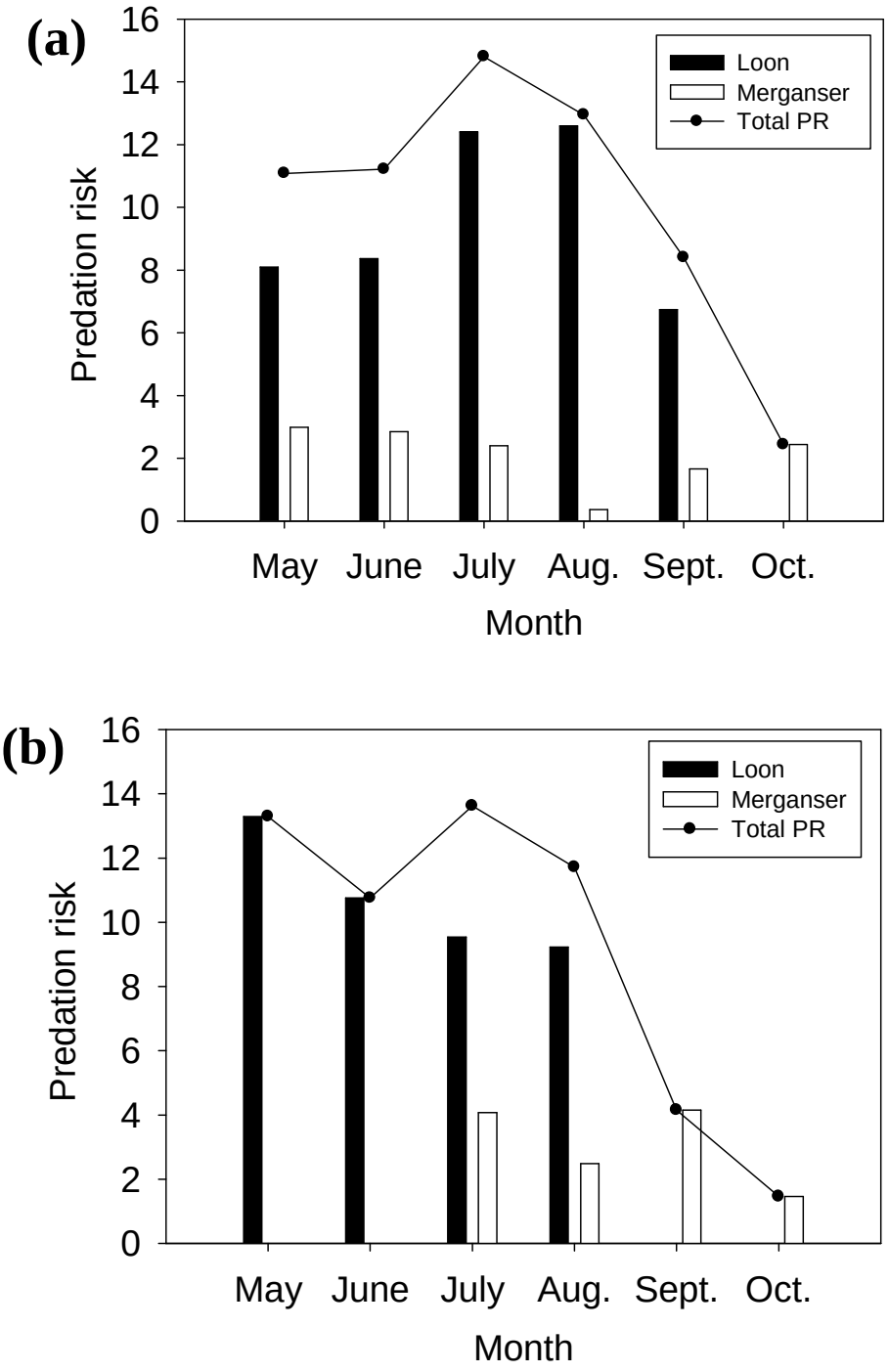

Figure 3.11 Predation risk as calculated by presence/absence and foraging activity data for loons and mergansers in Lake 304 (a) and Lake 303 (b) at the Experimental Lakes Area in 2011. Dive times were not calculated for Lake 303 mergansers and averaged Lake 304 foraging activity data was used to calculate Lake 303 merganser PR. The equation for predation risk is modified from Beckmann et al. (2006).

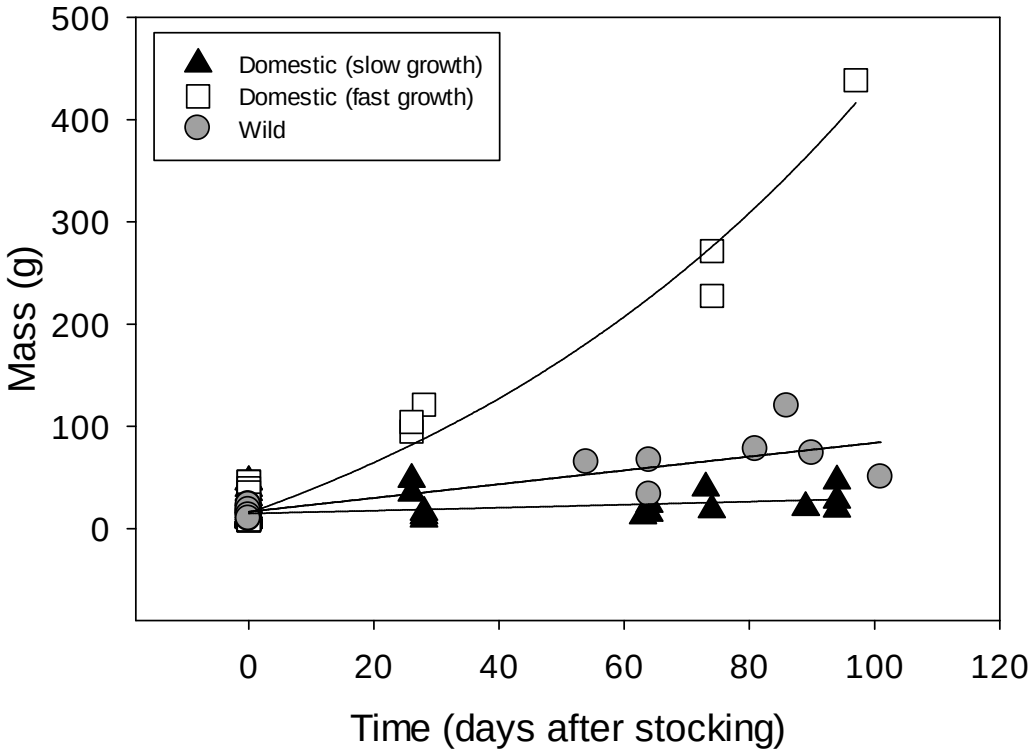

Figure 3.12 Body mass of wild and domestic stocked rainbow trout in Lake 304 at the Experimental Lakes Area in 2011. Domestic rainbow trout were further divided into fast-growing and slow-growing groups.

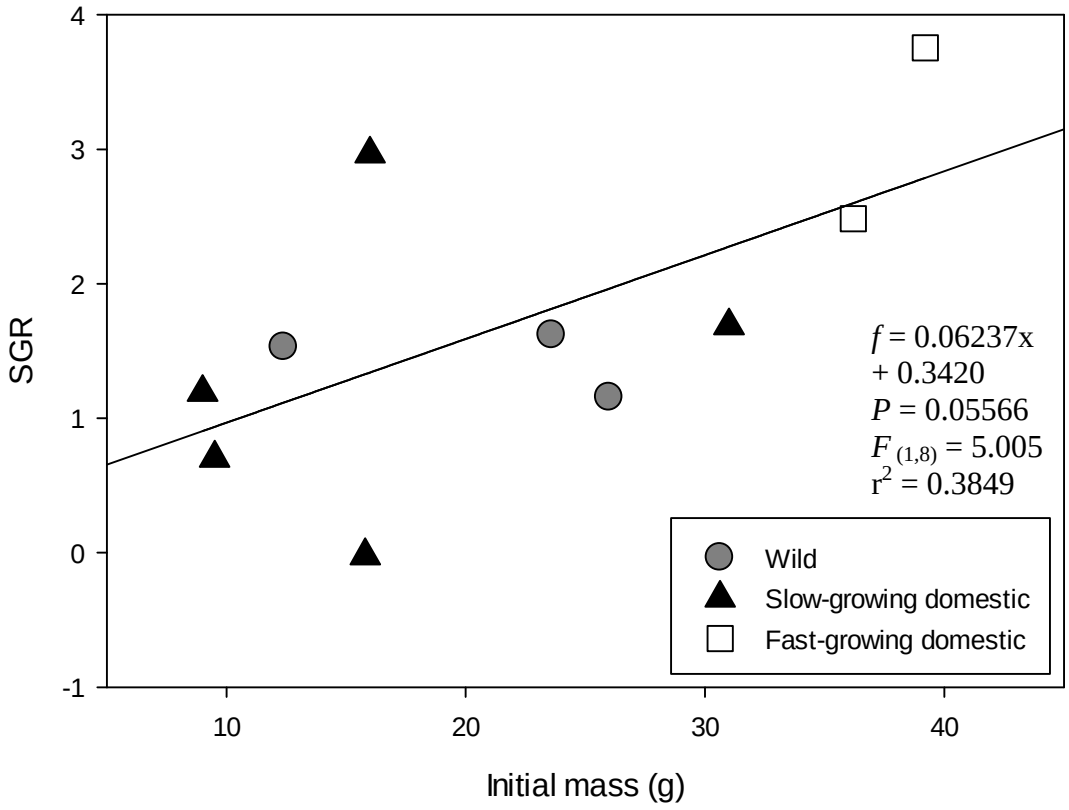

Figure 3.13 Specific growth rate (SGR) as a function of initial mass (g) as measured from wild and domestic rainbow trout recovered from Lake 304 at the Experimental Lakes Area in 2011, retaining decimal coded wire tags.

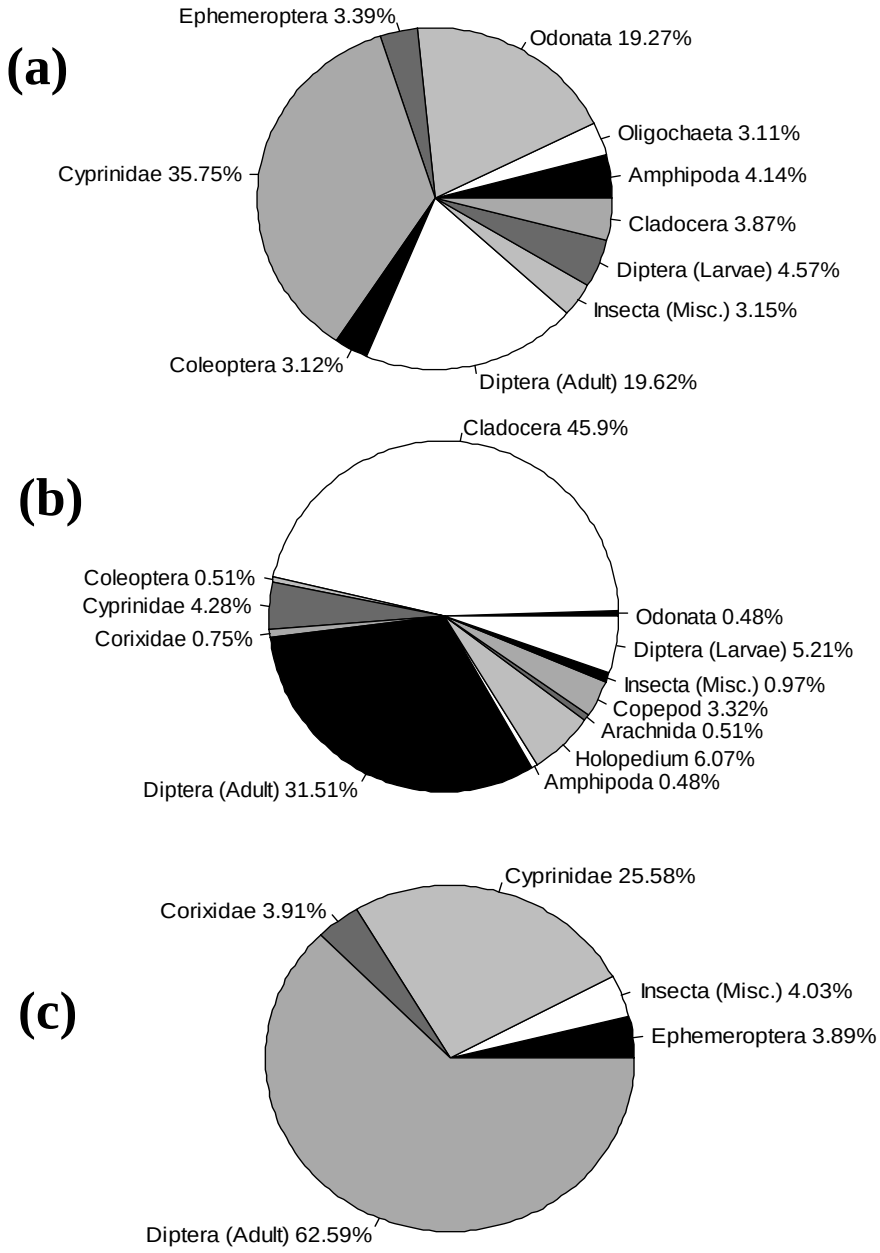

Figure 3.14 Relative Importance (RI) index of prey items identified in fast-growing domestic (n=6) (a), slow-growing domestic (n=11) (b), and wild (n=8) (c) rainbow trout stocked in Lake 304 at the Experimental Lakes Area from 2011 (and one wild rainbow trout from 2012).

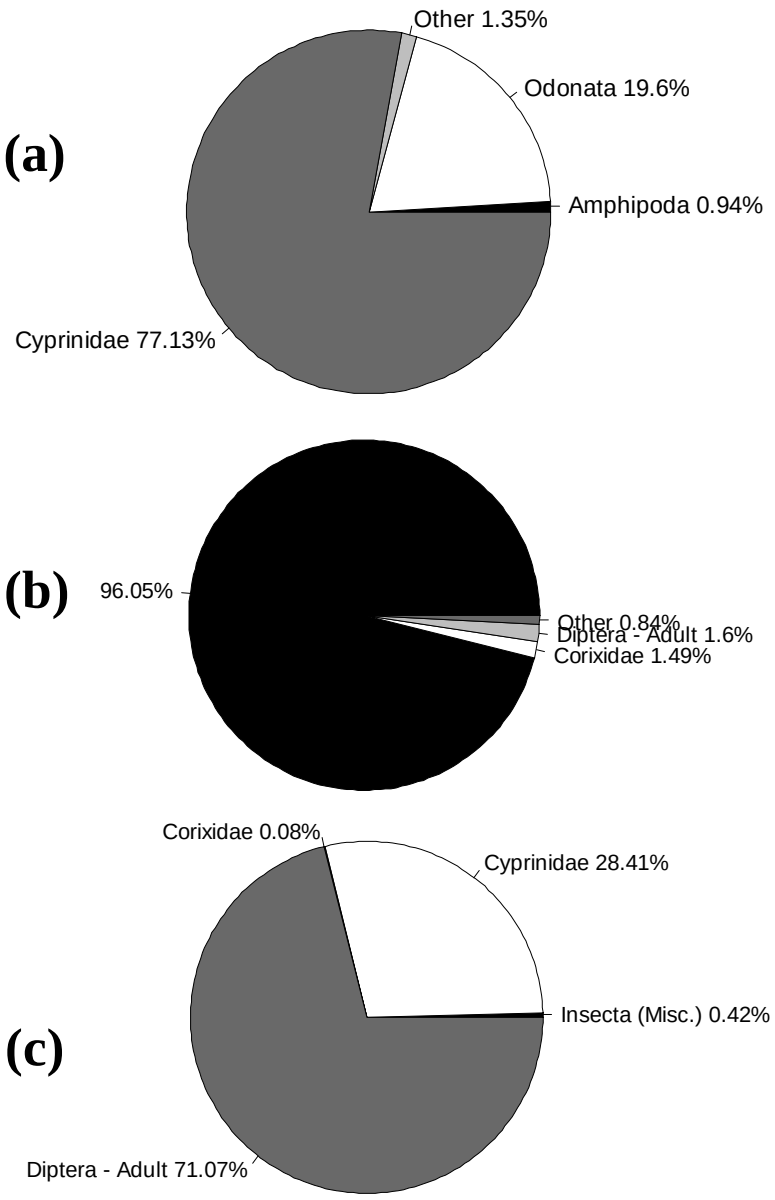

Figure 3.15 Percentage of stomach contents by prey weight identified in fast-growing domestic (n=6) (a), slow-growing domestic (n=11) (b), and wild (n=8) (c) rainbow trout stocked in Lake 304 at the Experimental Lakes Area from 2011 (and one wild rainbow trout from 2012).

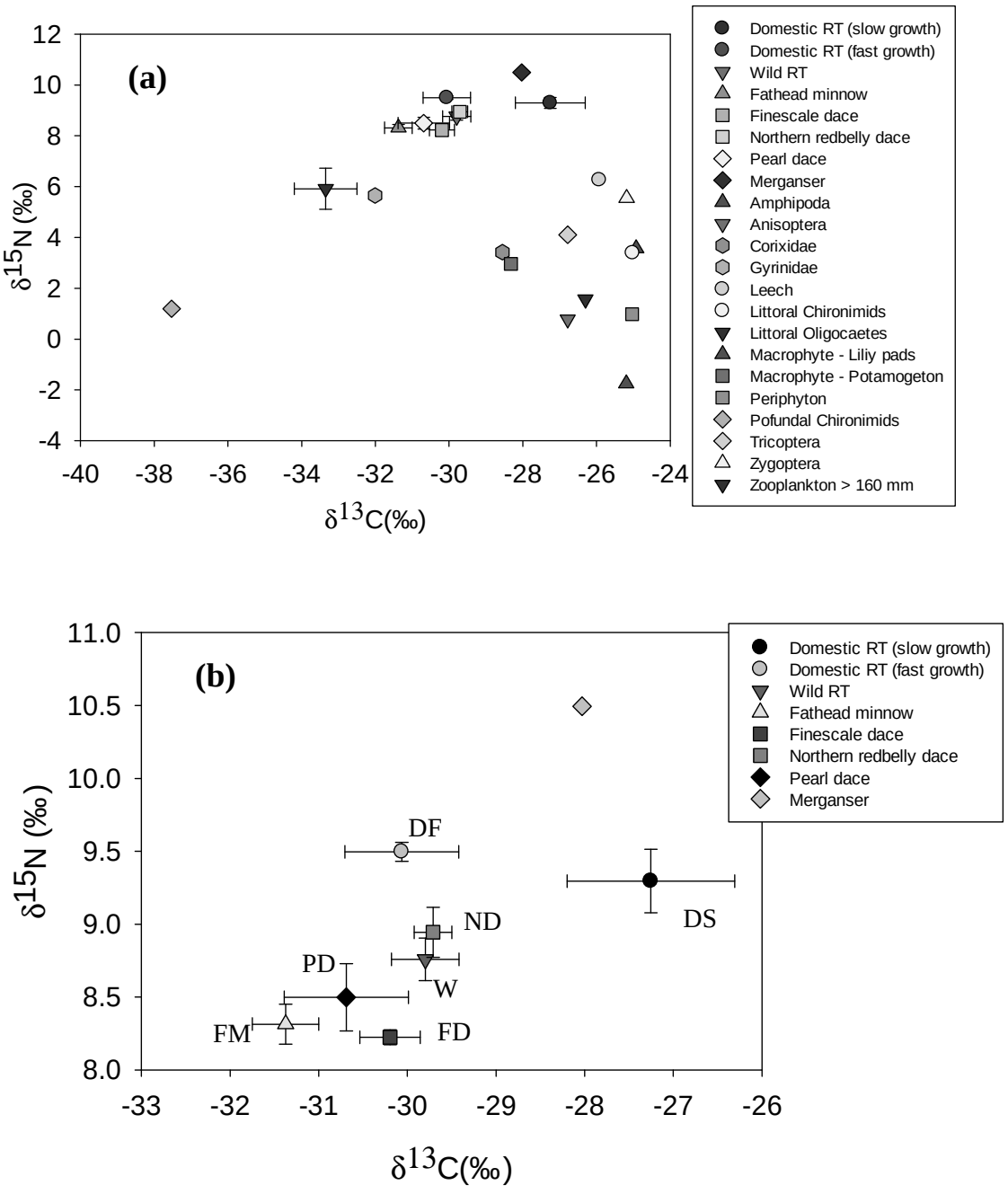

Figure 3.16 $\delta^{13}C$/ $\delta^{15}N$ ± 1 S.E. stable isotope biplot of all major food components (a) and top level consumers (b) obtained from Lake 304 at the Experimental Lakes Area in 2011. Note: Rainbow trout and merganser stable isotope values were obtained from liver samples.

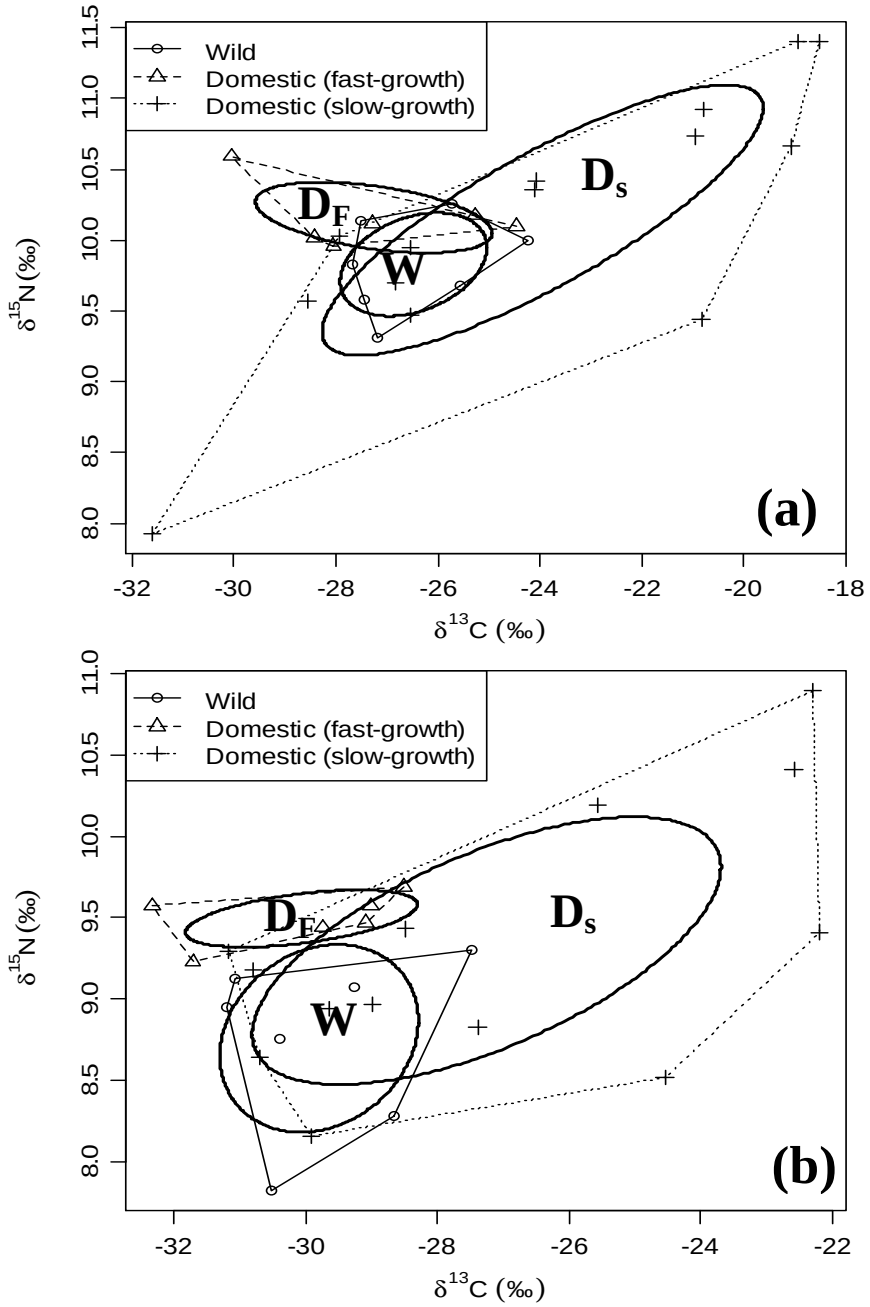

Figure 3.17 Standard ellipses and convex hulls inscribed around $\delta^{13}C$ and $\delta^{15}N$ muscle (a) and liver (b) of wild (W), fast-growing (D_F) and slow-growing (D_S) rainbow trout in Lake 304 at the Experimental Lakes Area in 2011.

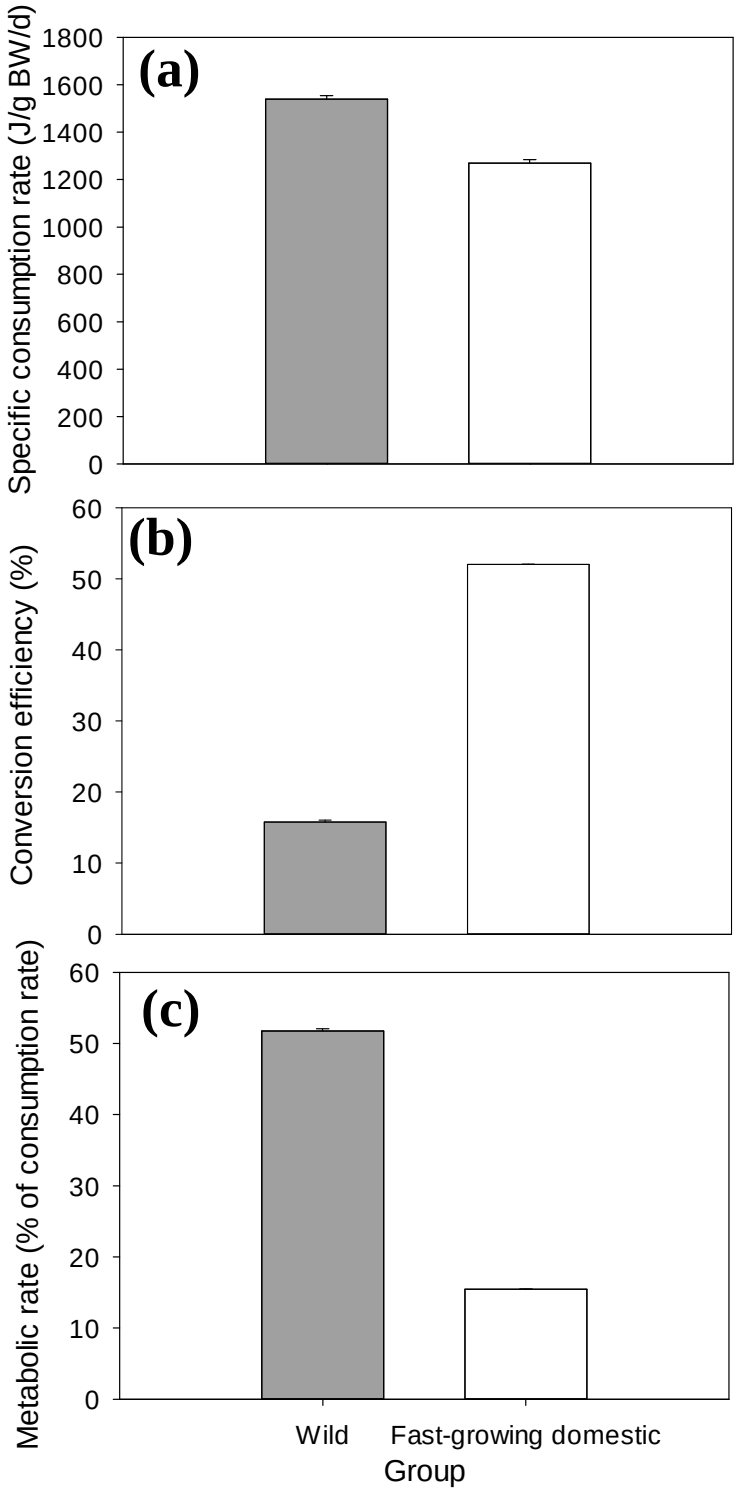

Figure 3.18 Comparison of specific consumption rates (a), conversion efficiency of consumption to growth (b) and metabolic rate (c) between wild (grey bars) and fast-growing domestic (white bars) rainbow trout in Lake 304 at the Experimental Lakes Area in 2011 as predicted by the BM/MMBM. Error bars represent ±1 S.E.

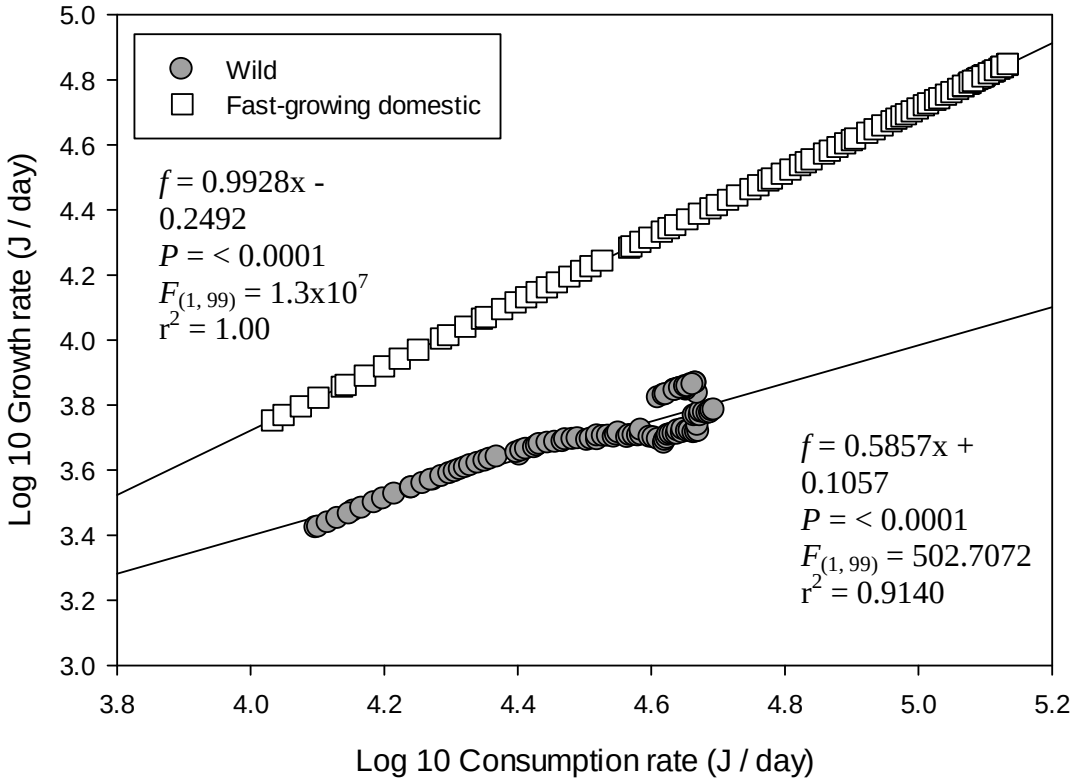

Figure 3.19 The log-log relationship between growth rate and consumption rate for wild and fast-growing domestic rainbow trout in Lake 304 at the Experimental Lakes Area in 2011 as predicted by the BM/MMBM.

Chapter 4. Synthesis

Rainbow trout is the most common species reared in Canadian freshwater aquaculture (DFO 2007a, 2007b). The majority of production occurs at open-pen operations in Lake Huron, with regular incidences of farmed fish escaping from holding facilities (Podemski and Blanchfield 2006; Patterson 2010). To date, no studies have examined the potential risk escaped farmed rainbow trout pose to the naturalized strains present in the Laurentian Great Lakes (LGL), yet this information is critical to the assessment of this industry. In this thesis, I took an integrative approach to compare the growth and survival of size-matched domestic and naturalized rainbow trout in replicated laboratory and whole-lake experiments. Conclusions drawn from these experiments allow for the interpretation of the comparative growth and mortality of both LGL strains of rainbow trout, and how they may relate to success and competition in the wild.

4.1 Survival

The outcome of the field experiment demonstrates the need for whole-lake studies that encompass the variables in nature that are not present in laboratory or semi-natural settings. Specifically, the controlled environment of the growth trials resulted in almost no mortality over the 100 d experiment. With no risk of predation and ideal abiotic factors, survival is a negligible variable in laboratory studies. In contrast, very few stocked rainbow trout were recaptured from the study lakes (3% from Lake 304, and 0% from Lake 303). Some initial mortality could be attributed to handling stress; however, it appears that warm water

temperatures could have been responsible for directly and indirectly influencing mortality rates. Water temperatures in the study lakes did approach near lethal limits for rainbow trout (26˚C), which could have been a source of direct stress and mortality. More likely, is that warm water temperatures forced fish to seek refuge in cooler, open-water areas where they became highly susceptible to avian predation. In Lake 303, the shallow lake (Z_{max} = 3 m), the absence of deep water for temperature and predation refugia may explain why no fish were captured from this lake. Nonetheless, the level of avian predators on our study lakes are consistent with other studies (Post et al. 1999; Biro et al. 2004a, 2006; Beckmann et al. 2006) and alone could explain the near extirpation of rainbow trout with similar levels of total daily consumption. In contrast to other studies examining the mortality of wild and domestic salmonids in lakes, there were no observed differences in survival between strains in the present study, although recapture rates were low. This phenomenon can be attributed to wild and domestic rainbow trout sharing the same littoral habitat throughout the field season in Lake 304. Ultimately, mortality rates can be extreme in natural settings with non-ideal environmental parameters and high levels of predation, which in this study appeared to affect domestic and wild strains of salmonids equally.

4.2 *Growth*

A major concern of escapes of farmed rainbow trout is their potentially higher growth rate than wild conspecifics (eg. Ayles and Baker 1983; Biro et al. 2004a, 2006). To date, no studies have examined whether growth differences exist

between naturalized strains of fish from Lake Huron and the aquaculture strains of fish that are raised in those same waters.

I was able to show that in laboratory growth trials under optimal and competitive treatments, that the domestic strain of rainbow trout was able to achieve >2 times the growth rate of the wild strain. I attributed this finding to the enhanced feeding rates and efficiency as well as the physiology that the domestic strain possessed to exhibit these growth rates. Next, I conducted a replicated whole-ecosystem experiment in Lakes 303 and 304 at the Experimental Lakes Area (ELA) to quantify the growth and survival of these same two strains under the selective pressures of a natural environment. Under these conditions, some evidence exist that domestic rainbow trout may separate into a fast and a slow-growing group, although this finding is based on the recapture of few individuals. The fast-growing group had growth rates approximately 3 times that of wild conspecifics. The slow-growing group, however had growth rates that were lower than even wild rainbow trout and were observed to gain little to no mass throughout the experiment. Food item selection was quite variable among groups. The fast-growing domestic group was most associated with high energy food items (forage fish). This finding is corroborated by the stable isotope analysis (SIA) on muscle and liver tissue. The fast-growing domestic group has a niche space that is specialized with little overlap of the other groups. Using a mercury mass balance/bioenergetics model (MMBM/BM) optimized with data from Chapter 2, I was able to quantify the metabolism budgets of each group of rainbow trout from the field experiment. Although wild rainbow trout had the greatest mass-specific

consumption rates, they also possessed the greatest metabolic costs among the groups, indicating that fast-growing domestics possessed the most efficient metabolic strategy for growth among groups.

In addition, growth rates varied greatly in domestic and wild rainbow trout between lab trials (TGC = 3.35 and 2.58, respectively) and field experiments (TGC = 1.31/2.96 and 1.43, respectively). Although the commercial feed used in the tank trial experiments was relatively high energy, rainbow trout in Lake 304 were able to constantly forage for food. This is the reason specific consumption rates in the field are orders of magnitude greater than in the laboratory study. Although the feeding regime in the satiation treatment of the growth trial was designed to emulate maximum feeding, it was discontinuous and may not fully satiate all of the individuals in a given tank. Perhaps in the future, automatic feeders would better fulfill a group of fish's desire to feed. Consumption rates are more similar between wild and domestic strains in the laboratory experiment compared to fast-growing domestic and wild rainbow trout in the field. A possible reason for this result is the proximity of each fish in the confines of a tank. Growth of teleost fishes is also negatively affected by densities such as that in the laboratory study, limiting the maximum potential for fish to grow. In a natural setting, a greater variety of resources and space is available for rainbow trout to take advantage of allowing for the potential for enhanced growth rates. Stocking of rainbow trout in higher density regimes has been shown to limit growth rates of fish in temperate lakes (Bohlin et al. 2002; Frazer 1969; Jenkins et al. 1999). Therefore it could be surmised that potential growth of a rainbow trout correlates

with population density in the wild. Water temperature and time of season are two other confounding variables affecting fish growth. Because there is a clear relationship between temperature and metabolic rate in ectotherms, and particularly for teleost fishes, the declining temperature in the tank trial is a major impedance to fish completely reaching their potential for energy assimilation. At the point when temperatures reach 6°C by days 80-101 in the tank trials, metabolism and growth rate would be at its lowest. Conversely, water temperature in Lake 304 for the majority of the experiment remained between 15 - 25°C within the water column, potentially allowing greater muscle turnover and opportunity to assimilate food into body mass.

The main themes that can be inferred from the data presented in this thesis are: This strain of domestic rainbow trout is able to achieve superior growth rates compared to wild conspecifics in both laboratory and field settings. Inter- and intra-strain variation of growth between wild and domestic rainbow trout strains can be described successfully by prey selection and metabolic differences between strains.

While acknowledging the constraints of this study, associated with small lake size (<10 ha) and the few fish captured, it is still important to attempt to extrapolate these findings to real-world situations. Overall, the occupation of littoral habitat by wild and domestic strains of rainbow trout suggests that opportunities for selective predation and potential food sources are similar for both strains. However, fast-growing domestics had a greater reliance on forage fish species compared to wild and slow-growing domestic rainbow trout. If patterns of habitat

use by aquaculture and naturalized strains of rainbow trout in Lake Huron are similar to that observed in this study, it is possible that escaped rainbow trout could outcompete wild trout for littoral forage. Furthermore, previous studies have underestimated the divergence in growth rates of selectively-bred fish versus naturalized or wild. Most studies suggest a 2-3 fold growth advantage in domestic salmonids, while evidence from this field study suggest that the difference for LGL rainbow trout strains could be even greater.

4.3 Future direction

A manipulative, replicated field experiment of this magnitude has the ability to generate realistic, meaningful data, but also has several facets that should be adjusted for future studies. A major limitation of this field study was the small sample size from few fish recaptures. Recovering 28 of 1000 fish stocked in Lake 304 and zero of >2000 fish in Lake 303 highlights the challenges associated with conducting large-scale studies under natural conditions. As previously described, it is not uncommon to see a large decline in rainbow trout abundance as a result of predation, stocking and environmental stresses. However, some changes can be applied to the design of future growth studies. First, natural mortality is typically greatest at early life stages in fishes (Biro et al. 2005; Garvey et al. 1998). Perhaps stocking larger rainbow trout in each lake that are less susceptible to predation and more tolerant to environmental stresses would lend to greater recapture numbers at the conclusion of the experiment. Larger size classes would also be more amenable to monitoring for movement (i.e., telemetry methods). In this way, the relative selection of micro habitat, both vertically and spatially (inshore

vs. offshore) could be accurately quantified as well as greater confidence in determining total mortality. Another possible improvement to future studies is to select more suitable lakes for rainbow trout habitat. Both Lakes 303 and 304 are relatively low volume and shallow compared to the majority of lakes in which rainbow trout are stocked (or reared). These lakes are characteristic of lower heat capacities and will reach high temperatures and low dissolved oxygen relatively quickly. Lake 303 has no stratification, so these extreme temperatures are mixed throughout the water column for a great proportion of the ice-free season. In future, deeper, larger lakes provide a better scenario to measure survival for rainbow trout. Rainbow trout would have more space to avoid nets in larger lakes; however, refuge from temperature and predation would potentially be more available for stocked fish.

Designing an experiment which includes prior residence of a rainbow trout population is another possible option for the future. By establishing a population of naturalized rainbow trout in a lake, size-matching, initial mortality and coordinating simultaneous stocking of both strains could be avoided. Adding aquaculture fish to an already established, naturalized fish community would also provide another layer of realism to the experiment. This design scheme would simulate a pulse influx of aquaculture rainbow trout to a naturalized population, mimicking an escape from aquaculture in the context of a real-life scenario. This would also provide the advantage of establishing the ecosystem effects both before and after a perturbation by an invasive species to a system. The problem with such a design is that a sizable time investment would be required in stocking

and maintaining the health of a rainbow trout population prior to a domestic fish release.

A natural progression from a study in an experimental lake would be one that establishes the degree of competition between wild and domestic rainbow trout in close proximity to aquaculture facilities. Work has been done monitoring survivorship and site fidelity in experimental aquaculture sites (Blanchfield et al. 2009) and from farms on Lake Huron (Patterson 2010); however, relative competition between rainbow trout conspecifics has not been directly established. In addition to wild rainbow trout, it would be interesting to quantify the degree of trophic interactions other common fish species would have to aquaculture strains, such as lake trout (*Salvelinus namaycush*) in boreal shield lakes.

This research on the difference in growth and survival between a wild and a growth-enhanced (domestic) strain of rainbow trout has provided a precursor to assessing the risk of natural fish populations to transgenic (genetically engineered) salmonids. The approval of a strain of transgenic Atlantic salmon for human consumption (AquAdvantage) is now in the first draft of approval under the American Food and Drug Administration (FDA). Preliminary findings indicate that there is no substantial effect of genetically altered salmon on human or environmental health (Conner, 2012). In its final stage of approval, transgenic fish would initially be reared in biologically contained inshore facilities. A natural progression from this operation would be to initiate production in fish farms that would eventually be in contact with the natural environment much like current domestic salmonid farms. Because the specific ecological implications of a

genetically engineered genotype of fish on the health of a native environment have not been previously experienced, the experimentation of these strains as it contributes to our knowledge base would be essential. A "Phase 2" project to this study at the ELA would be the experimentation of wild versus an anthropogenically growth-enhanced salmonid. This growth enhancement can be achieved by the administration of recombinant bovine somatotropin to individual fish. This growth hormone stimulation is known to closely mimic the growth and physiology of transgene-enhanced salmonids (Devlin et al. 2001). The eventual goal would be to provide enough expertise in successfully experimenting with growth-enhanced strains that a comparison between transgenic and wild salmonids could be accomplished at a whole-ecosystem scale. By enhancing our knowledge base on the interactions of selectively-bred and wild salmonids, researchers can more confidently design and implement experiments that look to compare fish that possess human-induced rapid growth rates on natural populations.

Appendix 1. Model input calculations and stable isotope sample list

This appendix details the methods of calculating the inputs into the mercury mass balance/bioenergetcs model. It also provides a listing of all of the biotic samples analyzed for stable isotope analysis.

A1.1 Methyl-mercrury (MeHg) calculation

For each group of rainbow trout (wild, slow-growing domestic and fast-growing domestic) I calculated the caloric and methyl-mercury (MeHg) values from their prey items. Using data analyzed from stomach contents, prey items were subdivided into: zooplankton, benthic/terrestrial invertebrates and cyprinids. Bulk zooplankton MeHg were collected monthly (April – October) in 2011 and an average value ($\bar{x}$ ± 1 S.D.) was used (53.12 ± 25.17 ng • g^{-1} d.w.; n = 6). Benthic/terrestrial invertebrate MeHg concentrations were averaged from ambient literature values from Lakes 658 and 239 at the ELA from 2000 – 2004 (Paterson et al. 2006; Harris et al. 2007). These groups of invertebrates included: *Dogielinotidae, Ceratopogonidae, Chironomidae, Ephemeroptera, Hydracarina* and *Pisidiidae*. A mean value was calculated as 52.31 ± 21.21 ng • g^{-1} d.w. (n = 6). Cyprinids were averaged from Lake 304, August 2011 samples consisting of pearl dace (*Margariscus margarita*), northern redbelly dace (*Phoxinus eos*), fathead minnow (*Pimephales promelas*) and finescale dace (*Phoxinus neogaeus*). The mean concentration of MeHg from these samples was 269.58 ± 98.89 ng • g^{-1} w.w. (n = 20). For model consistency, zooplankton and benthic/terrestrial

invertebrate MeHg values were converted to wet weight assuming an average dry: wet moisture (%) from literature values (Table A5).

A1.2 Energy density calculation

I determined the caloric content of prey items consumed by rainbow trout using bomb calorimetry. Values for bulk zooplankton averaged, 21280.28 ± 960.59 J • g^{-1} d.w. (n = 3). Benthic/terrestrial invertebrates were averaged based on samples from Lake 304. These groups included *Ephemeroptera*, *Anisoptera*, *Corixidae* and *Zygoptera*. Average caloric content of benthic/terrestrial invertebrates was, 18677.7828 ± 2753.138 J • g^{-1} d.w. (n = 4). The duplicate value was within 10%. The same 4 minnow species analysed were from Lake 304 as for MeHg. Average caloric content of minnows was, 25489.07 ± 1932.652 J • g^{-1} d.w. (n = 8). The duplicate value was within 5%. Dry weight values were converted to wet weight using the same ratios as for MeHg.

Table A.1 Biota collected from Lake 304 at the Experimental Lakes Area in 2011 and analyzed for $\delta^{15}N$ and $\delta^{13}C$ stable isotopes. Note: replicate samples are not included in n value.

Species	Tissue	n
Fish		
Rainbow trout – Wild (t_0)	Muscle	10
	Liver	10
Rainbow trout – Wild (t_x)	Muscle	7
	Liver	7
Rainbow trout – Domestic (t_0)	Muscle	10
	Liver	10
Rainbow trout – Domestic (t_x)	Muscle	20
	Liver	20
Pearl dace	Muscle	11
Northern redbelly dace	Muscle	10
Fathead minnow	Muscle	9
Finescale dace	Muscle	10
Invertebrates		
Amphipoda	Whole - homogenized	1
Ansioptera	Whole - homogenized	1
Corixidae	Whole - homogenized	1
Gyrinidae	Whole - homogenized	1
Leech	Whole - homogenized	1
Littoral chironomids	Whole - homogenized	1
Littoral Oligochaetes	Whole - homogenized	1
Profundal chironomids	Whole - homogenized	1
Trichoptera	Whole - homogenized	1
Zygoptera	Whole - homogenized	1
>160 μm bulk zooplankton	Whole - homogenized	6
Aquatic primary producers		
Periphyton	Whole - homogenized	1
Lily pads	Whole - homogenized	1
Potamogeton	Whole - homogenized	1
Birds		
Merganser	Liver	1
	Muscle	1

Table A.2 Input parameters for mercury mass balance/bioenergetics model to determine metabolic rates for wild, fast-growing domestic and slow-growing domestic rainbow trout in Lake 304 at the Experimental Lakes Area in 2011. * = 2 outliers were removed. Values are estimated over 100 d (June 25 - October 3, 2011).

Parameters	Wild	Domestic (fast growth)	Domestic (slow growth)
Mass (g)			
t_0	14.2	12.7	12.7
t_{100}	83.8	438.0	28.7
MeHg (ng • g^{-1} w.w.)			
t_0	22.49	14.15	14.15
t_{100}	233.00	346.10	251.90*
Prey	32.81	111.55	0.08
Prey energy (j • g^{-1} w.w.)	4041.35	4248.52	3696.296

Table A.3 NRCC (National Research Council of Canada) and NIST (National Institute of Standards and Technology) measured and standard values of total mercury (THg) as detected by a DMA-80 instrument.

Reference standard	Measured values			Standard values		Citation
	Average value ($ng \bullet g^{-1}$)	Standard deviation	n	Average value ($ng \bullet g^{-1}$)	Standard deviation	
DOLT-2 (Dogfish liver)	2.0071	0.0000	1	2.1400	0.2800	NRCC
DORM-3 (Fish protein)	0.3595	0.0316	4	0.3550	0.0530	NRCC
TORT-2 (lobster hepatopancreas)	0.2346	0.0482	8	0.2700	0.0600	NRCC
1566 (Oyster Tissue)	0.0347	0.0064	3	0.0371	0.0013	NIST
2976 (Mussel Tissue)	0.0600	0.0095	8	0.0610	0.0036	NIST

Table A.4 NRCC (National Research Council of Canada) and NIST (National Institute of Standards and Technology) measured and standard values of methyl mercury (MeHg) as analysed by acid digestion/ethylation-GC-CVAFS detection.

	Measured values			**Standard values**		
Reference standard	Average value ($ng \cdot g^{-1}$)	Standard deviation	n	Average value ($ng \cdot g^{-1}$)	Standard deviation	Citation
DORM-3 (Fish protein)	130.96	0.86	2	152.00	13.00	NRCC
TORT-2 (lobster hepatopancreas)	25.48	2.17	2	28.09	0.31	NRCC
2976 (Mussel Tissue)	402.59	6.04	2	355.00	56.00	NIST

Table A.5 Weight ratios (%) for dry: wet mass (±1 S.D.) used for converting wet weight values of MeHg and caloric content in fish.

Group	Dry: wet mass (%)	Source
Zooplankton		
Rotifer	10	Downing and Rigler 1984
Copepoda	11 - 14	Schindler et al. 1971; Dumont et al. 1975; Downing and Rigler 1984
Cladocera	10 - 12	Dumont et al. 1975; Hewett and Johnson 1992
Average	11.2 ± 1.0	
Benthic invertebrates		
Ephemeroptera	22 - 24	Cummins and Wuycheck 1971; Driver et al. 1974
Diptera larvae	5 - 12	Cummins and Wuycheck 1971; Hewett and Johnson 1992
Average	15.8 ± 10.3	
Fish		
Yellow perch	19.5-19.9	Van Walleghem 2006; Orihel et al. 2007
Average	19.7 ± 0.3	

Table A.6 Calculations of caloric and MeHg content of prey items in wet weight. Mass is based on the sum of all stomachs from that group of rainbow trout recaptured from Lake 304 at the Experimental Lakes Area in 2011. MeHg values are based on detections from DMA-80 (fish), aqueous phase ethylation (zooplankton) and literature values (benthic/terrestrial invertebrates). Caloric values are based on bomb calorimetry analysis.

Strain		Prey Item			
		Bulk zooplankton	Invertebrates	Cyprinids	Total
Domestic (fast growth)	Mass (g)	0.0017	0.0519	2.4846	2.5382
	% Mass	0.07	2.04	97.89	100.00
	MeHg (ng • g^{-1})	0.0017	0.0715	111.4750	111.5482
	Energy (J • g^{-1})	1.60	70.60	4176.33	4248.52
Domestic (slow growth)	Mass (g)	0.0009	0.0848	0.0383	0.1240
	% Mass	0.73	68.39	30.89	100.00
	MeHg (ng • g^{-1})	0.0004	0.0539	0.0244	0.0783
	Energy (J • g^{-1})	17.30	2361.22	1317.77	3696.30
Wild	Mass (g)	0.0000	0.3685	0.9637	1.3322
	% Mass	0.00	27.66	72.34	100.00
	MeHg (ng • g^{-1})	0.0000	0.3807	32.4283	32.8090
	Energy (J • g^{-1})	0.00	955.06	3086.29	4041.35

Table A.7 Equations for growth curves based on mass to fit wild, fast-growing domestic and slow-growing domestic rainbow trout recaptured from Lake 304 at the Experimental Lakes Area in 2011.

	Wild	Domestic (fast growth)	Domestic (slow growth)
Growth Equation	5 parameter exponential growth $f = y_0+a^{bx}+c^{dx}$		Linear regression $f = y_0+a*x$
Coefficients			
a	$7.3688x10^{-18}$	182.2704	0.1590
b	0.0222	0.0120	NA
c	83112.6941	$1.9274x10^{-18}$	NA
d	$8.1088x10^{-6}$	$2.0817x10^{-17}$	NA
y_0	-83096.3454	-167.1840	12.7820
r^2	0.7761	0.9711	0.1170

Table A.8 Equations for curves fit for body mass to MeHg concentrations of wild, fast-growing domestic and slow-growing domestic rainbow trout recaptured from Lake 304 at the Experimental Lakes Area in 2011.

	Wild	Domestic (fast growth)	Domestic (slow growth)
MeHg Equation	$f=y_0+a*x$ Linear	$f=y_0+a*\ln(x)$ Logarithm	$f=y_0+a*(1^{-b(x)})$ Exponential rise to max.
Coefficients			
a	2.3819	101.0074	411.6000
b	NA	NA	0.1649
y_0	-6.9851	-268.2202	-308.4000
r^2	0.9012	0.8648	0.1566

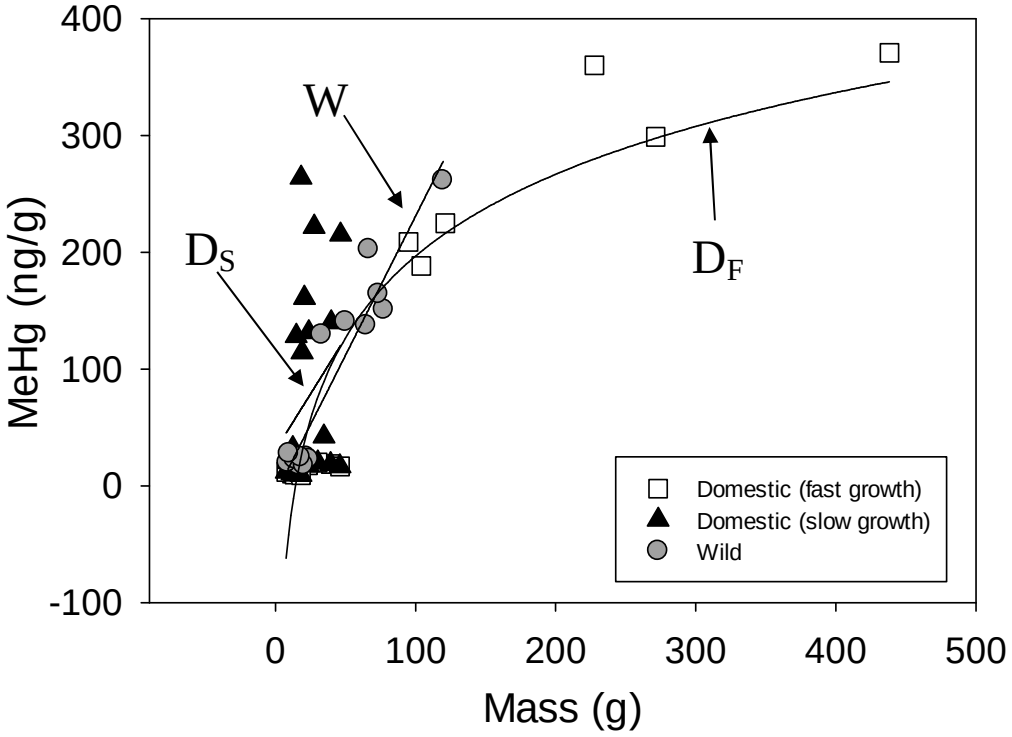

Figure A.1 Methyl mercury (MeHg) values regressed over body mass of wild (W), fast-growing (D_F) and slow-growing (D_S) domestic rainbow trout at t_0 and recaptured in Lake 304 at the Experimental Lakes Area in 2011.

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