

PHYSIOLOGICAL TRADE-OFFS UNDERLYING GROWTH VARIATION FROM INDIVIDUALS
TO SPECIES: CONSEQUENCES FOR INTEGRATED PHENOTYPIC DIFFERENTIATION AND
ECOLOGICAL DIVERSIFICATION IN JUVENILE SALMONIDS

by

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Abstract

The purpose of my thesis research was to explore the physiological and behavioural correlates and trade-offs associated with faster growth, and their consequences for phenotypic and ecological differentiation along productivity gradients in salmonids. I examined variation in behavioural, digestive and bioenergetic strategies between different ecotypes and species of wild salmonids that differ in ecological requirements. Juveniles of large-bodied piscivore vs. small-bodied insectivore rainbow trout differed sharply in bioenergetics and behaviour. Relative to insectivore fry, piscivores presented a pattern of faster growth, higher food intake, higher basal metabolism, higher growth efficiency and larger digestive organs, but also more proactive behaviours and greater digestive efficiency (e.g., lower digestive metabolism). Faster piscivore growth was traded-off against lower aerobic scope and presumably against lower survival when facing predators. Piscivore and insectivore integrated phenotypes largely differentiated along the slow-fast continuum of the Pace-Of-Life Syndrome framework (Réale et al., 2010). This result was consistent with specialization of the two ecotypes to rearing habitats that differ in productivity – insectivores occur in tributaries with low prey availability relative to the piscivore rearing environment. The coherence between fast growth and high habitat productivity also emerged as a key driver of the post-emergence dispersal of piscivore fry along a productivity gradient in the Lardeau River. Juvenile steelhead trout and coho salmon also differed in growth and bioenergetics. Relative to coho salmon, faster-growing steelhead trout had higher food consumption and digestive metabolism but lower growth efficiency, which differentiated the two species along an energy-maximizing (steelhead) vs. efficiency-maximizing (coho) continuum (Rosenfeld et al., 2020). This pattern was largely consistent with their specialization to adjacent habitats (pools for coho, riffles for steelhead) along increasing prey flux gradient in coastal streams where the species co-occur. Steelhead trout presented higher aerobic scope than coho salmon, which compensated for their elevated digestive metabolism and ultimately resulted in the convergence of aerobic budgets between the two species. Overall, I demonstrate: i) the existence of multiple sets of physiological trade-offs associated with growth differentiation in salmonids from individuals to populations and species; and ii) the consequences of integrated phenotypic differentiation for ecological specialization along natural productivity gradients.

Lay summary

Growth rate is an important biological attribute that allows organisms to develop from juveniles to adults as part of their life cycle. Although growth rate is known to vary among individuals, populations and species, the physiological and behavioural characteristics that allow organisms to ultimately grow at different rates are unclear. To better understand how physiology and behaviour determine growth rate, I compared behavioural, digestive and bioenergetic characteristics among populations and species of salmon and trout that are known to grow at different rates as a result of their specialization to different environments. In essence, I demonstrated that achieving faster growth is facilitated by a suite of physiological and behavioural characteristics that allow organisms to maximize energy assimilation and support faster growth in nature.

Preface

The research presented in this thesis was designed and carried out in the Department of Zoology at the University of British Columbia, Vancouver campus. All experimental protocols presented in this thesis were approved by the University of British Columbia's Animal Care Committee under Animal Use Protocol #AUP A17-0036. Guidance and advice regarding hypotheses, experimental design, data analysis, and writing edits was kindly provided by Dr. Jordan Rosenfeld and Dr. Jeffrey Richards.

A version of Chapter 1 has been published [Monnet G, Rosenfeld JS, Richards JG. Adaptive differentiation of growth, energetics and behaviour between piscivore and insectivore juvenile rainbow trout along the Pace-of-Life continuum. *J. Anim. Ecol.*, 89(11), 2717-2732, 2020]. Drs. Jordan Rosenfeld and Jeffrey Richards were the lead investigators responsible for all major areas of concept formation. I was responsible for data collection and analysis, as well as manuscript composition. All authors contributed to manuscript edits.

A version of Chapter 2 has been published [Monnet G, Rosenfeld JS, Richards JG. Behavioural variation between piscivore and insectivore rainbow trout *Oncorhynchus mykiss*. *J. Fish Biol.*, 99(3), 955-963, 2021]. I was the lead investigator, responsible for all major areas of concept formation, data collection and analysis, as well as the majority of manuscript composition. Drs. Jordan Rosenfeld and Jeffrey Richards were involved in the early stages of concept formation and contributed to manuscript edits.

I was the lead investigator for the projects located in Chapters 3, 4, and 5 where I was responsible for all major areas of concept formation, data collection and analysis, as well as the majority of manuscript composition. Drs. Jordan Rosenfeld and Jeffrey Richards were involved in the early stages of concept formation and contributed to manuscript edits. The data presented in Chapters 3, 4 and 5 have been submitted for review before publication in various scientific journals.

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List of abbreviations

AE	Assimilation efficiency
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AS	Aerobic scope
CT _{max}	Critical thermal maximum
FC	Food consumption
GE	Growth efficiency
GRT	Gut residence time
MMR	Maximum metabolic rate
PCA	Principal Component Analysis
POLS	Pace-Of-Life Syndrome
SDA	Specific dynamic action
SDA _{dur}	Duration of specific dynamic action
SDA _{peak}	Peak specific dynamic action
SGR	Standard growth rate
SMR	Standard metabolic rate

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General introduction

Salmonids: a taxon of remarkable ecological diversity in the Pacific Northwest

Salmonids are a remarkably diverse group of teleost fishes that have adapted to various aquatic habitats throughout the Northern Hemisphere where multiple populations and species of salmon, trout, and their relatives now coexist sympatrically. The Pacific Northwest of North America is within the native range of five species of Pacific salmon (coho: *Oncorhynchus kisutch*; chum: *O. keta*; pink: *O. gorbuscha*; chinook: *O. tshawytscha*, and sockeye: *O. nerka*) and multiple closely related species of trout, including rainbow/steelhead (*O. mykiss*), cutthroat (*O. clarkii*), bull trout (*Salvelinus confluentus*) and Dolly Varden (*S. malma*). This great diversity of species is superimposed on an equivalent (if not greater) diversification at the intraspecific level since, within the same species, multiple populations have repeatedly specialized to adjacent ecological niches and differentiated into distinct genetic lineages. For example, the post-glacial recolonization of Icelandic rivers and lakes by ancestral populations of Arctic char (*Salvelinus alpinus*) has initiated the ecological speciation of these populations into multiple morphs (i.e., small benthic, large benthic, planktivorous, and piscivorous) that strongly differ in their phenotypic and ecological attributes along a benthic-limnetic axis of habitat use (e.g., Doenz et al., 2019). Similar ecological specialization has occurred in many freshwater lakes of Northwestern North America (including in British Columbia) where distinct but sympatric rainbow trout ecotypes have repeatedly differentiated along a large-bodied piscivore vs. small-bodied insectivore axis of adult morphology and feeding ecology (Keeley et al., 2005, 2007).

Post-glacial specialization to distinct aquatic habitats (e.g., coastal rivers, inland lakes, estuaries) by Pacific salmonids has resulted in adaptive suites of molecular, physiological, morphological, behavioural and life-history traits, and promoted the differentiation of integrated phenotypes among individuals, populations, and species (e.g., Rosenfeld et al., 2020). In particular, specialization to habitats with distinct trophic characteristics has contributed to the evolution of alternative dietary preferences (e.g., planktivorous, insectivorous, piscivorous, or generalist diets) or feeding capacity (e.g., low vs. high prey consumption) among populations and species of salmonids, which have then often diverged in a number of developmental attributes including, but not limited to, juvenile growth and/or adult body size (e.g., Nicieza et al., 1994; Martens et al., 2014;

Hughes et al., 2019). However, the nature of the physiological and ecological drivers of growth variation among individuals, populations and species of salmonids and fish in general remains poorly understood. In addition, the implications of growth variation to integrated phenotypic differentiation (e.g., through its associations with energetics and behaviour) and habitat use remain unclear.

Ecological and evolutionary drivers of juvenile growth

Growth rate (i.e., the capacity to produce an absolute mass of somatic tissues per unit time) is a key life-history attribute that often differentiates life-history strategies among individuals, populations and species (Arendt, 1997; Dmitriew, 2011) through its associations with other fitness correlates (e.g., reproduction). Growth rate constitutes, in part, an adaptive response to evolutionary (e.g., direct selection to increase adult body size: Sibly et al., 2015) and ecological contexts (e.g., prey abundance: Diehl, 1993), and may therefore vary genetically or plastically among and within taxa (Allen et al., 2016). The mechanisms underlying growth variation in nature, however, remain unclear, particularly since the factors that constrain growth may vary in response to idiosyncrasies in ecological and evolutionary contexts that drive adaptive differentiation at local scales.

Faster growth is an effective developmental response to multiple ecological stressors occurring at different time horizons in aquatic ecosystems. For instance, after leaving their nests (“emergence”), maximizing post-emergence growth to rapidly achieve minimum size thresholds that ensure overwinter survival (Quinn & Peterson, 1996; Post & Parkinson, 2001) is an effective response to shorter growing seasons in high latitude freshwater habitats (Conover & Schultz, 1995). Such countergradient variation in growth induced by seasonality differentiates populations of Atlantic silverside (*Menidia menidia*) along a latitudinal gradient of fast (Nova Scotia)-to-slow (South Carolina) juvenile growth and energetics (Billerbeck et al., 2000, 2001). Maximizing growth within a fixed time frame appears to be the most effective developmental trajectory for reaching sexual maturity earlier and targeting larger adult body size (Czarnoleski et al., 2008; Rosenfeld et al., 2015; Hughes et al., 2019), which may increase ultimate fecundity and total reproductive output. Identifying the mechanisms underlying divergent growth trajectories may therefore allow a better

assessment of reciprocal constraints linking juvenile and adult life histories and improve our understanding of the evolutionary and ecological drivers of growth variation in nature.

Phenotypic adaptations supporting fast growth: benefits and costs

Faster growth is determined, in part, by strong selection that maximizes performance associated with energy intake and allocation to growth and other processes. Evidence for such selection includes a variety of adaptations ranging from the physiological, to anatomical, and behavioural levels with the shared result of maximizing growth.

Physiological adaptations

Many physiological adaptations contribute to the emergence of growth variation among individuals, populations and species, including partitioning available energy differently within individual energy budgets. Standard metabolic rate (SMR), the minimum metabolic expenditure for general body maintenance in non-growing, non-digesting, and inactive ectotherms, is a key component of fish energy budgets (Chabot et al., 2016; Metcalfe et al., 2016). A substantial fraction of individual energy budgets (Finstad et al., 2007) is encompassed by SMR which conditions, in part, the amount of energy left over for additional physiological processes including immunity or reproduction. Because SMR is determined by extrinsic (e.g., food availability: Auer et al., 2015b; Allen et al., 2016) and intrinsic factors (e.g., size of digestive organs: Elia, 1992), elevated SMR often promotes growth in productive habitats where food is abundant or can be monopolized (e.g., Cutts et al., 1998). By contrast, elevated SMR becomes maladaptive in low prey flux environments where foraging opportunities are insufficient to maintain positive energy budgets with high basal energy demand (Armstrong & Schindler, 2011).

Individual aerobic budgets are largely determined by maximum metabolic rate (MMR, i.e. the maximum capacity of energy supply to tissues in aerobic conditions) that can exceed SMR by multiple orders of magnitude (Chabot et al., 2016). Maximum metabolic rate is primarily contingent on mitochondrial performance (e.g., mitochondrial activity or density) and respiratory efficiency (Weibel et al., 2004), that is, the oxygen supply capacity of the cardiovascular and respiratory systems. In fish, high MMR is often associated with elevated swimming performance (Van Leeuwen

et al., 2011) rather than high capacity for growth (Allen et al., 2016), although maximizing MMR may largely increase aerobic scope (i.e., the residual aerobic capacity left over after paying maintenance costs) and subsequent energy allocation to growth (Auer et al., 2015b). However, the existence of a positive association between high aerobic scope and elevated growth remains uncertain due to the complexity of bioenergetic metabolic pathways associated with growth.

Anatomical adaptations

Growth variation among taxa may also arise from underlying variability in anatomical design associated with feeding capacity and efficiency (Piersma & Lindtröm, 1997). For instance, intestinal residence time is a key bottleneck for energy assimilation (Nicieza et al., 1994), as processing larger meals in support of faster growth may require evolving shorter transit times that maximize consumption rates but decrease assimilation efficiency (Sibly, 1981). Shortening transit duration to allow more rapid extraction of labile energy may therefore be advantageous in habitats with high prey fluxes where energy assimilation is optimized by maximizing consumption rather than digestive efficiency (Hart & Gill, 1992; Armstrong & Schindler, 2011). By contrast, lengthening transit time to increase assimilation efficiency may be more adaptive in contexts of low food availability (e.g., in unproductive habitats, or between intermittent food pulses: Armstrong & Bond, 2013). Evolving larger digestive tracts is another potential adaptation to maximize nutrient assimilation at the intestinal surface, but potentially at the cost of elevated basal metabolism (i.e., SMR: Secor & Diamond, 2000) because of the comparatively high maintenance costs of digestive tissues (Armstrong & Schindler, 2011; Neubauer & Andersen, 2019).

Behavioural adaptations

Achieving faster growth also requires evolving proactive behaviours that maximize prey consumption during foraging activity. Greater capacity for exploration or risk-taking are typical markers of proactive behaviour that often positively correlate with greater prey consumption (Sih et al., 2004b; Mittlebach et al, 2014), thereby illustrating the contribution of high risk-high reward behaviours to the achievement of faster growth (Lankford et al., 2001; Biro et al., 2004; Stamps, 2007). Proactive behaviours also often correlate with high active metabolic capacity, as prolonged

foraging activity and agonistic interactions associated with competition for prey are likely to elevate total energy demands (Vøllestad & Quinn, 2003). Few studies have, however, examined the nature of the multivariate relationships among growth, metabolism and behaviour, as well as their implications in a context of adaptive phenotypic differentiation among and within taxa.

Adaptive trade-offs

Despite multiple adaptations that result in faster growth, growth is often limited to optimal rather than physiologically maximal rates by adaptive trade-offs with other essential phenotypic traits (e.g., immune response). Although morphological and behavioural trade-offs have been widely explored (e.g., cranial vs. fin morphology: Gilbert et al., 2021; shy vs. bold behaviours: Stamps, 2007), cryptic physiological trade-offs and their impacts on fitness correlates including growth are less well studied, despite their likely contribution to phenotypic and ecological differentiation in nature (Rosenfeld et al., 2020). Physiological trade-offs associated with higher prey consumption and growth (e.g., Sadowska et al., 2013) may alter a variety of metabolic processes including a bottom-up reduction of aerobic scope by an increase in basal metabolism (i.e., SMR: Allen et al., 2016), or the saturation of aerobic budgets by the elevation of digestive metabolism following large meals (Auer et al., 2015b; Norin & Clark, 2017; McLean et al., 2018).

Achieving faster growth can also induce anatomical trade-offs among organ systems, whereby a preferential investment in metabolically expensive digestive organs that facilitate energy assimilation may result in a reduction in size of competing cardiovascular and neural tissues (e.g., a larger digestive tract vs. smaller brain trade-off, as suggested by the “expensive-tissue” hypothesis: Aiello & Wheeler, 1995). In addition, maximizing consumption to elevate growth may require faster gut clearance to allow rapid consumption of new food (Sibly, 1981), which may in turn reduce nutrient assimilation and growth efficiency (i.e., high growth vs. low growth efficiency trade-off: Rosenfeld et al., 2020).

Trade-offs can also occur between faster growth and proactive behaviours associated with riskier foraging activity. For instance, evolving riskier behaviours (e.g., greater exploration or boldness) to maximize prey consumption and growth may come at the cost of reduced survival

through an elevation of predation risk and associated mortality (Lankford et al., 2001; Biro et al., 2004).

Integrated phenotypic differentiation along productivity gradients

Multivariate trade-offs associated with growth are central in holistic frameworks that summarize trait associations expected to differentiate phenotypes along productivity gradients, like the Pace-Of-Life Syndrome (Réale et al., 2010) or the rate-maximizing vs. efficiency-maximizing axis of energy use (Finstad et al., 2011; Rosenfeld et al., 2020).

The Pace-Of-Life Syndrome framework (Réale et al., 2010) summarizes key positive trait associations and trade-offs expected to differentiate physiological, behavioural and life-history attributes between fast phenotypes (i.e., fast growth, high metabolism, and proactive behaviours) providing higher fitness in highly productive environments, and slow phenotypes (i.e., slow growth, low metabolism, and reactive behaviours) that would be more competitive in habitats with lower productivity. The validity of trait associations presented by the Pace-Of-Life framework has been tested empirically on a variety of terrestrial and aquatic taxa by multiple studies that generate conflicting conclusions. For instance, the comparison of different breeds of domestic dogs (Careau et al., 2010) reported strong correlations among bolder personalities, higher metabolism and lower survival as predicted by the Pace-Of-Life Syndrome; in contrast, the comparison of different populations of Eastern mosquitofish (*Gambusia holbrooki*) identified positive associations among slower life histories, higher metabolism, and bolder behaviours (Polverino et al., 2018). Such conflicting results leave uncertainties regarding the universality of the Pace-Of-Life Syndrome hypothesis and its capacity to accurately depict phenotypic differentiation along productivity gradients.

Alternative axes of phenotypic differentiation such as a growth vs. growth efficiency trade-off are not accounted for in the Pace-Of-Life Syndrome framework, but are central to trade-offs between rate-maximizing vs. efficiency-maximizing strategies of energy use (Finstad et al., 2011; Rosenfeld et al., 2020). A rate- vs. efficiency maximizing trade-off generates the expectation that energy-maximizing phenotypes presenting a pattern of fast growth, high consumption, short gut residence time, low nutrient absorption, low growth efficiency and high digestive costs (e.g., SDA)

would be more adaptive in productive habitats with high prey flux. In contrast, efficiency-maximizing phenotypes manifesting slow growth, low consumption, high transit duration, high nutrient assimilation, high growth efficiency and low digestive costs may be more competitive in less productive habitats. Energy-maximizing vs. efficiency-maximizing strategies of energy use have been suggested to represent a significant axis of adaptive differentiation in salmonids and fish in general (Finstad et al., 2011; Van Leeuwen et al., 2011). The coherence among bioenergetics, physiology, and habitat use remains, however, unclear because traits that affect energy budgets and growth are rarely compared in an adaptive differentiation framework.

Productivity (i.e., prey availability) gradients are pervasive in nature and can differentiate productive from unproductive habitats at the microhabitat scale (e.g., focal locations within a stream) or at the macrohabitat scale (e.g., between streams or watersheds). Because food availability largely conditions juvenile bioenergetics and growth (Van Leeuwen et al., 2011; Martens et al., 2014; Hughes et al., 2019), characterizing the extent of phenotypic differentiation that occurs along natural productivity gradients provides an opportunity for understanding how related taxa specialize to adjacent ecological niches with distinct prey fluxes.

Fish models

Piscivore and insectivore rainbow trout (*Oncorhynchus mykiss*) are sympatric but ecologically divergent salmonid ecotypes that have repeatedly evolved in freshwater habitats of Northwestern North America. Piscivorous trout typically achieve remarkable adult body size (5-15 kg; Irvine, 1978a) by shifting from an invertebrate diet (as juveniles) to a piscivorous diet (as adults) of kokanee salmon (landlocked sockeye salmon, *Oncorhynchus nerka*; Keeley et al., 2005, 2007; for Kootenay Lake, see Andrusak & Parkinson, 1984). In contrast, the insectivore ecotype is genetically distinct from the piscivore (Taylor et al., 2019) and typically matures at smaller adult size (<2-3 kg) on a largely invertebrate-opportunistic diet. Insectivore populations are somewhat genetically differentiated (Taylor et al., 2019) but are primarily demographically independent or close to independent; despite probable gene flow amongst these populations, population trends are likely not related among insectivore streams. Larger adult size in the piscivore ecotype may be most effectively attained by a steeper juvenile growth trajectory (Czarnoleski et al., 2008; Sibly et al.,

2015), thereby creating the potential for reciprocal constraints between adult (i.e., larger body size) and juvenile life histories (i.e., faster growth). The extent to which piscivore and insectivore ecotypes differ in adult morphology as a result of underlying variation in juvenile growth, physiology, and behaviour is, however, unknown. In addition, the degree to which the distribution of piscivore and insectivore phenotypes along a natural gradient of low (insectivores)-to-high rearing habitat productivity (piscivore) matches predictions of the Pace-Of-Life Syndrome (Réale et al., 2010) and the energy (rate)-maximizing vs. efficiency-maximizing axis of energy use (Rosenfeld et al., 2020) remains unclear.

Steelhead trout (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) are sympatric species of anadromous salmonid in many coastal streams of Northwestern North America. Due to differences in spawning schedules between species, coho salmon emerge earlier than steelhead trout and the two species therefore compete for territories in preferred lower-velocity pool habitat (Young, 2004) where growth can be maximized at low swimming cost (Hartman, 1965; Young, 2001). Steelhead trout are spring spawners and coho salmon are fall spawners, so that steelhead trout emerge from spawning redds a month after coho salmon parr. Prior residence and resulting size advantage associated with earlier emergence allow coho salmon to effectively outcompete and displace smaller steelhead trout into shallow, high-velocity riffle habitats (Johnston, 1970; Bugert & Vjornn, 1991; Young, 2004). In riffle habitats prey encounter rates and swimming costs are elevated compared to low-velocity pools (Hayes et al., 2000), hence creating a longitudinal low-to high prey flux gradient across pool-riffle sequences. This habitat partitioning is expected to generate matching physiological and morphological adaptations that allow steelhead trout to exploit riffle habitats including, relative to coho salmon, elevated food intake and growth (Sullivan et al., 2000), lower growth efficiency (Rosenfeld et al., 2020), and higher active metabolism (Van Leeuwen et al., 2011), which would be consistent with maximizing energy intake in highly productive but energy-demanding habitats. Despite these expectations, the existence of variation in digestive physiology underlying the ecological specialization of steelhead trout and coho salmon along low-to-high prey flux gradients remains unclear.

Comparing divergent populations (i.e., piscivore vs. insectivore ecotypes) and species of juvenile salmonids (i.e., coho salmon vs. steelhead trout) that presumably differ in growth trajectories and bioenergetics provides an opportunity to identify the physiological basis of growth

variation from individuals to species, and to evaluate the consequences of integrated phenotypic differentiation for habitat use and ecological diversification in juvenile salmonids.

Thesis objectives

My thesis focuses on understanding the mechanisms of multivariate adaptation in physiology, anatomy and behaviour underlying growth variation, and their consequences for integrated phenotypic differentiation and ecological specialization in juvenile salmonids. The main objectives are i) to understand the mechanisms of multivariate adaptation and trade-offs associated with the evolution of faster growth by comparing behavioural, digestive and bioenergetic strategies between wild ecotypes and species of juvenile salmonids; ii) to evaluate the consistency between observed phenotypic variation and theoretical trait associations predicted by holistic frameworks of phenotypic specialization (e.g., the Pace-Of-Life Syndrome); and iii) to determine the coherence of integrated performance in energy use with niche differentiation along productivity gradients.

This thesis work will provide a better understanding of the physiological and behavioural mechanisms underlying growth variation and integrated phenotypic differentiation in nature. It will also provide a better understanding of multivariate adaptation as an integrated response to habitat use and its contribution to shaping biodiversity patterns at local scales.

Chapter 1: Differentiation in growth, energetics and behaviour between piscivore and insectivore juvenile rainbow trout (*O. mykiss*) along the Pace-Of-Life continuum

1.1 Introduction

Variation in life-history strategies among individuals, populations and species reflects the ecological and evolutionary contexts that drive adaptive differentiation (Stearns, 1989b). Recent studies suggest that contrasting life-history strategies reflect divergence in physiological performance (e.g., Van Leeuwen et al., 2011) and behaviour (e.g., Biro et al., 2004), which collectively define the integrated phenotype of an individual. Integration refers to the fact that the adaptive significance of a phenotype is best understood by considering the multivariate associations and inherent trade-offs among constituent traits (Réale et al., 2010; Careau & Garland, 2012). Despite the emergence of the integrated phenotype concept, it remains unclear whether there are generalizable patterns in multivariate adaptation (Careau & Garland, 2012), and how such patterns map onto environmental gradients. To address this gap, Réale et al. (2010) proposed the Pace-Of-Life Syndrome (POLS) as a framework summarising the expected trade-offs and positive trait associations among suites of physiological, behavioural and life-history traits along a productivity gradient. The framework distinguishes slow phenotypes (e.g., low metabolism, growth, reactive behaviours) providing higher fitness in low-productivity environments from fast phenotypes (e.g., elevated metabolism, growth, and proactive behaviours), presumed to be more competitive in highly productive systems. Empirical tests of this framework generate contrasting results, with some providing clear support (e.g., Näslund, 2015) while others do not (e.g., White et al. 2016), so that the generality of the POLS remains unclear.

Although adaptive differentiation involves multiple trait dimensions of the integrated phenotype, growth rate is a fundamental life-history attribute with significant ecological consequences (e.g., for habitat use or mortality), and constitutes a key adaptive axis in the phenotypic differentiation of individuals, populations and species (Arendt, 1997). Achieving optimal growth that confers maximal fitness may induce strong trade-offs with other life-history traits (Dmitriew, 2011), such as lower survival due to extended foraging activity to support high growth (Lankford et al., 2001; Biro et al., 2004). Many factors select for high growth rate in the wild,

including direct selection to increase body size in a fixed time frame (Sibly et al., 2015), which may indirectly constrain juveniles to a rapid high-growth trajectory (Rosenfeld et al., 2005; Hughes et al., 2019). In a broader evolutionary context, trait selection at one life-history stage may also constrain adaptation at another (Rosenfeld et al., 2015); for instance, high juvenile growth rates may require high environmental productivity (i.e., prey availability), leading to the prediction that evolution of large adult body size may be contingent on high-productivity juvenile rearing environments.

Trade-offs among competing physiological, behavioural or life-history traits are inevitable consequences of limited energy budgets and constitute fundamental mechanisms for the emergence and coexistence of specialized phenotypes in nature (Garland, 2014). Multivariate trade-offs are also central in concepts like the Pace-Of-Life Syndrome (Réale et al., 2010), and occur for example between growth vs. active metabolism, where elevated growth supported by larger, metabolically costly digestive organs (e.g., larger digestive tract) may elevate resting metabolism (i.e., SMR) and potentially reduce energy allocation towards organs involved in oxygen intake and delivery, thus lowering aerobic potential. Metabolic trade-offs are common in vertebrates, ranging from mice (Sadowska et al., 2013) to juvenile salmonids (Van Leeuwen et al., 2011; Rosenfeld et al., 2020). Many populations that demonstrate this growth vs. active metabolism trade-off are, however, artificially selected for fast growth, (e.g., Reinbold et al., 2009; Allen et al., 2016), leaving its relevance to wild populations unclear. Anatomical trade-offs may also occur following a preferential investment in digestive organs with high metabolic demand rather than competing cardiovascular and neural tissues (Álvarez & Metcalfe, 2007), as predicted by the “expensive tissue hypothesis” (Aiello & Wheeler, 1995). The existence of organ-system trade-offs in fish taxa, however, remains uncertain (Allen et al., 2016), as suggested for example by the positive correlation between heart and intestine-stomach mass among individual brown trout (*Salmo trutta*: Norin & Malte, 2012). Similarly, behaviour constitutes another key, but somewhat neglected, dimension of the integrated phenotype concept (Careau & Garland, 2012). Although behavioural proactivity (e.g., higher boldness, exploration and fidelity to routines) is predicted to co-vary with growth and metabolism by the POLS (Réale et al., 2010), the nature of multivariate associations among growth, metabolism, anatomy and behaviour remains somewhat speculative since these three categories of traits are rarely measured simultaneously (Mittlebach et al., 2014).

Piscivore and insectivore rainbow trout constitute divergent ecotypes that have repeatedly evolved in freshwater habitats of Northwestern North America, including Kootenay Lake (British Columbia, Canada) where they occur sympatrically. Piscivore juveniles feed exclusively in the Lardeau River for 1-2 years before migrating to Kootenay Lake where they achieve large adult body sizes (5-15kg) on a diet of kokanee salmon (*Oncorhynchus nerka*; Keeley et al., 2005, 2007; for Kootenay Lake, see Andrusak & Parkinson, 1984; Andrusak & Andrusak, 2006). The nature of the environmental constraints that select for large piscivore adult body size is unclear, and may include morphological and internal anatomical requirements for piscivory (e.g., developing a larger mouth: Keeley et al., 2007) or moving larger substrate for egg deposition while spawning in the Lardeau River. By contrast, the insectivore ecotype, genetically distinct from the piscivore (Taylor et al., 2019), includes multiple discrete populations feeding in smaller tributary streams around Kootenay Lake for 1-2 years before maturing in the lake at typical sizes - of 2-3kgs - on a largely insectivore diet. Insectivore populations are somewhat genetically differentiated (Taylor et al., 2019) but are primarily demographically independent or close to independent; despite probable gene flow amongst these populations, population trends are likely not related among insectivore streams. Larger size at maturity in piscivores can be achieved through a combination of later age at maturity (i.e., 4-6 years) that may prolong juvenile growth relative to small insectivores (2-3 years: J. Burrows, pers. comm.), or by faster juvenile growth rate within a fixed time frame (Czarnoleski et al., 2008; Rosenfeld et al., 2015; Sibly et al., 2015), which may constrain metabolic performance, internal anatomy and behaviours related to energy acquisition (Fig. 1.1). Piscivore and insectivore ecotypes thus provide a compelling model for understanding the reciprocal adaptive constraints between juvenile and adult life stages, i.e. whether selection for larger adult body size in the piscivore ecotype indirectly selects for higher juvenile growth (Fig. 1.1; Rosenfeld et al., 2015; Sibly et al., 2015), as well as the factors that influence the covariation of multiple traits within a POLS context.

In this study we examined associations among growth, metabolic, anatomical and behavioural traits in juveniles of piscivore vs. insectivore rainbow trout from Kootenay Lake across a gradient of food availability. We predicted that i) selection for larger adult body size would result in higher juvenile growth at the cost of lower active metabolic performance (Fig. 1.1); ii) faster-growing piscivores would be constrained to habitats with higher prey availability, and exhibit proactive foraging behaviour to support elevated growth; and iii) that general patterns of multivariate trait

associations would match the predictions of the POLS (Fig. 1.1). To test these predictions, we simulated a productivity gradient in the laboratory by rearing fish from a piscivore and insectivore population at three feeding treatments (low, mid and high rations) for two weeks under common garden conditions, and measured traits related to individual growth (standard growth rate [SGR], food consumption [FC], growth efficiency [GE]), metabolism (SMR, MMR, and AS) and anatomy (relative stomach, brain and heart masses and gut length). We additionally measured the transition time to feeding on novel prey items and fright response as key behavioural traits relevant to energy acquisition, and tested for differences in invertebrate drift biomass between natal piscivore and insectivore streams. To assess whether differences between piscivore and insectivore ecotypes were consistent over time and space, we replicated the measurement of SGR, SMR and invertebrate drift in the original piscivore and insectivore populations across years, as well as two additional insectivore populations from different tributary streams (replication was not possible for the single piscivore population in the lake).

1.2 Material and Methods

1.2.1 Fish collection and ecotype contrasts

Detailed insectivore and piscivore comparison (2017)

Young-of-the-year rainbow trout were collected in their natal streams around Kootenay Lake in August 2017, less than two months after hatching to minimize potential effects of a prolonged exposure to different ecological conditions on their early development. Piscivores were collected in the upper Lardeau River (LAR, UTM 5593885N 481874E, see Appendix), a moderate-sized gravel-cobble river (approximately 70m bankfull width, 2m bankfull depth, $58\text{m}^3\cdot\text{s}^{-1}$ Mean Annual Discharge) flowing for 60kms before its junction with the Duncan River, the main northern tributary to Kootenay Lake. Insectivorous fry were collected from Redfish Creek (RED, UTM 5495734N 496548E), a smaller (approximately 5m bankfull width, 1m bankfull depth) cobble-boulder forested channel in the west arm of Kootenay Lake. Fish collection was performed using dip-netting methods and occurred during the day or after dusk when fry moved to quiescent marginal habitats. Once

captured, fry were held in flow-through plastic bins placed in Redfish Creek for 1-2 days prior to their transfer to aquatic facilities at The University of British Columbia (UBC: Vancouver, Canada).

Piscivore vs. multiple insectivore population comparison (2018)

To test for temporal consistency in trait differences among ecotypes, collection of insectivore and piscivore fry from Redfish Creek and the Lardeau River was repeated in 2018 and fish were transported back to the laboratory as in 2017. Using the same sampling techniques as in 2017, fry were also collected from two additional insectivore populations - Hendryx and Duhamel Creeks - located on the eastern and western sides of Kootenay Lake, respectively. This provided replication for the insectivore ecotype to allow statistical inference for whether insectivore traits overlap with piscivore traits.

1.2.2 Fish rearing and ration treatments

At UBC, fish were transferred into a walk-in environmental chamber with a 12h:12h day:night cycle and $14 \pm 0.4^\circ\text{C}$ temperature. Prior to experiments, all fish were quarantined (4ppm saltwater) for one week while fed freeze-dried chironomids twice a day on a near maintenance ration (1% wet body mass). A 1600L flow-through system composed of two connected housing tanks (245x60x40cm each) with multiple compartments was used to allow individual rearing under common garden conditions; individual rearing was intended to minimize any potential effects of dominance on subsequent growth and metabolism. Tanks were divided transversely into two rows of eight compartments using plastic mesh screen, allowing water flow and visual contact while preventing movement of fish or food between the 16 compartments in each tank. Our experimental design included low, mid and high (satiation) ration levels for both piscivore and insectivore fry, for a total of six experimental treatment combinations. Ten fish were randomly chosen for each treatment combination, stocked in individual rearing compartments, and fed 1.2 mm commercial trout pellets (BioPro2, Bio-Oregon, Washington, USA) on a ration slightly above maintenance (~1% wet mass per day) for seven days to standardize initial fish energetics. After seven days of acclimation, average body mass was $3.02 \pm 1.11\text{g}$ (mean \pm SD) for piscivores and $1.30 \pm 0.67\text{g}$ for insectivores. Fish were then placed on either low ration (i.e., complete food deprivation), mid ration

(i.e., 1% wet body mass) or high ration (i.e., *ad libitum*) for two weeks before subsequent measurements of individual growth, metabolism and anatomy. Although there was some degree of overlap in body size, piscivores had higher initial masses than insectivores at both low (Tukey's HSD (Honest Significant Difference); $P < 0.01$), mid (Tukey's HSD: $P < 0.001$) and high rations (Tukey's HSD: $P < 0.001$), reflecting their larger initial size on collection. Initial body masses within ecotypes did not differ across feeding treatments ($P > 0.05$). Ration treatments were limited to a 2-week duration to minimize mortality in the fasting treatment. Fish on mid and high rations were delivered pellets at a constant rate over 8h daily using automatic feeders: high ration feeders were loaded with a satiation ration of pellets every morning which distributed food to each compartment, leaving the fish free to self-feed. To prevent accumulating food from biasing the ration levels to which fish were assigned, each rearing tank was gently siphon vacuumed with a two-day interval to minimize disturbance to individual feeding rates, which were measured on days 7 and 14. All fish were measured for weight (g) and fork length (mm) at the beginning of each treatment and weekly thereafter. Mid-ration was readjusted every week after weighing.

Piscivore vs. multiple insectivore population comparison (2018)

Using the same sampling and rearing protocols as described above for 2017, 10 piscivorous (Lardeau River) and 10 insectivorous fry from each of the three insectivore populations (i.e., Redfish, Hendrix and Duhamel Creeks, $N = 30$ insectivores) were maintained for two weeks on a high-food ration, which was identified in 2017 as the treatment maximizing differences among populations. Invertebrate drift, SGR, and SMR were measured and analyzed for each of the four populations following the protocols described below.

1.2.3 Standard Growth Rate

To determine whether juvenile piscivores and insectivores differ in growth, every fish was weighed in water using a digital scale after one and two weeks of treatment, and individual standard growth rate (SGR, %wet body mass·day⁻¹) was calculated as

$$SGR = \frac{\ln M_{final} - \ln M_{initial}}{t} \times 100$$

where $M_{Initial}$ is initial body mass (g), M_{Final} is final body mass (g), and t is growth interval in days. Individual SGR was calculated as the average of observed growth at one and two weeks.

1.2.4 Food Consumption

To assess potential differences in energy intake between ecotypes, individual food consumption (FC) was measured after one and two weeks of feeding for fish on mid and high rations, the later being equivalent to the maximum amount of food a fish can ingest per day. The bottom of each tank was siphon vacuumed at 08:00 AM to remove leftover food and faeces before automatic feeders were loaded with food (in excess at high ration) and fish allowed to feed for a day. Each tank was vacuumed again the next day to collect remaining food and faeces, which were then carefully separated, and uneaten pellets were dried for one week at 60°C and weighed. The mass of pellets distributed was considered as dry mass; the difference in moisture content between distributed and remaining (i.e., dried) pellets was assumed to be negligible, since all pellets were exposed to similar air humidity during processing prior to weighing. Mass-specific food consumption was estimated as

$$FC = \frac{F_{Available} - F_{Remaining}}{M_{Fish}} \times 100$$

where FC is the amount of dry food consumed (%dry body mass), $F_{Available}$ the dry mass (g) of pellets distributed, $F_{Remaining}$ the dry mass (g) of food remaining after feeding, and M_{Fish} the dry mass of the fish (g). Calculations of M_{Fish} assumed an average body water content of 76% (Allen, et al., 2016). Average individual FC was calculated as the average of consumption estimates at one and two weeks.

1.2.5 Growth Efficiency

Growth Efficiency (GE) of fish on mid and high rations was calculated after one and two weeks as

$$GE = \frac{M_{Gained}}{F_{Consumed}}$$

where M_{Gained} is mean daily mass increment (%dry body mass) and $F_{Consumed}$ the daily amount of dry food consumed (%dry body mass). Average individual GE was calculated as the mean of GE

measured after one and two weeks. Fish were fed for the last time after 14 days of treatment at 05:00 PM, and experiments on individual metabolism performed the next day.

1.2.6 Oxygen Consumption Rates

To evaluate potential differences in energy metabolism between ecotypes, MMR and SMR were measured the day after feeding treatments ended, i.e. after a continuous period of 15 (MMR) and 36 hours (SMR) of fasting. To prevent any residual food consumption that might cause a postprandial elevation of metabolism (Alsop & Wood, 1997), each tank was siphoned free of food at 08:00 AM prior to respirometry. Measurements of MMR occurred a minimum of five hours after the beginning of the day cycle, to minimize any effect due to circadian rhythm. Each fish was placed in a 12-L bucket filled with water at ambient temperature and chased to exhaustion, which was reached when fish no longer reacted to a gentle push with the hand. Once exhausted, each fish was placed into a plastic closed respirometer fixed with an oxygen sensor (NeoFox, Ocean Insight, Dunedin, Florida, USA) that recorded the drop in oxygen tension from approximately 90% to 60% air saturation, while a small stir bar inside the respirometer ensured mixing. To account for the lag in response time of the oxygen probe, the first minute of every oxygen trace was discarded and the 60-second period with the highest drop in oxygen tension was used to calculate maximum oxygen consumption rate as

$$\dot{M}O_2 = \frac{V_W \cdot \Delta C_W O_2}{\Delta t}$$

where $\dot{M}O_2$ is the rate of oxygen consumption ($\mu\text{mol}O_2 \cdot \text{h}^{-1}$), V_W the volume of water in the respirometer (L), $\Delta C_W O_2$ the change in oxygen tension in the respirometer, and Δt the 60-sec period over which the drop in oxygen tension was measured. Barometric pressure, recorded at each trial date, and oxygen solubility coefficient in water α_{O_2} ($\mu\text{mol}O_2 \cdot \text{L}^{-1} \cdot \text{kPa}^{-1}$) were used to correct dissolved oxygen concentration in the respirometers. Finally, oxygen consumption rates were divided by body mass to estimate mass-specific oxygen consumption rates.

Immediately after the measurement of MMR, each fish was transferred to an individual glass flow-through respirometer (13cm long, 3cm in diameter) connected to a head tank supplying 80-90% air-saturated water. Oxygen concentration in the head tank was recorded continuously with an

optical sensor, and oxygen-depleted water exiting each respirometer was directed to a closed glass vial where an oxygen probe could be inserted. Effluent water was returned to a sump for denitrification and reoxygenation before being redirected to the head tank. Ten glass respirometers were connected in parallel to allow simultaneous measurement of SMR in 10 fish and were covered with black plastic to minimize stress and activity. Flow rates through respirometers were adjusted to ensure 80-90% air saturation and estimated by weighing the amount of water discharged in one minute. Fish were allowed to acclimate inside their respirometers for a minimum of 12 hours before SMR measurement, i.e. a minimum of 36 hours after last feeding. SMR was determined between 03:00 AM and 06:00 AM, a period of low oxygen consumption, as observed in previous studies (Van Leeuwen et al., 2011; Allen et al., 2016) and pilot respirometry experiments on piscivore and insectivore fry (G. Monnet, personal obs.). Initial oxygen concentration was measured in the head tank, and five replicate oxygen measurements were collected from the outflow vials below the 10 respirometers, providing five points of oxygen consumption per respirometer. Oxygen consumption rates were retained for each fish and averaged to calculate individual SMR as

$$\dot{M}O_2 = V_W \cdot \Delta C_W O_2$$

where V_W is the flow rate through the respirometer ($L \cdot h^{-1}$). Following SMR measurement, fish were euthanized before being frozen at $-60^\circ C$. The difference between MMR and SMR was calculated for each fish to obtain aerobic scopes (AS).

1.2.7 Organ sizes

After euthanasia, fish were dissected and organs associated with both cognitive (i.e., brain), cardiovascular (i.e., heart) and digestive (i.e., stomach, intestines) performance were excised. Brain (neural cord excluded), heart and stomach (peripheral fats excluded) were weighed (mg) and their relative masses expressed as %wet body weight. Intestine length (from stomach to anus) was measured (mm) and its relative length expressed as %fork length of each fish.

1.2.8 Behavioural traits: capacity to break routines and fright response

To test whether piscivores and insectivores differ in foraging behaviours, both ecotypes were tested for their capacity to switch to a novel food item (see Kotrschal et al., 2013) by transitioning from feeding on bloodworms (freeze-dried chironomids) to 1.2 mm commercial food pellets. This was intended as an assay of the capacity of different ecotypes to break routine behaviour, which is predicted to be slower for more proactive personalities (Bolhuis et al., 2004; Kotrschal et al., 2013). Twenty-five size-matched individuals of each ecotype were weighed (g), divided into five replicate groups of five fish each and fed on a 1% wet mass ration for a day prior to each experiment. Over six days, each group was given two meals a day (1.5% wet mass ration each) with the composition gradually increasing from 50% pellets (day 1) by daily increments of 10% to 100% pellets on day 6. Each group was allowed to feed for 10 minutes after each meal delivery. Uneaten bloodworms and pellets were collected by vacuuming, carefully separated from faeces and dried at 60°C for a week. The proportion of pellets consumed by a group during each meal was calculated as the ratio of pellets consumed to pellets added, where consumption is the difference between pellets mass added and recovered.

Five days after being transitioned to pellets, size-matched fish from both ecotypes were tested for their sensitivity to a frightening stimulus, characterized by the fall of a novel object into the aquarium. Group compositions within each ecotype were randomized by reassigning fish to new groups of five individuals. To startle fish, a 4-cm long dense plastic cylinder was dropped into each of the five 20-L aquariums in a standardized fashion, and the behavioural responses of the five fish in each replicate group was recorded using a video camera (SJ4000, SJCAM, China) mounted above the tanks. Twelve screenshots taken five seconds apart were extracted from the first minute of recording following each drop, and the distance between the pectoral insertion on each fish to the center of gravity of the cylinder was measured on each screenshot using ImageJ2 software (v.2.0.0; Rueden et al., 2017). Following a similar protocol, the average number of corners visited over one minute over all groups of fish following the drop was measured as an additional behavioural response. This test was repeated twice, three days apart.

1.2.9 Invertebrate drift abundance in piscivore vs. insectivore streams

To determine whether different prey availabilities in natal rearing streams have the potential to contribute to early phenotypic differentiation of piscivorous and insectivorous fry, we sampled invertebrate drift for one day in July and August 2017 in both the Lardeau River and Redfish Creek. Five 250 μ m-mesh drift nets were installed per stream to filter drift for approximately one hour. To estimate volume filtered (m^3), velocity and depth in each net were measured at the start and end of each set using a Marsh McBirney digital flow meter (Hach Flow Company, Colorado, USA). Collected invertebrates were preserved in 70% ethanol; the total number of macroinvertebrate larvae (i.e., orders Diptera, Trichoptera, Plecoptera, Ephemeroptera and Coleoptera) present in each sample (N_{Larvae}) was counted using a binocular microscope, and drift concentration was calculated as the ratio of total invertebrates to volume filtered. Because the Lardeau river drains Trout Lake, zooplankton were common in the Lardeau River drift samples (while absent in Redfish Creek); zooplankton abundance was estimated by counting invertebrates (i.e., orders Cladocera, Cyclopoida and Calanoida) present in a two milliliters subsample of each drift sample under a binocular microscope, after normalizing volumes to 50mL with water.

Drift biomass in 2017 was estimated by taking digital images of 15 random individuals of the most abundant taxa for each river and month. Individual body length (mm) was then measured using ImageJ2 software and taxa-specific biomass were estimated using length-weight relationships from the literature, before being applied to sample counts to estimate total invertebrate and zooplankton biomass.

1.2.10 Statistical analysis

Although phenotypic integration is better understood when multivariate traits are analyzed simultaneously (i.e., non-independently), traits were first analyzed independently to assess their degree of variation between ecotypes and their sensitivity to ration level and body mass. To test for differences between ecotypes in growth-related (SGR, FC, GE), metabolic (SMR, MMR, AS) and anatomical traits (gut length and relative brain, heart, and stomach masses), we performed analyses of covariance (two-way ANCOVAs) using R software (v.1.1.456; RCore Team, 2018). Distribution normality and variance homoscedasticity were tested using Shapiro-Wilk and Bartlett tests, respectively. We used model selection (Burnham & Anderson, 2002) to assess the response of each

of the ten phenotypic traits (e.g., SGR) to a standard set of explanatory variables including ecotype (i.e., piscivore, insectivore), ration (i.e., low, medium, high), body mass, and all their interactions. Body mass was included as a covariate to account for initial differences in body mass between populations and allometric variation of some traits (e.g., SGR). Because food consumption and growth efficiency were not measurable in fasting fish, models for these traits only included data for fish on mid and high rations. Automatic model selection (dredge function, MuMin package) was used to determine the most parsimonious set of explanatory variables for each phenotypic trait. An analysis of variance (ANOVA) was then performed on the best model for each trait (i.e., with the lowest AIC) to evaluate the effects of retained fixed effects. When a significant ecotype x ration interaction was detected, data were analyzed by ration using Tukey's HSD (Honest Significance Difference) tests (glht function, multcomp package). Because of fish mortalities over the course of the study, sample sizes were variable and ranged from a high of 60 for SGR to 36 for SMR out of a maximum of 60 observations with no mortality (i.e., 10 fish per ration, three rations, two populations).

To better understand how multivariate trade-offs and positive trait associations differentiate piscivore and insectivore integrated phenotypes, all traits were then analyzed simultaneously by Principal Component Analysis (PCA). The dataset used for this analysis was restricted to fish for which all ten traits were measured. Sample size for multivariate analysis was then reduced to 36 fish, including 23 piscivores (6 fish at low, 7 at mid and 10 at high ration) and 13 insectivores (4 fish at low, 5 at mid and 4 at high ration). Such low and unbalanced sample sizes in 2017 were suspected to potentially weaken strength of inference and motivated, in part, the collection of fish from replicate insectivore populations from additional tributary streams in 2018. The results obtained in 2018 with balanced sample sizes showed similar multivariate trait associations (see below), suggesting that unequal sample sizes in 2017 did not bias results. To account for differences in body mass among ecotypes and rations, the fitted regressions described above were used to standardize trait values to the mean final body mass (2.97g) of the 36 fish. Two normalized and centered PCAs were then performed on standardized traits (dudi.pca function, ade4 package). The first PCA included only eight traits (i.e., SGR, SMR, MMR, AS, Relative brain/heart/stomach masses and gut length) including all three ration levels (i.e., low, mid and high) for both ecotypes. The second PCA included all ten traits but excluded fish on the fasting ration because measurements of food

consumption and growth efficiency were absent for this treatment. Strength and significance of emerging patterns of trait associations revealed by the two PCAs were assessed through ordination plots and correlations among individual traits (*rcorr* function, *Hmisc* package). The P-values from multiple correlations were adjusted for false discovery rate with a Benjamini-Hochberg correction.

Behavioural data (habituation to a new prey and fright response) could not be transformed to meet assumptions of normality and homoscedasticity. Consequently, non-parametric Kruskal-Wallis tests were performed using average daily pellet consumption as the habituation response variable and either day, ecotype, or a day x ecotype interaction as fixed effects. Mann-Whitney tests were used for *post-hoc* multiple comparisons. This protocol was repeated for fright response data to evaluate the effects of ecotype and sequence (i.e., first or second test) on both fish-object distance and the number of corners visited.

To test whether invertebrate drift was higher in the piscivore rearing stream (Lardeau River), we modelled 2017 drift concentration (individuals·m³) and biomass (mg·m³) as a function of stream (i.e., Lardeau River or Redfish Creek), month (i.e., July or August) and a stream x month interaction. Both concentration and biomass met the assumptions of normality and homoscedasticity after log-transformation. ANOVAs and *post-hoc* Tukey's HSD tests were performed on the two best models (i.e., with the lowest AICs) to evaluate the significance and magnitude of stream effects on invertebrate drift abundance.

The same analysis was performed for 2018 SGR, SMR and drift data. Only drift concentration was measured in 2018 to test whether differences in prey abundance were consistently lower in replicate insectivore streams (Redfish, Duhamel and Hendryx Creeks) relative to the Lardeau River, and whether differences in drift between natal streams were consistent across years (i.e., 2017 vs. 2018).

1.3 Results

1.3.1 Standard Growth Rate

As expected, standard growth rate increased with ration for both ecotypes (ANCOVA: $P < 0.001$; Fig. 1.2a). There was a significant ecotype x ration interaction (ANCOVA: $P < 0.001$), with piscivore

growth increasing faster at mid (Tukey's HSD: $P < 0.001$) and high ration levels (Tukey's HSD: $P < 0.001$), but no difference among ecotypes in the fasting treatment. Contrary to allometric expectations that mass-specific growth should decrease with body mass (Allen et al., 2016), SGR also increased with body mass (ANCOVA: $P < 0.001$; Fig. 1.2a).

1.3.2 Food Consumption

Food consumption was significantly higher at high ration (ANCOVA: $P < 0.001$, Fig. 1.2b), and showed a strong ecotype by ration interaction (ANCOVA: $P < 0.001$) with the piscivore ecotype exhibiting a much larger increase in maximum ration at high food (Tukey's HSD: $P < 0.001$, Fig. 1.2b). Although FC results at satiation were consistent with our expectations, the lower food consumption of piscivores compared to insectivores at mid ration were unexpected given their higher absolute growth. Food consumption did not significantly vary with body mass.

1.3.3 Growth Efficiency

Growth efficiency significantly declined with ration (ANCOVA: $P < 0.001$), for both ecotypes (Fig. 1.2c), as expected, i.e. lower food intake at mid ration may be digested more efficiently, resulting in higher GE relative to satiation. GE of piscivores exhibited a stronger decrease at high food (Tukey's HSD: $P < 0.001$), resulting in a significant ecotype x ration interaction (ANCOVA: $P < 0.001$). Despite this decline piscivores retained higher GE at both rations (Tukey's HSD: $P < 0.01$; Fig. 1.2c). Growth efficiency did not significantly change with body mass. Differences in growth and growth efficiency between ecotypes suggest higher performances of piscivores relative to insectivores at mid and high rations.

1.3.4 Standard Metabolic Rate

Standard metabolic rates increased marginally but significantly (ANCOVA: $P < 0.001$) with ration for both ecotypes (Fig. 1.3a). SMR significantly differed between ecotypes (ANCOVA: $P < 0.001$).

1.3.5 Maximum Metabolic Rate

Maximum metabolic rates of both ecotypes significantly decreased with ration (ANCOVA: $P < 0.05$; Fig. 1.3b), although *a posteriori* differences between were only significant between low and high ration for piscivores (Tukey's HSD: $P < 0.01$; Fig. 1.3b). In contrast with SMR, there was no consistent difference in MMR between ecotypes.

1.3.6 Aerobic Scope

As expected, AS mirrored MMR (Fig. 1.3c) and declined along with increasing ration levels (ANOVA: $P < 0.001$). Piscivores showed lower AS than insectivores at mid and high ration (Tukey's HSD: $P < 0.01$; Fig. 1.3c). Overall, differences in energy metabolism suggest that faster growth of piscivore juveniles requires higher maintenance budgeting (SMR) and is achieved at the cost of lower aerobic scope (AS).

1.3.7 Relative size of organs

Relative stomach mass of both ecotypes slightly but significantly increased with ration (Fig. 1.4a). Following our predictions, the piscivore ecotype demonstrated overall larger stomachs (ANCOVA: $P < 0.001$), although only differences between piscivores and insectivores at low ration were significant (Tukey's HSD: $P < 0.01$). Relative gut length showed similar trends (Fig. 1.3b), increasing with ration level (ANCOVA: $P < 0.01$), with piscivores showing longer guts than insectivores at each ration level (ANCOVA: $P < 0.001$; Tukey's HSD: $P < 0.01$; Fig. 1.5b).

Relative heart mass did not significantly change with ration. Consistent with MMR results, insectivores showed significantly greater heart masses than piscivores at low, mid and high rations (Tukey's HSD: $P < 0.01$; Fig. 1.4c).

Relative brain mass of both ecotypes decreased with ration (ANCOVA: $P < 0.01$; Fig. 1.4d), although brain mass of insectivores showed a stronger decline with body mass, resulting in a significant ecotype x mass interaction (ANCOVA: $P < 0.001$). This interaction suggests that initially smaller relative brain sizes in piscivore fry do not persist as body size increases, in part because relative brain size may be disproportionately reduced by faster somatic growth in the piscivore ecotype, without any actual change in energy allocation to brain development. Analyses of variance

(ANOVAs) performed at each ration level to remove the effects of ration and body mass on relative brain size reported a significant difference in relative brain size between ecotypes at low ($P < 0.01$), mid ($P < 0.001$), and high ration ($P < 0.001$).

Overall, anatomical results suggest the possibility of an organ-size trade-off among organs with competing energy costs. Faster-growing piscivores demonstrated greater energy investments into organs associated with food processing and digestion, while insectivores invest more in the development of organs involved in cardio-vascular or cognitive performances. However, the existence of a strong ecotype \times mass interaction for relative brain size and the lack of overlap in body size between ecotypes limit inference, and these results must therefore be interpreted with caution.

1.3.8 Multivariate associations between growth, metabolism and anatomy

The PCA that included all eight traits measured at low, mid and high rations (Fig. 1.5, left panel) identified three principal clusters of traits. The first cluster included moderate (Pearson, $r = 0.49$) to strong (Pearson, $r = 0.82$, see Appendix for details) positive correlations among growth-related traits, including growth rate (i.e., SGR), routine metabolism (i.e., SMR) and size of organs involved in digestion (i.e., stomach mass, gut length). As expected, growth-related traits were negatively correlated with a second cluster of strongly correlated (Pearson, $r = 0.89$, $P < 0.001$) traits related to active metabolism (i.e., MMR, AS), resulting in growth vs. active metabolism as a major axis of differentiation between ecotypes. A third and independent cluster loading heavily on PC2 included relative brain and heart masses, positively but moderately correlated (Pearson, $r = 0.58$, $P < 0.001$). Both ecotypes showed similar patterns of phenotypic differentiation between ration levels (Fig. 1.5), from low growth/high active metabolism at low food to high growth/low active metabolism at high food. This differentiation across rations was, however, of greater magnitude in piscivores, increasing the degree of divergence in growth and metabolism between ecotypes at higher ration levels.

The second PCA (ten traits, fasting ration dropped: Fig. 1.5, right panel) identified similar multivariate trait associations. As predicted, FC appeared strongly (Pearson, $r = 0.89$) positively correlated with SGR, and the rest of growth-related traits (i.e., SMR, relative stomach mass and gut length, see Appendix for details).

1.3.9 Behavioural traits: capacity to break routines and fright response

Unexpectedly, piscivores were quicker to break a behavioural routine than insectivores, as shown by their earlier transition from feeding on bloodworms to pellets (Fig. 1.6a). Piscivores reached a threshold of 95-100% pellets consumption after two days, while the equivalent was achieved by insectivores after six days, revealing a significant ecotype x day interaction (Kruskal-Wallis: $P < 0.001$).

Piscivores also exhibited greater confidence than insectivores when exposed to a frightening stimulus (Fig. 1.6b). Piscivores generally approached the foreign object more closely than insectivores, although the difference between ecotypes was only statistically significant in the second replicate trial (Fig. 1.6b), resulting in significant ecotype x trial interaction (Kruskal-Wallis, $P < 0.001$). Surprisingly, piscivores and insectivores did not differ in the number of corners visited after being startled (Fig. 1.7c), although this number uniformly and significantly decreased between tests (Kruskal-Wallis, $P < 0.01$), suggesting some degree of acclimation. Overall, results of group behaviour experiments suggest a more proactive personality type in piscivorous fry, which might contribute to maximizing food intake and subsequent growth rate in the wild.

1.3.10 Invertebrate drift abundance

As predicted, the natal stream of the piscivorous ecotype showed a higher productivity relative to the natal stream of insectivores. Drift densities and biomass in the Lardeau River exceeded those in Redfish Creek in both July (Tukey's HSD: $P < 0.01$) and August 2017 (Tukey's HSD: $P < 0.001$, Fig. 1.7). Although macroinvertebrate drift abundance increased during the summer in both streams, the increase in prey biomass between July and August 2017 was greater in the Lardeau River than in Redfish Creek (Fig. 1.7b), resulting in a significant stream x month interaction (ANOVA: $P < 0.05$). Differences in concentration and biomass of available prey between streams became even more significant after inclusion of plankton data from the Lardeau River (Fig. 1.7a, 1.6b), which were much higher in early summer (mean \pm SD: 128 ± 30 individuals \cdot m³ in July vs. 18 ± 7 individuals \cdot m³ in August 2017).

Overall, prey abundance data highlights the higher relative productivity of the Lardeau River, which would facilitate higher food intake and associated growth rate of piscivorous fry.

1.3.11 2018 replicate sampling of additional insectivore populations

As in 2017, piscivorous fry collected in the Lardeau River showed higher growth (i.e., SGR: Fig. 1.8a) and maintenance metabolism (i.e., SMR: Fig. 1.8b) than insectivores from each of the three insectivorous populations (i.e., Redfish, Hendryx and Duhamel Creeks) sampled in 2018.

Macroinvertebrate drift collected in 2018 also confirmed the higher prey availability in the Lardeau River relative to the insectivore streams (Fig. 1.8c). Invertebrate drift in 2018 significantly differed among streams (ANOVA: $P < 0.001$) and between months (ANOVA: $P < 0.001$). Prey availability greatly differed between July and August among streams, resulting in a significant stream x month interaction (ANOVA: $P < 0.001$; Fig. 1.8c). In July, prey availability was higher in the Lardeau River compared to Redfish (Tukey's HSD: $P < 0.001$) and Hendryx ($P < 0.001$) but not Duhamel Creek (Tukey's HSD: $P = 0.06$). In August, prey availability was higher in the Lardeau River compared to Redfish (Tukey's HSD: $P < 0.01$) and Duhamel ($P < 0.001$) but not Hendryx Creek (Tukey's HSD: $P = 0.07$). Regardless of monthly interactions, absolute prey availability was consistently higher in the Lardeau River.

Overall, consistency in fish performance and rearing habitat productivity among the multiple insectivore populations sampled in 2018 supports the inference that the Redfish Creek population is representative of the insectivore ecotype, and strengthens the inference of trait differentiation between insectivore and piscivore ecotypes of rainbow trout based on the 2017 data.

1.4 Discussion

1.4.1 Reciprocal constraints between juvenile and adult life histories

Measuring growth performance in divergent ecotypes of rainbow trout allowed the exploration of reciprocal constraints between adult morphology and juvenile growth. As expected, fry of large piscivorous adults grew consistently faster than fry of smaller-bodied insectivores across a gradient of increasing food availability, supporting the inference that achieving a large adult body size within

a fixed time frame is most effectively attained by a steeper juvenile growth trajectory (Czarnoleski et al., 2008; Rosenfeld et al., 2015; Sibly et al., 2015). This effect appears to be independent of a presumed later age at maturity for the piscivore trout (4-6 years against 2-3 years for insectivores; J. Burrows, pers. comm) which could also contribute to their larger adult body size. Such reciprocity between larger adult morphology and faster juvenile growth illustrates the strong potential for selection on adult life-histories to indirectly constrain adaptation at the juvenile life stage. These results are consistent with previous work (Allen et al., 2016) that identified higher juvenile growth rates in domesticated rainbow trout compared to slower-growing wild insectivorous individuals. Although the piscivore ecotype is known to achieve a larger size at age than the insectivore (Irvine, 1978a), the nature of ecological factors selecting for different adult body sizes between ecotypes (and by inference different juvenile growth rates) remains somewhat speculative. For instance, a larger adult morphology may be a prerequisite for piscivory (Mittelbach & Persson, 1998), or to move the larger substrates during spawning below lake outlets (Riebe et al., 2014), while smaller adult body size may minimize exposure to predators in shallow stream spawning habitats (Quinn et al., 2001).

1.4.2 Metabolic trade-offs associated with high growth

Comparing juvenile growth, energetics and anatomy from divergent ecotypes of rainbow trout facilitated the identification of key trait associations (Careau & Garland, 2012) and trade-offs (Careau, 2017) differentiating their integrated phenotypes. Ordinations identified a strong positive covariance among traits that support high growth (i.e., FC, GE, SMR, relative stomach mass and gut length), as well as a major trade-off between these growth-related traits and aerobic scope. As expected, piscivore fry showed higher SMR and lower resulting aerobic capacities (i.e., AS) than insectivores, since MMR was similar between ecotypes. Despite small sample sizes in 2017, the replication of this study in 2018 showed similar multivariate trait associations, strengthening the inference of a major growth vs. active metabolism adaptive axis in the phenotypic differentiation of piscivore and insectivore ecotypes. Similar trade-offs between growth and active metabolism have been repeatedly observed in fish including Atlantic silversides (*Menidia menidia*: Billerbeck et al., 2000), sea bass (*Dicentrarchus labrax*: Killen et al., 2014) and rainbow trout (*Oncorhynchus mykiss*:

Allen et al., 2016), but do not appear to be universal. For instance, recent studies observed a positive covariance between growth and swimming capacity (i.e., U_{crit} , MMR, AS) in steelhead trout (*Oncorhynchus mykiss*: Van Leeuwen et al., 2011; Rosenfeld et al., 2020), and a positive correlation between aerobic scope and maximum food consumption in brown trout (*Salmo trutta*: Auer et al., 2015a). Collectively, these contrasting outcomes suggest that metabolic trade-offs may be highly context-specific (Careau & Garland, 2012; Careau et al., 2014), with local idiosyncrasies in ecological and evolutionary contexts driving variability in trait associations that underlie presumably adaptive differentiation of populations.

Negative effects of elevated growth on aerobic budgets (i.e., AS) are most likely mediated by an increase in resting metabolism (i.e., SMR). Elevation of apparent SMR is a common physiological response of animals reared on high rations or artificially selected for high growth (Arnott et al., 2006; Rosenfeld et al., 2015), and is predicted by the “expensive tissue hypothesis” (Aiello & Wheeler, 1995) to result from a preferential investment in digestive organs with high metabolic demand rather than competing cardiovascular and neural tissues (Álvarez & Metcalfe, 2007). The resulting potential for organ-system trade-offs (Rosenfeld et al., 2015; Allen et al., 2016) appear consistent with correlations among organ sizes observed between ecotypes. The larger stomachs and longer guts of piscivores would facilitate food processing and subsequent growth, but be of high energetic demand, potentially selecting for smaller brains and hearts sizes as suggested in our study and elsewhere (e.g., guppies: see Kotrschal et al., 2013). The role of organ-size trade-offs in selection for high growth, however, remains somewhat equivocal (Chappell et al., 1999; Réale et al., 2010; Careau & Garland, 2012); for example, the well-documented phenomena of smaller relative brain size in domesticated animals (O’Regan & Kitchener, 2005) may arise simply as a consequence of selection for faster somatic growth, rather than any direct selection on brain size, which could also be the case in our piscivore-insectivore comparison. In addition, the existence of clear organ-system trade-offs involving brain size remains equivocal in our piscivore-insectivore comparison due to different relationships between relative brain size with body mass associated with marginal overlap in body size between ecotypes. A growth vs. growth efficiency trade-off is another potential axis of adaptive differentiation between fast and slow-growing phenotypes (Allen et al., 2016; J. Rosenfeld, unpublished data). Because rainbow trout on a high ration typically have reduced growth efficiency (Allen et al., 2016), one might expect fast growing ecotypes to also have lower growth efficiency.

The piscivore ecotype, however, simultaneously achieved both high growth and high efficiency, suggesting that elevated growth efficiency is an adaptive mechanism for achieving higher growth as observed in other taxa (e.g., snails: Czarnoleski et al., 2008). Higher growth efficiencies, coupled with higher food consumptions by piscivores, indicate that differences in resource acquisition and use efficiency might be major cryptic drivers of ecological differentiation in salmonids, as in other taxa (e.g., Price et al., 2015).

1.4.3 Role of behaviour and prey abundance in ecotype divergence

Differences in foraging-related behaviours and natal stream productivities emerged as major elements of divergent resource acquisition strategies between piscivore and insectivore ecotypes. As expected, piscivores showed more confident responses to a threatening stimulus than insectivores, by staying closer to the unknown item dropped in their environment, indicating lower reactivity to potential threats. Diverging responses to fear between piscivores and insectivores may, however, also result from different learning or habituation capacities, or be confounded with different curiosities or boldness between ecotypes. Fear is a defining response to new and risky situations, and along with boldness and risk-taking is a major behavioural dimension of foraging strategies. For instance, risk-taking is known to mediate a widespread growth-mortality trade-off, whereby intense foraging activity supporting higher food consumption and growth comes at the cost of elevated predation risk and subsequent mortality (Lankford et al., 2001; Biro & Stamps, 2008). Similarly, a lower sensitivity to threatening stimuli may constitute a key behavioural adaptation of piscivore juveniles to support high growth in the wild. Alternatively, the greater confidence of piscivores may result from a lower capacity to identify the unknown item as a threat, rather than higher risk-taking exclusively. Piscivores also demonstrated higher behavioural flexibility by transitioning to a novel food item (i.e., commercial pellets) faster than insectivores. Although lower routine fidelity (Kotrschal et al., 2013) was suggested to be more characteristic of reactive behavioural types (Sih et al., 2004b; Kotrschal et al., 2013), a greater capacity to shift from one prey type to another may constitute a key component to facilitate higher food consumption and growth of proactive piscivorous fry, through diet diversification. Overall, behavioural assays suggest that there is some differentiation of foraging-related behaviours between piscivore and insectivore

ecotypes along a proactive-reactive axis (Sih et al., 2004b). Such differentiation may be a key element of an integrative, and presumably adaptive, response to differential selection on juvenile growth in the wild. Although group experiments may constitute less accurate indicators of animal personality than tests on individuals, these results are consistent with a 2018 side study in which piscivores were observed to be bolder, more exploratory and more active than most of three insectivore populations (Chapter 2).

Different prey abundances between piscivore and insectivore natal streams also support divergent resource acquisition strategies between ecotypes. As hypothesized, the Lardeau River - the piscivore natal stream - showed greater abundances and biomass of drifting macroinvertebrates than insectivore tributary streams (i.e., Redfish, Duhamel and Hendryx Creeks), providing favorable environmental conditions for the faster juvenile growth of piscivores. Unlike insectivore tributary streams, the Lardeau River also provided high densities of zooplankton, further supplementing prey availability, particularly for immediately post-hatch fry. Different juvenile trout densities between the Lardeau River and tributary streams may also influence realized *per capita* prey availabilities. Although fish densities were not explicitly estimated in this study, they are not expected to be significantly different among streams. The high fecundity of piscivore adults (Andrusak & Andrusak, 2006) and resulting high fry numbers are expected to mitigate the higher habitat availability in the Lardeau River, and lead to similar fish densities as in the insectivore streams. In addition, invertebrate drift was only measured in each stream over one hour in both July and August 2017 and 2018. Because invertebrate drift varies over time in response to ecological context (e.g., predation risk, temperature), future studies would benefit by measuring invertebrate drift more frequently over the growing season (i.e., from fry emergence in June-July to the beginning of winter) to better assess the temporal consistency of variation in prey abundance between piscivore and insectivore streams.

1.4.4 Consistency of multivariate trait associations with predictions of the Pace-Of-Life Syndrome

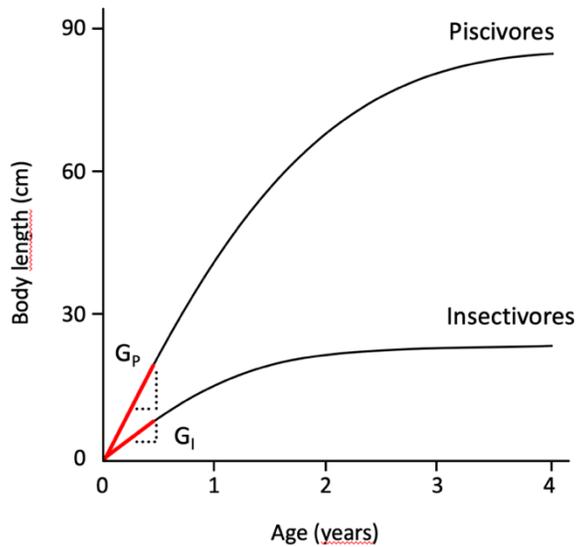
Multivariate trait associations largely differentiated piscivore and insectivore integrated phenotypes along the slow-fast continuum of the POLS. Positive covariance between elevated resting metabolism (i.e., SMR), more proactive behaviour (i.e., reduced response to fear, lower

routine fidelity) and elevated growth-related traits (i.e., SGR, FC, GE) is consistent with theoretical predictions. Although the POLS does not include explicit predictions with respect to anatomy, the suggestion of an organ-system trade-off between size of digestive (i.e., stomach mass, gut length), cardiovascular (i.e., heart) and neural (i.e., brain) tissues - differentiating piscivores and insectivores along a digestive-cardiovascular and brain size axis - appears partly consistent with differentiation along the POLS slow-fast metabolic continuum. Active metabolism (i.e., MMR, AS) was clearly traded-off against growth, partially undermining the accuracy of POLS predictions for traits associations related to growth and energetics. Despite this, the overall piscivore phenotype including high growth, high basal metabolism and proactive behaviours is generally consistent with a fast phenotype at the productive end of the POLS, associated with highly-productive environments, as supported by the greater prey abundance observed in the Lardeau River. Conversely, the insectivore phenotype with low growth, low basal metabolism and more reactive behaviours is consistent with a slow phenotype providing higher fitness in low-productivity environments at the other end of the POLS continuum (i.e., the smaller insectivore rearing streams tributary to Kootenay Lake).

This integrative study suggests that the POLS (Réale et al., 2010) is generally an accurate conceptual framework for predicting trait divergence in wild populations along a productivity gradient. This conclusion needs to be tempered, however, by the proviso that major trade-offs between traits supporting growth vs. active metabolism may increase variability in multivariate associations defining the integrated phenotype. Refining the predictions of the POLS with respect to growth vs. active metabolism may thus enhance its capacities and foster a better understanding of the evolutionary mechanisms involved in the adaptive differentiation of wild populations.

Identifying the evolutionary mechanisms underlying phenotypic and ecological variability is of fundamental importance to understanding the emergence and maintenance of taxonomic diversity in nature. Comparison of divergent ecotypes has provided landmark studies in ecotypic differentiation (Schluter, 1993, 1996a; Lowry et al., 2014), but these often focused on a limited subset of traits. By explicitly demonstrating the multidimensional divergence in growth, metabolism, anatomy and behaviour between piscivore and insectivore ecotypes of rainbow trout, our study confirms the value of contrasting ecotypes for understanding evolutionary patterns of phenotypic differentiation. Multidimensional ecotypic divergence, omnipresent in taxa presenting

large variation in life-history strategies such as salmonids (Morinville & Rasmussen, 2003; Keeley et al., 2005; Hughes et al., 2019), appears to be of major significance in the emergence and maintenance of local diversity.



Hypotheses	References	Traits/Factors measured	Predictions	
			Piscivore	Insectivore
(H1) Selection for larger adult body size (piscivore ecotype) indirectly selects for faster juvenile growth at the cost of lower active metabolism	Sibly et al. (2015) Allen, Rosenfeld & Richards (2016)	Growth rate *	High	Low
(H1a) Selection for traits associated with high juvenile growth come at the cost of lower scope for active metabolism	Billerbeck, Lankford & Conover (2001) Reinbold, Thorgaard & Carter (2009)	FC *	High	Low
		GE *	High	Low
		SMR *	High	Low
		MMR ^o	Low	High
		AS *	Low	High
(H1b) Selection for a large digestive tract to support high juvenile growth trades-off against other organ systems	Rosenfeld <i>et al.</i> (2015) Kotrschal <i>et al.</i> (2013)	Relative stomach mass *	High	Low
		Relative brain mass *	Low	High
		Relative heart mass *	Low	High
		Relative gut length *	High	Low
(H2a) Piscivorous fry support their faster growth by exhibiting more proactive foraging behaviours	Lankford, Billerbeck & Conover (2001) Biro <i>et al.</i> (2004)	Response to fear *	Low	High
		Routine fidelity *	High	Low
(H2b) Piscivorous fry support faster growth by benefiting from higher environmental prey availability	-	Stream productivity *	High	Low
(H3) Multivariate associations among growth, metabolic and behavioural traits are consistent with the predictions of the POLS	Réale <i>et al.</i> (2010)	All of the above phenotypic traits †	Fast phenotype	Slow phenotype

* indicates support for the hypothesis; ^o indicates no support; † indicates equivocal support

Figure 1.1. Summary of hypotheses relating to the phenotypic differentiation of piscivore and insectivore ecotypes of rainbow trout from Kootenay Lake. Note that larger size at maturity in piscivores can be achieved by a combination of later age at maturity and faster juvenile growth.

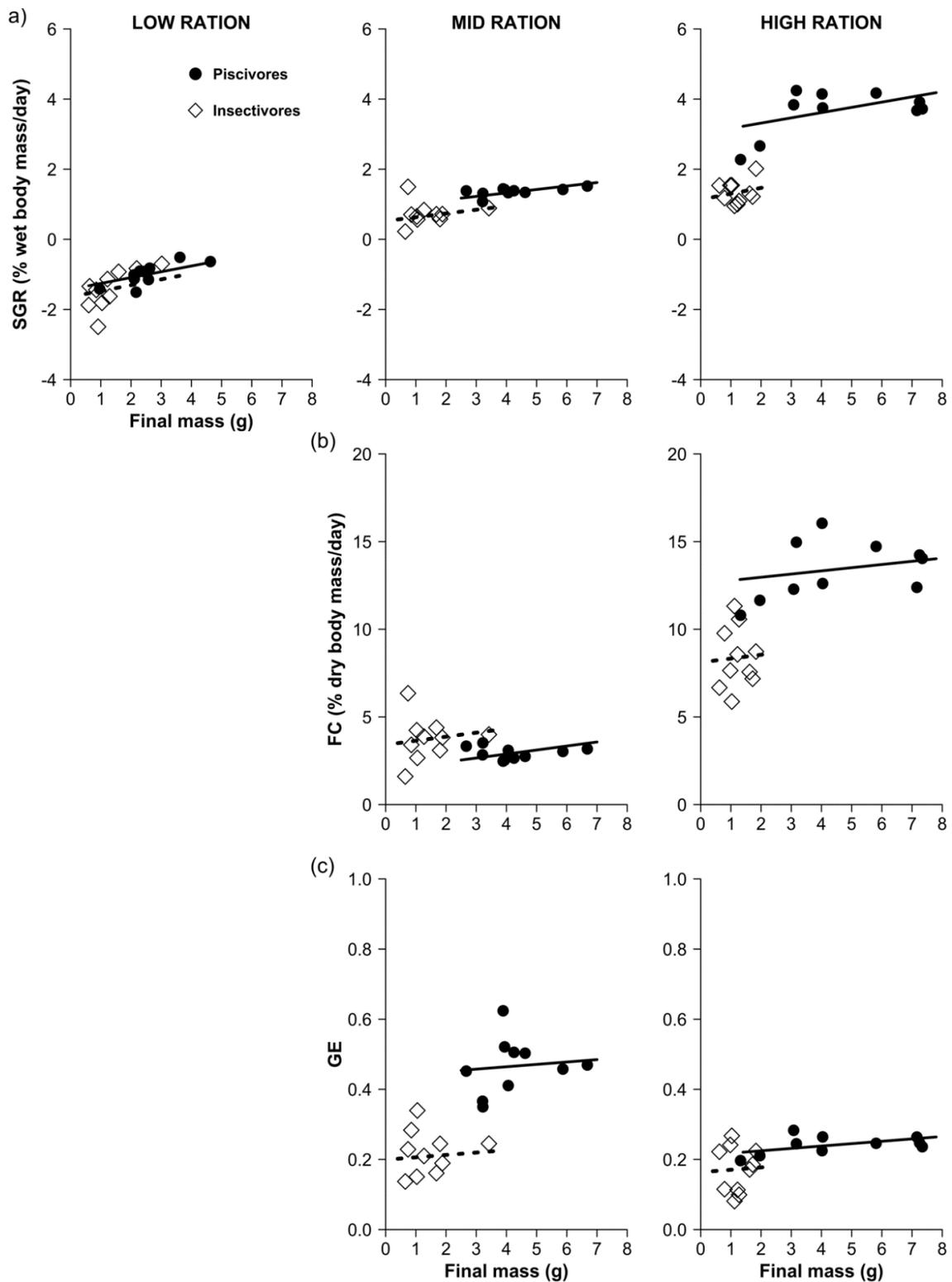


Figure 1.2. Effects of ecotype and mass on Standard Growth Rate (a), Food Consumption (b) and Growth Efficiency (c), at low (left), mid (middle) and high ration (right panel). Filled circles and solid lines represent piscivorous fry (Lardeau River), while empty diamonds and dashed lines represent insectivorous fry (Redfish Creek).

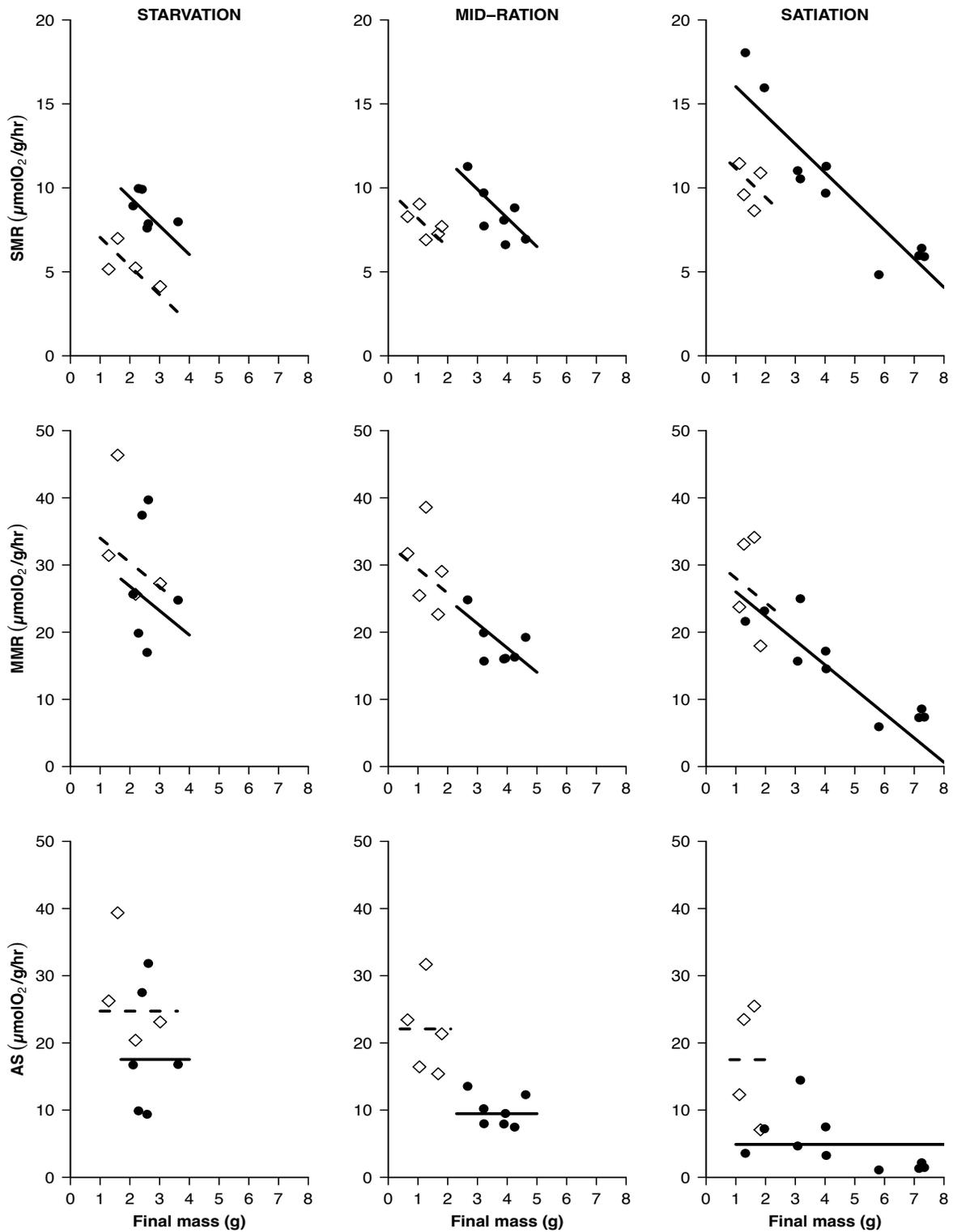


Figure 1.3. Effects of ecotype and mass on Standard Metabolic Rate (a), Maximum Metabolic Rate (b) and Aerobic Scope (c), at low (left), mid (middle) and high ration (right panel). Filled circles and solid lines represent piscivorous fry (Lardeau River), while empty diamonds and dashed lines represent insectivorous fry (Redfish Creek).

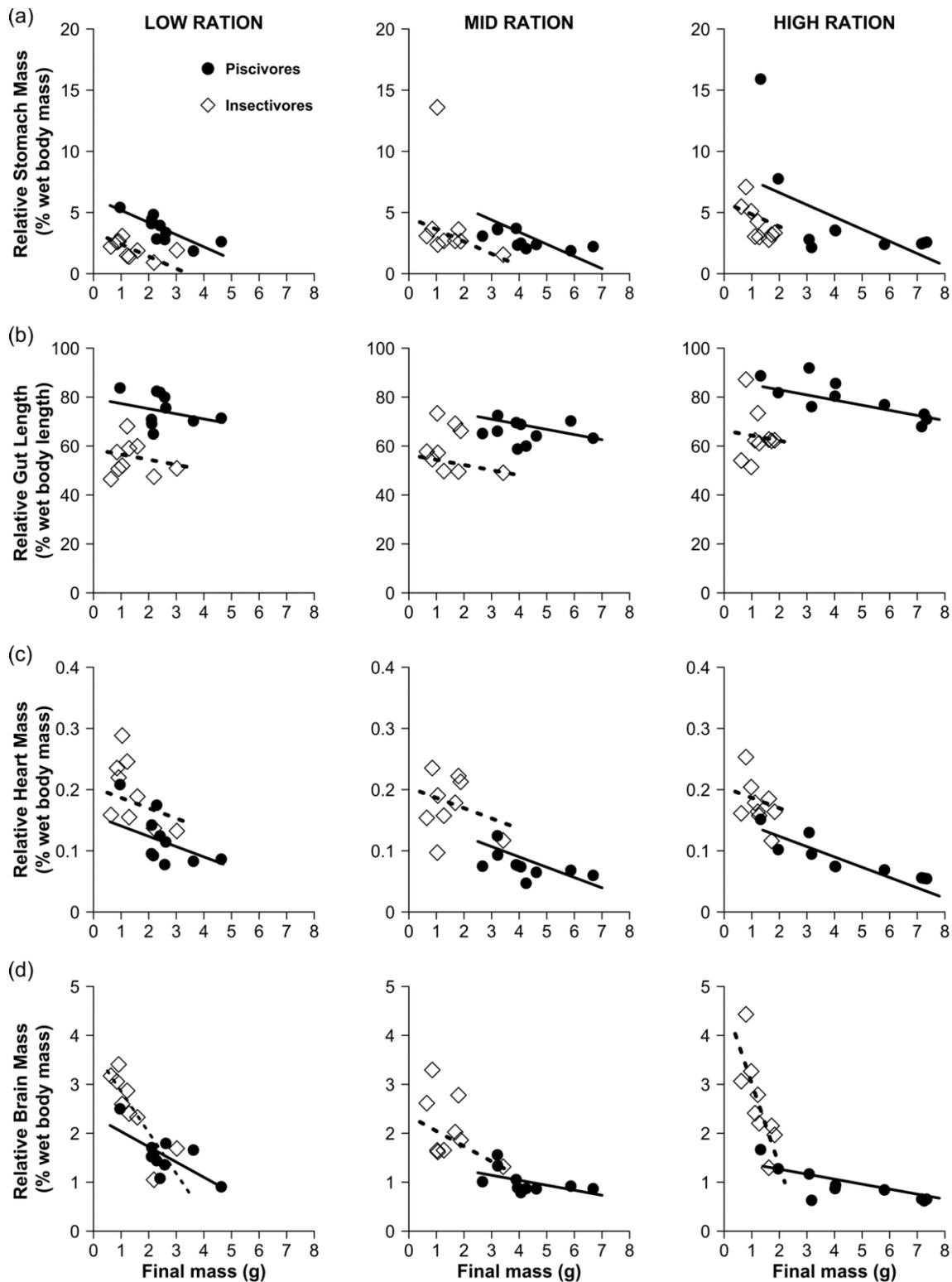


Figure 1.4. Effects of ecotype and mass on Relative Stomach mass (a), Gut length (b), Heart mass (c) and Brain mass (d) at low (left), mid (middle) and high ration (right panel). Filled circles and solid lines represent piscivorous fry (Lardeau River), while empty diamonds and dashed lines represent insectivorous fry (Redfish Creek).

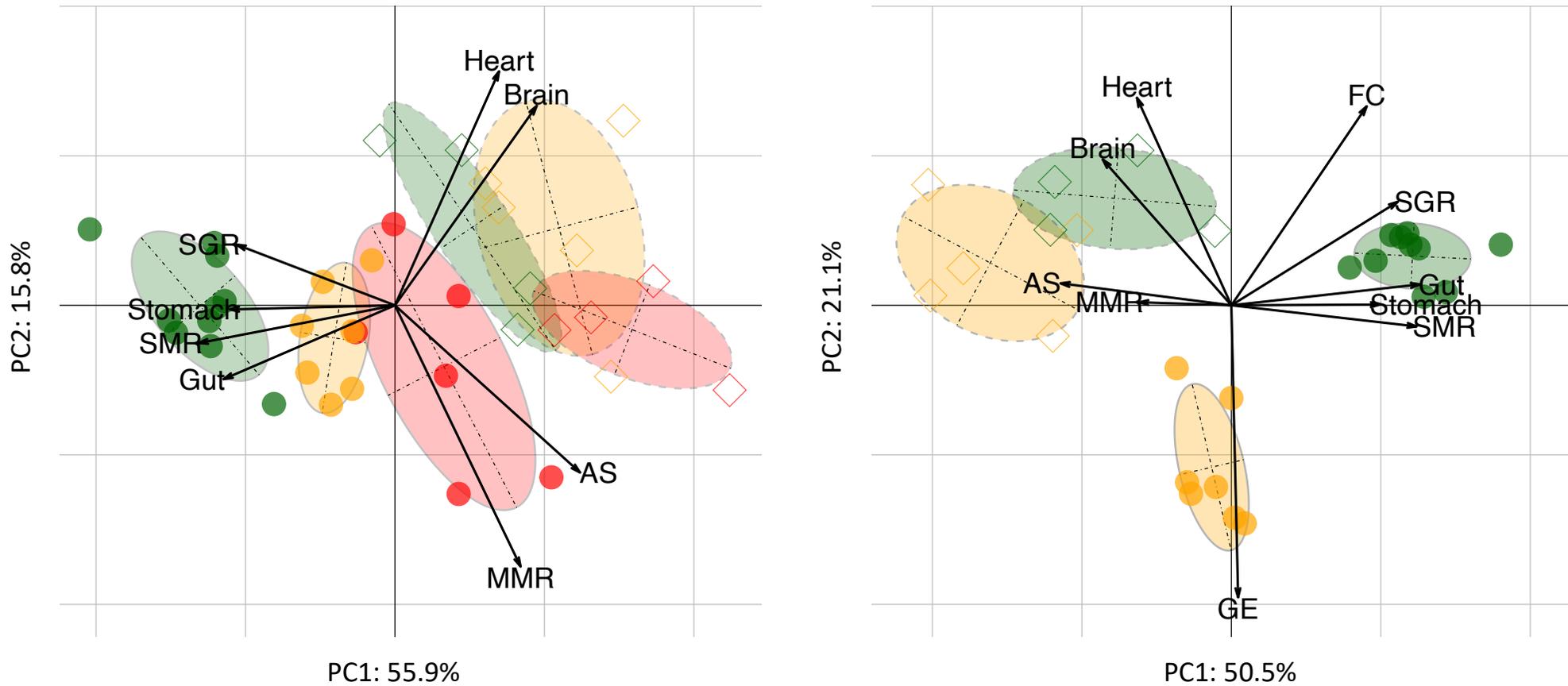


Figure 1.5. Principal Components Analyses (PCAs) including growth-related, metabolic and anatomical traits with size-adjusted values. The left panel includes the eight traits measured at the three ration levels; low (red), mid (yellow) and high (green ellipses). The right panel includes all ten traits measured at two ration levels (mid and high). Filled circles and solid lines represent piscivorous fry (Lardeau River), while empty diamonds and dashed lines represent insectivorous fry (Redfish Creek). The center of each inertia ellipse is the center of gravity of the associated point cloud, and the orientation of the ellipse is a graphical summary of the dispersion of the point cloud; the length of the axes of each inertia ellipse is equal to 1.5 times the square root of the eigenvalues of the associated covariance matrix.

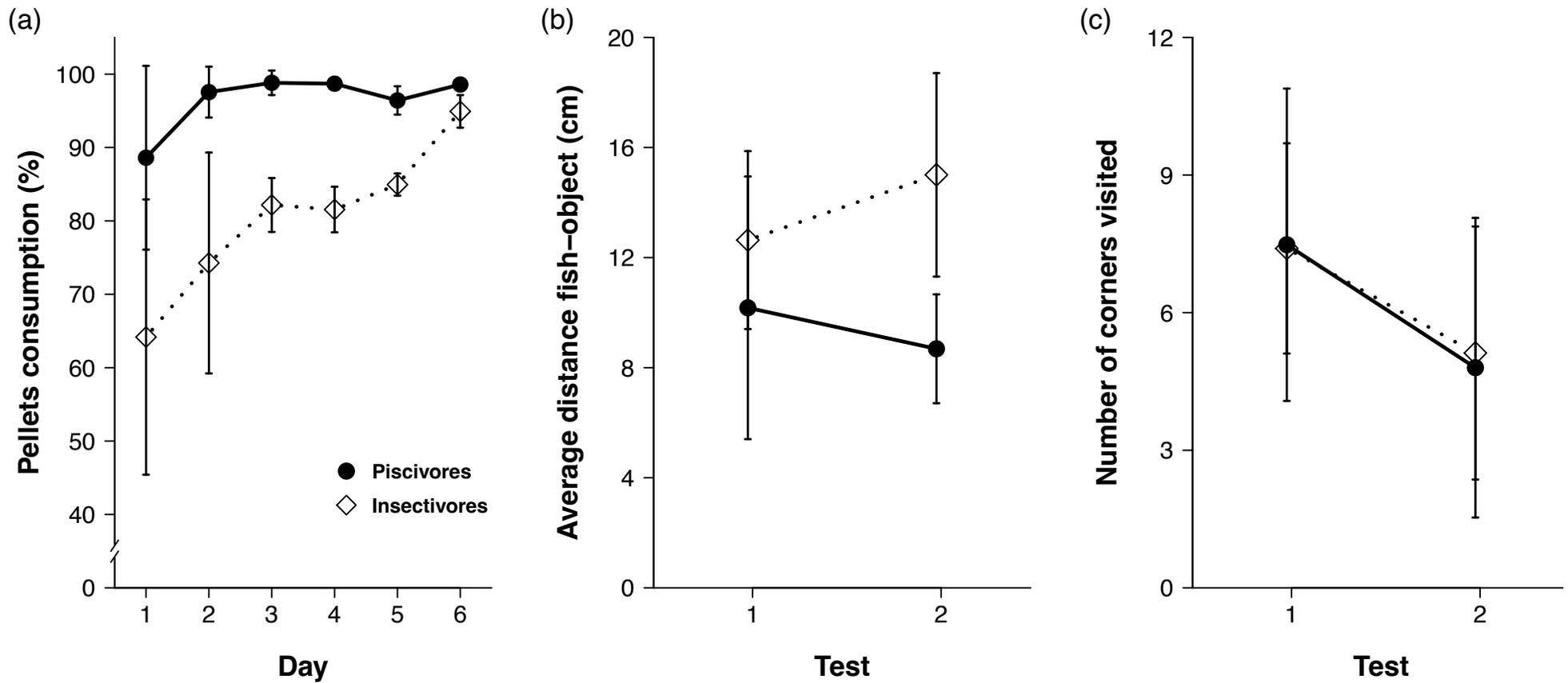


Figure 1.6. Effects of ecotype and time on transition to a novel food source ((a), mean \pm SD, N = 5 groups per ecotype), and effects of ecotype and sequence on average distance fish-object ((b), mean \pm SD, N = 5 groups per ecotype) and number of corners visited ((c), mean \pm SD, N = 5 groups per ecotype). Filled circles and solid lines represent piscivorous fry (Lardeau River), while empty diamonds and dashed lines represent insectivorous fry (Redfish Creek).

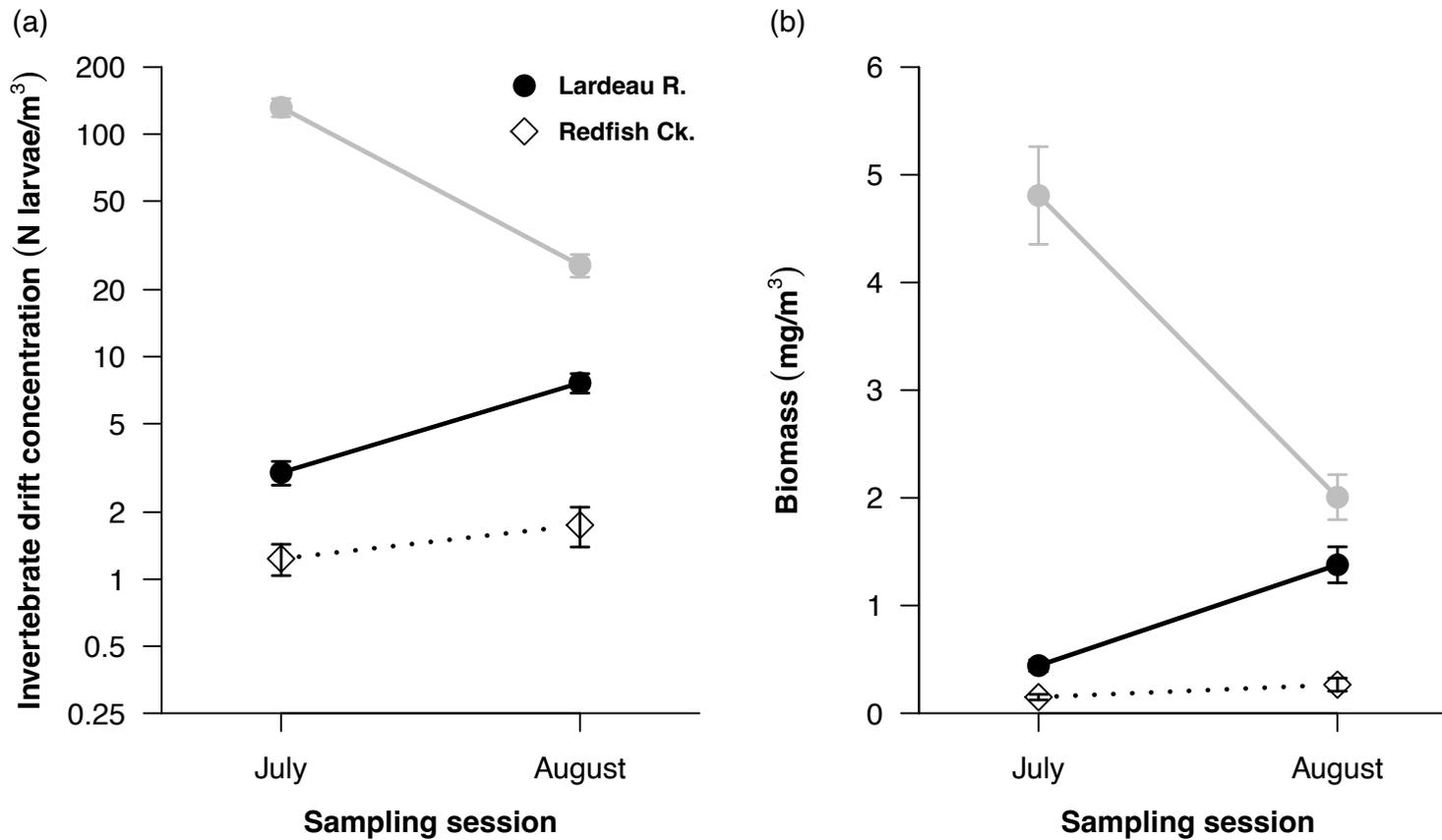


Figure 1.7. Estimates of invertebrate drift concentration (a; mean \pm SE) and biomass (b; mean \pm SE) for the Lardeau River and Redfish Creek in July and August 2017. Filled circles and solid lines represent the Lardeau River, while empty diamonds and dashed lines represent Redfish Creek. Grey circles and solid lines represent total productivity of the Lardeau River after inclusion of plankton data. Estimates were derived from drift or plankton samples collected for one hour a single day at five different stations in each month.

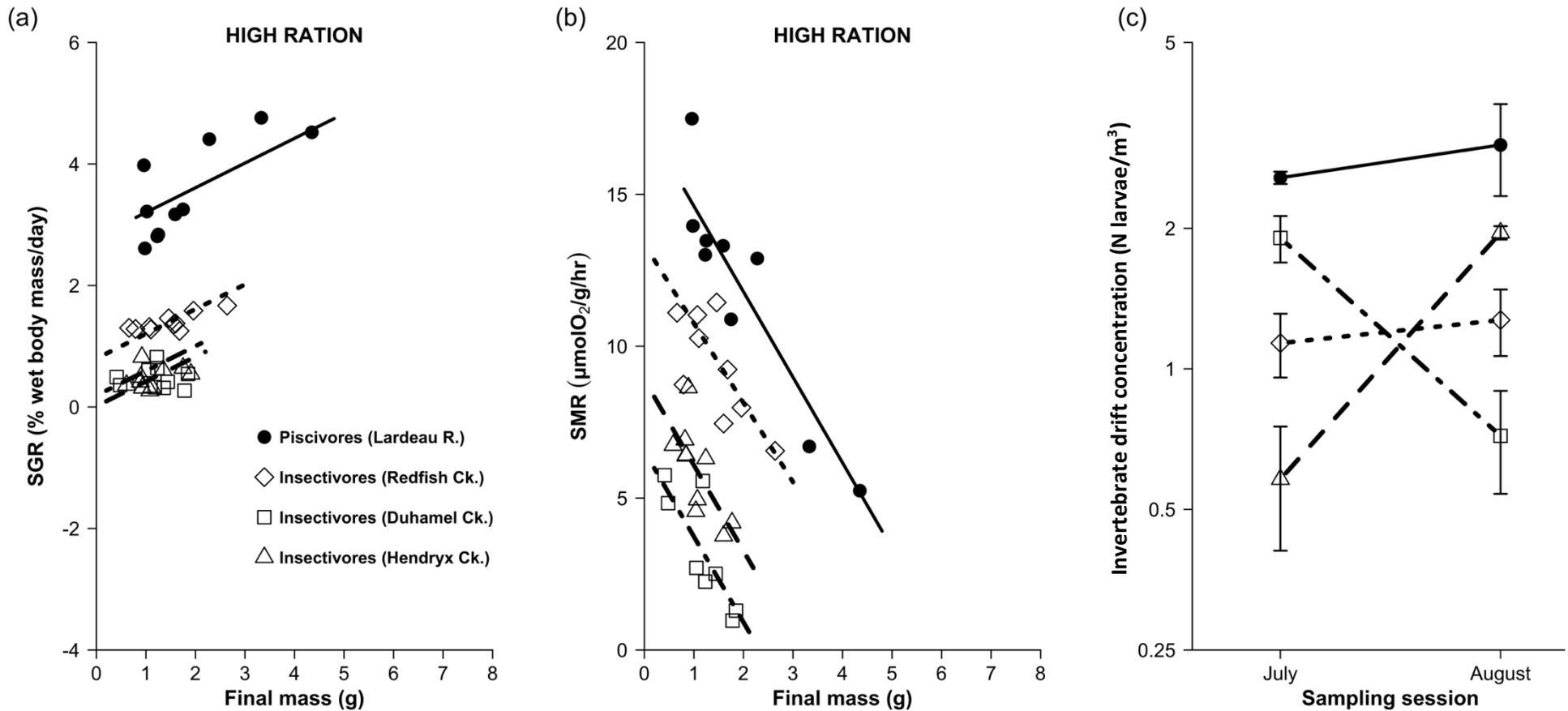


Figure 1.8. Effects of ecotype and mass on Standard Growth Rate (a) and Standard Metabolic Rate (b), and estimates of invertebrate drift concentration (mean \pm SE) for the Lardeau River and multiple insectivore populations (Redfish, Duhamel and Hendryx Creeks). Filled circles and solid lines represent piscivorous fry and the Lardeau River, while empty diamonds and dashed lines represent insectivorous fry and Redfish Creek, empty squares and long-short dashed lines represent insectivorous fry and Duhamel Creek, empty triangles and dotted lines represent insectivorous fry and Hendryx Creek. Estimates were derived from drift samples collected for one hour a single day at five different stations in each month.

Chapter 2: Behavioural variation between piscivore and insectivore rainbow trout *Oncorhynchus mykiss*

2.1 Introduction

Like many vertebrate taxa, fish exhibit substantial inter-individual variation in behaviour including boldness (Kotrschal et al., 2013), aggression (Vøllestad & Quinn, 2003) or feeding motivation (Mota Silva et al., 2010). This multidimensional variation in behavioural expression among individuals may have significant ecological consequences for individual performance (e.g., growth) or fitness (e.g., survival), and may ultimately contribute to the broader phenotypic differentiation of taxa along a taxonomic hierarchy from individuals to populations, ecotypes and species. To date, many studies have reported behavioural differences among individuals (e.g., Van Der Bilj et al., 2015), populations (e.g., Di-Poi et al., 2014), ecotypes (e.g., Baran & Streelman, 2020) or species of fish (e.g., White et al., 2020), yet few have assessed the consistency of expression in multiple behaviours across different levels of biological organization.

The proactive-reactive continuum has been suggested as a major axis of behavioural differentiation among individuals, populations and species (Koolhaas et al., 1999; Réale & Festa-Bianchet, 2003; Villegas-Ríos et al., 2018), where proactive individuals (e.g., bolder, more exploratory) are predicted to be more efficient at acquiring and processing information in stable environments, but slower to alter routine behaviours when environments or ecological circumstances change (Bolhuis et al., 2004). In contrast, reactive individuals (e.g., shy, less exploratory) are expected to scrutinize their environment more carefully, make slower yet well-informed decisions, and be better adapted to decision-making in changing environments (e.g., under variable predation risk). For example, proactive Tilapia (*Oreochromis niloticus*) were observed to adapt more quickly to and feed more efficiently in a simple T-maze system than reactive individuals (Mesquita et al., 2016). A proactive-reactive behavioural axis, although well supported at the individual level in domesticated vertebrates (e.g., mice: Benus et al., 1991; chickens: Van Hierden et al., 2002), remains less studied among wild populations, ecotypes and species (but see Di-Poi et al., 2014 for example).

The potential adaptive significance of contrasting proactive vs. reactive behaviours can be understood in light of trade-offs with other dimensions of the integrated phenotype, including life history (e.g., bold behaviour to maximize growth at the cost of survival: Lankford et al., 2001; Stamps,

2007) or metabolism (e.g., bold behaviour to maximize energy intake vs. the high energy demands of metabolically costly organs that maximize growth: Mittelbach et al., 2014). The apparent consistency among behavioural, metabolic and life-history continua has led to the emergence of holistic concepts like the Pace-Of-Life hypothesis (Réale et al., 2010), which posits the phenotypic differentiation of slow vs. fast phenotypes along a gradient of increasing environmental productivity. In this context, assessing how behavioural associations differ among individuals, populations and species, and correlate with other phenotypic dimensions (e.g., life-history traits, metabolism) is fundamental to understanding the integrated nature of ecological diversification.

Piscivore and insectivore ecotypes of rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) repeatedly evolved in a small number of freshwater systems in Northwestern North America, including the Kootenay Lake basin (B.C., Canada), and constitute a compelling model for understanding behavioural differentiation and its underlying ecological and evolutionary mechanisms. Piscivore rainbow trout exclusively spawn and feed for 1-2 years in the Lardeau River, the second-largest northern tributary of the lake, before migrating to Kootenay Lake to mature at large body sizes (5-15 kg) on a piscivorous diet of kokanee salmon (Keeley et al., 2005, 2007; for Kootenay Lake, see Andrusak & Andrusak, 2006; Andrusak & Parkinson, 1984). This piscivore ecotype is genetically differentiated from the insectivore ecotype (Taylor et al., 2019), which includes multiple discrete populations that spawn and rear for 1-2 years in smaller tributary streams around Kootenay Lake, before maturing in the lake where they achieve smaller adult body sizes (2-3 kg) on a largely insectivore diet. Contrasting adult body size between ecotypes has recently been identified as a major developmental constraint driving the potential adaptive differentiation in growth, energetics and behaviour of piscivore and insectivore juveniles (Monnet et al., 2020). A diminished fear response (Monnet et al., 2020) suggests that piscivores are more proactive than insectivores, which may be a requirement to support their faster juvenile growth; nonetheless, the full extent to which broader behavioural variation differentiates piscivore and insectivore individuals, populations and ecotypes based on a common proactive-reactive axis remains speculative.

In this study we evaluated the extent of behavioural differentiation among piscivore and insectivore individuals, populations and ecotypes by measuring an expanded suite of behaviours, including emergence time, activity, exploration and predator inspection in wild-caught juveniles from three insectivore populations and the single piscivore population in Kootenay Lake. Based on the expectation of divergence along a proactive-reactive axis, we predicted that ecotypes would present

major behavioural differences, with piscivores presenting shorter emergence time, and greater activity, exploration and predator inspection than insectivores, consistent with their broader ecotypic differentiation in growth and energetics (Monnet et al., 2020).

2.2 Material and Methods

2.2.1 Collection of piscivore and insectivore fry

Piscivore and insectivore *O. mykiss* trout were collected from their natal streams tributary to Kootenay Lake (B.C., Canada) in August 2018, less than 2 months after hatching to minimize any effects of exposure to different rearing environments on their behaviour. Piscivore fry were captured in the upper Lardeau River (LAR: UTM 5593885N 481874E), whereas insectivore fry were collected from three discrete insectivore populations around the lake, including Duhamel (DUH: UTM 5491977N 483444E), Hendryx (HEN: UTM 5512904N 510274E) and Redfish Creeks (RED: UTM 5495734N 496548E; see Monnet et al., 2020 for details). Fish collection occurred at dusk, when low light intensity and nocturnal movement of fry towards marginal habitats facilitated their detection with flashlights and capture with dip-nets. Because fish collection required several days to complete, captured fry were temporarily held for 1 to 2 days in a nearby spawning channel before their transfer to aquatic facilities at the University of British Columbia (Vancouver, Canada).

2.2.2 Fish rearing

Piscivore and insectivore fry were transferred into a walk-in environmental chamber with controlled temperature ($14.3 \pm 0.5^\circ\text{C}$, mean \pm SD) and day:night cycle (12h light:12h darkness). To minimize adverse effects of competition and variation in temperature or light on fish behaviour, each of the four populations of fish was divided among three 200L rearing tanks ($N_{\text{total}} = 12$ tanks), each stocked with 12-15 fish and randomly located in the environmental chamber. Densities of fish per aquarium were largely similar to densities observed in nature at this life stage, minimizing the potential of rearing conditions to affect behaviour. Immediately after being assigned to a rearing tank and before any experimental treatment, fish were quarantined in 5ppm saltwater for a week to minimize the potential for disease transfer, while transitioning from a near-maintenance ration of freeze-dried chironomids (slightly above 1% wet body mass, twice a day) to commercial pellets (BioPro2, Bio-Oregon, Washington, USA).

Body mass (mean \pm SD) of fish after quarantine was 1.16 ± 0.43 g (LAR), 0.97 ± 0.23 g (DUH), 0.97 ± 0.43 g (HEN) and 1.23 ± 0.39 g (RED); differences in body mass among populations were not significant (ANOVA: $F_{[3,44]} = 1.46$, $P = 0.23$).

2.2.3 Measuring multiple behaviours

To evaluate the extent to which piscivores and insectivores differed in behaviour, we measured emergence time, exploration and activity in 12 fry from the piscivore population (i.e., Lardeau River) and 12 fry from each of the three insectivore populations (i.e., Duhamel, Hendryx and Redfish Creeks: $N_{\text{insectivores}} = 36$; $N_{\text{total}} = 48$) within a week of the end of the quarantine period. Fish were selected based on body mass, while making sure to maintain individual variation in body mass and overall size-matching across populations; this method minimized the risk of non-random selection of bold or slow-swimming fish, and statistical control for any potential variation of behaviour with body mass. Similar to Weinersmith et al. (2009), we created a 50L observation arena supplied with oxygenated and chilled (13-14°C) water, marked with a grid of 18 equal-area sectors (10×12 cm each) visible to the observer, with the bottom covered in gravel and embellished with three artificial plants to create an unfamiliar yet realistic environment conducive to exploration (Fig. 2.1a). A 15 cm-diameter opaque acclimation chamber, equipped with a removable door, was fixed in one of the 18 sectors (which it fully occupied) to hold fish before their release into the arena. Water depth in the arena was set to 10 cm to minimize vertical movement, and movement of each tested fish in a two-dimensional plane was recorded using a small video camera (SJCAM 4000, SJCAM Company, Shenzhen, China) mounted above the arena (Fig. 2.1a). Fish were fasted for 24h before the start of experiments to maximize swimming motivation, and were tested between 11:00 AM and 04:00 PM, that is, 5-10h after the beginning of the daylight cycle to minimize effects of circadian rhythm on individual behaviour; because swimming activity rapidly decreases with fasting duration in rainbow trout (Simpkins et al., 2004), 24h of fasting appeared as the best compromise to standardize hunger levels while minimizing the risk of decreased swimming motivation in the fish.

To estimate emergence time, exploration and activity, each fish was individually placed inside the acclimation chamber (door closed) for 45min, before the door of the chamber was opened to allow exploration of the open field arena for 15min. As per Weinersmith et al. (2009), individual emergence time was measured as the latency of a fish to exit the acclimation chamber (i.e., the delay in seconds

to exit the chamber after the door was raised). Although emergence time from a shelter has been criticized for the ambiguity of the behaviour involved in this response (i.e., boldness vs. anxiety in confined spaces: see Carter et al., 2013 and Roche et al., 2016 for review), most sources of stress that may induce anxiety in fish were minimized (e.g., relatively short stay in the confined shelter, capacity to move freely in the unfamiliar arena).

Exploration was determined by the number of sectors out of 17 (expressed as a percentage) visited by a fish within 3min after exiting the acclimation chamber, and was intended as an index of area inspected. Activity was determined by the total number of transitions among sectors within 3min after exiting the acclimation chamber, independent of the number of sectors visited. Because a fish cannot visit different sectors without transitioning between them, activity and exploration are bound to be correlated to some extent in this experiment, although the strength of this correlation could greatly vary among individuals based on their capacity to gather environmental information; individuals with good information collection capabilities may explore thoroughly with few transitions, whereas other individuals with a lower capacity to gather information may display greater activity for a similar exploration of their environment. Exploration and activity as measured here may also represent different steps of the decision-making process, where exploration mostly refers to the capacity of gathering information on the surrounding environment (i.e., before making a decision), whereas activity may correlate with the motor response following decision-making. Transitions across adjacent sectors have also been used as a relevant proxy for movement behaviour (Rosemberg et al., 2011). Fish were classified as entering a sector when the front half of the body (snout to pectoral fin) crossed the line delimiting two adjacent sectors. After each test, each fish was weighed to the nearest 0.01g and returned to its rearing tank. To prevent the accumulation of olfactory cues from influencing the behaviour of subsequent fish, individuals from different populations were tested sequentially and water was changed after each series of three tests.

Predator inspection behaviour is a measure of risk-taking and represents the capacity of an individual to take risks in a non-novel situation (Réale et al., 2007; Van Der Bilj et al., 2015). To evaluate whether ecotypes differ in predator inspection behaviour, 12 piscivores and 12 insectivores from each of two insectivore populations (i.e., Duhamel and Hendryx Creeks: $N_{\text{insectivores}} = 24$; $N_{\text{total}} = 36$) were tested for predator inspection, using a separate batch of fish due to time and space constraints. A 110L experimental tank supplied with cold oxygenated water was divided transversely into four adjacent compartments with opaque plastic partitions (Fig. 2.1b). At one end of the tank, the first compartment

(approximately 40×20×15 cm) housed a shoal of conspecific *O. mykiss* to confer visual security because of the anti-predatory benefits of shoaling (Landeau & Terborgh, 1986). A mesh partition separated this compartment from an adjacent one of the same dimensions used as an acclimation area for test individuals. This acclimation area was in turn separated from a third observation compartment (approximately 40×20×15 cm) by an opaque and removable plastic partition. Finally, this central observation area was separated by a solid partition from the last section (approximately 40×20×15 cm) at the opposite end of the tank, which served as a predator compartment. To minimize the potential for bias associated with circadian rhythm, predator inspection trials took place between 11:00 AM and 04:00 PM. In predator-present trials, a shoal of three randomly selected rainbow trout fry of the same ecotype was placed in the first compartment of the experimental set-up, whereas a 20cm cutthroat trout (*Oncorhynchus clarkii*) was placed in the predator section at the opposite end of the tank to acclimate for 15 min. Predator activity and behaviour was relatively constant across trials; although the larger trout was visible to experimental fish, it did not initiate attack behaviours or become highly active. A randomly selected test fish was then placed in the acclimation (second) compartment with the opaque plastic partition lowered (i.e., preventing visual contact between the test fish and predator), and left to acclimate for 30min. The opaque partition was then raised mechanically, and fish movements within the observation area (central compartment) were recorded for 10min using a small video camera mounted above the tank. After the test, each fish was weighed to the nearest 0.01g before being returned to its rearing tank. *Post-hoc* video analysis allowed the determination of predator inspection as the shortest distance between fish and predator (in centimetres) within the first 5min of exploration measured using ImageJ2 software (v.2.0.0; Rueden et al., 2017).

2.2.4 Data analysis

To evaluate differences in behaviour between ecotypes and populations, we tested for differences in emergence time, activity, exploration and predator inspection between ecotypes and populations by performing ANCOVAs using R software (v.1.1.456; RCore Team, 2018). For the ecotype comparison, we assessed the response of each behaviour (e.g., activity) to ecotype (i.e., insectivore, piscivore), body mass and their interaction. Although piscivore and insectivore fry did not significantly differ in body size, body mass was nonetheless included as a covariate in the study models to account for marginal allometric variation in individual behaviours among populations. Low densities of fish in rearing tanks,

the minimal exposure to lab conditions before testing, and the random selection of piscivore and insectivore fry from multiple rearing tanks prior to the experiment are believed to have minimized the potential for tank or social effects to influence behavioural expression. Model selection was then used to identify whether the covariate (body mass) and its interaction with the factors ecotype or population were to be retained, before ANOVA was performed to estimate the significance of each fixed effect on the response variable of interest. Prior to analysis, emergence time data were transformed so that individuals that exited the acclimation chamber earlier (i.e., the boldest) were attributed the highest scores for emergence time; this was achieved by calculating the latency to exit the chamber (in seconds) for each individual relative to the maximum latency observed across all fish (i.e., 735s), expressed as a score from 0 (shy) to 1 (bold). As an example, a fish exiting 10s following door removal would have a score of $(735-10)/735 = 0.98$.

The same protocol was used for the population comparison, although in this case the initial set of fixed effects in regression included population [i.e., LAR (piscivore), HEN, DUH and RED (insectivores) for activity, emergence time and exploration; LAR, HEN and DUH for predator inspection], body mass and all their interactions. Note that each behavioural comparison among populations and between ecotypes was performed using the unique piscivore population of Kootenay Lake as a single replicate; by contrast, insectivore populations included two (i.e., for predator inspection: HEN, DUH) or three replicates (i.e., for emergence time, exploration, activity: HEN, DUH, RED), considered as either independent populations (i.e., population-level comparison) or as replicate ecotypes. Although the lack of piscivore population replication inevitably introduces some degree of pseudo-replication in the results, the singular behaviours displayed by the piscivore population relative to most of the three insectivore populations nonetheless constitute a relevant outlier on a behavioural continuum; nonetheless, the low number of populations within each of the two ecotypes considered in this study inherently limits statistical power.

To assess whether the range of individual variation in behaviour differed among populations or ecotypes, we calculated the variance of each behaviour (i.e., emergence time, activity, exploration, predator inspection) within every population (except Redfish Creek that only included data for emergence time, activity and exploration), and tested for overall differences in behavioural variation among populations and ecotypes. As variance data did not meet assumptions of normality and homogeneity after transformation, differences in variance across populations and ecotypes were

compared by performing non-parametric Kruskal-Wallis tests, followed by *post hoc* multiple Mann-Whitney comparisons and Holm-Bonferroni corrections for the population comparison.

2.3 Results

2.3.1 Behavioural variation among ecotypes, populations and individuals

Piscivore and insectivore ecotypes showed strong differences in behavioural expression. Latency to leave a shelter, distance to predator and proportion of sectors visited significantly differed between ecotypes (Fig. 2.1; Table 2.1). Differences between ecotypes in the number of transitions between sectors were not significant ($P = 0.07$; Table 2.2). Body mass and the ecotype x body mass interaction were never retained in our models following the model selection process. The correlation between the proportion of sectors visited and the number of transitions/minute was moderate and non-significant in the LAR (Pearson: $r = 0.37$, $P = 0.23$), RED (Pearson: $r = 0.52$, $P = 0.09$) and DUH populations (Pearson: $r = 0.29$, $P = 0.35$); nonetheless, this correlation was significant in the HEN population (Pearson: $r = 0.83$, $P < 0.001$), suggesting the existence of a strong autocorrelation between underlying behaviours in the HEN population. It is worth noting that different trait variance between piscivore and insectivore ecotypes may somewhat reduce the significance of our results. When compared with non-parametric tests (i.e., Kruskal-Wallis test), both latency to leave a shelter and distance to predator remained significantly different between ecotypes ($P < 0.001$ and $P < 0.001$, respectively). The difference in the proportion of sectors visited between ecotypes, however, became marginally insignificant ($P = 0.06$), while differences between ecotypes in the number of transitions between sectors remained marginally insignificant ($P = 0.06$).

Ignoring ecotype, the four individual populations differed in proportion of sectors visited, number of transitions between sectors and distance to predator (Fig. 2.2; Table 2.1). The piscivore population (LAR) explored a higher proportion of sectors and showed a higher number of transitions per minute than the HEN and RED populations, but the difference was only significant with the HEN population (Tukey's HSD: $P < 0.01$ and $P < 0.01$). The piscivore population also showed shorter distance to predator than the HEN and DUH populations, but the difference was only significant with the HEN population (Tukey's HSD: $P < 0.05$; Table 2.1). Differences in latency to leave a shelter among populations were marginally non-significant ($P = 0.07$; Table 2.2). Body mass and the population x body mass interaction

were never retained in our models following model selection. It is worth noting that piscivore and insectivore populations sometimes presented similar behaviours (e.g., proportions of sectors visited and number of transitions/min appear similar between LAR and DUH), suggesting that these populations differ in terms of inter-individual behavioural variation rather than mean population behaviour. Again, unequal trait variation between piscivore and insectivore populations (e.g., F-test for latency to exit a shelter: LAR-RED: $P < 0.001$; LAR-HEN: $P < 0.001$; LAR-DUH: $P < 0.001$) may somewhat reduce the significance of our results. When compared with non-parametric tests (i.e., Kruskal-Wallis tests), both proportion of sectors visited, number of transitions between sectors and distance to predator remained significantly different among populations (Kruskal-Wallis tests: $P < 0.01$, $P < 0.01$, and $P < 0.01$ respectively). By contrast, differences in latency to leave a shelter among populations became significant (Kruskal-Wallis; $P < 0.001$).

The magnitude of behavioural variation differed among piscivore and insectivore individuals (i.e., within populations and ecotypes). Unexpectedly, piscivore fry showed little inter-individual variation in behavioural expression, as suggested by the low variance observed for both latency to leave a shelter ($\sigma^2 = 29.96$, Fig. 2.1a) and distance to predator ($\sigma^2 = 7.93$, Fig. 2.1b); by contrast, each of the three insectivore populations presented large behavioural variances, indicating their greater diversity of individual behaviours (Fig. 2.2). Consequently, piscivore fry showed lower behavioural variance across all behaviours studied (mean \pm SD; $\sigma^2 = 23.0 \pm 13.4$) compared to each of the three insectivore populations (mean \pm SD; RED: $\sigma^2 = 5400 \pm 9100$; HEN: $\sigma^2 = 7430 \pm 14470$; DUH: $\sigma^2 = 10350 \pm 20620$); differences were significant between ecotypes (Kruskal-Wallis: $\chi^2 = 5.522$, $P = 0.018$), but marginal among populations (Kruskal-Wallis: $\chi^2 = 6.325$, $P = 0.09$).

2.4 Discussion

2.4.1 Behavioural variation among ecotypes, populations and individuals

Comparing multiple behaviours from piscivore and insectivore *O. mykiss* allowed the identification of strong contrasts in their behavioural expression at the population and ecotype levels. As predicted, piscivores presented a broadly consistent pattern of more proactive behaviours relative to insectivores (i.e., shorter emergence time, and greater exploration, activity and predator inspection) in both the population and ecotype comparisons; nonetheless, the degree of behavioural differentiation between piscivores and insectivores was reduced for some traits (e.g., proportion or sectors visited, number of

sectors visited) and population contrasts (e.g., between LAR and DUH populations), suggesting that behavioural differentiation between these populations may represent a continuum with multiple traits expressed to varying degrees among populations depending on the selective environment.

Although contrasting behaviours have repeatedly been observed among hatchery (e.g., Sneddon, 2003; Andersson et al., 2013; Øverli et al., 2011) or hatchery vs. wild populations of rainbow trout (Biro et al., 2004), fewer studies (e.g., Elias et al., 2018) have demonstrated the existence of differentiation in behaviour among wild populations of rainbow trout. The greater proactivity exhibited by piscivore fry in this study is consistent with previous work that reported lower fidelity to behavioural routines and a reduced fear response in piscivore fry relative to insectivores (Monnet et al., 2020). More broadly, the divergence of insectivore and piscivore ecotypes along a reactive-proactive behavioural axis is consistent with the predictions of the behavioural dimension of the Pace-Of-Life Syndrome (Réale et al., 2010) and contributes - along with parallel divergence in metabolism and life histories (Monnet et al., 2020) - to the broader differentiation of insectivore and piscivore ecotypes along a slow-to-fast phenotypic gradient.

Piscivore and insectivore trout also strongly differed in terms of inter-individual variation in behavioural expression. Unexpectedly, emergence time, exploration, activity and predator inspection behaviour showed relatively low variation among piscivore fry but differed substantially among individuals within each of the three insectivore populations, suggesting a wider domain of behavioural expression in the insectivore ecotype. Presumably, higher consistency in behavioural expression among piscivore individuals may be the result of stronger selection on proactive behaviours to maximize foraging efficiency and support their faster growth in nature (Monnet et al., 2020). By contrast, the potential adaptive significance of the large behavioural variation observed within insectivore populations remains somewhat unclear. A wider range of insectivore personalities may provide greater plasticity to environmental variation (Roche et al., 2016) in the diverse rain- and snow-fed insectivore streams of Kootenay Lake in which flow, temperature and associated prey abundance are presumably more variable compared to the larger, lake-headed Lardeau River where piscivore fry rear. Alternatively, a wider range of insectivore behaviours may mostly become adaptive at later life stages, i.e. after fry migrate from their rearing streams to mature in Kootenay Lake; nonetheless, this scenario assumes that individual behaviours remain stable over time, which has rarely been demonstrated in fish other than over short time scales (e.g., days: White et al., 2015; weeks: Boulton et al., 2014, Baker et al., 2018; months: Wengström et al., 2016). These results also highlight the necessity of sampling

multiple populations within an ecotype or species to capture the full range of individual behaviours and allow the correct interpretation of potential differences between ecotypes or taxonomic units. For instance, sampling additional piscivore populations may provide better estimates of inter-individual variation in behaviour within this ecotype; this variation could increase with the inclusion of additional piscivore populations, unless multiple piscivore populations maintain highly consistent behavioural expression among individuals, due to intense selection on specific behaviours that maximize foraging efficiency and ultimately support faster growth in this ecotype (Monnet et al., 2020).

2.4.2 Potential mechanisms underlying behavioural variation between ecotypes

The emergence and maintenance of multivariate variation in behaviour between ecotypes may result from different mechanisms, including strong trade-offs with fitness-related traits. Selection for different adult body sizes between ecotypes (i.e., 2-3 kg for insectivores vs. 5-15 kg for piscivores) drives the differentiation of insectivore and piscivore fry along a slow-to-fast gradient of juvenile growth (Monnet et al., 2020). The mapping of the proactive vs. reactive behavioural axis reported in this study onto this gradient suggests that behaviour may mediate a potential energy gain vs. survival trade-off, whereby proactive piscivore fry maximize their food intake and growth at the cost of higher predation risk (Stamps, 2007). Although this trade-off has been repeatedly observed in fish (Lankford et al., 2001; Vøllestad & Quinn, 2003; Biro et al., 2004), it remains somewhat speculative in this system as little is known about differences in predation rates and agents (e.g., type of predator) in piscivore vs. insectivore rearing streams; in addition, the existence of large behavioural variation among insectivore populations somewhat reduces the coherence between the proactive vs. reactive behavioural axis and the fast vs. slow growth axis that differentiate piscivore from insectivore ecotypes.

Reduced variation in behavioural expression among piscivores may also result from differences in selection on their underlying physiology. Behaviours that maximize growth may be associated with narrower metabolic requirements (e.g., higher standard metabolic rate, large metabolically costly organs to support growth: Gallagher et al., 1998; Kotrschal et al., 2013; Mittelbach et al., 2014). The physiological demands associated with a fast-growing lifestyle to achieve large adult body sizes (i.e., the piscivore ecotype: Rosenfeld et al., 2015; Monnet et al., 2020) may constitute a strong constraint on behaviour resulting in low variation in piscivore behaviour. By contrast, populations where selection on growth is less intense may present greater physiological and behavioural diversity, associated with greater variation in life-history traits.

2.4.3 Consequences of contrasting behaviours on diet specialization

Contrasting behaviours between piscivore and insectivore trout may also contribute to later trophic specialization as lake-resident adults. Shifting from an invertebrate-based diet as juveniles to a piscivorous one as adults (i.e., preying on juvenile kokanee salmon) may require piscivore fry to develop greater foraging capacities, by gathering information faster concerning prey location/number/condition or better anticipating movement of fish prey. Few studies have investigated the variability in personalities underlying habitat partitioning, although Elhinger and Wilson (1988) observed a strong foraging polymorphism in bluegill sunfish *Lepomis macrochirus* (Rafinesque 1819) that differentiated into benthic (i.e., foraging on damselfly nymphs) vs. limnetic feeders (i.e., zooplankton) when reared in laboratory conditions. The contribution of juvenile behaviour to the diet specialization of piscivore and insectivore adults assumes that behaviour remain stable over ontogeny, which remains unclear (Sih et al., 2004b). Defining key behavioural characteristics of piscivore and insectivore ecotypes at the adult life stage may help understand the traits associated with their diet specialization and the differentiation of their adult morphology and ecology.

Table 2.1. Population means (\pm SE, N = 12 for each behaviour and population) for the four behaviours measured, i.e. latency to leave a shelter, proportion of sectors visited, number of transitions per minute, and shortest distance to predator.

	Piscivore		Insectivores	
	<i>Lardeau R.</i>	<i>Duhamel Ck.</i>	<i>Hendryx Ck.</i>	<i>Redfish Ck.</i>
Trait				
Latency to leave a shelter (sec)	3.33 \pm 1.71	162.16 \pm 58.64	88.91 \pm 48.92	115.66 \pm 36.42
Proportion of sectors visited (%)	94.11 \pm 1.77	93.13 \pm 2.15	73.03 \pm 5.39	84.31 \pm 4.71
Number of transitions/minute	24.75 \pm 1.17	24.61 \pm 1.75	16.52 \pm 2.39	21.33 \pm 1.37
Shortest distance to predator (cm)	7.58 \pm 0.81	13.04 \pm 1.62	16.62 \pm 3.65	-

Table 2.2. Significance of behavioural trait differences among ecotypes and populations of piscivore and insectivore *Oncorhynchus mykiss*.

Best fit model	Terms	df	F	P
Ecotype comparison				
Boldness	Ecotype	47	6.023	0.018
Exploration	Ecotype	47	4.565	0.037
Activity	Ecotype	47	3.208	0.07
Predator inspection	Ecotype	35	6.306	0.017
Population comparison				
Boldness	Population	47	2.489	0.07
Exploration	Population	47	6.493	< 0.001
Activity	Population	47	4.932	< 0.001
Predator inspection	Population	35	3.746	0.034

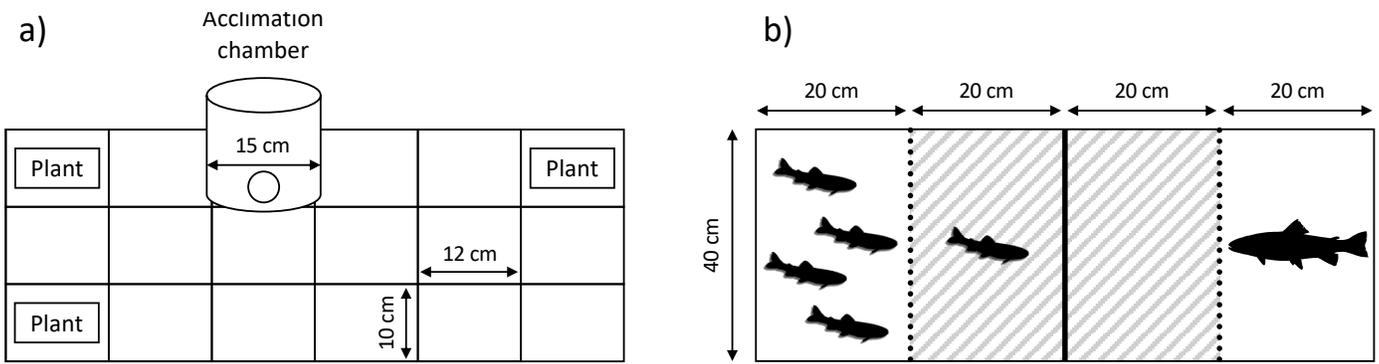


Figure 2.1. Experimental arenas used to measure boldness, activity and exploration (a), and predator inspection (b). Dashed lines on panel b represent meshed partitions, the straight line represents the removable partition, and the grey area represents the exploration area.

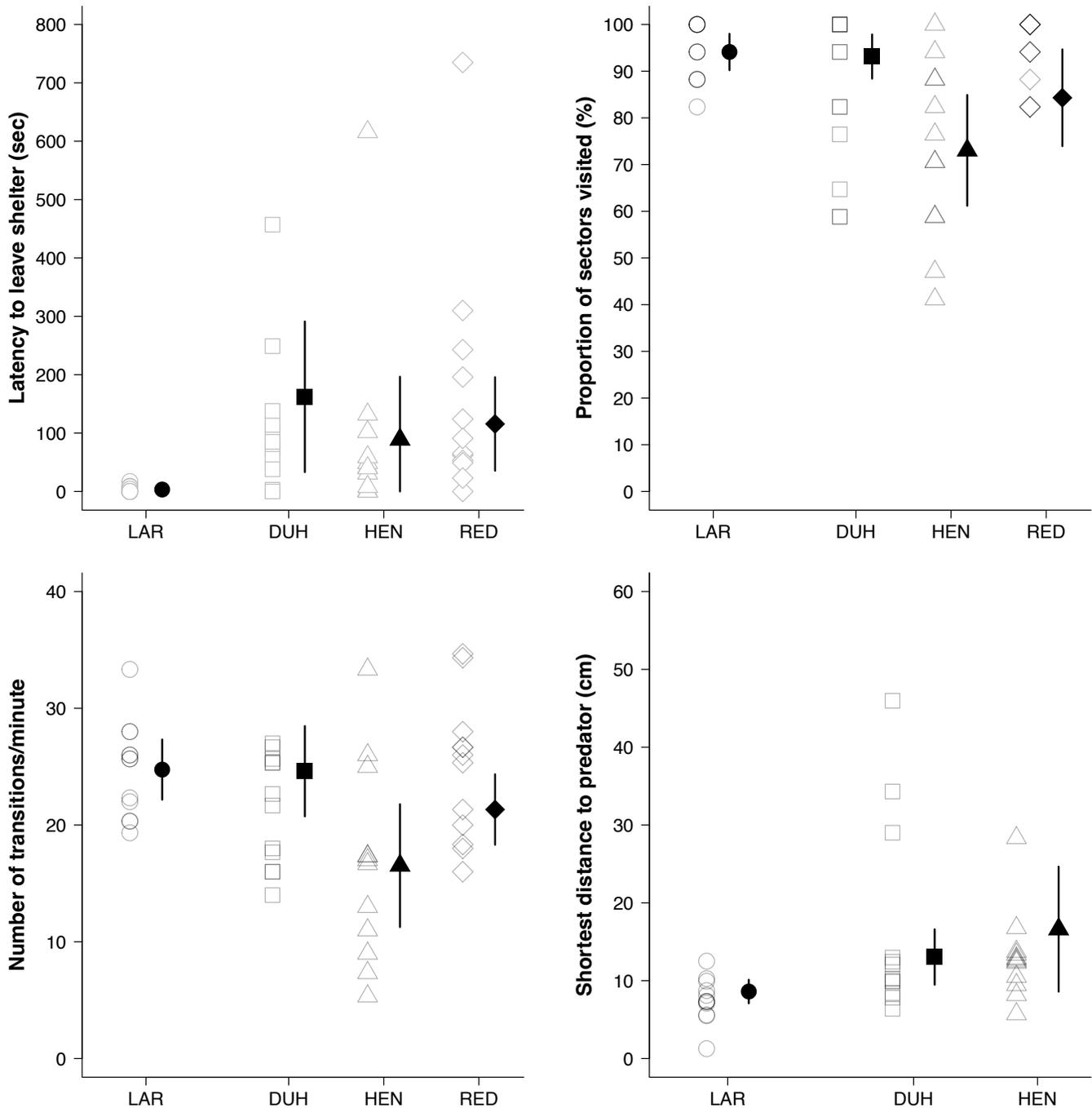


Figure 2.2. Individual boldness (top left, N = 12 per population), exploration (top right, N = 12 per population), activity (down left, N = 12 per population) and predator inspection (down right, N = 12 per population) of piscivore (circles) and insectivore (squares, triangles, diamonds) *Oncorhynchus mykiss* fry. Filled symbols represent population means, while vertical lines represent 95% confidence intervals.

Chapter 3: Growth variation along a dispersal gradient in rainbow trout

3.1 Introduction

Juvenile dispersal is a fundamental ecological process during which organisms migrate from a natal site to successive habitat patches that match changing habitat needs through ontogeny. The requirement for dispersal largely depends on landscape constraints on the adjacency of spawning and rearing habitats (e.g., habitat connectivity: Tonkin et al., 2018) and intensity of competition at the natal site (Dunning et al., 1992; Metcalfe & Thorpe, 1992). In riverine fishes, dispersal after emergence from nest sites along longitudinal river gradients has historically been considered a passive transport phenomenon driven by unidirectional flow (e.g., Lechner et al., 2016), whereby newly-hatched fry typically enter the current to drift from their natal site to downstream habitats with lower competition from conspecifics (Humphries, 2005) or more optimal conditions for development (e.g., temperature and prey availability: Schiemer et al., 2003; Kaylor et al., 2021). Many studies, however, have emphasized the active nature of dispersal and the effects of intrinsic drivers (e.g., sex, age, body condition: Stevens et al., 2010; Archer et al., 2019) and individual variation in performance (e.g., growth, metabolism, behaviour) on juvenile movement (Fraser et al., 2001; Pavlov et al., 2008; Cote et al., 2010; Aparicio et al., 2018; Comte & Olden, 2018). For instance, individuals exhibiting more proactive behaviours (e.g., greater boldness: Myles-Gonzales et al., 2015), larger body size (Bradford & Taylor, 1997), or higher energy metabolism (Sloat & Reeves, 2014) often disperse over longer distances than their more reactive, smaller-bodied conspecifics. The mechanisms and final outcome of dispersal for the spatial distribution of phenotypes, however, remain unclear. Dispersal may result in homogenization of phenotypes across a landscape, or sorting of phenotypes whereby traits match conditions encountered during successive steps of dispersal. In particular, it is unclear whether phenotypic differentiation of dispersers along environmental gradients reflects the attributes that favour movement during dispersal (e.g., greater exploratory behaviour: Cote et al., 2010) vs. the attributes that maximize fitness in the terminal habitat.

Dispersal capacity is also determined by ecological context (e.g., prey abundance, temperature) both in the natal and destination habitats (Rasmussen & Belk, 2017; Kaylor et al., 2021). The linear nature of freshwater lotic systems often generates longitudinal gradients that may result in a diversity of ecological pressures on life-history, metabolic, and behavioural traits mediating active dispersal, and may ultimately promote the spatial differentiation of individual phenotypes (Comte & Olden, 2018).

For example, increasing density-dependent competition at the site of fry emergence may result in emigration of phenotypes that are poor competitors; reduced competitive ability is often associated with subordinate behaviour and lower growth rates (Metcalf & Thorpe, 1992; Lahti et al., 2001), leading to an expectation that dispersers should present reduced growth performance and less aggressive personalities. Alternatively, if resources become depleted by high conspecific densities at the natal site, then faster-growing phenotypes may be the individuals that disperse to reach downstream habitats with higher food availability *per capita* (Kahler et al., 2001; Einum et al., 2011; Brundson et al., 2017). Longitudinal gradients in resource availability may, however, override density effects; for instance, if habitat is more productive at the natal site then faster-growing phenotypes should not disperse, whereas faster-growers should disperse if habitat productivity increases downstream. Alternatively, dispersal may occur along natural thermal gradients (Northcote, 1969, 1984) and spatially differentiate individuals based on their temperature preferences. For example, higher water temperature at the site of fry emergence (e.g., shallow, low-velocity pool) relative to cooler downstream habitats may result in emigration of temperature-intolerant individuals (e.g., with a lower critical thermal maximum, CT_{max}). This scenario generates an expectation that individual phenotypes should differentiate along an upstream-downstream gradient of decreasing CT_{max} matching the gradual decrease of water temperature in downstream microhabitats. Longitudinal matching of habitat characteristics and dispersing phenotypes should therefore be highly contingent on environmental gradients below the spawning site. Few studies have, however, investigated the degree to which spatial differentiation of juvenile phenotypes matches habitat attributes along ecological gradients following post-emergence dispersal.

The population of piscivore rainbow trout (*Oncorhynchus mykiss*) from Kootenay Lake, British Columbia, Canada, exclusively spawns and rears in the Lardeau River, a lake-headed moderate-sized gravel-cobble river that flows south for approximately 60 kms before entering Kootenay Lake (see Fig. 3.1 for map). Piscivore adults only spawn in a discrete 300-m long reach immediately below the outlet of Trout Lake, and fry typically emerge between June and August before most individuals disperse from the natal site towards Kootenay Lake within a few weeks of emergence (Northcote, 1969). Previous studies (Northcote, 1969; Irvine, 1978) indicate decreasing downstream gradients of temperature and prey abundance; the lake-influenced upper section of the river is warmer (up to 19-20°C in July: Cartwright, 1961) with four-fold higher macroinvertebrate abundance (Cartwright, 1961) relative to the colder lower section (maximum 14-15°C in July) where water temperature and prey concentration

progressively decrease with the addition of large volumes of cold water from the many glacial-fed tributaries joining the Lardeau along its course to Kootenay Lake. In rainbow trout, CT_{max} typically ranges from 27°C to 32°C (Chen et al., 2015), which exceeds the range of water temperatures present in the Lardeau River. Social stress, induced for example by increasing density-dependent competition at the site of fry emergence may, however, reduce thermal tolerance in less competitive individuals (LeBlanc et al., 2011) and initiate their downstream dispersal. Prioritizing a rapid assessment of CT_{max} over more sensitive but experimentally challenging indicators of thermal performance (e.g., optimal temperature for growth) hence allows a preliminary exploration of variation in temperature tolerance associated with juvenile dispersal along a thermal gradient in the Lardeau River. The downstream dispersal of piscivore fry along a 60 km reach characterized by multiple ecological gradients (i.e., distance from the emergence area, water temperature, and prey abundance) therefore offers an opportunity to assess whether dispersers are behaviourally and physiologically unique relative to stayers, and for investigating the degree of spatial differentiation in integrated phenotype following post-emergence dispersal in a riverine fish.

In this study, we evaluated the extent of inter-individual differentiation in growth, metabolic performance (i.e., active metabolism [MMR], temperature tolerance [CT_{max}]) and behaviour (i.e., emergence time, exploration, sociability) associated with downstream dispersal of juvenile trout along the Lardeau River. We collected fish from three locations below their natal site and measured performance in identical environments in the laboratory, to evaluate whether variation in individual integrated phenotypes matched ecological gradients of distance travelled during dispersal, water temperature, and prey abundance. We predicted that i) faster-growing fry would remain in the upper Lardeau River where higher prey abundance supports growth maximization, while downstream-dispersing fry would exhibit lower intrinsic growth rates; ii) individuals with elevated CT_{max} would remain in the warmer upper Lardeau River; and iii) dispersal distance would be positively correlated with traits related to active movement, so that fish with higher MMR, greater boldness, higher exploration, and lower sociability would disperse further downstream.

3.2 Material and Methods

3.2.1 Productivity and thermal gradients along the Lardeau River

The magnitude of prey availability and thermal gradients along the Lardeau River was assessed by sampling invertebrate drift and water temperature three times between July and September 2019 in each of the three sampling sites (i.e., Tenderfoot Creek, Poplar Creek, and Howser locality; Fig. 3.1). Four 250 μ m-mesh drift nets were installed in each site to filter drifting aquatic invertebrates for approximately one hour. Volume filtered (in m³) was estimated for each net by measuring water velocity and depth at the start and end of each set using a Marsh McBirney digital flow meter (Hach Flow Company, Colorado, USA). Collected invertebrates were preserved in 70% ethanol, before prey abundance was determined by counting the total number of macroinvertebrate larvae (primarily orders Diptera, Ephemeroptera, Plecoptera, and Coleoptera) present in each sample using a binocular microscope. Zooplankton abundance was also estimated for each drift sample by counting invertebrates (primarily orders Cladocera, Calanoida, and Cyclopoida) present in a 5mL-subsample under a binocular microscope after standardizing volumes to 150mL with water. Drift concentrations ($N_{\text{larvae}} \cdot \text{m}^{-3}$) were calculated as the ratio of total invertebrate abundance (N_{larvae}) to volume filtered (m³) for each drift sample and invertebrate type (i.e., macroinvertebrates and zooplankton).

Water temperature was measured in the centre of the water column next to each drift net (N=4) for each sampling date and area using a digital thermometer.

3.2.2 Fish collection along an increasing gradient of downstream dispersal

Juvenile piscivore rainbow trout (*Oncorhynchus mykiss*) were collected in the Lardeau River on two consecutive days in September 2019, within a maximum interval of three months after emergence to allow sufficient time for post-emergence downstream dispersal while minimizing potential effects of a prolonged exposure to different ecological conditions (e.g., temperature, prey abundance) on inter-individual phenotypic differentiation. Fish were collected in each of three sampling sites distributed along an increasing gradient of distance downstream of the emergence area (i.e., towards Kootenay Lake), ranging from 6 kms (TEN) to 18 kms (POP) and 36 kms (HOW, see Fig. 3.1). Fish collection occurred at dusk, when the nocturnal movement of trout fry towards marginal habitats facilitated their capture with dip nets. Once captured, all fish were temporarily held for 1-2 days in flow-through plastic bins placed in a small tributary stream to Kootenay Lake before their transfer to The University of British Columbia (UBC, Vancouver, Canada).

3.2.3 Fish rearing

At UBC, all fish were transferred to an environmentally regulated chamber with 12h light: 12h dark cycle and ambient temperature of $13.3 \pm 0.4^{\circ}\text{C}$ (mean \pm SD), before being quarantined in separate closed 150L-tanks with 4ppm saltwater for seven days to minimize potential for disease transfer among individuals. Following quarantine, all rearing tanks were connected to a general recirculating water system equipped with a biological filtration system supplying each tank with denitrified and oxygenated water in a common garden fashion. Elements of decor (e.g., rocks, artificial plants) were also added to each tank to create a realistic and stimulating rearing environment for the fish. Once in their rearing tanks, all fish were fed freeze dried chironomids twice a day on a near maintenance ration ($\sim 1\%$ wet body mass), before a week-long transition to a near maintenance ration of 1.2 mm commercial food pellets (BioPro2, Bio-Oregon, Washington, USA) delivered by automatic feeders twice a day. This week of dietary transition also allowed the standardization of fish energetics before subsequent experiments.

Before the start of experiments, 20 fish from each subpopulation (i.e., TEN, POP, HOW) were randomly selected and stocked in separate 150L rearing tanks connected to the general recirculating water system. Each tank was covered with mesh screen to prevent fish escape and equipped with automatic feeders distributing pellets at a constant rate over eight hours. Once stocked in their respective rearing tanks, all fish from each subpopulation were maintained on a satiation ration (i.e., food *ad libitum*) for two weeks to maximize the likelihood of detecting baseline differences in growth and metabolic performance among the three subpopulations based on previous comparisons of similar rainbow trout populations (Allen et al., 2016; Monnet et al., 2020). All fish were weighed (g) at the beginning of the two-week treatment and weekly thereafter. The average body mass of fish (mean \pm SD) at the beginning of the two-week feeding treatment was $1.46 \pm 0.79\text{g}$ (TEN), $1.66 \pm 0.65\text{g}$ (POP), and $1.45 \pm 0.66\text{g}$ (HOW); differences in initial body mass among subpopulations were not significant (ANOVA: $P = 0.38$).

3.2.4 Standard growth rate (SGR)

Baseline differences in growth among subpopulations distributed along the Lardeau River were characterized by measuring individual standard growth rates after one and two weeks at satiation. Each fish was weighed in water using a digital scale, and SGR ($\%\text{wet body mass}\cdot\text{day}^{-1}$) was calculated as

$$SGR = \frac{\ln M_{final} - \ln M_{initial}}{t} \times 100$$

where M_{final} is the final body mass (g), $M_{initial}$ is the initial body mass (g), and t is the growth interval in days. The average of observed growth rates after one and two weeks on satiation was determined as the individual SGR. Fish were fed for the last time after two weeks of treatment at 05:00 PM, before the bottom of each tank was gently cleaned to remove leftover food and faeces and prevent residual postprandial metabolism from influencing MMR measured the next day.

3.2.5 Maximum oxygen consumption rate (MMR)

To test whether piscivore fry differ in active metabolism in relation to dispersal along the Lardeau channel, individual maximum metabolic rate (MMR) was measured the day following the end of the two-week feeding treatment. Maximum metabolic rate was measured a minimum of 18 hours after the last meal and 5 hours after the beginning of the day cycle to ensure fasting conditions and prevent circadian rhythm from influencing MMR. Each fish was placed in a 20L-bucket containing water at ambient temperature and chased by hand until exhaustion, which was assumed to be reached when fish no longer reacted to a gentle chase or push with the hand. Once exhausted, each fish was placed in a custom-made plastic respirometer whose volume (20-50mL) was adjusted to the size of the fish and equipped with an oxygen sensor (Neofox, Ocean Insight, Florida, USA) to record the drop in oxygen tension from ~95% to ~65% air saturation. The 60s-period with the highest drop in oxygen tension was then used to calculate maximum oxygen consumption rate as

$$\dot{M}O_2 = \frac{V_W \cdot \Delta C_W O_2}{\Delta t}$$

where $\dot{M}O_2$ is the oxygen consumption rate of the fish ($\mu\text{mol O}_2 \cdot \text{h}^{-1}$), V_W is the volume of water in the respirometer (L), $\Delta C_W O_2$ is the change in oxygen tension in the respirometer, and Δt is the 60s-period over which the decrease in oxygen tension was measured. Barometric pressure on each trial date and oxygen solubility coefficient in water αO_2 ($\mu\text{mol O}_2 \cdot \text{L}^{-1} \cdot \text{kPa}^{-1}$) were used to correct dissolved oxygen concentration in the respirometers. Finally, oxygen consumption rates were divided by body mass to estimate mass-specific oxygen consumption. Measurements from the last 8 individuals of the HOW

population were excluded from analysis because of technical issues with the oxygen sensor that generated unreliable data.

3.2.6 Temperature tolerance (CT_{max})

To test whether inter-individual variation in dispersal performance along the Lardeau channel is associated with temperature tolerance, the three subpopulations were tested sequentially for critical thermal maximum (CT_{max}) seven days after the completion of the MMR experiment. Following standard protocols for fish (Beitinger et al., 2000), 12 individuals were randomly selected from each subpopulation before being weighted (in g) and placed in an experimental 40L-tank equipped with aquarium heaters increasing water temperature at a constant rate of 0.3°C per minute. The tank was also equipped with two air stones for oxygenation and mixing, to ensure homogeneous conditions of temperature and oxygen availability throughout the tank. After a 45min acclimation period, water was heated until fish displayed a loss of equilibrium (LOE: Jung, 2018; Frommel et al., 2020); the temperature at which LOE occurred was recorded for each fish using a digital thermometer, before all fish were promptly returned to their rearing tank following LOE to rest for three days before the start of behavioural experiments. No mortality occurred during or following the determination of CT_{max} .

3.2.7 Measuring multiple behaviours (emergence time, exploration, and sociability)

To test whether piscivore trout differ in behaviour in relation to dispersal along the Lardeau River, we measured emergence time from a start box, exploration, and sociability in 20 fish from each subpopulation within 10 days after the end of the CT_{max} experiment. To estimate emergence time and exploration, each fish was randomly selected from its rearing tank, before being placed in a custom-made 50L observation arena (approximately 10cm deep, i.e. half-full) supplied with oxygenated water at ambient temperature. This arena was marked with a grid of 18 equal-area sectors (10x12cm each) visible to the observer and embellished with gravel substrate and artificial plants to create an unfamiliar yet realistic environment conducive to exploration (see Weinersmith et al., 2009 for details). Each tested fish was first placed in an opaque acclimation chamber equipped with a removable door for 45min before being released into the open field where a small video camera (SJCAM 4000, SJCAM Company, China) mounted above the arena recorded movement of the fish in a two-dimensional plane within three minutes following departure from the acclimation chamber. As per Weinersmith et al.

(2009), emergence time was determined as the delay to exit the acclimation chamber (in seconds) after the door was removed, while exploration was determined as the proportion of sectors visited out of 17 (i.e., 18 sectors minus the sector occupied by the acclimation chamber; expressed as a percentage); exploration was measured only for three minutes after the fish exited the acclimation chamber to prevent excessive habituation to the arena. Each fish was weighted (in g) after the end of the test before being returned to its rearing tank, and water inside the arena was changed after each series of three tests to prevent persistent chemical cues released by previously tested fish from biasing the behaviour of subsequent fish.

To determine the extent to which social behaviour differed among dispersing individuals, 20 fish from each subpopulation were tested for sociability two days after the end of the open field experiment. A 40L-tank containing oxygenated water at ambient temperature was divided into two unequal compartments (i.e., representing approximately one third and two thirds of the volume of the tank) by a mesh partition. A shoal of three rainbow trout fry from a given subpopulation was placed in the smaller of the two compartments at the beginning of the experiment to create a group stimulus conducive to social behaviours. The second (and largest) compartment of the observation arena was marked into three equal-area sectors visible to the observer and representing a gradient of physical distance from the shoal of conspecifics (i.e., with the first sector being closest to the shoal, the second sector intermediate, and the third sector farthest from the shoal). This compartment was used to acclimate each tested fish for 10min before estimating its degree of sociability by measuring the amount of time spent in each sector (in seconds) within five minutes after the end of the start of the experiment to prevent excessive habituation to the arena. A control version of this experiment was performed by measuring the same behavioural response in 20 other fish from each subpopulation in the absence of conspecifics in the small compartment of the arena (i.e., tested fish were alone in the arena and no longer received social cues from conspecifics). All fish were weighted (in g) after each test before being returned to their respective rearing tanks.

3.2.8 Data analysis

All phenotypic traits (growth rate, MMR, CT_{max} , emergence time, exploration, and sociability) were compared among sampling sites (TEN, POP, HOW) using R software (v.1.3.1093). Distribution normality and variance homoscedasticity were tested using Shapiro-Wilk and F-tests, respectively: when these

two assumptions were met, trait values were compared among subpopulations by performing analyses of variance (ANOVA) and *post-hoc* Tukey's Honest Significant Difference (Tukey's HSD) tests; when normality and homoscedasticity were not verified even after log transformation, trait values were compared using nonparametric tests (i.e., Kruskal-Wallis and *post-hoc* Mann-Whitney tests). Because the three subpopulations had similar ranges of body mass throughout the chain of experiments (TEN: 0.31-5.89g; POP: 0.74-5.36g; HOW: 0.44-4.65g), all traits were standardized to the same body mass across all subpopulations and experiments (i.e., 1.91g). Although body mass did not significantly differ among subpopulations, this standardization was intended to better account for potential allometric trait variation, and an analysis on mass-uncorrected traits (i.e., without individual mass as a covariate) yielded similar conclusions in terms of the significance of main effects (see Table 3.S1, Appendix). Mass standardization was achieved by determining the equation of the linear regression between each phenotypic trait and body mass for each subpopulation (i.e., TEN, POP, HOW); obtained equations were then used to adjust individual trait-values to a common body mass of 1.91g for each subpopulation. Prior to analysis, emergence time data were transformed so that individuals that exited the acclimation chamber earlier were attributed the highest scores for emergence time; the latency to exit the chamber (in seconds) of each individual was expressed relative to the maximum latency observed across all fish (i.e., 235 seconds); for example, a fish exiting 10s following door removal would be attributed a score of $(235-10)=225$. Sociability data was also expressed as a score by multiplying the amount of time (in seconds) spent in the first area (i.e., close to the shoal) by 2, in the second area (i.e., at mid-distance from the shoal) by 1 and in the third area (i.e., far from the shoal) by 0 before dividing the result by the 5-min duration (i.e., 300s) of the experiment for each individual. For example, an individual that spent 50s in the first area, 150 in the second area, and 100 in the third area would be attributed a sociability score of $(50 \times 2 + 150 \times 1 + 100 \times 0) / 300 = 0.83$. Individual sociability scores were then corrected by body mass as described above, before subtracting the average sociability score of the control experiment (i.e., across all the 20 control individuals) from the sociability score of each tested fish and for each subpopulation.

Differences in water temperature and prey abundance among sampling sites were compared using a similar statistical protocol. After verifying data normality and homoscedasticity, temperature and prey abundance values were compared among subpopulations and sampling sessions by performing parametric (i.e., ANOVA and *post-hoc* Tukey's HSD tests) or nonparametric analyses (i.e., Kruskal-Wallis and *post-hoc* Mann-Whitney tests).

3.3 Results

3.3.1 Gradients of temperature and prey abundance along the Lardeau River channel

Water temperature significantly differed among sampling sites in early July (ANOVA: $P < 0.001$), late July (Kruskal-Wallis: $P < 0.01$), and September (ANOVA: $P < 0.001$; Fig. 3.1), and was always significantly higher in the upper Lardeau River (TEN), closer to the outlet of Trout Lake, relative to the two downstream sites (i.e., POP and HOW; Tukey's HSD and Mann-Whitney tests: $P < 0.05$). Water temperature was also higher in late July relative to early July and September for each of the three sampling sites (+4.5°C on average in TEN; +4.3°C on average in POP; +2.1°C on average in HOW).

As expected, prey abundance strongly declined along the river gradient for both macroinvertebrates and zooplankton in early July (Kruskal-Wallis tests: $P < 0.01$), late July (ANOVA: $P < 0.05$ and $P < 0.001$, respectively), and September (ANOVA: $P < 0.001$; Kruskal-Wallis: $P < 0.01$, respectively; Fig. 3.1). Collectively, these results support the existence of decreasing downstream gradients of temperature and prey abundance along the Lardeau River channel.

3.3.2 Standard growth rate (SGR)

Growth rate significantly declined along an upstream-to-downstream gradient of subpopulations within the Lardeau River (Kruskal-Wallis: $P < 0.001$; Fig. 3.2a), although differences in SGR were only significant between TEN and HOW (Mann-Whitney: $P < 0.001$) and between POP and HOW (Mann-Whitney: $P < 0.01$).

3.3.3 Maximum metabolic rate (MMR)

The measures of MMR did not significantly vary among subpopulations (Kruskal-Wallis: $P = 0.41$; Fig. 3.2b).

3.3.4 Critical thermal maximum (CT_{max})

Measures of CT_{max} did not significantly vary among subpopulations along the Lardeau River (ANOVA: $P = 0.23$; Fig. 3.2c).

3.3.5 Behavioural traits (emergence time, exploration, sociability)

Emergence time marginally increased along the river gradient, but differences among subpopulations were not significant (Kruskal-Wallis: $P = 0.24$; Fig. 3.2d).

Similarly, exploration and sociability did not significantly differ among subpopulations (Kruskal-Wallis for exploration: $P = 0.14$; Fig. 3.2e; ANOVA for sociability: $P = 0.58$; Fig. 3.2f).

3.4 Discussion

3.4.1 Phenotypic differentiation following juvenile dispersal

Comparing key life-history, metabolic, and behavioural traits among rainbow trout individuals distributed along a natural gradient of dispersal distance allowed the evaluation of the contribution of multidimensional performance to dispersal in riverine fish. Contrary to our predictions, with the exception of growth rate which significantly decreased downstream, individuals from the three sampling sites only marginally differed in their overall integrated phenotype. In contrast with growth, metabolic (i.e., MMR, CT_{max}) and behavioural traits (i.e., emergence time, exploration, sociability) did not significantly differ among individuals, indicating minimal relationship between active metabolism or behaviour and active dispersal unlike suggested elsewhere (Fraser et al., 2001; Dingemanse et al., 2003; Cote et al., 2010). Previous studies on juvenile dispersal in this population have suggested positive effects of lower water temperatures on downstream movement (Northcote, 1969), which is not consistent with the minimal variation in CT_{max} that we observed along a decreasing gradient of water temperature in the Lardeau River. Apparent homogeneity in CT_{max} may result from the relatively small difference in water temperature between upstream and downstream habitats during the summer (maximum of 4.1°C in late July). This difference in water temperature is likely insufficient to determine habitat selection based on individual thermal maxima in cold water specialists like salmonids, where CT_{max} typically increases by as little as 2°C over a 15°C acclimation temperature range (Brett, 1956). In addition, CT_{max} characterizes an upper limit of thermal tolerance, and may misrepresent more subtle and positive effects of water temperature on juvenile movement and microhabitat selection. For

instance, juvenile trout in the Lardeau River may select rearing microhabitats with a thermal regime that matches their optimum temperature for digestion and growth rather than CT_{max} , as suggested in other salmonid taxa (Armstrong & Schindler, 2013).

Despite our prediction that long-distance dispersal should be associated with higher active metabolism, MMR did not significantly vary among sampling sites, suggesting that riverine dispersal is largely independent of maximum metabolic capacity. Because downstream migration can last anywhere from days to months (Northcote, 1969; Pavlov, 2017), other indicators of metabolic performance less extreme than MMR (e.g., sustained swimming capacity) may better represent the contribution of active metabolism to juvenile dispersal (Comte & Olden, 2018). The negative association between growth rate and distance travelled during dispersal is consistent with previous work (Ronce, 2007; Ronce & Clobert, 2013; Comte & Olden, 2018) demonstrating strong associations between dispersal estimates and life-history traits (e.g., body length). By contrast, the apparent absence of a relationship between dispersal distance and potential metabolic and behavioural correlates of dispersal contrasts with previous studies (Rehage & Sih, 2004; Myles-Gonzalez et al., 2015; Baptista et al., 2019); this suggests that the realized phenotypic differentiation of dispersers may reflect selection for attributes that maximize fitness in the terminal habitat (e.g., growth) rather than attributes that promote active dispersal (e.g., metabolism and behaviour). The small sample sizes used in this study (i.e., 20 fish at each site) may, however, limit our capacity to detect more subtle phenotypic variation associated with downstream dispersal. In addition, the effects of individual condition or emergence timing on decisions to disperse may contribute to variation in dispersal among individuals, unless dispersal is mostly contingent on passive transport phenomena (Lechner et al., 2016; Keckeis et al., 2017).

3.4.2 Longitudinal matching of habitat characteristics and dispersing phenotypes

The existence of substantial ecological variation along the Lardeau River allowed the exploration of the coherence between dispersing phenotypes and ecological gradients, with the upper Lardeau presenting warmer waters with higher prey abundance relative to the lower sections of the river. As predicted, the decrease in individual growth rates along the river channel largely mapped onto an upstream-to-downstream gradient of decreasing prey availability, whereby fry present in the high-productivity habitats of the upper Lardeau River grew faster than individuals present in downstream habitats with

lower prey abundance. In contrast with growth differences, inter-individual variation in temperature tolerance (i.e., CT_{max}) and traits associated with active movement (i.e., MMR, boldness, exploration, sociability) did not map onto downstream gradients of water temperature and distance from the spawning area, providing no evidence that temperature tolerance and active movement capacity are major drivers of active dispersal as suggested elsewhere (Fraser et al., 2001; Cote et al., 2010; Myles-Gonzales et al., 2015; Batista et al., 2019). Because we measured growth at a satiation ration in the laboratory, these differences should mostly reflect genetic differences in growth potential of individuals among locations, rather than a downstream difference in prey availability among sites. The potential role of a plastic (i.e., environmentally-induced) response to ambient food availability, however, cannot be eliminated with our study design. Ideally, dispersers (and non-dispersers) would need to be collected immediately after their decision to emigrate to test for differences in growth or behavioural traits in common laboratory environments. We collected fish three months after emergence to allow full downstream dispersal, and this time interval may have been sufficient for dispersers to plastically downregulate their phenotype (including growth rate) in response to lower prey abundance and temperature in their terminal habitat. Similarly, it is possible that dispersers were behaviourally differentiated from non-dispersers immediately post-hatch, but trait expression was homogenized over time in the terminal habitat; or that initial dispersal was simply driven by external density-dependent factors rather than trait differences; finally, the existence of maternal effects may also explain, in part, the existence (or absence) of phenotypic variation among dispersers.

3.4.3 Ecological implications of the spatial differentiation of individual growth phenotypes

While the piscivore rainbow trout ecotype generally exhibits faster growth (Monnet et al., 2020), diversity in growth phenotypes may be favoured by temporally fluctuating selection (Siepielski et al., 2009) or bet-hedging strategies (Gremer et al., 2016), which could increase the relative fitness of slow growers in low productivity years or when densities of juveniles are unusually high. The number of piscivore spawners varies annually from <200 to >1000 adults, which is expected to increase densities of juveniles from >500,000 to >2,500,000 (assuming 7200 eggs deposited per female, a 1:1.3 female to male ratio, and an average egg-fry survival rate of 80%; Irvine, 1978). While the ecological consequences of lower downstream growth are unclear, long-range dispersers could benefit from lower density-dependent competition for food, which may compensate for lower prey abundance in downstream habitats and allow dispersers to achieve a similar autumn body size as faster-growing

upstream individuals. Considering growth in isolation may also paint an incomplete picture of the fitness consequences of different growth trajectories, since trade-offs between growth and survival may allow divergent life-history strategies to have similar fitness outcomes (Laughlin et al., 2020).

Determining the mechanisms and outcomes of dispersal is fundamental to understanding how organisms select and use habitats patches that match changing ecological requirements through ontogeny. This study highlights the coherence between growth phenotypes and a prey availability gradient along a freshwater lotic system and suggests that phenotypic differentiation of dispersers reflects the attributes that maximize fitness in the terminal habitat rather than the traits that favour active dispersal. Future studies should consider i) the degree to which individual dispersal is related to intrinsic differences in dispersal propensity vs. density-dependent effects; and ii) whether habitat matching of attributes like growth represents a phenotypic sorting process at the dispersal stage, or is the outcome of developmental plasticity or differential survival in the terminal habitat.

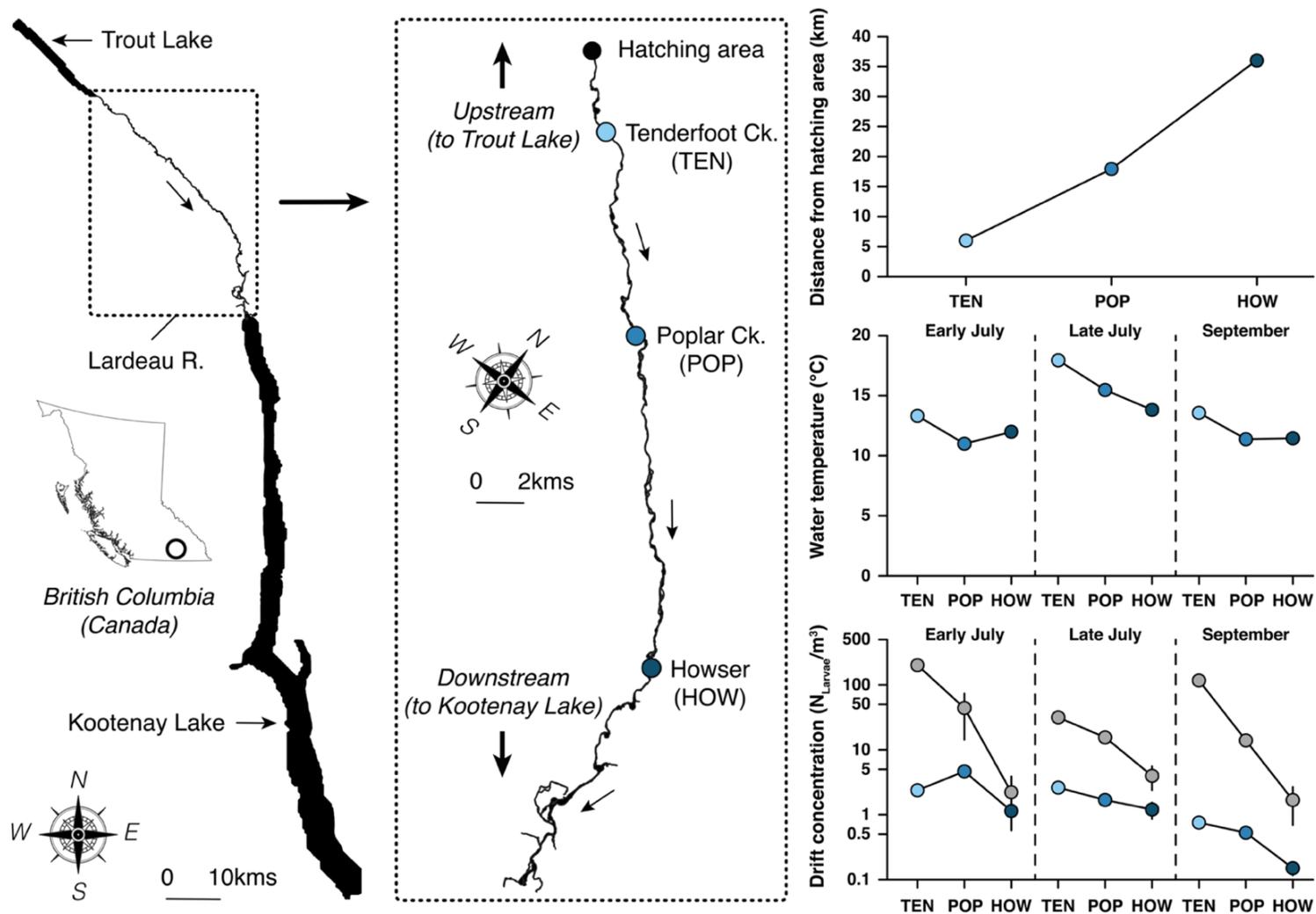


Figure 3.1. Location of the three sampling sites (i.e., TEN, POP, and HOW) within the Lardeau River, and summary of the three environmental gradients (i.e., downstream distance from emergence area, water temperature, prey abundance) present along an upstream-to-downstream river gradient. For prey abundance data, blue dots represent estimates of macroinvertebrate drift concentrations, while grey dots represent plankton data. Vertical bars represent standard errors; note the log scale for invertebrate drift data. Estimates were derived from four temperature and drift samples collected on the same day at the TEN, POP, and HOW stations in each month.

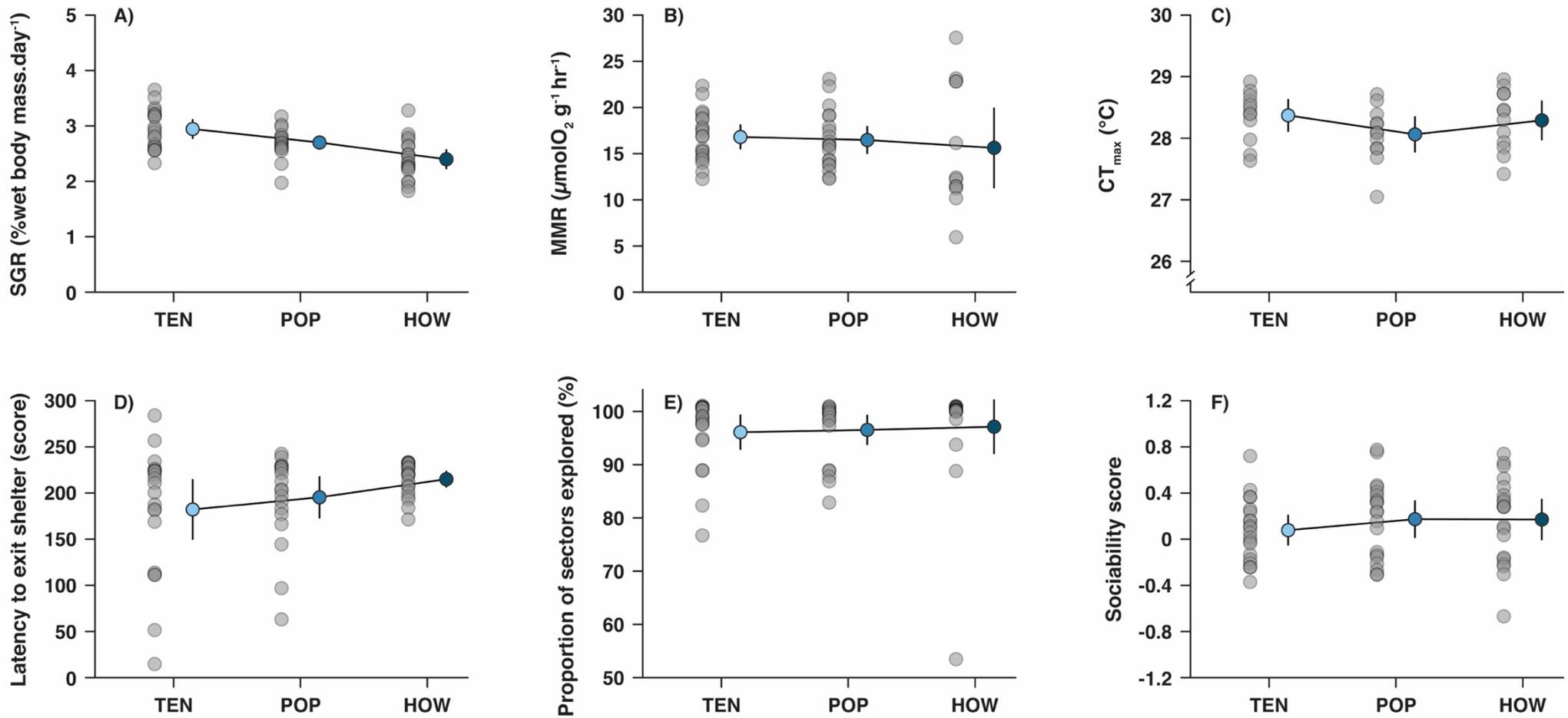


Figure 3.2: Differences in standard growth rate SGR (a), maximum metabolic rate MMR (b), CT_{max} (c), emergence time (d), exploration (e), and sociability (f) among subpopulations of piscivore fry distributed along an upstream (TEN)-to-downstream (HOW) gradient of distance from the emergence area in the Lardeau River. All individual values presented are corrected to a common body mass of 1.91g. Vertical bars represent standard errors.

Chapter 4: Selection for high growth displaces costs of digestion from SDA to SMR in divergent ecotypes of rainbow trout

4.1 Introduction

Growth rate is a fundamental life-history attribute with strong implications for the ecological and phenotypic differentiation of individuals, populations and species (Arendt, 1997; Dmitriew, 2011). A mosaic of evolutionary pressures may select for faster growth in nature, including direct selection to increase body size in a fixed time frame (Sibly et al., 2015), higher prey availability (Diehl, 1993), and selection to maximize performance associated with feeding capacity and digestion (e.g., Kotrschal et al., 2014). Physiological constraints on food intake and growth (e.g., digestive costs and gut residence time: Millidine et al., 2009; Nieceza et al., 1994) constitute key dimensions of the multi-trait integrated phenotype (Hume, 1989; Van Gils et al., 2007), but the nature of trade-offs in digestive strategies and how they relate to broader integrated trait differentiation among taxa remains poorly understood.

Optimizing energy acquisition depends significantly on digestive performance, a serial process that spans handling prey to absorbing nutrients. For instance, maximizing food intake to support faster growth (e.g., Allen et al., 2016) often requires developing larger digestive organs that increase gut capacity (Armstrong & Bond, 2013), but elevate both basal metabolism (i.e., standard metabolic rate SMR: Elia, 1992; Wang et al., 2001) and postprandial oxygen demands (characterized by the energy costs of digestion, commonly referred to as total specific dynamic action SDA; its maximum intensity SDA_{peak} ; and duration SDA_{dur} : Jobling, 1981). The positive relationship between metabolic demands and capacity for growth and consumption is widely observed in vertebrate taxa (fish: Millidine et al., 2009; Norin & Clark, 2017; reptiles: Bessler et al., 2010; birds: Bech & Præsteng, 2004). Maximizing food intake may also constrain other dimensions of digestive performance (Sibly, 1981), including the balance between evolving a shorter gut residence time (GRT) to maximize food turnover and consumption vs. a longer GRT to maximize assimilation and growth efficiency (Rosenfeld et al., 2020; we define GRT as the time interval between feeding and excretion, assimilation efficiency [AE] as the fraction of nutrients assimilated from a meal, and growth efficiency [GE] as the ratio of mass gained to food consumed). Despite a general understanding of the significance of digestive performance for growth, the nature of multivariate trade-offs among food intake, postprandial metabolism (i.e., SDA, SDA_{peak} , SDA_{dur}), GRT, AE and growth remains

unclear, and these various axes of digestive performance provide multiple degrees of freedom for the evolution of alternative digestive strategies.

Integrating multiple dimensions of digestive performance may inform how variation in digestive strategy facilitates the coexistence of ecologically contrasting taxa along environmental gradients. Previous studies (e.g., Finstad et al., 2011; Rosenfeld et al., 2020) have suggested the existence of rate-maximizing vs. efficiency-maximizing strategies, where energy (rate) maximizers are characterized by high food intake, short GRT, low AE, low growth efficiency and high digestive costs and may be constrained to high-prey availability environments supporting elevated food intake. Alternatively, efficiency maximizers that manifest lower food intake, high GRT, high AE, high growth efficiency and low digestive costs may be better adapted to less productive habitats with lower prey flux. However, overlap between digestive performance and habitat productivity remains poorly understood since digestive traits that affect energy budgets are rarely compared in an adaptive differentiation framework. In addition, the extent to which contrasting digestive strategies map onto the differences in growth among ecologically divergent taxa along productivity gradients is unclear, particularly in fish (see Billerbeck et al., 2000 for an exception).

Piscivore and insectivore rainbow trout (*Oncorhynchus mykiss*) are sympatric but ecologically divergent salmonid ecotypes that have repeatedly evolved in freshwater habitats of Northwestern North America, including Kootenay Lake in British Columbia, Canada. In this system, the unique piscivore population rears for 1-2 years in the Lardeau River that drains into the north arm of the lake (Irvine, 1978), where high availability of invertebrate prey and proactive foraging behaviours allow piscivore fry to maximize food intake, growth, and growth efficiency (Monnet et al., 2020) before migrating to the lake where they achieve large adult body size as piscivores (up to 15kgs: Andrusak & Andrusak, 2006). In contrast, the genetically distinct insectivore ecotype (Taylor et al., 2019) includes multiple discrete populations that rear for 1-2 years in smaller and less productive tributary streams around the lake where lower prey availability limits juvenile growth and ultimate adult size achieved on a largely insectivorous diet (2-3 kgs: Andrusak & Andrusak, 2006; Monnet et al., 2020). The higher growth rates of piscivores likely require optimizing digestive performance to maximize energy intake in a high-productivity rearing habitat to facilitate the evolution of large adult body size; however, the cryptic nature of digestive performance obscures the extent to which piscivore and insectivore juveniles differ in digestive adaptations as part of their integrated phenotypic differentiation.

In this study, we examined associations among food consumption, digestive metabolism, food processing efficiency, and growth in juveniles of piscivore and insectivore rainbow trout ecotypes from Kootenay Lake. We predicted that i) the greater food intake of piscivore fry would result in higher absolute SDA, SDA_{peak} and SDA_{dur} relative to insectivores, but lower relative costs of digestion (i.e., per g consumed), as suggested in Millidine et al. (2009); we label this the “economies of scale” hypothesis for food consumption and growth efficiency, whereby incremental increases in consumption have decreasing costs; and ii) the greater growth efficiency of faster-growing piscivore fry would result from a longer GRT and higher AE.

4.2 Material and Methods

4.2.1 Collection of juvenile piscivore and insectivore trout

Young-of-the-year rainbow trout (*Oncorhynchus mykiss*) were collected from their rearing streams around Kootenay Lake in July 2020, within one month of emergence to minimize the potential for differences in water temperature and prey abundance between streams to influence their early development. Piscivore fry were collected in the upper Lardeau River (UTM 5593885N 481874E), while insectivore fry were collected from two discrete insectivore populations around the lake, Hendryx Creek (UTM 5512904N 510274E) and Redfish Creek (UTM 5495734N 496548E). Fish collection occurred at dusk, when nocturnal use of low-velocity marginal habitats facilitated fry capture with dip-nets. Following capture, all fish were temporarily held for 1-2 days in flow-through plastic bins placed in Redfish Creek before their transfer to aquatic facilities at The University of British Columbia (UBC, Vancouver, Canada).

4.2.2 Fish rearing

Piscivore and insectivore fry were transferred to an environmentally regulated chamber with a 12h day: 12h night cycle and ambient temperature of $13.6 \pm 0.6^\circ\text{C}$ (mean \pm SD). Each of the three populations (i.e., one piscivore, two insectivores) was subdivided into three 200L glass tanks (nine tanks in total), each stocked with ~15 fish to approximate fish densities in their rearing stream, before being quarantined and treated with 3ppm saltwater for five days to minimize potential for disease transfer across populations. The nine rearing tanks were initially operated as separate closed systems during the quarantine period, before being connected to a recirculating water system equipped with biological filtration and supplying

each tank with denitrified, oxygenated water in a common garden fashion. Rocks and artificial plants were added to all tanks to create a realistic and enriched rearing environment. Because piscivore and insectivore fry differed slightly in body mass at the time of collection, experiments with each of the three populations were performed sequentially (Redfish Ck., Lardeau R., Hendryx Ck.) to allow smaller individuals to grow to a similar average size across all populations before the start of experiments. Following transfer to laboratory tanks, all fish from each population were fed freeze dried chironomids twice a day on a near maintenance ration (~1% wet body mass) for three days, before being transitioned to a near maintenance ration of commercial food pellets (BioPro2, Bio-Oregon, Washington, USA) delivered by automatic feeders twice a day.

Before the start of experiments, 12 fish from each population were randomly selected and stocked in a separate experimental system allowing individual rearing, which was intended to eliminate the potential for agonistic interactions that would affect individual energy budgets. This experimental system consisted of a 300L glass tank connected to a sump ensuring water denitrification and reoxygenation, and was subdivided transversely into two rows of six individual compartments (12 compartments in total) using plastic mesh screen preventing transfer of food between adjacent compartments. This experimental tank was equipped with automatic feeders ensuring the distribution of food pellets in each compartment at a constant rate over 8 hours. Once individually housed in the system, the 12 fish were fed a ration slightly above maintenance (~1% body mass) for seven days to standardize fish energetics, before being placed on a satiation ration (i.e., *ad libitum*) for two weeks and tested for growth, basal metabolism, and digestive performance. The satiation ration was chosen to maximize contrast in energetics between ecotypes, and the duration of the feeding treatment (i.e., two weeks) was determined based on previous comparisons of energetics between similar rainbow trout ecotypes (Allen et al., 2016; Monnet et al., 2020). All fish were weighed to the nearest 0.01g at the beginning of the two-week feeding treatment and weekly thereafter. Average body mass (mean \pm SD) at the beginning of the feeding protocol was 0.56 ± 0.21 g for piscivores, 0.60 ± 0.15 g for insectivores from Redfish Creek and 0.56 ± 0.15 g for insectivores from Hendryx Creek; differences in body mass were not significant (ANOVA: $F_{[2,33]} = 0.23$, $P = 0.79$).

4.2.3 Standard growth rate (SGR)

To characterize baseline differences in growth between ecotypes, individual standard growth rates were determined after one and two weeks at satiation by weighing fish in water using a digital scale, and SGR (%wet body mass·day⁻¹) was calculated as

$$SGR = \frac{\ln M_{final} - \ln M_{initial}}{t} \times 100$$

where $\ln M_{final}$ is the natural logarithm of the final body mass (g), $\ln M_{initial}$ is the natural logarithm of the initial body mass (g), and t is the growth interval in days. Individual SGR was determined as the average of observed growth rates during the first and second week of the feeding treatment. Fish were fed for the last time after two weeks of treatment at 04:00 PM, and the bottom of each individual compartment was gently cleaned soon after to remove leftover food and faeces and to start a period of fasting allowing complete gut clearance before measuring gut residence time the next day.

4.2.4 Gut residence time (GRT) and maximum food consumption

To evaluate potential variation in food processing capacity between ecotypes, all fish were tested for gut residence time the next day after 10:00 PM, i.e. after a minimum period of 30 hours of fasting, which was determined from pilot experiments as a sufficient time to allow complete gut clearance. Each fish was fed a satiation ration of food pellets; individual food consumption was recorded, before each tank was siphoned to prevent subsequent feeding. All fish were left to digest overnight until 6:00 AM the next day, where two daytime cameras mounted above the experimental tank provided a live view of the time of excretion for each fish from outside the experiment room. The overnight determination of gut residence time was a necessity imposed by the relatively long gut transit times (up to 20 hours) observed in piscivore and insectivore fry. All faeces were collected after a minimum period of 36 hours post-feeding, before being frozen at -60°C for later analysis of assimilation efficiency. All fish were fed two meals of pellets on the day following faeces collection (second meal at 05:00 PM), before leftover food was removed by siphon vacuuming each compartment to start a continuous period of fasting of 48 hours prior to respirometry experiments.

Measuring gut residence time in satiated fish informs the maximum capacity of piscivore and insectivore ecotypes to process a single large meal of food when they are acclimated to a maximum ration (i.e., with plastic upregulation of gut capacity). To assess whether any observed differences in gut passage time between ecotypes were persistent at lower rations, gut residence time for a single meal

was also estimated in a separate batch of piscivore and insectivore fry acclimated to feeding on a maintenance ration (i.e., 1% wet body mass of commercial pellets using the same methods).

4.2.5 Oxygen consumption rates (*SMR*, *SDA_{peak}*, *SDA_{dur}*, *SDA*)

To test whether piscivore and insectivore ecotypes differ in digestive metabolism, we sequentially measured SMR and SDA on individual fish. Forty-eight hours after last feeding, each fish was placed into an individual glass flow-through respirometer connected to a head tank supplying oxygenated and denitrified water at room temperature. An optical oxygen sensor (Neofox, Ocean Insight, Florida, USA) placed in the head tank continuously recorded dissolved oxygen concentration before distribution to three respirometers, and deoxygenated water exiting each respirometer was redirected to a glass vial in which another oxygen sensor could be inserted to record oxygen tension following fish respiration. Effluent water was then evacuated to a sump to be denitrified and reoxygenated, before being redirected to the head tank. Flow rate into each respirometer was adjusted to the oxygen demand of the fish using an external valve to maintain a minimum of 90-100% oxygen saturation, and the adjusted flow rate was determined by weighing the volume of water discharged in one minute using a digital balance. After transfer of fish, all respirometers were covered with black plastic to minimize activity and stress. Fish acclimated inside their respirometer for 10 hours before their SMR was measured between 03:00 AM and 06:00 AM (i.e., 58-61 hours after last feeding), a period of low oxygen consumption in juvenile salmonids (Van Leeuwen et al., 2011; Allen et al., 2016). Continuous traces of oxygen tension were obtained from the head tank and each respirometer, before oxygen consumption rates ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{h}^{-1}$) were calculated as

$$\dot{M}O_2 = V_W \cdot \Delta C_W \cdot O_2$$

where V_W is the flow rate through the respirometer ($\text{L} \cdot \text{h}^{-1}$), and $\Delta C_W \cdot O_2$ is the change in oxygen tension in the respirometer. Dissolved oxygen concentrations in the respirometers were determined by correcting PO_2 (partial pressure of oxygen) for barometric pressure and oxygen solubility α_{O_2} ($\mu\text{mol O}_2 \cdot \text{L}^{-1} \cdot \text{kPa}^{-1}$) at 13°C. For each fish, individual SMR was determined as the lowest 10th percentile of oxygen consumption rates recorded over three hours, before being divided by the body mass of the fish to determine mass-specific SMR ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$).

Immediately after the measurement of SMR, fish were transferred to individual plastic intermittent-flow respirometers to acclimate for a minimum of three hours before feeding and the start

of SDA measurements. The SDA respirometers were custom-made from 170mL snapware containers and connected to the recirculation water system described above, in parallel to the flow-through respirometers previously used to measure SMR. The posterior side of the lid of each chamber was equipped with a sealed rubber stopper through which an optical oxygen sensor could be inserted to measure oxygen consumption rate during digestion. A three-way plastic connector placed directly upstream of the chamber allowed fish to be fed *in situ* by injecting food pellets one at a time into the water inlet of each respirometer. Solenoids connected upstream of each respirometer and controlled by a repeat cycle timer ensured the continuous repetition of two-step cycles composed of 5 minutes during which the system was closed to measure oxygen consumption rates, followed by 10 minutes of flushing during which the system was opened again to replace water and restore ambient oxygen levels inside each respirometer. Finally, a small stir bar inside each chamber ensured gentle mixing during measurements of oxygen consumption. Following feeding, all chambers were covered with black plastic to minimize stress and individual oxygen consumption rates were continuously recorded for a minimum of 30 hours. Background microbial respiration was recorded in each chamber for two measurement cycles (i.e., 30-45 minutes) before each respirometry session. Oxygen consumption rates ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{h}^{-1}$) were calculated during each intermittent-flow cycle following feeding as

$$\dot{M}O_2 = \frac{V_W \cdot \Delta C_W \cdot O_2}{\Delta t}$$

where V_W is the volume of water in the respirometer (L), $\Delta C_W \cdot O_2$ is the change in oxygen tension in the respirometer, and Δt is the 5-min period over which the drop in oxygen tension was recorded. Individual oxygen consumption rates were corrected for background respiration, barometric pressure and oxygen solubility in water at 13°C, before being divided by body mass to determine mass-specific, background-corrected oxygen consumption rates ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). SDA_{dur} was calculated as the time interval (in hours) between feeding and the first time where $\dot{M}O_2$ returned to previously measured SMR levels; SDA_{peak} was calculated as the highest value of $\dot{M}O_2$ ($\mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) between feeding and the endpoint of SDA_{dur} ; finally, SDA ($\mu\text{mol O}_2 \cdot \text{g}^{-1}$) was estimated as total oxygen consumption over the duration of SDA less SMR, by integrating the area under the fitted regression of background-corrected, mass-specific oxygen consumption rates using the *fishMO2* R package (Chabot, 2020). Although measuring multiple dimensions of postprandial metabolism (i.e., duration: SDA_{dur} ; maximum intensity: SDA_{peak} ; total costs: SDA) may seem redundant, different associations among SDA_{dur} , SDA_{peak} and SDA may generate alternative metabolic strategies (e.g., long, low-intensity vs. short, high-intensity digestion) with distinct

consequences for individual aerobic budgets. Simultaneous measurement of SDA_{dur} , SDA_{peak} and SDA may therefore allow a better understanding of integrative variation in digestive phenotypes among and within taxa.

4.2.6 Assimilation efficiency (AE)

To evaluate potential differences in assimilation efficiency between ecotypes, nutrient uptake capacity was measured using the faeces previously collected during the experiment on gut residence time and stored at -60°C . Assimilation efficiency (AE, in %) was calculated as

$$AE = \frac{M_{pellets} - M_{faeces}}{M_{pellets}} \times 100$$

where $M_{pellets}$ is the dry mass of pellets consumed by each fish (mg), and M_{faeces} is the dry mass of faeces collected (mg). This protocol may overestimate nutrient assimilation efficiency since leaching of dissolved organics from faeces are not accounted for, nor are alternative elimination pathways such as urine; nevertheless, faeces still constitute a relevant indicator of nutrient assimilation during digestion as it is the main path of waste evacuation.

4.2.7 Data analysis

All traits related to growth (SGR), basal metabolism (SMR), digestive metabolism (SDA , SDA_{peak} , SDA_{dur}) and food processing efficiency (GRT, AE) were compared between piscivore and insectivore ecotypes by performing analyses of variance (ANOVAs) and analyses of covariance (ANCOVAs) using R software (v.1.3.1093). First, distribution normality and variance homogeneity were verified using Shapiro-Wilk and F-tests, respectively; phenotypic traits that did not meet these two assumptions were log-transformed. To account for differences in body mass between piscivore and insectivore juveniles at the end of our experiments and their effects on trait variation, all traits were adjusted to the mean final body mass of the 36 fish (i.e., 1.0g) by performing separate fitted regressions against body mass for each trait and population, and adjusting individual values to that of a 1g fish.

To account for reluctance of fish to feed to satiation inside their respirometers and its effects on magnitude of digestive metabolism (i.e., SDA , SDA_{peak} , SDA_{dur}) among individuals, each ration-dependent trait (i.e., SDA , SDA_{peak} , SDA_{dur} , GRT, AE) was also adjusted to the maximum measured food consumption

of each fish (in mg), which was determined as the higher of the two food consumption estimates determined at the beginning of GRT and SDA experiments. Linear regressions between each ration-dependent trait and observed meal size were used to adjust each individual trait value up to the maximum meal size observed for the corresponding fish, and therefore standardize all fish responses to a maximum ration. A second set of linear regressions between body mass and traits adjusted to maximum ration were then used to control for variation in body size among individuals, i.e., to adjust each trait for each fish to the mean final body mass of the 36 fish (1.0g). Corrected values of SDA, SDA_{peak} , and SDA_{dur} were then used to calculate relative (i.e., meal-controlled) digestive traits, hereafter referred to as Rel-SDA, Rel- SDA_{peak} , and Rel- SDA_{dur} .

Following trait correction, separate linear models were used to compare each phenotypic trait (SGR, SMR, SDA, SDA_{peak} , SDA_{dur} , GRT and AE) between piscivore and insectivore populations. Models for traits that did not directly depend on ration size (i.e., SGR, SMR) only included population (i.e., Lardeau River [piscivore], Hendryx Creek [insectivore #1], and Redfish Creek [insectivore #2]) as a fixed effect. Models that compared ration-dependent responses (i.e., SDA, SDA_{peak} , SDA_{dur} , GRT, AE, Rel-SDA, Rel- SDA_{peak} , and Rel- SDA_{dur}) between ecotypes also included log meal size and population x log meal size as covariates, where meal size is observed maximum ration for a 1.0g fish. Automatic model selection (MuMin package) was then used to identify the model with the most parsimonious set of fixed effects (i.e., determined as the model with the most optimal combination of lowest AIC and highest AIC weight), before an ANOVA was performed on the best model retained. Because gut residence time may have a controlling influence on costs of digestion and assimilation efficiency, we also modelled SDA and AE as a function of population, GRT, and population x GRT. Finally, Tukey's HSD (Honest Significant Difference) tests were used to evaluate the significance of trait variation among populations.

Patterns of multivariate associations among food intake, growth, routine metabolism and digestive traits were evaluated by Principal Component Analysis (PCA), a multivariate approach commonly used to increase interpretability of patterns in trait covariation while minimizing information loss. A normalized and centered PCA (ade4 package) was performed on all corrected traits (maximum FC, SGR, SMR, SDA, SDA_{peak} , SDA_{dur} , GRT and AE). Strength and significance of emerging patterns of differentiation in digestive strategies between ecotypes revealed by the PCA were then assessed through an ordination plot and pairwise correlation tests among all phenotypic traits, after adjusting P-values for false discovery rate using a Benjamini-Hochberg correction.

4.3 Results

4.3.1 Standard growth rate and standard metabolic rate (SGR, SMR)

Consistent with previous studies (Monnet et al., 2020), piscivore fry had significantly higher growth rates ($F_{[2,33]} = 121.9$, $P < 0.001$; Fig. 4.1a) and SMR ($F_{[2,33]} = 20.4$, $P < 0.001$; Fig. 4.1b) compared to insectivore juveniles; differences in SGR and SMR between insectivore populations were not significant (Tukey's HSD: $P = 0.08$ for SGR, $P = 0.10$ for SMR).

4.3.2 Digestive metabolism (SDA , SDA_{dur} , SDA_{peak})

There was a significant increase in SDA with meal size ($F_{[1,32]} = 30.4$, $P < 0.001$) for both ecotypes (Fig. 4.2a). Unexpectedly, piscivore fry presented with much lower absolute SDA than the two insectivore populations ($F_{[2,32]} = 50.9$, $P < 0.001$) despite them having consumed larger meal sizes. Differences in SDA between ecotypes persisted when expressed on a per g of food basis ($F_{[2,33]} = 225.4$, $P < 0.001$), with no increase in Rel-SDA with increasing meal size in piscivores (Fig. 4.2b). Differences in costs of digestion also persisted when the incrementally higher SMR of piscivores was added to their SDA costs (assuming that the higher piscivore SMR entirely represents investment in a larger digestive tract; Fig. 4.2a, 4.2c, grey dots).

Measures of SDA_{peak} significantly increased with meal size ($F_{[1,32]} = 32.3$, $P < 0.001$) for both ecotypes (Fig. 4.2c). Rel- SDA_{peak} marginally increased with meal size ($F_{[1,32]} = 2.7$, $P = 0.11$; Fig. 4.2d) and was significantly lower in the piscivore ecotype ($F_{[2,32]} = 23.3$, $P < 0.001$).

Finally, SDA_{dur} significantly decreased with ration size for both ecotypes ($F_{[1,32]} = 5.5$, $P = 0.03$; Fig. 4.2e), and piscivore fry presented shorter SDA_{dur} despite higher food intake compared to insectivores from Hendryx Creek (Tukey's HSD: $P < 0.001$) but not Redfish Creek (Tukey's HSD: $P = 0.21$). Rel- SDA_{dur} strongly decreased with meal size ($F_{[1,32]} = 29.4$, $P < 0.001$; Fig. 4.2f), and was significantly lower in piscivore fry compared to insectivore fry from both Hendryx Creek (Tukey's HSD: $P < 0.001$) and Redfish Creek (Tukey's HSD: $P < 0.001$). Although no ANCOVA model retained a significant population x meal size interaction, different relationships between each trait of interest and meal size (i.e., different slopes) among populations may somewhat reduce their comparison. Separate analyses of variance (ANOVAs)

discarding the effects of meal size on each trait, however, did not alter the significance of main effects (Table 4.S2, Appendix).

4.3.3 Food processing efficiency (GRT, AE)

There was an increase in GRT from maintenance to satiation ration diets for each population (Fig. 4.S1, Appendix). At satiation, GRT did not significantly vary with meal size (Fig. 4.3a) but differed among populations ($F_{[2,33]} = 101.4$, $P < 0.001$). Contrary to our hypothesis that higher piscivore AE would be associated with longer GRT, piscivore fry showed shorter food passage time compared to insectivores from Redfish Creek (Tukey's HSD: $P < 0.001$) but not from Hendryx Creek (Tukey's HSD: $P = 0.25$).

AE did not significantly vary with ration size (Fig. 4.3b). As expected, however, piscivore fry presented higher AE compared to the two insectivore populations ($F_{[2,33]} = 17.4$, $P < 0.001$) despite similar or shorter GRT. Contrary to our prediction that shorter GRT would reduce costs of digestion and AE, both the costs of digestion (SDA, Fig. 4.4a) and AE (Fig. 4.4b) were insensitive to GRT.

4.3.4 Multivariate associations among digestive metabolism, food processing efficiency and growth

The PCA with absolute values of all digestive traits showed strong differentiation between ecotypes on PCA1 (52.9% of explained variation, Fig. 4.5), with a strong negative relationship between digestive metabolism (i.e., SDA, SDA_{dur}) and a cluster of traits related to food consumption (i.e., FC), basal metabolism (i.e., SMR), assimilation efficiency (i.e., AE), and growth rate (i.e., SGR; see Table 4.S1, Appendix for strength and significance of univariate correlations). The second principal component axis (25.5% of explained variation) mostly differentiated individuals within each population based on gut residence time (i.e., GRT) and intensity of postprandial metabolism (i.e., SDA_{peak}). Overall, this PCA highlighted the strong differentiation of digestion strategies between ecotypes along a fast growth-low SDA metabolic expenditure (piscivores) vs. slow growth-high SDA metabolic expenditure (insectivores) axis.

4.4 Discussion

4.4.1 Effects of food intake on postprandial metabolism

Assessing the degree of divergence in digestive physiology between fast- and slow-growing rainbow trout ecotypes allowed for the exploration of digestive constraints on early development. Consistent with previous studies (Monnet et al., 2020), piscivore fry presented a pattern of higher growth and associated basal metabolism (i.e., SMR) relative to insectivores, which was associated with maximized feeding capacity (i.e., higher maximum food consumption).

Variation in feeding capacity between ecotypes, however, resulted in unexpected patterns in costs of digestion that allowed faster-growing piscivore fry to achieve both higher maximum consumption and higher digestive efficiency, which is inconsistent with the hypothesized trade-off along a slow-growth, high efficiency vs. high-growth, low efficiency metabolism axis. Although absolute SDA typically increased with meal size among individuals within each population, piscivores presented higher digestive efficiency than insectivores but consistently lower digestion costs despite larger meal sizes (i.e., lower absolute SDA; similar absolute SDA_{peak} and SDA_{dur} , but lower SDA_{peak} and SDA_{dur} per g of food consumed). Assessing SDA alone, however, provides an incomplete picture of divergence in digestion costs between ecotypes; the elevated SMR of the piscivore ecotype is likely related, at least in part, to a larger digestive tract that allows a higher maximum ration (Monnet et al., 2020). This larger digestive tract and associated higher SMR can also be considered an enhanced cost of digestion relative to insectivores, and to some extent represents a displacement of costs of digestion from SDA to SMR. Even if increased SMR is included as part of digestive metabolism, however, costs of digestion in piscivores remains lower than in the insectivore ecotype (Fig. 4.2a, 4.2c).

Reduction in overall costs of digestion in piscivores, and subsequent increased growth efficiency (Monnet et al., 2020), may be realized by at least three possible pathways. First, the evolution of a permanently larger digestive tract that may reduce the costs associated with handling larger meals compared to cyclically upregulating a smaller digestive system. Second, the greater surface area of an enlarged digestive tract may allow greater paracellular (passive) absorption of nutrients across the gut wall. Finally, economies of scale in the physiology underlying SDA may greatly decrease the marginal costs associated with increased ration. These mechanisms are considered in more detail below.

4.4.2 Displacement of SDA costs to SMR: cyclic upregulation vs. stable overhead costs

All animals cyclically upregulate their digestive tract to some extent during feeding, or over longer time scales when resources are abundant and stable (Hammond & Diamond, 1992; Piersma & Lindstrom,

1997). This is typically achieved by rapidly increasing the surface layer of epithelial cells in the intestine that are responsible for enzyme excretion and nutrient absorption (Hammond & Diamond, 1992; Secor et al., 1994). Because upregulating the digestive tract is metabolically costly (Secor et al., 1994; Armstrong & Schindler, 2011), these processes are reversed after digestion; the degree to which animals downregulate their digestive tract between successive meals is a major axis of digestive differentiation, and is strongly related to feeding periodicity (Secor & Diamond, 2000; Secor, 2009). For instance, snakes with an inactive ambush predator lifestyle may experience months between meals and will massively downregulate the size of their digestive system in response to fasting conditions (Secor & Nagy, 1994; Secor et al., 1994) to minimize overhead costs associated with digestive tract maintenance (i.e., SMR). In contrast, active snakes that are constantly feeding typically present with higher SMR and a more limited capacity to downregulate their digestive tract (Secor & Diamond, 2000). Because the energetic costs of upregulation are significant, once meals are sufficiently frequent it becomes more efficient to pay the maintenance costs of a larger gut and reduce the frequency and costs of up-and down-regulating the digestive system. A similar trade-off may be operating in the divergent digestive strategies of the insectivore and piscivore ecotypes; the repeated costs of upregulating a smaller gut to accommodate a large ration may exceed the stable maintenance costs of a larger digestive tract, which manifests as an elevated SMR in the piscivore ecotype. The lower SDA in the piscivore ecotype is consistent with a more stable and elevated capacity for digestion, where a larger gut may increase SMR (maintenance costs), but reduce the need for cyclic upregulation (i.e., SDA).

4.4.3 Passive vs. active strategies to increase digestion efficiency

In addition to reducing costs of digestion, piscivore fry also reduced gut transit times at high ration while maintaining higher assimilation efficiency relative to insectivores in this study. Previous studies have suggested a trade-off between a short gut residence time to maximize food intake vs. maximizing assimilation efficiency, which increases with GRT (e.g., Sibly, 1981). Piscivores appear to have mitigated this trade-off by evolving a larger gastro-intestinal tract (approximately 85% of their body length vs. 65% in insectivores for a 1g fish: Monnet et al., 2020; G. Monnet, unpublished data), which would allow piscivore fry to maintain high assimilation efficiency despite having a shorter or similar gut residence time than their insectivore conspecifics. This pattern might extend beyond the piscivore vs. insectivore comparison, since previous studies that compared the anatomical characteristics of eight rainbow trout ecotypes reported that piscivores were one of the ecotypes with the largest digestive organs (see Keeley

et al., 2005). While a larger gut may elevate SMR because of the comparatively high maintenance costs of digestive tissues (Armstrong & Schindler, 2011; Neubauer & Andersen, 2019), net energetic savings may be possible if a larger gut lowers postprandial metabolic costs. Caviedes-Vidal et al. (2008) demonstrated that birds and bats have evolved smaller digestive tracts to minimize flight costs, but maintain a large digestive capacity by increasing their intensity of low-energy paracellular gut assimilation relative to non-flying mammals. Active transcellular absorption of nutrients requires some level of energy expenditure to transport nutrients across epithelial cells lining the gut (Karasov et al., 2011), while paracellular transport is a diffusive process through inter-cellular junctions driven by concentration gradients with minimal energy demand (Pappenheimer, 1993; Kiela & Ghishan, 2016). A larger gut with increased surface area greatly increases the scope for low energy paracellular transport of digested nutrients, without the potential constraints that might be associated with minimizing intestinal mass in flying animals. The potential for increased gut area to enhance paracellular transport may be a significant contributing factor in the ability of the piscivore digestive system to maintain high assimilation efficiency at low cost, while also lowering gut transit time to maximize consumption.

4.4.4 Economies of scale in digestion

Total assimilation efficiency in both ecotypes was largely insensitive to ration size, suggesting that optimal assimilation is relatively conserved for a given phenotype. These results are somewhat consistent with previous studies on vertebrate taxa where digestive efficiency either remained stable (in snakes: Cox & Secor, 2007; fish: Knight et al., 2021) or decreased with meal size over short- (Solomon & Brafield, 1972; Jobling et al., 1977; Legler et al., 2010) or long-term periods in fish (Kotrschal et al., 2014). Decreasing AE with increasing ration may therefore reflect the lower capacity of digestive enzymes to penetrate and degrade the center of larger ingested food masses. This is evident in the incremental increase in SDA costs per g of food consumed for the insectivore ecotype, but not in piscivore fry where SDA costs per g of food were stable or declining with increased consumption. Economies of scale in food consumption were most evident in the reduction of SDA_{dur} with increasing ration; similarly, GRT also decreased with ration in piscivore (but not insectivore) fry. Shorter SDA_{dur} and GRT are effective pathways for increasing growth efficiency because they allow faster re-feeding (i.e., higher maximum consumption) and lower total SDA costs if SDA_{peak} is independent of duration. Economies of scale in SDA or other costs typically arise when processes have high initial overhead costs that are effectively fixed; for instance,

initial costs of upregulation of the mucosal gut epithelia may be high but are a prerequisite for digestion irrespective of ration size. Economies of scale in the processes that underly growth, however, may not be widely realized because of balancing selection against high growth and consumption, either because of high predation risk (Lankford et al., 2001), or low prey abundance (Monnet et al., 2020).

4.4.5 Differentiation of digestive strategies and energy budgets along productivity gradients

Multivariate associations among digestive traits largely differentiated piscivore and insectivore digestive strategies, but this phenotypic differentiation did not clearly map onto an energy (rate)-maximizing vs. efficiency-maximizing axis as suggested by previous studies at higher levels of taxonomic organization (e.g., between species: Rosenfeld et al., 2020). The overall piscivore digestive phenotype included lower postprandial metabolism (i.e., SDA, SDA_{dur}) and higher digestive efficiency (i.e., low GRT, high AE) in response to their higher food intake supporting faster growth, when the rate-maximizing strategy predicted faster-growing taxa to trade-off higher food intake against higher digestive costs and lower assimilation efficiency. Conversely, the slower-growing insectivore phenotype exhibited higher digestive costs and lower digestive efficiency associated with lower food intake and growth, when the efficiency-maximizing strategy predicted higher assimilation efficiency.

By contrast, ecotypic variation in food consumption does map onto a low-to-high environmental productivity gradient in the wild, where higher availability of invertebrate prey in the piscivore rearing stream (i.e., the Lardeau River) coupled with proactive individual foraging behaviours (Monnet et al., 2020) allow piscivores to maximize food intake to fuel digestion and growth, allowing scope for the emergence of economies of scale in growth efficiency. Conversely, lower prey abundance in smaller insectivore rearing tributaries coupled with reactive foraging behaviours likely limits energy acquisition, digestion and early growth in the insectivore ecotype.

4.4.6 Ecological implications of variation in digestive physiology

The magnitude of postprandial metabolism (i.e., SDA) has important implications for other aspects of metabolic performance since SDA represents a significant fraction of individual aerobic budgets, i.e. aerobic scope (Fry, 1971; Chabot et al., 2016) defined as the difference between SMR (the minimum metabolic rate for maintenance) and MMR (maximum metabolic rate, the maximum capacity of oxygen supply to organs and tissues). For instance, previous studies demonstrated that faster-growing

populations of Atlantic silverside (*Menidia menidia*) with higher SDA were much more vulnerable to predation following feeding compared to slower growing populations (Billerbeck et al., 2000; Lankford et al., 2001). High metabolic allocation to digestion typically reduces leftover aerobic scope for additional physiological processes (e.g., active swimming to avoid predators: Norin & Clark, 2017), and resulting metabolic trade-offs may in turn decrease fitness. Displacement of digestive costs (SDA) to elevated SMR in piscivores may reduce their AS relative to insectivores when fasting; however, their postprandial aerobic scope would not be compromised, and could be potentially higher than that of an insectivore.

Digestive physiology is a multivariate process with major consequences for individual fitness (Booth, 1990; Hart & Gill, 1992) and trophic specialization (Knight et al., 2021). Comparison of digestive performance between divergent populations has contributed to landmark studies in adaptive differentiation at continental scales (e.g., latitudinal countergradient in growth: Billerbeck et al., 2000). By contrast, variation in digestive performance has also been proposed as a significant contributor to the maintenance of local taxonomic diversity and interspecific ecological partitioning along environmental gradients (Van Leeuwen et al., 2011; Rosenfeld et al., 2020). By explicitly demonstrating the divergence in digestive performance between piscivore and insectivore ecotypes of rainbow trout, and the consequences of this variation for differentiation of their early growth trajectories, our study strongly supports the integrated role of digestive physiology in phenotypic differentiation and the emergence of local diversity. This study also emphasizes that the costs of growth are allocated to both maintenance of digestive machinery (SMR) and its active upregulation during and after feeding (i.e., SDA); the relative allocation of metabolic power between these compartments is a major design feature of the digestive system, and manifests as a significant axis of phenotypic differentiation within the integrated phenotype.

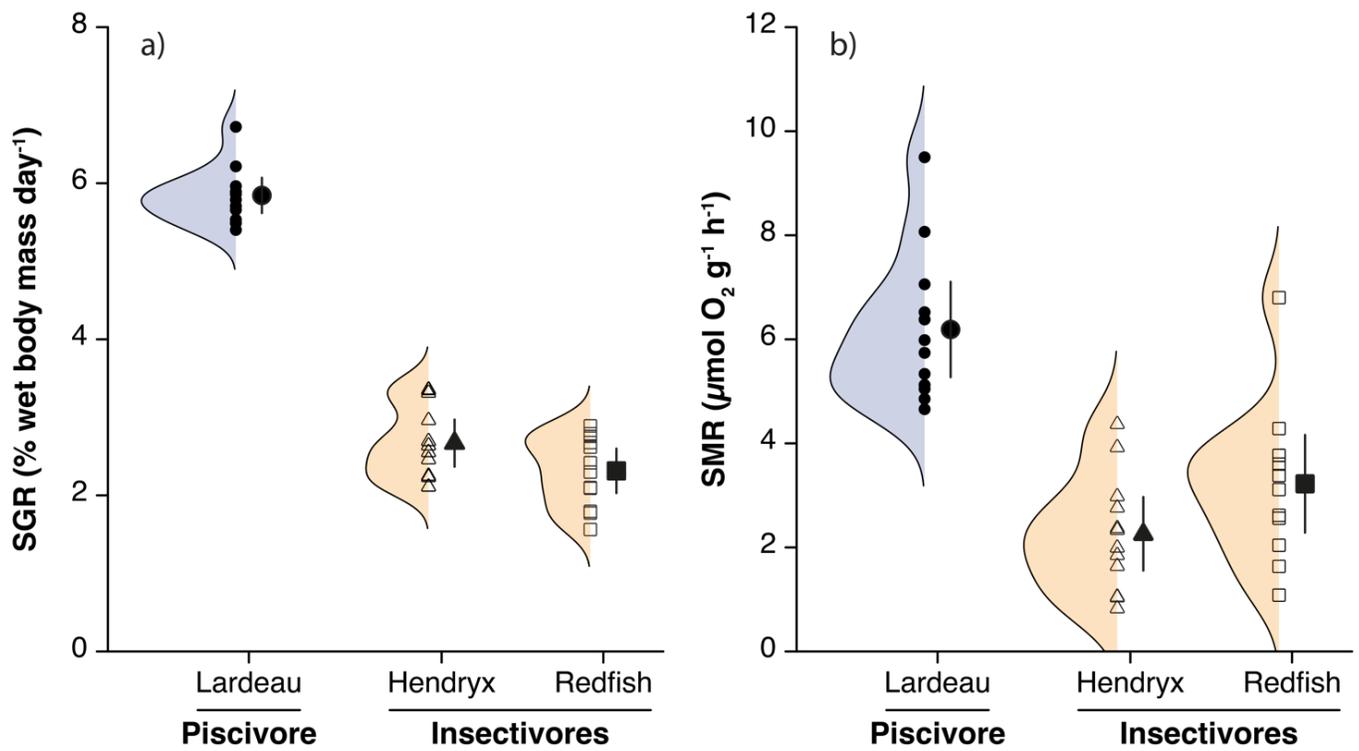


Figure 4.1: Differences in individual standard growth rate (SGR; a) and standard metabolic rate (SMR; b) between piscivore fry (dots) and insectivore fry from Hendryx Creek (triangles) and Redfish Creek (squares). Large black symbols represent population means, and black vertical lines represent 95% confidence intervals.

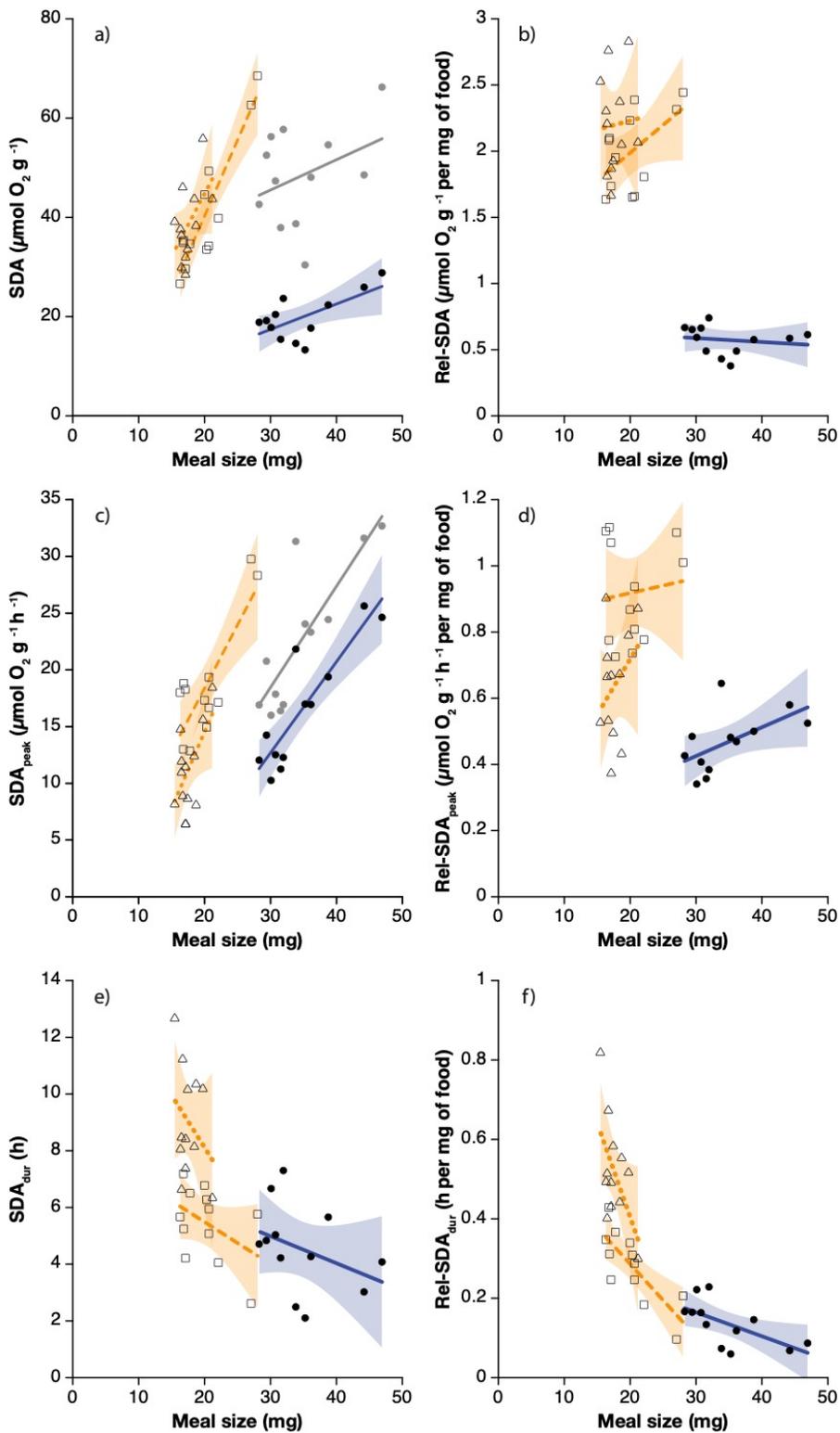


Figure 4.2: Relationships between meal size and absolute SDA (a), SDA_{peak} (c), SDA_{dur} (e); and between meal size and relative SDA (b), SDA_{peak} (d), and SDA_{dur} (f) for piscivore fry (dots and solid lines) and insectivore fry from Hendryx Creek (triangles and dotted lines) and Redfish Creek (squares and dashed lines). Trendlines with 95% CI are indicated for each population. Grey dots and lines on panels a) and c) represent total overhead costs (i.e., SMR+SDA) for the piscivore ecotype.

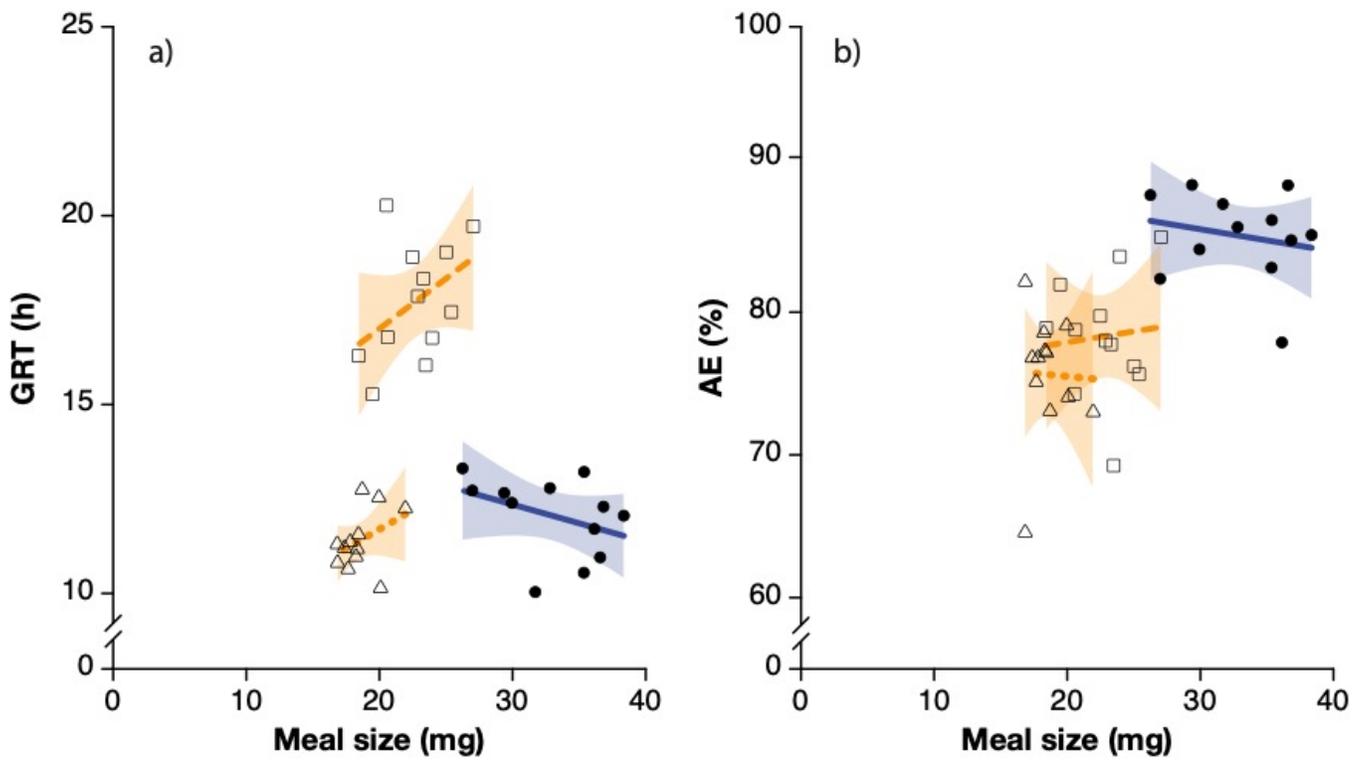


Figure 4.3: Relationships between meal size and absolute GRT (a) and AE (b) for piscivore fry (dots and solid lines) and insectivore fry from Hendryx Creek (triangles and dotted lines) and Redfish Creek (squares and dashed lines). Trendlines with 95% CI are indicated for each population.

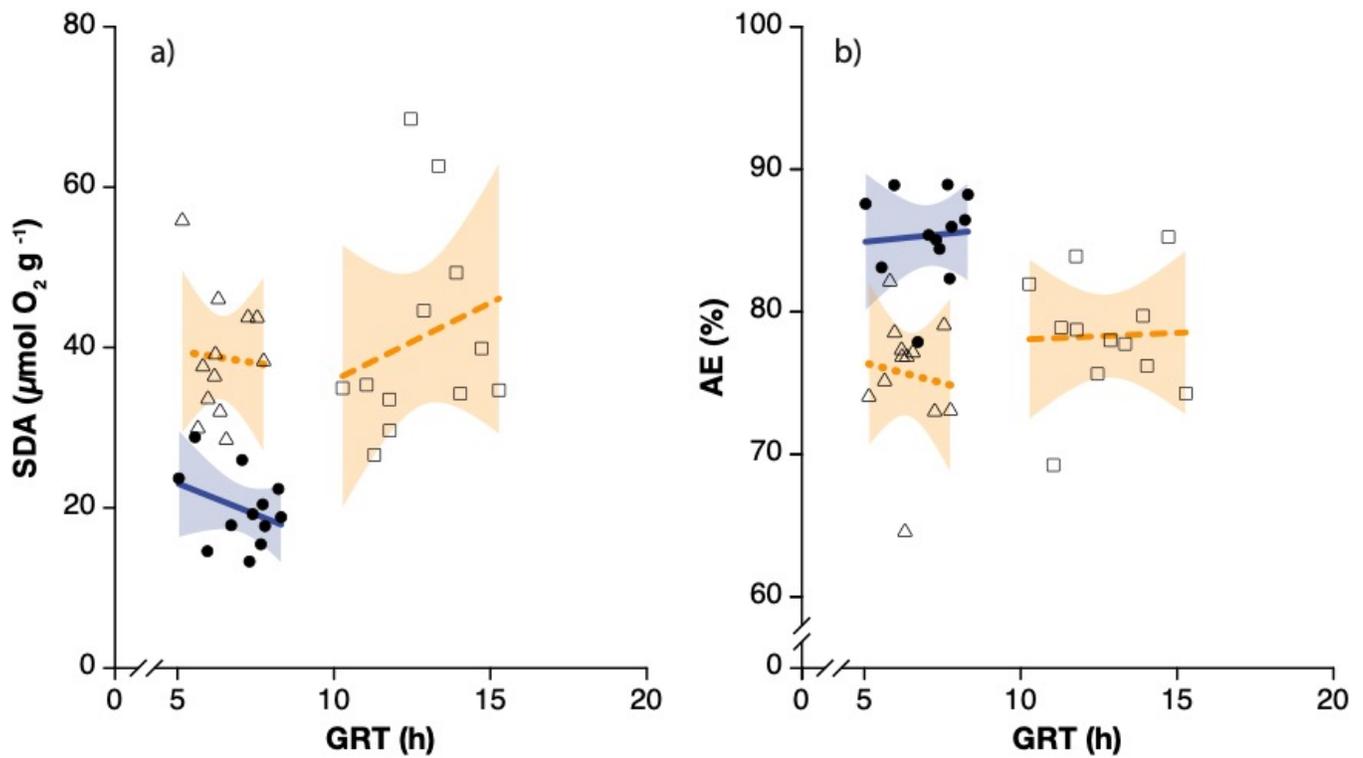


Figure 4.4: Relationships between GRT and individual SDA (a) and AE (b) for piscivore fry (dots and solid lines) and insectivore fry from Hendryx Creek (triangles and dotted lines) and Redfish Creek (squares and dashed lines). Trendlines with 95% CI are indicated for each population.

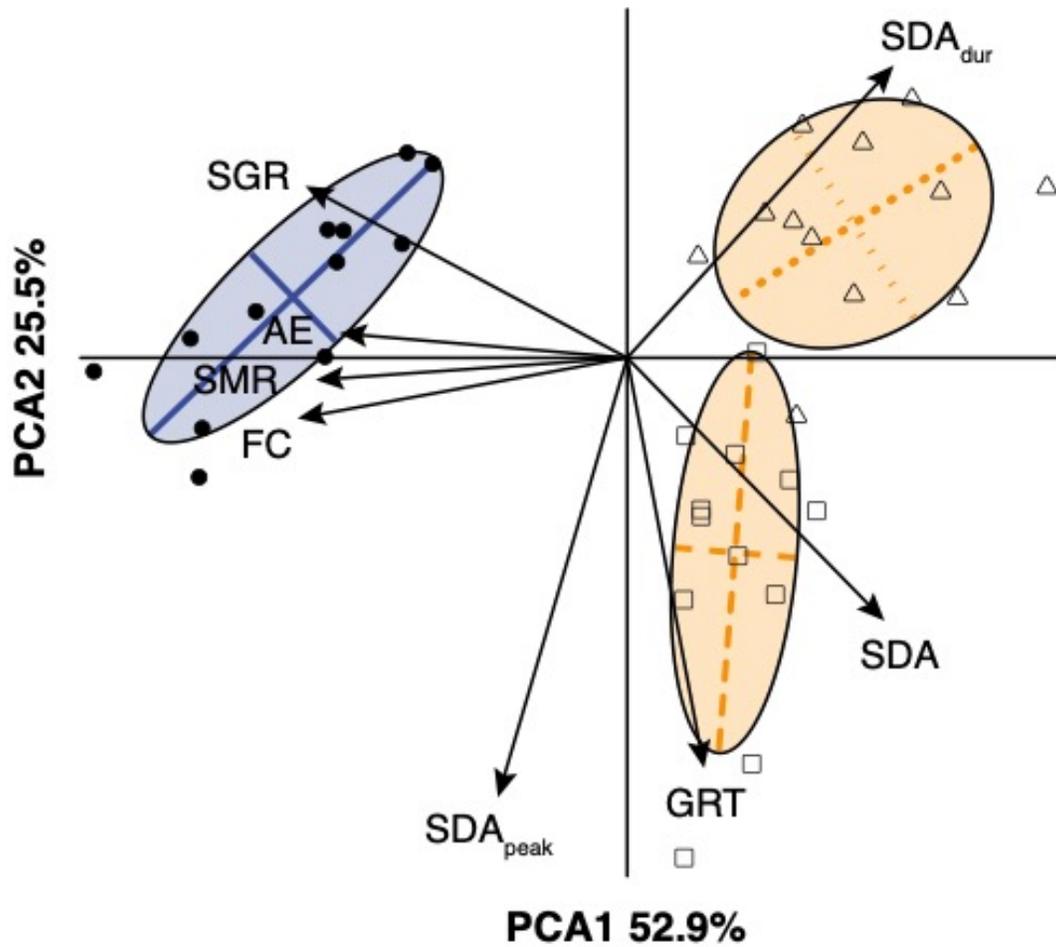


Figure 4.5: Principal Component Analysis (PCA) illustrating the multivariate associations among FC, SGR, SMR, and absolute SDA, SDA_{peak}, SDA_{dur}, GRT and AE for piscivore fry (dots, solid lines and blue ellipse) and insectivore fry from Hendryx Creek (triangles, dotted lines and orange ellipse) and Redfish Creek (squares, dashed lines and orange ellipse).

Chapter 5: Contrasting digestive strategies differentiate growth, energetics, and performance between coho salmon and steelhead trout following ecological specialization in coastal streams

5.1 Introduction

Adaptive trade-offs impose fundamental constraints on phenotypic evolution and shape biodiversity patterns by allowing the coexistence of taxa along environmental gradients (Careau et al., 2010; Finstad et al., 2011). Although morphological and behavioural trade-offs have received considerable attention (e.g., cranial vs. fin morphology: Gilbert et al., 2021; shy vs. bold behaviours: Stamps, 2007), physiological trade-offs and their impacts on fitness correlates (e.g., growth and survival: Monaghan et al., 2009) may also drive considerable phenotypic and ecological differentiation (Braendle et al., 2011; Careau & Garland, 2012). Cryptic trade-offs among competing physiological processes, however, are less well studied, despite the potential to generate significant biological diversity in nature (Agrawal et al., 2010).

Growth rate is a fundamental life-history attribute contributing to the adaptive differentiation of individuals, populations and species (Arendt, 1997; Dmitriew, 2011), and growth is often traded off against other life history traits. Many evolutionary pressures (e.g., direct selection for larger adult size: Sibly et al., 2015) and ecological factors (e.g., higher prey availability: Diehl, 1993) select for faster growth in nature, which is typically optimized well below physiological maximum rates via trade-offs with competing physiological processes (e.g., aerobic performance: Norin & Clark, 2017). Recent studies suggest that faster growth rates may be traded-off against lower growth efficiency (i.e., the ratio of mass gained to food consumed: Rosenfeld et al., 2020), because maximizing food intake as a pre-requisite for faster growth (Allen et al., 2016; Monnet et al., 2020) may require faster gut clearance to allow greater consumption, ultimately reducing nutrient uptake and growth efficiency. Growth and growth efficiency are often positively correlated within species (i.e., faster-growing populations are also more efficient: Lindgren & Laurila, 2005; Allen et al., 2016; Monnet et al., 2020). With greater scope for adaptive differentiation among species, however, alternative trade-offs may manifest between species maximizing the rate of energy intake vs. those maximizing growth efficiency (Rosenfeld et al., 2020). Rate-maximizing taxa would maximize gross energy intake and growth at the cost of elevated metabolism and lower growth efficiency (Ydenberg et al., 1994), and be dependent on high-productivity

environments (Finstad et al., 2011); in contrast, efficiency-maximizing taxa would optimize energy intake by minimizing metabolic costs and be better competitors in less productive habitats.

Contrasting energy consumption strategies should have matching effects on digestive metabolism. Maximizing food intake to support faster growth may increase postprandial metabolic demand to digest food (i.e., specific dynamic action SDA, its maximum intensity SDA_{peak} , and duration SDA_{dur}), as widely observed in ectotherms (e.g., Secor & Boehm, 2006; Millidine et al., 2009; Bessler et al., 2010). At the anatomical level, processing larger meals may also require optimizing the balance between evolving a short gut residence time (GRT, the time interval between feeding and excretion) to maximize consumption vs. a longer GRT to maximize assimilation efficiency (AE, the fraction of nutrients assimilated from a meal) and growth efficiency (Sibly, 1981).

Variation in postprandial metabolism (SDA) may also contribute to differentiation of aerobic budgets (i.e., the partitioning of aerobic capacity among competing processes such as activity, growth, digestion, or immune function) among individuals, populations and species (Chabot et al., 2016). Aerobic scope (AS) is defined as the aerobic capacity left over when standard metabolic rate (SMR, or maintenance costs) is subtracted from maximum metabolic rate (MMR, the maximum capacity of oxygen supply to tissues: Fry, 1971), and represents the residual aerobic capacity that can be directed to other functions like active metabolism. As SDA increases with meal size (Secor, 2009), a trade-off may arise between feeding vs. retaining aerobic scope for activity or predator avoidance (Auer et al., 2015a; Norin & Clark, 2017). Few studies, however, have investigated how this trade-off manifests in a context of phenotypic differentiation among species despite the strong ecological implications of SDA and aerobic budgets. Collectively, these multiple lines of evidence generate an expectation that the phenotypes of taxa adopting rate-maximizing vs. efficiency-maximizing strategies should show divergent metabolic, energetic, and digestive attributes in a multivariate trait space defining the integrated phenotype.

Juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*O. mykiss*) co-exist in many coastal streams in Northwestern North America and constitute a compelling model for exploring the relationships between digestive physiology, energetics, and the coherence between digestive physiology and habitat use along resource gradients. Although both species typically feed on drifting invertebrates and prefer deep, low-velocity pools where swimming costs can be reduced to maximize growth (Hartman, 1965; Young, 2001), coho salmon are generally found in deeper, low-velocity pools while steelhead trout can also exploit shallow, high-velocity riffle habitats (Johnston, 1970; Bugert &

Bjornn, 1991; Young, 2004). At the northern edge of their sympatric range, coho salmon fry emerge from redds earlier than steelhead trout and establish territories in preferred low-velocity pools (Young, 2004) where prior residence and associated size advantage allow coho salmon to effectively outcompete and displace late-emerging steelhead trout into adjacent riffles. We hypothesize that this habitat partitioning should select for divergent physiological and morphological adaptations that allow steelhead trout to exploit high energy flux riffle habitats where both prey encounter rates and swimming costs are elevated compared to low-velocity pools (Hayes et al., 2000). Relative to coho salmon, steelhead trout are known to exhibit elevated food intake and growth (Sullivan et al., 2000), lower growth efficiency (Rosenfeld et al., 2020), higher active metabolic capacity (Van Leeuwen et al., 2011) and more cylindrical body shape with shorter lateral fins (Bisson et al., 1988), which is consistent with maximizing energy intake in highly productive but energy-demanding habitats. Diversification of digestive strategies may be a key component of a growth vs. growth efficiency trade-off and may represent an overlooked dimension of adaptive differentiation in salmonids and fish in general (Rosenfeld et al., 2020).

In this study, we assessed the degree of differentiation in digestive performance, growth, energetics, and aerobic budgets between juvenile steelhead trout and coho salmon to relate observed differences (if any) to ecological diversification between the two species along an energy flux gradient. We predicted that i) the specialization of steelhead trout to high-velocity, high energy flux habitats would result in elevated food intake and faster growth at the cost of lower growth efficiency relative to coho salmon; ii) the two species would differentiate along a rate-(energy) maximizing (i.e., steelhead trout) vs. efficiency-maximizing (i.e., coho salmon) axis of digestive strategies matching their ecological lifestyle; and iii) the higher postprandial metabolic demand (SDA) associated with elevated food intake would occupy a greater fraction of the steelhead trout aerobic budget and reduce excess aerobic scope relative to coho salmon.

5.2 Material and Methods

5.2.1 Collection of juvenile steelhead trout and coho salmon

Sympatric young-of-the year steelhead trout and coho salmon were collected in each of two replicate coastal streams near Vancouver, B.C., Canada (the Coquitlam River [UTM 5464693N 516952E] and

Silverhope Creek [UTM 5468848N 611012E]) during two consecutive days in September 2020. Fish collection occurred at dusk, when the nocturnal shift of juvenile steelhead trout and coho salmon to quiescent marginal habitats facilitated their detection and capture with dip-nets. Once collected, fish were immediately transferred to aquatic facilities at The University of British Columbia (UBC, Vancouver, Canada).

5.2.2 Fish rearing

Fish were transferred into a walk-in environmental chamber with a day-night cycle of 12h daylight:12h darkness and ambient temperature of $13.4 \pm 0.5^\circ\text{C}$ (mean \pm SD). Each population (i.e., coho salmon and steelhead trout from the Coquitlam River and Silverhope Creek) was subdivided into five 200L glass tanks (10 tanks in total), each stocked with 20-25 individuals, before being quarantined with 3ppm saltwater for one week to minimize potential for disease transfer across populations. Rocks and artificial plants were placed in each tank for environmental enrichment. Because coho salmon and steelhead trout from Silverhope Creek had larger body mass upon collection relative to fish from the Coquitlam River, subsequent experiments on growth and digestive physiology were performed sequentially in order of decreasing initial body size to allow smaller fish to grow to a similar mean body size across all populations before the start of experiments. Once transferred to rearing tanks, each population was fed a maintenance ration (~1% wet body mass) of freeze-dried chironomids twice a day for six days while being transitioned to a near maintenance ration of commercial food pellets (BioPro2, Bio-Oregon, Washington, USA) delivered by automatic feeders twice a day.

To assess differences in digestive physiology and metabolism between species, a subset of 12 fish from each population were randomly selected from multiple tanks and individually stocked in an experimental rearing tank connected to a sump for water filtration and reoxygenation. Individual rearing was intended to minimize adverse effects of social interactions on individual feeding, metabolism and growth. The experimental rearing system was composed of a 300L glass tank divided transversely into two series of six compartments (12 compartments in total) using plastic mesh partitions allowing water flow and visual contact while preventing food transfer between adjacent compartments. The experimental tank was covered with a mesh screen to prevent fish escape and equipped with automatic feeders to distribute food pellets in each compartment at a constant rate over eight hours daily. Once stocked in their individual compartments, all fish were fed a ration slightly above maintenance (~1% body

mass) for seven days to standardize body condition and energetics, before being placed on a satiation ration (i.e., *ad libitum*) for two weeks and tested for growth, digestive performance, and aerobic metabolism. The satiation ration was intended to maximize the likelihood of detecting physiological differences between species, and the duration of the feeding treatment (i.e., two weeks) was determined based on previous comparisons of energetics in juvenile salmonids (Allen et al., 2016; Monnet et al., 2020).

All fish were weighed to the nearest 0.01g at the beginning of the two-week feeding treatment and weekly thereafter. Average body mass at the beginning of the two-week feeding treatment was 3.61 ± 0.75 g (mean \pm SD) for coho salmon from Silverhope Creek, 2.64 ± 1.04 g for coho salmon from the Coquitlam River, 3.28 ± 1.59 g for steelhead trout from Silverhope Creek and 2.46 ± 1.07 g for steelhead trout from the Coquitlam River; differences in initial body mass between species were not significant (ANOVA: $F_{[1,46]} = 0.52$, $P = 0.47$). Of the 48 fish stocked in the experimental rearing system at the start of the two-week feeding treatment (i.e., 12 fish from each of the four populations), 47 were available for final analysis: 1 coho salmon (Coquitlam River) died from unknown causes.

5.2.3 Standard growth rate (SGR)

To evaluate baseline variation in growth between coho salmon and steelhead trout, individual standard growth rates SGR (%wet body mass·day⁻¹) were calculated after one and two weeks of satiation as

$$SGR = \frac{\ln M_{final} - \ln M_{initial}}{t} \times 100 \quad (1)$$

where $\ln M_{final}$ is the natural logarithm of the final body mass (g), $\ln M_{initial}$ is the natural logarithm of the initial body mass (g), and t is the growth interval in days. Individual SGR was calculated as the average of observed growth rates at one and two weeks.

5.2.4 Food consumption (FC)

To evaluate potential differences in maximum food intake between species, food consumption was measured for each individual after one and two weeks of feeding at satiation. To estimate food consumption, the bottom of each individual compartment was siphon vacuumed in the morning to remove leftover food and debris, before automatic feeders were loaded with an excess ration of food pellets and fish allowed to feed for 24 hours. The next day, each tank was siphoned again to collect

unconsumed food pellets, which were then carefully separated from faeces before being dried for one week at 60°C and weighed. The mass of pellets distributed was considered as dry mass; the moisture contents of distributed and remaining (i.e., dried) pellets were assumed to be equivalent, since all pellets were exposed to similar air humidity during processing prior to weighing. Individual food consumption (FC, in %dry body mass) was then calculated as

$$FC = \frac{M_{food\ available} - M_{food\ remaining}}{M_{fish}} \times 100 \quad (2)$$

where $M_{food\ available}$ is the dry mass of food consumed (g), $M_{food\ remaining}$ is the dry mass of food remaining after feeding (g), and M_{fish} is the dry mass of the fish (g) assuming a mean 76% water content of fish body mass (Allen et al., 2016). Individual FC was calculated by averaging the two food consumption estimates obtained after one and two weeks of the satiation feeding treatment.

5.2.5 Growth efficiency (GE)

Differences in growth efficiency (GE) between juvenile coho salmon and steelhead trout were calculated using individual growth and food consumption estimates as

$$GE = \frac{M_{gained}}{F_{consumed}} \quad (3)$$

where M_{gained} is the mean daily increase in body mass (%dry body mass), and $F_{consumed}$ is the daily food intake (%dry body mass). Individual GE was calculated as the average of the two growth efficiency estimates obtained after one and two weeks of the satiation feeding treatment. Coho salmon and steelhead trout juveniles were fed for the last time after 14 days of satiation at 04:00 PM; 30 minutes after feeding, the bottom of each individual compartment was gently vacuumed to remove leftover food and faeces and to initiate a continuous period of fasting before measuring gut residence time the next day.

5.2.6 Gut residence time (GRT)

To assess potential differences in food processing capacity between species, gut residence time was measured in all fish on day 15 after 10:00 PM, that is, after a continuous period of 30 hours of fasting, which was determined from preliminary observations as a sufficient delay to allow complete gut clearance before consumption of new food. Automatic feeders delivered food in excess and fish were allowed to feed for 30 minutes before each tank was siphoned to prevent subsequent feeding. Fish were

left to digest overnight until 6:00 AM the next day, where two daytime cameras mounted above the experimental rearing tank allowed the experimenter to record the time of excretion for each individual from outside the experimental chamber. This overnight digestion step was imposed by the long intestinal transit times (up to 20 hours) reported in juvenile coho salmon and steelhead trout. After a minimum of 36 hours after feeding, all faeces were collected and frozen at -60°C for subsequent estimation of assimilation efficiency. Following faeces collection, all fish were fed twice on that day before individual compartments were cleaned to remove excess food and start a continuous period of fasting prior to respirometry experiments.

Because fish may plastically upregulate the size of their digestive tract on a satiation ration, (Allen et al., 2016), we also measured gut residence time in a separate batch of fish from each population reared on a maintenance ration of food pellets (~1% body mass); the intent was to assess whether any observed differences in gut passage time were persistent at lower rations.

5.2.7 Oxygen consumption rates (MMR, SMR, SDA components)

To evaluate differences in aerobic capacity between coho salmon and steelhead trout, maximum metabolic rate (MMR) and standard metabolic rate (SMR) were measured in all fish two days after measuring gut residence time. We measured MMR after a minimum of 8 hours after the beginning of the day cycle and after a continuous period of 44 hours of fasting, to prevent circadian rhythm and residual digestive activity from biasing MMR. Each fish was placed in a 20L bucket of water at ambient temperature (i.e., 13°C) and chased by hand until exhaustion, which was assumed to be reached when fish no longer reacted to a gentle flip or push with the hand. Although post-exercise respirometry has been suggested to underestimate MMR relative to swimming respirometry, recent comparative analyses (e.g., Killen et al., 2017) have found minimal difference between the two methods, and the use of post-exercise respirometry should provide a meaningful assay of variation in relative maximum metabolic output among individuals (e.g., Allen et al., 2016; Monnet et al., 2020). Once exhausted, fish were immediately placed in a plastic, custom-made respirometer equipped with a small stir bar to ensure mixing while an optical oxygen sensor (Neofox, Ocean Insight, Florida, USA) recorded the decrease in oxygen tension from ~95-100% to ~60-65%. Determination of MMR used the 60-s period of the oxygen trace over which the rate of oxygen consumption in the respirometer was maximal, and individual oxygen consumption rates ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{h}^{-1}$) were calculated as

$$\dot{M}O_2 = \frac{V_W \cdot \Delta C_W \cdot O_2}{\Delta t} \quad (4)$$

where V_W is the volume of water in the respirometer (L), $\Delta C_W \cdot O_2$ is the change in oxygen tension in the respirometer, and Δt is the 60-s period over which the drop in oxygen tension was recorded. Partial pressure of oxygen (PO_2) was corrected for barometric pressure (reported during each round of respirometry) and oxygen solubility coefficient αO_2 ($\mu\text{mol O}_2 \cdot \text{L}^{-1} \cdot \text{kPa}^{-1}$) in water at 13°C. Corrected oxygen consumption rates were then divided by body mass to calculate mass-specific MMR for each fish.

Immediately after the measurement of MMR, fish were placed in separate glass flow-through respirometers to measure SMR. Each respirometry chamber was connected to a head tank supplying oxygenated water at ~90-100% saturation, which was continuously recorded during the experiment with an optical oxygen sensor. Oxygen-depleted water exiting each respirometer was directed to a closed glass vial where a second oxygen probe continuously recorded variation in individual oxygen consumption rates ($\dot{M}O_2$); effluent water was then returned to a sump for denitrification and reoxygenation, before being redirected to the head tank. In all, three flow-through respirometers were connected in parallel to the head tank to allow simultaneous measurements of SMR on three fish each day. Respirometers were covered in black plastic to ensure visual isolation and minimize stress. Flow rates inside each respirometer were determined by weighing the volume of water discharged in one minute using a digital scale and adjusted to achieve equilibrium oxygen tensions of ~90-100% saturation. Fish acclimated inside their respirometer for 9-14 hours before SMR was determined the next morning, i.e., a minimum of 58 hours after last feeding. Standard metabolic rate was determined between 03:00 AM and 06:00 AM, a period of low oxygen consumption frequently reported in juvenile salmonids (Van Leeuwen et al., 2011; Allen et al., 2016). Continuous traces of oxygen tension were obtained from oxygen probes in the head tank and respirometers, and used to calculate individual oxygen consumption rates ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{h}^{-1}$) as

$$\dot{M}O_2 = V_W \cdot \Delta C_W \cdot O_2 \quad (5)$$

where V_W is the flow rate through the respirometer ($\text{L} \cdot \text{h}^{-1}$). Dissolved oxygen concentration in each respirometer was corrected for barometric pressure and oxygen solubility at 13°C. For each fish, individual SMR was determined as the lowest 10th percentile of oxygen consumption rates recorded over 3 hours, and divided by body mass to determine mass-specific SMR ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$).

Immediately after the measurement of SMR, fish were placed in individual plastic intermittent-flow respirometers connected to the recirculating water system to acclimate for 3 hours before the start of

SDA measurements. Intermittent-flow respirometers were made of 170mL polypropylene snapware containers equipped with a rubber stopper sealed on the posterior side of the lid, through which an optical oxygen sensor could be inserted to measure individual oxygen consumption rates ($\dot{M}O_2$) during digestion. Intermittent-flow cycles included a first step of five 5 minutes during which the system was closed to measure oxygen consumption rates, followed by a second step of ten minutes of flushing during which the system was opened to replace water and restore ambient oxygen levels. The continuous repetition of these cycles was ensured by solenoids connected upstream of each respirometer and controlled by a repeat cycle timer; a small stir bar inside each chamber ensured gentle mixing. At the end of the 3h acclimation period, fish were fed inside their respirometer by injecting commercial food pellets one at a time through a three-way valve placed upstream of each respirometer. Individual consumption of pellets was recorded; however, not all fish fed to satiation (as defined by earlier individual consumption rates), presumably because of differences in individual stress responses to placement in a respirometer. Following feeding, individual oxygen consumption rates were recorded during a continuous period of 24-36 hours. Microbial background respiration was recorded inside each respirometer over two measurement cycles (i.e., 30-45 minutes) before each respirometry session. Individual oxygen consumption rates ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{h}^{-1}$) were then calculated for each intermittent-flow cycle following feeding as described earlier for closed respirometry using equation (4).

Corrected oxygen consumption rates were then divided by body mass to calculate mass-specific oxygen consumption rates ($\dot{M}O_2$, in $\mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). A return to maintenance metabolism was used as the endpoint of SDA, and SDA_{dur} was therefore calculated as the time interval (in hours) between feeding and the first point of the oxygen trace to fall below SMR. For each individual, SDA_{peak} was calculated as the highest value of background-corrected, mass-specific $\dot{M}O_2$ (in $\mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) between feeding and the endpoint of SDA_{dur} . Finally, SDA ($\mu\text{mol O}_2 \cdot \text{g}^{-1}$) was estimated by integrating the area under the fitted regression of background-corrected, mass-specific oxygen consumption rates over SDA_{dur} minus SMR. Calculations of SDA_{dur} , SDA_{peak} , and SDA were made using the *fishMO2* R package (Chabot, 2020). The different dimensions of postprandial metabolism reflected by SDA_{dur} , SDA_{peak} and SDA (i.e., duration, maximum intensity, and total costs, respectively) may generate contrasting metabolic strategies (e.g., long, low-intensity digestion vs. short, high-intensity digestion) with distinct consequences for the partitioning of energy within individual aerobic budgets. Measuring SDA_{dur} , SDA_{peak} and SDA

simultaneously may therefore facilitate our understanding of the mechanisms that differentiate digestive phenotypes among and within taxa.

5.2.8 Assimilation efficiency (AE)

To test for differences in assimilation efficiency (AE) between juvenile coho salmon and steelhead trout, faeces previously collected during the gut residence time experiment were dried at 60°C for a week before being weighed with a digital scale. Assimilation efficiency (AE, %) was calculated as

$$AE = \frac{M_{pellets} - M_{faeces}}{M_{pellets}} \times 100 \quad (6)$$

where $M_{pellets}$ is the dry mass of pellets distributed (mg) and M_{faeces} is the dry mass of faeces collected (mg). This approach may overestimate AE because it does not consider leaching of dissolved organics from faeces or alternative elimination pathways such as urine excretion; however, mass of faeces produced remains a relevant comparative index of nutrient assimilation as it is the main path of waste evacuation.

5.2.9 Data analysis

All traits related to growth (i.e., SGR, FC, GE), aerobic metabolism (i.e., SMR, MMR, AS), digestive metabolism (SDA, SDA_{peak} , SDA_{dur}) and food processing (GRT, AE) were compared between coho salmon and steelhead trout using linear mixed models (LMMs; R software v.1.3.1093). All variables were first log-transformed to meet assumptions of normality and homoscedasticity, which were verified using Shapiro-Wilk and F-tests, respectively. To control for variation in body mass between steelhead trout and coho salmon at the end of our experiments and its effects on trait variation, all trait values were allometrically adjusted to the mean final body mass of the 47 fish (i.e., 4.091g) by performing separate fitted regressions of each trait against body mass for each population.

Reluctance of all fish to feed to satiation in the respirometer greatly increased variation in ration and therefore SDA responses among individuals. To control for this variation, all traits that directly depended on meal size (i.e., SDA, SDA_{peak} , SDA_{dur} , GRT, AE) were adjusted to the maximum measured food consumption of each fish (in mg). The maximum meal size of each fish was determined as the higher of the three food consumption estimates determined successively for each fish (i.e., during the two-week feeding treatment [first estimate], at the beginning of the GRT assay [second estimate], and during SDA

experiments [third estimate]). A linear regression between each trait (e.g., SDA) and observed meal size was used to first adjust each trait up to the maximum meal size observed for each individual, thereby standardizing all fish responses to a maximum ration while maintaining residual variation. To control for variation in body size among individuals, linear regressions between body mass and traits adjusted to maximum ration were then used to adjust each trait to the mean final body mass of the 47 fish (i.e., 4.091g).

Following standardization of traits to maximum ration and average body size as described above, separate linear mixed models were used to evaluate the effects of species (i.e., coho salmon vs. steelhead trout; fixed effect) on SGR, FC, GE, SMR, MMR and AS. For responses that were ration-dependent (SDA, SDA_{peak} , SDA_{dur} , GRT and AE), log of maximum meal size was included as a covariate, along with a species x log meal size interaction. Each model also included river (i.e., Coquitlam River or Silverhope Creek) as a random effect to account for potential trait variation between the two populations of each species. Automatic model selection was then used to identify the model with the most parsimonious set of fixed effects for each phenotypic trait (i.e., with the most optimal combination of lowest AIC and highest AIC weight), before an ANOVA was performed using the best mixed model retained (i.e., the model including the best set of fixed effects and river as a random effect). A similar linear mixed model approach was also used to evaluate differences in SDA between species, including GRT and a species x GRT interaction as fixed effects. Tukey's HSD (Honest Significant Difference) tests were used to evaluate significant trait differences among populations.

Because digestive physiology is a multivariate trait, patterns of association among growth, routine metabolism, digestive metabolism and food processing were determined using Principal Component Analysis (PCA), a multivariate analysis used for reducing the dimensionality of large datasets while minimizing information loss. The dataset used for this analysis included the mass-corrected values of nine traits (SGR, FC, GE, SMR, SDA, SDA_{peak} , SDA_{dur} , GRT, AE) for each of the 47 fish that were available for final analysis; MMR and AS were not included in this analysis due to the relative independence (low correlation) of active metabolism to growth and digestion observed in a preliminary PCA. A normalized and centered PCA was performed on standardized traits before the strength and significance of emerging patterns of trait associations revealed by the PCA were assessed through correlation tests among individual traits; P-values from multiple correlations were corrected for false discovery rate using a Benjamini-Hochberg correction.

To compare the fraction of aerobic capacity dedicated to combined digestive and maintenance metabolism between coho salmon and steelhead trout, the sum of SMR and average hourly postprandial oxygen consumption rate ($\mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$; obtained by dividing total SDA by SDA_{dur}) was expressed as a fraction of MMR (in %) for each individual. Three coho salmon from the Coquitlam River presented percentages above 100% and were discarded from the comparison; these outliers may potentially have resulted from an underestimation of MMR if these individuals failed to reach complete exhaustion during measurement of active metabolism, or from an overestimation of SDA or SMR. The percentage of MMR occupied by SMR and SDA combined for the 44 remaining individuals was then compared between species using a linear mixed model including species (i.e., coho salmon or steelhead trout) as a fixed effect and river (i.e., Coquitlam River or Silverhope Creek) as a random effect.

5.3 Results

5.3.1 Standard growth rate (SGR)

Steelhead trout grew significantly faster than coho salmon ($F_{[1,44]} = 432.3$, $P < 0.001$; Fig. 5.1a). Standard growth rate was also significantly higher in Silverhope Creek compared to the Coquitlam River for both coho salmon (+14% on average; Tukey's HSD: $P < 0.001$) and steelhead trout (+26% on average; Tukey's HSD: $P < 0.001$).

5.3.2 Food consumption (FC)

As expected, steelhead trout showed higher maximum FC than coho salmon in both the Coquitlam River and Silverhope Creek ($F_{[1,44]} = 337.1$, $P < 0.001$; Fig. 5.1b). Food consumption was significantly higher in steelhead trout from Silverhope Creek compared to steelhead trout from the Coquitlam River (+99% on average; Tukey's HSD: $P < 0.001$), but FC did not significantly differ between the two populations of coho salmon (Tukey's HSD: $P > 0.05$).

5.3.3 Growth efficiency (GE)

Growth efficiency of steelhead trout was significantly lower than GE of coho salmon ($F_{[1,44]} = 157.0$, $P < 0.001$; Fig. 5.1c). Growth efficiency was also significantly lower in steelhead trout from Silverhope

Creek compared to steelhead trout from the Coquitlam River (-38% on average; Tukey's HSD: $P < 0.001$), but was similar for the two populations of coho salmon (+8% on average in coho salmon from Silverhope Creek; Tukey's HSD: $P > 0.05$). Overall, differences in growth-related traits between species are consistent with the prediction that faster growth of juvenile steelhead trout is supported by maximizing food intake at the cost of lower growth efficiency.

5.3.4 Standard metabolic rate (SMR)

There was no significant difference in SMR between species ($F_{[1,45]} = 1.5$, $P > 0.05$; Fig. 5.1d) or between the two populations of each species (Tukey's HSD tests: $P > 0.05$).

5.3.5 Maximum metabolic rate (MMR)

Steelhead trout showed significantly higher MMR than coho salmon ($F_{[1,44]} = 27.1$, $P < 0.001$; Fig. 5.1e). By contrast, MMR did not significantly differ between populations of the Coquitlam River and Silverhope Creek for both coho salmon (Tukey's HSD: $P > 0.05$) and steelhead trout (Tukey's HSD: $P > 0.05$).

5.3.6 Aerobic scope (AS)

Steelhead trout showed higher AS than coho salmon ($F_{[1,44]} = 22.1$, $P < 0.001$), and the difference between species was similar in Silverhope Creek (+69% on average in steelhead trout; Fig. 5.1f) and in the Coquitlam River (+66%). Within species, steelhead trout and coho salmon had higher AS in Silverhope Creek (+9% and +7% on average, respectively) than in the Coquitlam River; differences between populations were not significant for each species (Tukey's HSD tests: $P > 0.05$).

5.3.7 SDA components (SDA , SDA_{peak} , SDA_{dur})

As expected, juvenile steelhead trout showed higher SDA than coho salmon in both populations ($F_{[1,44]} = 8.6$, $P < 0.01$; Fig. 5.2a). SDA significantly increased with meal size for all populations ($F_{[1,42]} = 21.1$, $P < 0.001$; Fig. 5.2a). There was generally no relation between SDA and gut residence time (GRT: $F_{[1,32]} =$

2.6, $P = 0.12$; Fig. 5.2b), although this may be a consequence of a narrow range of GRT for most populations.

There was no significant difference in SDA_{peak} between species, but SDA_{peak} increased with meal size ($F_{[1,45]} = 70.1$, $P < 0.001$; Fig. 5.2c). Finally, SDA_{dur} differently responded to meal size between coho salmon and steelhead trout, leading to a significant species x meal size interaction ($F_{[1,43]} = 4.3$, $P = 0.04$; Fig. 5.2d); an ANOVA excluding meal size as a covariate indicated a significant difference in SDA_{dur} between species ($F_{[1,44]} = 51.7$, $P < 0.001$).

5.3.8 Gut residence time (GRT)

Steelhead trout and coho salmon did not significantly differ in GRT (Fig. 5.2e). GRT increased with meal size, but this effect was not significant (Fig. 5.2e).

5.3.9 Assimilation efficiency (AE)

As predicted, coho salmon exhibited higher assimilation efficiency than steelhead trout ($F_{[1,44]} = 35.6$, $P < 0.001$; Fig. 5.2f) in both Silverhope Creek and the Coquitlam River. Assimilation efficiency also increased significantly with meal size for all populations ($F_{[1,44]} = 13.2$, $P < 0.001$) but did not significantly increase with GRT (Fig. 5.2g). Although only one ANCOVA model (i.e., SDA_{dur}) retained a significant species x meal size interaction, different relationships between each trait of interest and meal size (i.e., different slopes) between species may somewhat reduce their comparison. Separate analyses of variance (ANOVAs) without meal size as a covariate, however, produced the same results (Table 5.S1, Appendix).

5.3.10 Multivariate associations among growth, digestive metabolism and food processing capacity

The PCA ordination showed strong multivariate differentiation between species on PCA1 (62.5% of explained variation), with a strong negative relationship between GE and a cluster of traits related to postprandial metabolism (i.e., SDA , SDA_{peak} and SDA_{dur}), SGR, and FC (Pearson: from $r = -0.79$ to $r = -0.94$; Fig. 5.3). The second principal component axis (13.7% of explained variation) appeared to represent variation among individuals within a population, where individuals with longer gut residence time tended to have higher AE and lower SMR. The PCA highlighted the strong phenotypic differentiation in digestive physiology and energetics between the two species, with steelhead trout presenting a general suite of

traits related to high food consumption and growth, while coho salmon exhibited an opposite pattern of lower food intake, lower energy expenditure during digestion and lower growth, but higher growth efficiency. This conclusion needs to be tempered, however, by the proviso that strong positive correlations among the three SDA components (i.e., SDA, SDA_{dur}, and SDA_{peak}) may overweight the first axis of the PCA (i.e., PCA1) and disproportionately emphasize differences in digestive physiology and bioenergetics among populations. This effect, however, is likely marginal since a second PCA performed after excluding SDA_{peak} and SDA_{dur} from the dataset (i.e., to retain SGR, FC, SMR, GE, SDA, GRT, and AE) generated a similar pattern of phenotypic differentiation among populations (see Figure 5.S2; Appendix). The first PCA with all measured variables was therefore retained to provide a broader overview of integrated variation in digestive phenotype among populations of coho salmon and steelhead trout.

5.3.11 Differentiation of aerobic budgets between steelhead trout and coho salmon

Steelhead trout juveniles presented higher aerobic scope than coho salmon in both populations (Fig. 5.1f), which resulted from similar SMR between species (Fig. 5.1d) but higher MMR in steelhead trout (Fig. 5.1e). The higher SDA of steelhead trout relative to coho salmon (Fig. 5.2a) was largely compensated by their higher available aerobic scope (Fig. 5.4). As a result, the two species unexpectedly presented similar ratios of metabolic costs to maximum aerobic capacity (i.e., [SMR + SDA]:MMR) with $56 \pm 16\%$ (mean \pm SD) for steelhead trout from the Coquitlam River, $54 \pm 11\%$ for coho salmon from the Coquitlam River, $68 \pm 12\%$ for steelhead trout from Silverhope Creek, and $59 \pm 18\%$ for coho salmon from Silverhope Creek; differences between species were not significant.

5.4 Discussion

5.4.1 Growth vs. growth efficiency trade-off

Comparing growth performance between species of juvenile salmonids exploiting different points along a resource flux (invertebrate drift) gradient allowed the exploration of energetic constraints on early growth differentiation. As expected, a major growth vs. growth efficiency trade-off differentiated faster-growing steelhead trout with high food intake and low growth efficiency from slower-growing coho salmon with lower food intake and higher growth efficiency. Although a growth vs. growth efficiency

trade-off has been suggested as a major axis of phenotypic and ecological differentiation in fish including salmonids (Rosenfeld et al., 2020), its existence does not appear to be universal, particularly at within species levels of divergence. For instance, this trade-off appears to be absent among populations within a species where growth and growth efficiency are often positively correlated (Martens et al., 2014; Allen et al., 2016; Monnet et al., 2020), but a similar trade-off between growth and efficiency may underlie seasonal shifts in growth performance recently observed among individual steelhead trout (Myrvold & Kennedy, 2020). Collectively, these contrasting outcomes suggest that physiological trade-offs are highly context-specific (Careau & Garland, 2012; Careau et al., 2014; Montiglio et al., 2018), and that their expression among individuals, populations and species is contingent on ecological and evolutionary context.

Mechanistically, the lower growth efficiency of the faster-growing phenotype (i.e., steelhead trout) appears to be driven by higher postprandial metabolic costs (e.g., Billerbeck et al., 2000) and lower nutrient assimilation (e.g., Knight et al., 2021) associated in part with a shortened gut residence time. The lower AE and higher postprandial metabolism (i.e., SDA) that we observed in steelhead trout, however, may not fully account for the substantial differences in growth efficiency between species. Some of the decrease in growth efficiency in steelhead trout relative to coho salmon may potentially be explained by differences in the costs of tissue synthesis or protein turnover rate (Allen et al., 2016; Lee & Morishita, 2017), but evidence supporting these mechanisms is scarce. Energy expenditures associated with territorial behavior may also affect growth efficiency by reducing energy allocated to growth (Finstad et al., 2011). The potential for aggressive behaviour to affect energy budgets was largely eliminated in our experiments by rearing fish individually; however, more aggressive behaviour by coho salmon could reduce their growth efficiency in nature (Vøllestad & Quinn, 2003), although such an effect would be unlikely to decrease their growth efficiency below that of steelhead trout.

5.4.2 Multivariate differentiation of digestive strategies along an energy flux gradient

Multivariate associations among growth and digestive traits differentiated coho salmon and steelhead trout along a rate-maximizing vs. efficiency-maximizing continuum of energy acquisition, processing, and use as suggested by earlier studies (Van Leeuwen et al., 2011; Rosenfeld et al., 2020). Faster-growing juvenile steelhead trout emerged as typical rate-maximizers (Finstad et al., 2011) through the elevation of both food intake and postprandial metabolic expenditure, at the cost of lower food processing efficiency (i.e., short GRT, low AE). In contrast, slower-growing coho salmon demonstrated

an alternative suite of traits that was consistent with an efficiency maximizing strategy, with lower maximum food consumption and postprandial metabolism but higher assimilation and growth efficiency. This pattern is consistent with positive associations among food consumption, SDA, and growth previously reported for many fish taxa (Billerbeck et al., 2000; Millidine et al., 2009; Norin & Clark, 2017). This trend, however, was reversed in a recent comparison of digestive performance between piscivore vs. insectivore rainbow trout ecotypes where faster-growing piscivores demonstrated higher food intake and AE, but lower SDA at satiation (GM, manuscript in preparation). Multivariate trait associations between steelhead trout and coho salmon also suggest that increasing maximum food consumption may require a shorter turnover of gut contents to accommodate a larger daily ration. Although optimal digestion theory (Sibly, 1981) predicts that processing larger meals may require evolving shorter food retention times (i.e., GRT) at the cost of lower AE, AE only marginally decreased with shorter GRT in steelhead trout relative to coho salmon (~3%: Fig. 5.2g), despite the shorter steelhead trout GRT for a given ration (Fig. 5.2e). This indicates that decreasing GRT can be an effective strategy to maximize net energy intake when food is abundant, despite marginally lower AE, which appears to be conserved. A more rapid gut transit time may also decrease active transport costs from swimming with a full gut in the higher-velocity riffle habitats occupied by steelhead trout (Thorarensen & Farrell, 2006). More broadly, flexible phenotypic changes in gut length, volume, and resulting transit time may constitute simple and effective controls on growth and growth efficiency in response to variation in prey abundance (Piersma & Lindström, 1997; Nicieza et al., 1994; Armstrong & Bond, 2013).

These results need to be tempered, however, by the proviso that the two populations of steelhead trout did not form a cohesive cluster of phenotypes. Greater intraspecific variation in digestive physiology and bioenergetics in steelhead trout may be of genetic origin; however, it may also be the result, in part, of a less successful acclimation of steelhead trout from the Coquitlam River to laboratory conditions. Despite no apparent disease or mortality in this population throughout the experimental process, steelhead trout from the Coquitlam River appeared to be less active between the beginning and end of the two-week feeding treatment (G. Monnet, personal obs.), with no apparent consequence for food consumption (+25% increase in average FC from week one to week two, against +14% in steelhead trout from Silverhope Creek). Alternatively, the high phenotypic variance between steelhead trout from the Coquitlam River and Silverhope Creek could be affected by differences in life-history strategies between populations. Both rivers contain anadromous (i.e., steelhead) and resident

rainbow trout, and anadromous individuals may present faster growth and higher active metabolism than residents (Kendall et al., 2014). A preponderance of resident trout from the Coquitlam River vs. anadromous trout from Silverhope Creek could contribute to observed differences in growth, digestive physiology and energetics between these populations.

The strong differentiation of digestive strategies between juvenile coho salmon and steelhead trout is largely consistent with their habitat partitioning in the wild along a gradient of low-to-high energy flux habitats (coho salmon: pools; steelhead trout: riffles; Hartman, 1965; Bisson et al., 1988). Although rapid, less-efficient digestion as observed in steelhead trout may be maladaptive in low energy flux environments where prey abundance and foraging opportunities are limited (Armstrong & Schindler, 2011), more rapid extraction of labile energy may be advantageous in habitats with high food availability (Réale et al., 2010) such as riffles where higher velocities increase local flux of drifting invertebrates. In riffle habitats where prey availability is less limiting and energy assimilation is primarily contingent on digestive capacity and efficiency (Hart & Gill, 1992; Armstrong & Schindler, 2011), our results indicate that steelhead trout can overcome the higher metabolic expenditure imposed by foraging and digestion at higher velocities and ultimately grow faster than coho salmon. In contrast, more-efficient digestion as observed in coho salmon maximizes fitness in lower-cost, lower-energy flux habitats such as their preferred deep, low-velocity pools where coho salmon can counterbalance stochastic foraging opportunities with lower metabolic costs associated with foraging and digestion. Rate-maximizing vs. efficiency-maximizing strategies may also manifest in other ecological contexts associated with resource gradients, including ephemeral habitats with stochastic food pulses vs. stable habitats with predictable food incomes (Armstrong & Schindler, 2011; Armstrong & Bond, 2013).

5.4.3 Differentiation of aerobic budgets between steelhead trout and coho salmon

Covariation in digestive and active metabolism between juvenile steelhead trout and coho salmon unexpectedly resulted in a convergence of their aerobic budgets. On average, both species allocated about half of their total aerobic scope (MMR-SMR) to costs of digestion when fed to satiation. This pattern of aerobic budgeting is typical of active feeders that must retain aerobic capacity for locomotory activity (e.g., to avoid predators: Metcalfe et al., 2016), while ambush predators including lionfish (Stell et al., 2019) and sculpins (Sandblom et al., 2014) with less active lifestyles may allocate their entire aerobic capacity to digestion. These estimates, however, are based on the fraction of aerobic scope occupied by SDA_{peak} , which may be appropriate for the opportunistic consumption of a single large meal

by an ambush predator; in contrast, we estimated the proportion of aerobic scope occupied by time-averaged SDA (i.e., average oxygen demand over the duration of SDA) which better represents the somewhat constant reduction of residual aerobic capacity imposed on active swimmers like juvenile salmonids by the frequent consumption of smaller meals of drifting invertebrates. The higher aerobic scope of steelhead trout, which was mostly supported by higher MMR as reported elsewhere (Van Leuween et al., 2011) then emerges as an aerobic surplus to compensate for the increased aerobic demand associated with elevated food intake, thereby avoiding a trade-off between digestive costs and residual aerobic capacity (Auer et al., 2015b; Norin & Clark, 2017; McLean et al., 2018).

5.4.4 Ecological implications of digestive physiology

The ecological implications of digestive physiology in wild fish remain somewhat underappreciated despite their relevance to many ecological processes, including biological invasions (Steell et al., 2019), adaptive differentiation (Rosenfeld et al., 2020), or trophic specialization (Knight et al., 2021). By explicitly demonstrating the multivariate divergence of digestive strategies between juvenile coho salmon and steelhead trout, and how this divergence matches variation in growth and energetics, our study highlights the key role of digestive physiology in potential adaptive differentiation between species that have specialized to different ecological niches along a gradient of resource availability. Because productivity gradients are pervasive in nature, variation in digestive physiology within and across taxa may represent a significant but cryptic source of phenotypic and ecological diversity at a local scale.

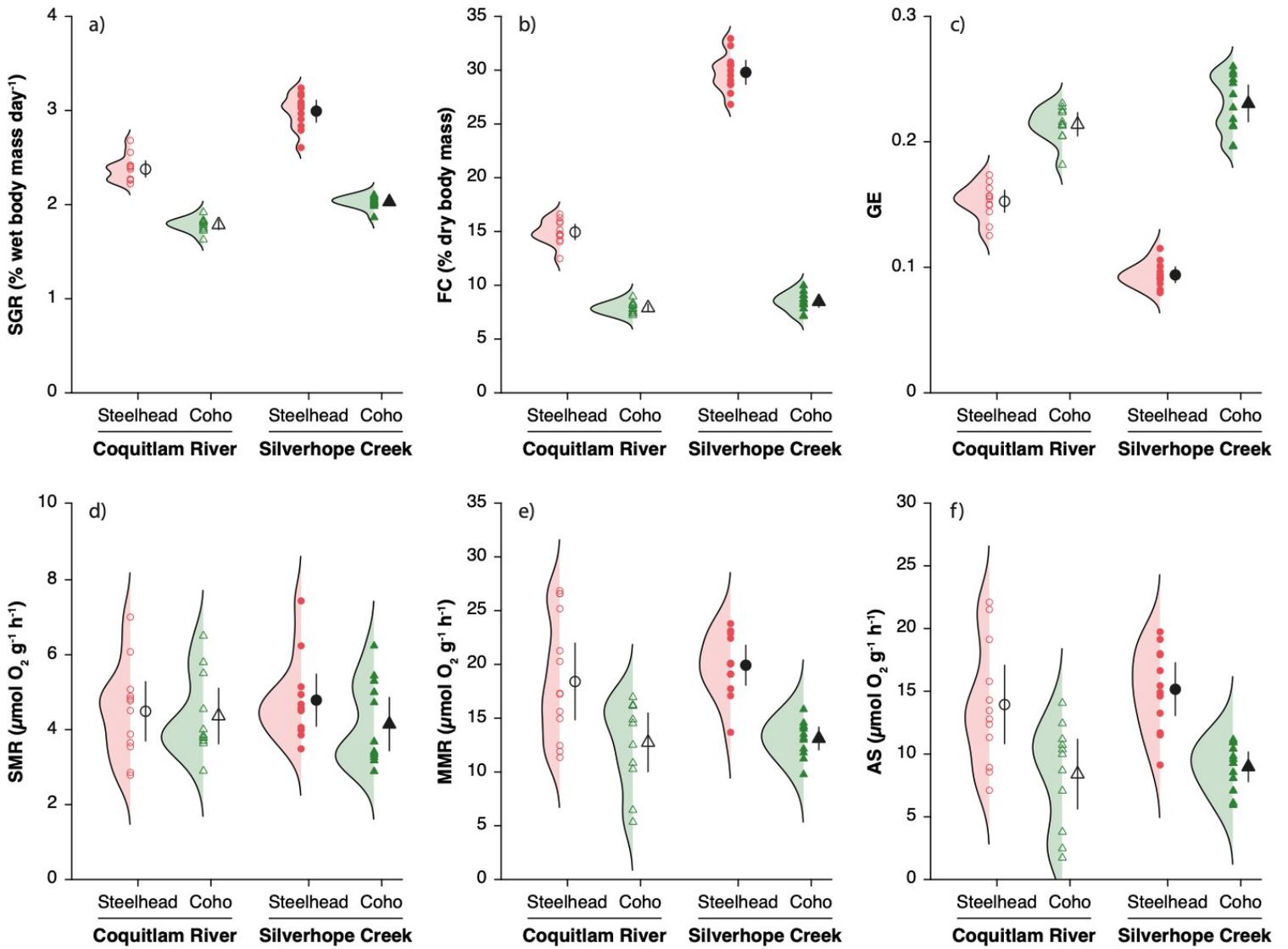


Figure 5.1: Differences in individual standard growth rate (a), food consumption (b), growth efficiency (c), standard metabolic rate (d), maximum metabolic rate (e) and aerobic scope (f) between steelhead trout (circles) and coho salmon juveniles (triangles). Black symbols represent population means, and black vertical lines represent 95% confidence intervals.

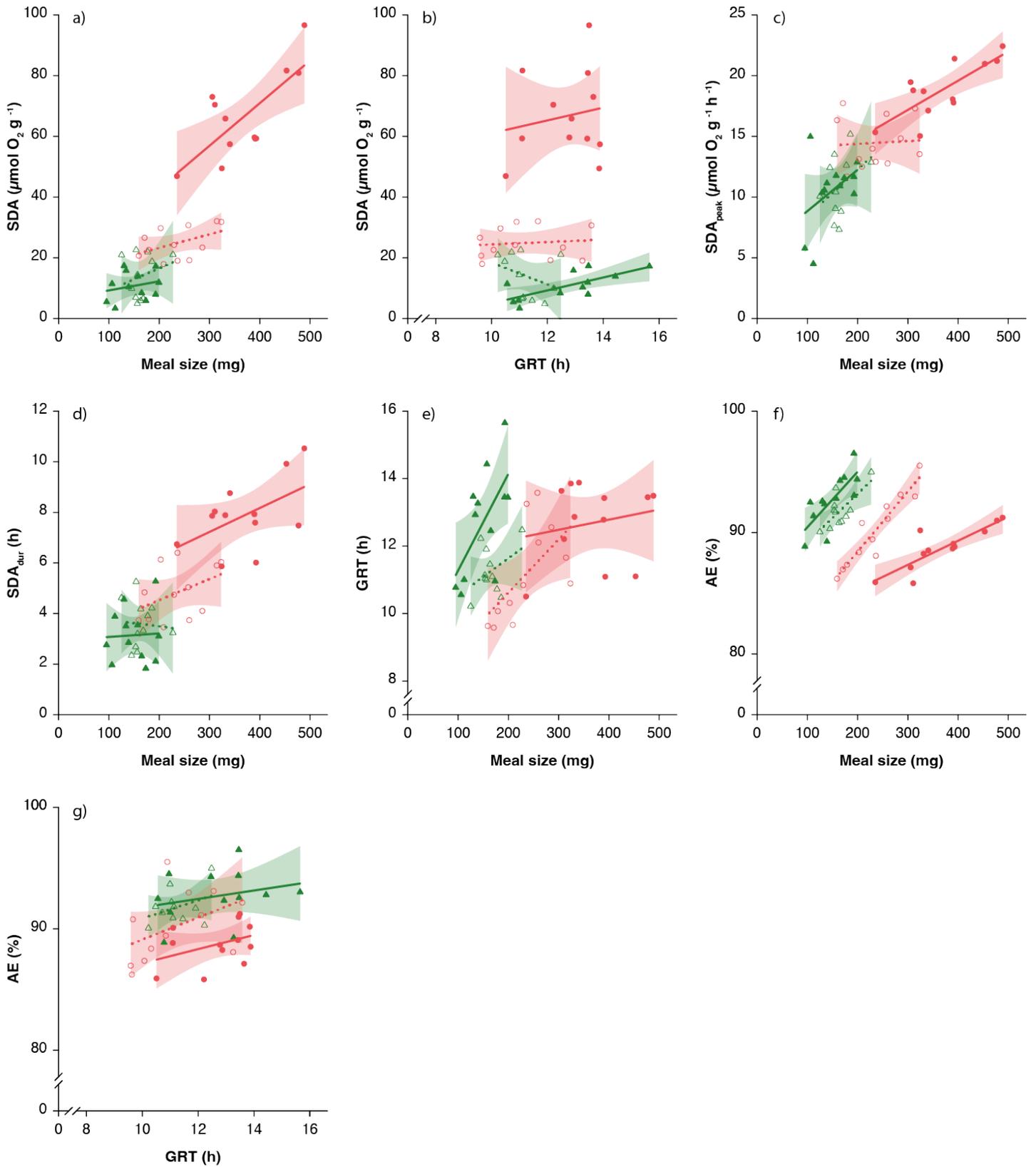
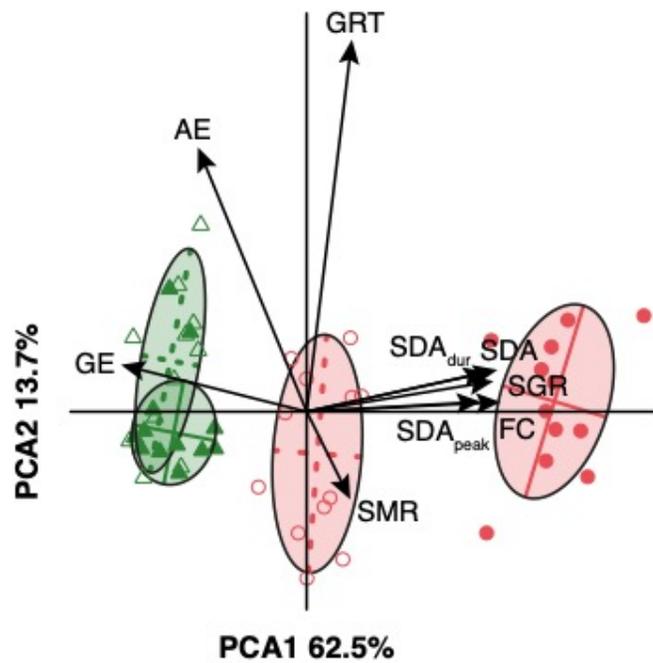


Figure 5.2: Relationships between meal size and SDA (a), SDA_{peak} (c), SDA_{dur} (d), gut residence time (e) and assimilation efficiency (f); and between GRT and SDA (b), and AE (g) for steelhead trout (circles) and coho salmon juveniles (triangles). Trendlines with 95% CI are indicated for each population.



Trait	SGR	FC	GE	SMR	SDA	SDA _{peak}	SDA _{dur}	GRT	AE
SGR	1								
FC	0.94 ***	1							
GE	-0.87 ***	-0.94 ***	1						
SMR	0.13	0.18	-0.19	1					
SDA	0.86 ***	0.93 ***	-0.86 ***	0.13	1				
SDA _{peak}	0.78 ***	0.80 ***	-0.79 ***	0.29	0.87 ***	1			
SDA _{dur}	0.82 ***	0.85 ***	-0.79 ***	0.08	0.92 ***	0.74 ***	1		
GRT	0.27	0.24	-0.08	0.02	0.25	0.16	0.26	1	
AE	-0.48 ***	-0.51 ***	0.51 ***	-0.14	-0.43 *	-0.34 *	-0.46 ***	0.23	1

Figure 5.3: Principal Component Analysis (PCA) including growth-related, metabolic and food processing-related traits with size-adjusted values for steelhead trout (circles) and coho salmon juveniles (triangles). The table indicates the strength and magnitude of emerging patterns of trait associations visible on the PCA; P-values from multiple correlations were corrected for false discovery rate with a Benjamini-Hochberg correction. *: P < 0.05; **: P < 0.01; ***: P < 0.001.

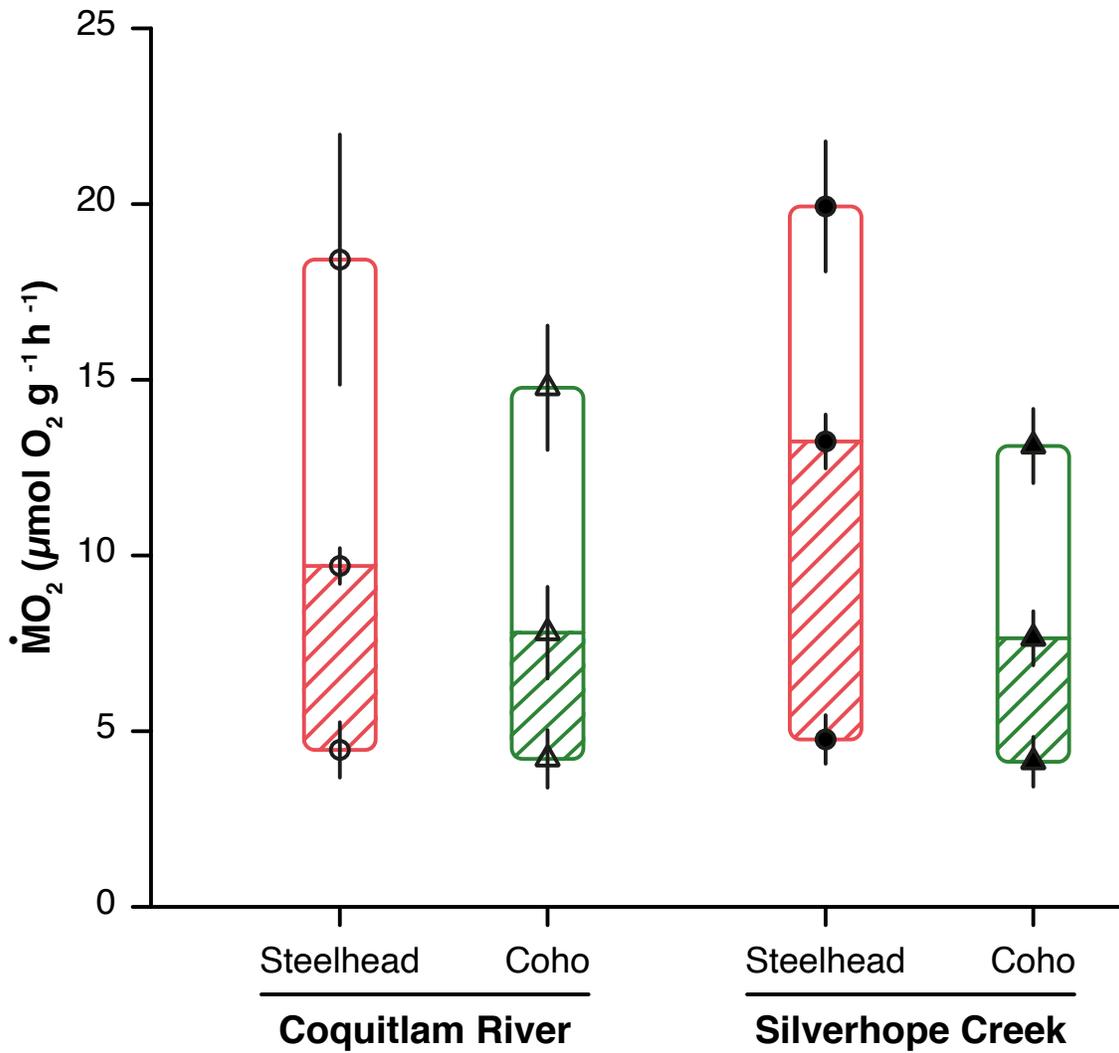


Figure 5.4: Aerobic budgets of steelhead trout (circles) and coho salmon juveniles (triangles). The symbols at the bottom and top of each bar represent SMR and MMR, respectively; the difference between MMR and SMR indicates aerobic scope (AS); the hatched area in each bar represents postprandial metabolism (i.e., SDA). Black symbols represent population means, while black vertical lines indicate 95% confidence intervals.

General discussion

The objectives of this thesis were to understand the mechanisms of multivariate adaptation and trade-offs associated with the evolution of faster growth by comparing behavioural, digestive and bioenergetic strategies of wild ecotypes and species of juvenile salmonids. In the discussion below, I briefly recap the literature on this area of research, before discussing how the findings of this thesis can be integrated into the existing literature and could serve as a basis for future axes of research related to animal bioenergetics.

Reciprocal constraints between juvenile and adult life histories

Growth variation in nature may be influenced by reciprocal constraints linking adult morphology and juvenile growth, whereby achieving larger adult body size within a fixed time frame may indirectly constrain juveniles to a rapid high-growth trajectory (Czarnoleski et al., 2008; Rosenfeld et al., 2015; Sibly et al., 2015; Hughes et al., 2019). Previous work (Martens et al., 2014; Allen et al., 2016) identified higher growth rates in juveniles of large, domesticated rainbow trout compared to juveniles of smaller insectivorous adults, yet the relevance of these results to wild populations of fish was unclear. My results from Chapters 1 and 4 demonstrate that, when reared in laboratory conditions, juveniles of large-bodied piscivorous rainbow trout grew significantly faster than juveniles of small-bodied insectivorous trout, supporting the inference that achieving a larger adult body size within a fixed time frame is most effectively attained by a steeper juvenile growth trajectory in wild fish populations.

Growth variation may also arise from delayed adult spawning and associated fry emergence (Quinn & Peterson, 1996; Post & Parkinson, 2001; Ebersole et al., 2006) which shortens the growing season in high latitude habitats and may select for faster juvenile growth (e.g., Billerbeck et al., 2000). These findings generate an expectation that species with sequential emergence schedules such as early-emerging coho salmon vs. late-emerging steelhead trout should differ along a slow-to-fast axis of juvenile growth. This prediction is consistent with previous studies (e.g., Van Leeuwen et al., 2011) and Chapter 5 of this thesis that experimentally demonstrates the existence of faster growth trajectories in steelhead trout relative to coho salmon and illustrates how variation in adult spawning schedule indirectly conditions growth performance in juvenile fish.

Post-emergence ecological context may override emergence effects to differentiate individual growth trajectories within a population (Metcalf & Thorpe, 1992; Einum & Fleming, 2000), as early-emerging fry may benefit from prior residence to establish territories in optimal rearing habitats where growth can be maximized (Kaylor et al., 2021). In line with previous observations (Irvine 1978a, 1978b), the decreasing gradient of individual growth rates observed along the Lardeau River following post-emergence dispersal largely matched ecological context (i.e., decreasing prey abundance: Chapter 3). Alternatively, decreasing individual growth rates along the Lardeau may also be explained, in part, by inter-individual variation in emergence time that may in turn influence dispersal and access to rearing habitats with different prey availability.

Physiological, anatomical, and behavioural adaptations that promote faster growth

Faster growth can be achieved through suites of adaptations occurring at successive levels of biological organization ranging from physiological processes to behaviours associated with foraging activity (Réale et al., 2010; Careau & Garland, 2012; Rosenfeld et al., 2020). At the physiological level, faster growth often positively correlates with elevated basal metabolism (i.e., standard metabolic rate SMR in ectotherms: Allen et al., 2016, or basal metabolic rate BMR in endotherms: Sadowska et al., 2013), which is known to vary genetically (i.e., within and among taxa: Finstad et al., 2007) and plastically in response to environmental factors including food availability (Van Leeuwen et al., 2011). Because SMR typically increases with ration size and growth performance (Secor & Diamond, 2000; Álvarez & Nicieza, 2005), elevated SMR emerges as adaptive in contexts where food is abundant (e.g., in productive environments: Rosenfeld et al., 2015; Auer et al., 2015b) but maladaptive when prey abundance is limited (Armstrong & Schindler, 2011). This general understanding is consistent with the positive correlations among elevated consumption, fast growth, and high SMR observed in the piscivore vs. insectivore ecotype contrast, (Chapters 1 and 4). Relationships among consumption, growth and SMR were more equivocal at the species level (Chapter 5); although steelhead trout fry presented higher consumption and growth relative to coho salmon when reared at satiation, the two species only marginally differed in SMR as suggested by previous studies (Van Leeuwen et al., 2011). These conflicting results suggest that maintaining high basal metabolism (i.e., SMR) is a key physiological adaptation to support faster growth at the

ecotype level, whereas achieving faster growth may be relatively independent of SMR at the species level. The existence of positive effects of SMR on growth, however, may depend on the capacity of fast-growing taxa to displace the costs of digestion associated with high postprandial metabolism (i.e., SDA: Secor, 2009) to SMR as in the piscivore ecotype that presented low SDA but high SMR at satiation (Chapter 4); in contrast, the different strategy evolved by steelhead trout (i.e, high SDA but low SMR: Chapter 5) may somewhat explain the absence of a positive correlation between growth and SMR at the species level.

Faster growth can also be supported by optimizing anatomical design associated with food processing capacity and efficiency. Typical pathways to maximize energy allocation to growth include evolving larger gastrointestinal tracts to maximize nutrient assimilation (Allen et al., 2016), shorter gut residence time to maximize consumption of new food (GRT: Sibly, 1981; Nicieza et al., 1994), or a larger digestive tract with more stable maintenance demand to reduce overhead costs of digestion (Hammond & Diamond, 1992; Piersma & Lindström, 1997). In line with these expectations, faster-growing piscivore fry with elevated consumption at satiation demonstrated a pattern of longer gastrointestinal tracts, and shorter (or similar) GRT relative to insectivores (Chapters 1 and 4), suggesting that minimizing food retention times and a permanently larger gut to reduce overhead costs of digestion are effective strategies to accommodate larger rations in support of faster growth. Although shortening GRT is expected to decrease cumulative absorption efficiency (AE: Sibly, 1981), piscivores with shorter (or similar) GRTs also presented higher AE than insectivores, suggesting the existence of additional adaptations (e.g., greater paracellular absorption rates: Caviedes-Vidal et al., 2008) that maximize nutrient assimilation despite rapid intestinal transit in the piscivore ecotype. Relationships among food consumption, GRT, and AE at the species level were largely consistent with expectations generated by the literature (Sibly, 1981; Rosenfeld et al., 2020) since, relative to slower-growing coho salmon, faster-growing steelhead trout presented similar GRT but lower AE in response to elevated food (Chapter 5). These results support the suggestion that minimizing GRT is a key adaptation to maximizing energy assimilation and growth when food is abundant, and illustrate how flexible phenotypic changes in gut structure and function constitute simple and effective controls on growth in response to variation in prey abundance as suggested elsewhere (Piersma & Lindström, 1997; Nicieza et al., 1994; Armstrong & Bond, 2013).

Achieving faster growth also requires developing proactive personalities (e.g., greater risk-taking or aggressiveness: Vøllestad & Quinn, 2003; Réale et al., 2010) to maximize foraging activity (Biro & Stamps, 2008; Mittlebach et al., 2014). This general understanding is consistent with the differentiation of slower-growing insectivore and faster-growing piscivore ecotypes along a broad reactive vs. proactive behavioural axis (Koolhaas et al., 1999; Sih et al., 2004b); relative to insectivores, more proactive piscivore fry presented an overall pattern of lower response to fear and routine fidelity (Chapter 1), but also greater boldness and risk-taking (Chapter 2). This conclusion, however, depends on the trait and population considered, and the existence of large behavioural variation among insectivore populations somewhat reduces the coherence of proactive vs. reactive behavioural axis and the fast vs. slow growth axis that differentiate piscivore from insectivore ecotypes. Although these results illustrate the positive effects of behavioural proactivity on growth, proactive behaviours may in turn compromise other fitness correlates not explicitly studied in this thesis, and may for example increase predation risk and associated mortality through a fast growth vs. low survival trade-off (Lankford et al., 2001; Biro et al., 2004; Stamps, 2007).

Physiological trade-offs associated with faster growth

Identifying physiological trade-offs underlying growth variation was a major motivation of this thesis. I explored five trade-offs and identified three as key sources of variation in energy use and growth within or among taxa. I address each of these below.

Fast growth vs. low aerobic scope trade-off

Fast-growing taxa typically present elevated basal metabolism (e.g., SMR) as a common physiological response to high food availability or selection for high growth (Arnott et al., 2006; Rosenfeld et al., 2015). High basal metabolism may in turn reduce aerobic scope (AS, the residual aerobic capacity after accounting for basal metabolic costs: Chabot et al., 2016) by mediating a fast growth vs. low aerobic scope trade-off as repeatedly suggested in fish (e.g., Billerbeck et al., 2000; Killen et al., 2014). A fast growth vs. low aerobic scope trade-off largely differentiated rainbow trout ecotypes since faster-growing piscivore fry presented higher SMR and reduced AS relative to slower-growing insectivores (Chapter 1). Consistent with previous studies on similar fish models (Reinbold

et al., 2009; Allen et al., 2016), these results illustrate the detrimental effects of high growth performance on active metabolic capacity at the population level through an elevation of basal metabolism (Careau & Garland, 2012). Interestingly, a fast growth vs. low aerobic scope trade-off was not clearly present at the species level where faster-growing steelhead trout presented similar SMR but higher active metabolic capacity (i.e., higher MMR and AS: Chapter 5) relative to slower-growing coho salmon. The rather neutral effects of growth on active metabolism at the species level conflicts with conclusions at the ecotype level, which illustrates the high context-dependence of physiological trade-offs (Careau, 2017; Montiglio et al., 2018).

High consumption vs. low residual aerobic scope trade-off

Maximizing food consumption to support faster growth (Cox & Secor, 2007; Allen et al., 2016) typically elevates digestive metabolism (i.e., specific dynamic action SDA: Billerbeck et al., 2000; Millidine et al., 2009; Norin & Clark, 2017). As SDA increases with meal size, a trade-off may arise between feeding vs. retaining aerobic scope for additional physiological processes (e.g., activity or predator avoidance: Auer et al., 2015a; Norin & Clark, 2017). Differences in digestive metabolism between ecotypes diverged from this general understanding since piscivores with greater consumption presented an unexpected pattern of lower SDA relative to insectivores, which likely resulted from the capacity of piscivore fry to displace digestive costs from SDA to SMR (Chapter 4). Although SDA and aerobic scope were not measured simultaneously, which prevented a clear comparison of aerobic budgets between ecotypes, maintaining a low SDA despite greater consumption (Chapter 4) may somewhat compensate for a lower aerobic scope in the piscivore ecotype (Chapter 1). Overall, these results do not clearly support the existence of a high consumption vs. low residual aerobic scope trade-off (Auer et al., 2015b; Norin & Clark, 2017; McLean et al., 2018) at the ecotype level. A high consumption vs. low residual aerobic scope trade-off was also not clearly present between species since, relative to coho salmon, the higher aerobic scope of steelhead trout reported in Chapter 5 and elsewhere (Van Leeuwen et al., 2011) largely compensated for higher SDA associated with elevated food intake. High postprandial metabolism therefore emerges as a strong counterpart to faster growth at the species level, yet the fraction of aerobic budgets allocated to digestive metabolism (SDA) can be compensated by a higher aerobic

scope. However, the existence of large phenotypic variation between the two populations of steelhead trout in Chapter 5 somewhat complicates the interpretation of differences in digestive physiology between coho salmon and steelhead trout, and suggests the potential for substantial variation in digestive performance within a species.

Organ-size trade-offs

Differences in size and activity among organs determines their contribution to basal metabolism (Elia, 1992; Wang et al., 2012). Competing metabolic costs among organs creates a potential for organ-system trade-offs (Rosenfeld et al., 2015; Longman et al., 2017) where, for example, animals may preferentially invest in larger gastrointestinal tracts with high metabolic demand (Secor et al., 1994; Secor & Diamond, 2000) at the cost of competing cognitive and cardiovascular tissues (Aiello & Wheeler, 1995; Álvarez & Metcalfe, 2007). Organ-system trade-offs appeared to differentiate piscivore and insectivore ecotypes since, relative to insectivores, piscivores presented a pattern of larger stomachs, longer guts, and smaller hearts (Chapter 1), which is consistent with their overall strategy to maximize feeding capacity and efficiency at the cost of lower aerobic scope. The existence of clear differences in brain size between piscivore and insectivore rainbow trout, however, remains uncertain due to different relationships between relative brain size and body mass associated with marginal overlap in body size between ecotypes (Chapter 1). More broadly, the role of organ-system trade-offs in selection for high growth remains somewhat equivocal (Careau & Garland, 2012) since smaller relative organ size may arise simply as a consequence of selection for faster somatic growth rather than any direct selection on organ size (O'Regan & Kitchener, 2005). Because organ size was not explicitly measured in the coho salmon-steelhead trout comparison, it remains unclear whether organ-system trade-offs also occur at the species level, especially since organ-system trade-offs were not clearly observed in other vertebrates (e.g., in birds: Chappell et al., 1999; and mammals: Sadowska et al., 2013) including fish (e.g., among individual brown trout: Norin & Malte, 2012).

High consumption vs. low nutrient absorption trade-off

Maximizing consumption to support faster growth (Reinbold et al., 2009; Martens et al., 2014) may also induce trade-offs in anatomical design associated with digestion (Afik & Karasov, 1995). For instance, optimal digestion theory (Sibly, 1981) predicts that distinct digestive strategies may arise from optimizing the balance between short gut residence time (GRT) to accommodate larger meals vs. increasing food retention time to maximize assimilation efficiency (AE: Nicieza et al., 1994; Price et al., 2015). These predictions are not supported by differences in digestive physiology between ecotypes since food consumption and AE were relatively insensitive to GRT; as a result, piscivores with elevated food consumption presented similar GRT and higher AE relative to insectivores (Chapter 4). Piscivores may have mitigated a short or similar retention time vs. low nutrient absorption trade-off by evolving larger gastrointestinal tracts relative to insectivores (Chapter 1) which, along with shorter GRT, may increase passive nutrient uptake while reducing transit duration and associated food turnover. In contrast, a short retention time vs. low nutrient absorption trade-off somewhat differentiated digestive strategies between coho salmon and steelhead trout since elevated food intake in steelhead trout juveniles resulted in similar GRT and marginally lower AE. Overall, these results illustrate how selection for faster growth may require flexible phenotypic changes in gut structure and function (e.g., minimizing intestinal transit) to maximize energy assimilation at the potential cost of lower nutrient assimilation as suggested elsewhere (Piersma & Lindström, 1997; Nicieza et al., 1994; Armstrong & Bond, 2013).

Fast growth vs. low growth efficiency trade-off

Faster growth often correlates with lower growth efficiency (i.e., the ratio of mass gained to food consumed: Afik & Karasov, 1995; Rosenfeld et al., 2020), whereby superficial nutrient extraction from large meals in support of fast growth may reduce growth efficiency. Consistent with previous studies on similar fish models (Martens et al., 2014; Allen et al., 2016), a high growth vs. low growth efficiency trade-off was not present between rainbow trout ecotypes since piscivore fry presented both higher growth and higher growth efficiency relative to insectivores (Chapter 1). As expected, a growth vs. growth efficiency trade-off differentiated steelhead trout and coho salmon along a fast growth, low efficiency vs. slow growth, high efficiency axis of bioenergetics (Chapter 4), thereby illustrating the lower efficiency with which faster-growing phenotypes (e.g., steelhead trout)

typically process and convert acquired energy in support of faster growth due to underlying metabolic and physiological constraints associated in part with higher food consumption (e.g., higher postprandial metabolic costs: Billerbeck et al., 2000; or lower nutrient assimilation: Cox & Secor, 2007).

Integrated phenotypic differentiation along productivity gradients

Multivariate trade-offs among physiological, anatomical, behavioural, and life-history traits are central in holistic concepts like the Pace-Of-Life Syndrome framework (Réale et al., 2010) or the energy (rate)-maximizing vs. efficiency-maximizing continuum of energy use (Rosenfeld et al., 2020). The predictions of the Pace-Of-Life Syndrome were largely consistent with the positive associations among fast growth, high basal metabolism, and overall proactive behaviours that differentiated piscivore and insectivore integrated phenotypes (Chapters 1, 2, and 4). However, the low active and digestive metabolisms observed in the piscivore ecotype (Chapters 1 and 4) conflicted with the predictions of the Pace-Of-Life framework where faster-growing phenotypes are expected to present high metabolism, irrespective of the metabolic pathways involved. In addition, the Pace-Of-Life framework underappreciate alternative axes of phenotypic variation such as the growth vs. growth efficiency trade-off (Rosenfeld et al., 2020) that may contribute to differentiate integrated phenotypes among and within taxa (e.g., Chapter 5). Although the Pace-Of-Life framework appears to be an accurate conceptual framework for predicting trait divergence between specialized phenotypes, refining its predictions with respect to growth vs. active metabolism or growth vs. growth efficiency may foster a more complete understanding of the evolutionary mechanisms underlying adaptive phenotypic differentiation.

Overall, integrated performance in energy use was largely coherent with niche partitioning along natural productivity gradients at the ecotype level, since faster-growing and proactive piscivore fry with high bioenergetics (i.e., fast phenotype) rear in a productive habitat with high prey availability that supports elevated energy assimilation and use (Chapter 1). In contrast, lower prey abundance in smaller tributaries around Kootenay Lake where insectivores feed coupled with reactive foraging behaviours appear to drastically limit juvenile bioenergetics and growth in the insectivore ecotype.

The predictions of the energy (rate)-maximizing vs. efficiency-maximizing continuum of energy use suggested by earlier studies (Finstad et al., 2011; Rosenfeld et al., 2020) were broadly consistent with the associations among fast growth, high digestive metabolism, reduced GRT at high ration, low AE, and low growth efficiency that differentiated coho salmon and steelhead trout integrated phenotypes (Chapter 5). Steelhead trout emerged as typical rate-maximizers (Van Leeuwen et al., 2011; Finstad et al., 2011) prioritizing elevated food intake and faster growth at the cost of higher digestive metabolism, lower nutrient uptake, and lower growth efficiency. In contrast, slower-growing coho salmon demonstrated an alternative suite of traits that was consistent with an efficiency maximizing strategy (Finstad et al., 2011; Rosenfeld et al., 2020), which matched their habitat partitioning along natural gradients of low-to-high energy flux habitats in coastal streams (coho salmon: pools; steelhead trout: riffles; Hartman, 1965; Bisson et al., 1988). Rapid extraction of labile energy therefore appears to be advantageous in habitats with high food availability (Réale et al., 2010) such as riffles where higher velocities increase local flux of drifting invertebrates and allow steelhead trout to overcome the higher metabolic expenditure imposed by foraging and digestion at high velocities and ultimately grow faster than coho salmon. In contrast, more-efficient digestion as observed in coho salmon maximize fitness in lower-cost, lower-energy flux habitats (Armstrong & Schindler, 2011; Armstrong & Bond, 2013) such as preferred deep, low-velocity pools where coho salmon can benefit from lower metabolic expenditure associated with foraging and digestion.

Overall, the ecotype and species comparisons illustrate the benefits of matching integrated phenotypes with bioenergetic performance and habitat specialization. Environmental productivity (i.e., prey availability) therefore emerges as a strong driver of phenotypic specialization in relation to energy assimilation and use within and among taxa.

Post-emergence dispersal along multiple environmental gradients in the piscivore rearing streams marginally differentiated integrated phenotypes among piscivore individuals with contrasting dispersal capacity (Chapter 3). The significant decrease of growth performance along an upstream-to-downstream gradient of prey availability in the Lardeau River, however, supports the major contribution of environmental productivity to growth variation at the individual level.

Stability of observed trade-offs from populations to species

None of the different trade-offs explored during this thesis was systematically present between populations and species of salmonids. This lack of consistency suggest that physiological trade-offs are highly context-specific as suggested elsewhere (Careau & Garland Jr., 2012; Careau et al., 2014; Montiglio et al., 2018), with local idiosyncrasies in ecological and evolutionary context driving variability in trait associations that underlie adaptive differentiation of individuals, populations and species. The existence of different physiological trade-offs may therefore result from selection on alternative life history axes irrespective of growth, including landlocked (i.e., piscivore and insectivore ecotypes) vs. anadromous (i.e., coho salmon and steelhead trout) reproductive cycles. Further research aiming to evaluate the evolutionary mechanisms underlying physiological adaptation across successive levels of taxonomic organization should therefore integrate other axes of life-history differentiation in addition to growth variation among populations and species.

Caveats and limitations

Comparing the degree of phenotypic divergence between piscivore and insectivore ecotypes was somewhat limited by the lack of replication in the piscivore ecotype that only included one population (i.e., Kootenay Lake). Although piscivore rainbow trout are present in multiple lakes throughout British Columbia (e.g., Arrow, Babine, and Okanagan lakes: Parkinson & Arndt, 2014), collecting piscivore fry in these lakes was compromised by local constraints (e.g., small populations, lack of access). As a result, it remains unclear whether the phenotypic divergence reported in this thesis between piscivore and insectivore rainbow trout from Kootenay Lake also applies to populations from other systems, or if the existence of phenotypic variance within the piscivore ecotype reduces its phenotypic and ecological differentiation from the insectivore ecotype. Exploring the existence of similar patterns in phenotypic divergence among piscivore and insectivore populations from other lake systems would provide insights into the consistency of the selective pressures that underlie their phenotypic differentiation.

The extent to which phenotypic variation observed between ecotypes (i.e., piscivores vs. insectivores) and species of juvenile salmonids (i.e., steelhead trout vs. coho salmon) under laboratory conditions would be maintained in the wild is also uncertain. Recent studies have reported some degree of mismatch between behavioural trends measured in artificial vs. natural

settings (e.g., Larsen et al., 2015), so that measuring key bioenergetic and behavioural traits in the field would help assess the ecological relevance of trait variation observed under laboratory conditions. Although measuring metabolism in the field is a challenge for aquatic organisms including free swimming fishes, recent technological advances (e.g., biotelemetry and accelerometry: Treberg et al., 2016; determination of the isotopic composition of otoliths: Chung et al., 2019) should facilitate our ability to estimate metabolic rates under field conditions and are worth exploring.

This thesis only characterized phenotypic variation at the juvenile life stage. The degree to which observed phenotypic differentiation is maintained over ontogeny (i.e., from juveniles to adults) remains speculative, and the experimental demonstration of such phenotypic stability over longer time scales is costly and challenging. Performing similar bioenergetic and behavioural comparisons between adults of piscivore vs. insectivore rainbow trout, and between adults of steelhead trout vs. coho salmon would provide meaningful insights into the persistence of phenotypic variation over ontogeny, and by extension into the stability of selective pressures differentiating integrated phenotypes across successive life stages.

Interpretation of the adaptive nature of integrated trait variation observed among individuals, populations and species of salmonids in this thesis must be somewhat qualified. Assessing how physiological, behavioural and life-history traits differentiate integrated phenotypes among and within taxa in response to variation in ecological context (e.g., prey availability) is a first and necessary steps towards a better understanding of phenotypic evolution. The extent to which observed phenotypic variation is truly adaptive, that is, heritable and resulting in greater fitness, remains, however, a question to be explored in more detail. Repeated observations of multivariate phenotypic differentiation among individuals, populations and species of salmonids reared in common environment (e.g., Van Leeuwen et al., 2011; Allen et al., 2016; G. Monnet, this thesis) provides preliminary support for the genetic determinism of adaptive variation associated with growth and bioenergetics. Confirming the adaptive nature of functional variation between ecotypes and species of salmonids would require performing comparative genetic studies (see Grummer et al., 2021 for example). An alternative solution would be to undertake similar measures and comparisons following reciprocal transfer experiments in the field, or between pure parental types and hybrids reared in common garden conditions from the egg stage.

Finally, the use of analyses of covariance (ANCOVAs) throughout this thesis must be tempered by the existence of biological constraints that somewhat reduced the comparability of trait values between ecotypes and species. For instance, limited overlap in body size between ecotypes initially limited their comparison (Chapter 1, 2017 data), before comparing piscivore and insectivore populations across similar ranges of body size in 2018. The degree of overlap in maximum meal size at satiation was also reduced between piscivores and insectivores (Chapter 4) and between coho and steelhead (Chapter 5). Nevertheless, individuals from different ecotypes or species with overlapping ration sizes often greatly differed in digestive performance as suggested by the minimal overlap (if any) of their 95%CI (for example, see differences in SDA between insectivores from Redfish Ck. and piscivores at a meal size of ~27mg, Fig. 4.2.a, Chapter 4). Altogether, the limited gap in meal size (if any) between ecotypes or species and the minimal overlap of 95%CI (if any) for traits that were detected as significantly different between ecotypes and species may marginally reduce the capacity of ANCOVA to detect trait differences between ecotypes or species. In addition, significant interactions between the categorical variable (e.g., population) and the covariate (e.g., meal size) may complicate interpretation of main effects. Such interactions were detected for three different traits throughout this thesis, including relative brain size (Chapter 1), SDA_{dur} (Chapter 5) and SDA (with GRT as a covariate: Chapter 5). These interactions were alleviated, in part, by performing one-way ANOVAs after discarding the covariate (i.e., body mass or meal size) from the initial models and by tempering my conclusions. Finally, in several instances I also used non-statistically significant linear regressions to correct trait values before their comparison between ecotypes or species. Although some of these regressions were not significant for some populations (e.g., $P = 0.53$ for AE in the piscivore population, Fig. 4.3b, Chapter 4), using these regressions to correct individual trait values to a common body mass or to maximum meal size was still valuable to account for marginal differences in mass or underfeeding on measured trait values.

A retrospective look at my thesis

Ecological specialization and associated phenotypic differentiation are generally approached from a habitat use perspective where the adaptive nature of phenotypes is assessed by their ability to occupy different habitats, particularly in salmonids (e.g., Young, 2004). Although this method is

effective at identifying morphological (Morinville & Rasmussen, 2006) and behavioural trade-offs (Naman et al., 2021), a habitat use approach may undervalue the contribution of cryptic physiological trade-offs to phenotypic differentiation and among taxa. In line with previous work (Van Leeuwen et al., 2011; Allen et al., 2016), this thesis used a comparative physiology approach to explore the degree of bioenergetic differentiation between taxa and how it related to habitat use. I proceed below to a quick retrospective assessment of my decisions throughout my PhD.

The nature of my research and the high mortality of wild juvenile fish under laboratory conditions required me to measure multiple traits (e.g., growth rate) multiple times on separate batches of fish. Although performing recurrent measurements was time consuming, this effort was somewhat mitigated by my rapid gain in efficiency associated with repeating similar rearing and experimental protocols in my successive PhD chapters.

Similarly, measuring a variety of phenotypic traits in a relatively large number of fish was an interesting way for me to diversify my scientific interests and skills, but was also a long and tedious task; I believe in retrospect that measuring multiple overlapping traits (e.g., exploration and activity, or boldness and risk-taking) could have been avoided to save time and explore alternative research topics, such as the genetic basis of functional variation between piscivore and insectivore rainbow trout ecotypes (e.g., Grummer et al., 2021). More time could also have been dedicated to better replicate the artificial productivity gradient with three ration levels (Chapter 1) in Chapter 4 and 5 to ameliorate my understanding of the plasticity of digestive performance in response to food availability.

Lastly, my thesis included limited fieldwork aside from fish and invertebrate sampling; in retrospect, I believe that incorporating more field experiments into my thesis would have allowed me to further diversify my skills as a scientist and would have increased the applicability and ecological relevance of my results.

General conclusions and applications

This thesis assesses the contribution of multiple physiological and behavioural adaptations to growth variation among individuals, populations and species of salmonids. Multiple physiological trade-offs associated with growth emerged as fundamental mechanisms driving differentiation of

integrated phenotypes along productivity gradients, and therefore represent significant sources of phenotypic and ecological diversity within and among taxa. The absence of universal trade-offs across intra- and interspecies comparisons, however, suggests that the expression and magnitude of physiological trade-offs directly depends upon local variation in evolutionary and ecological contexts.

This research can inform the conservation and management of the piscivore ecotype of rainbow trout from Kootenay Lake. This rare population of large-bodied trout is of interest for local recreational fisheries that generate \$5-10 million a year to the local economy (Andrusak & Andrusak, 2012), and the low abundance of piscivore adults currently observed raises a need for a better understanding of the evolutionary and ecological mechanisms that drive their juvenile development. This thesis has presented evidence that piscivore trout have evolved to optimize all the compartments of their phenotype to maximize juvenile growth, and that the rapid development of piscivore juveniles is contingent on high rearing habitat productivity in the Lardeau River. Future research should consider investigating the degree to which piscivore and insectivore ecotypes differ at the biochemical level (e.g., digestive enzymes, tissue composition) to complement the phenotypic screening started in this thesis; alternatively, piscivore and insectivore rainbow trout ecotypes may constitute relevant fish models to determine the effects of the gut microbiome on early growth, as recently suggested in zebrafish (Falcinelli et al., 2015).

From a theoretical perspective, this thesis provides a better understanding of the evolutionary mechanisms that underlie the emergence, maintenance and evolution of contrasting growth trajectories, bioenergetic strategies and overall phenotypic variation among individuals, populations and species of wild vertebrates. It also provides insights into the potential adaptive nature of bioenergetic variation by exploring how related taxa specialize to adjacent habitats along ecological gradients of resource availability that match their integrated performance in energy use. Because productivity gradients are pervasive in nature, physiological trade-offs and associated bioenergetic variation within and among taxa may therefore represent cryptic but significant sources of phenotypic and ecological diversity at local scales.

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Appendix

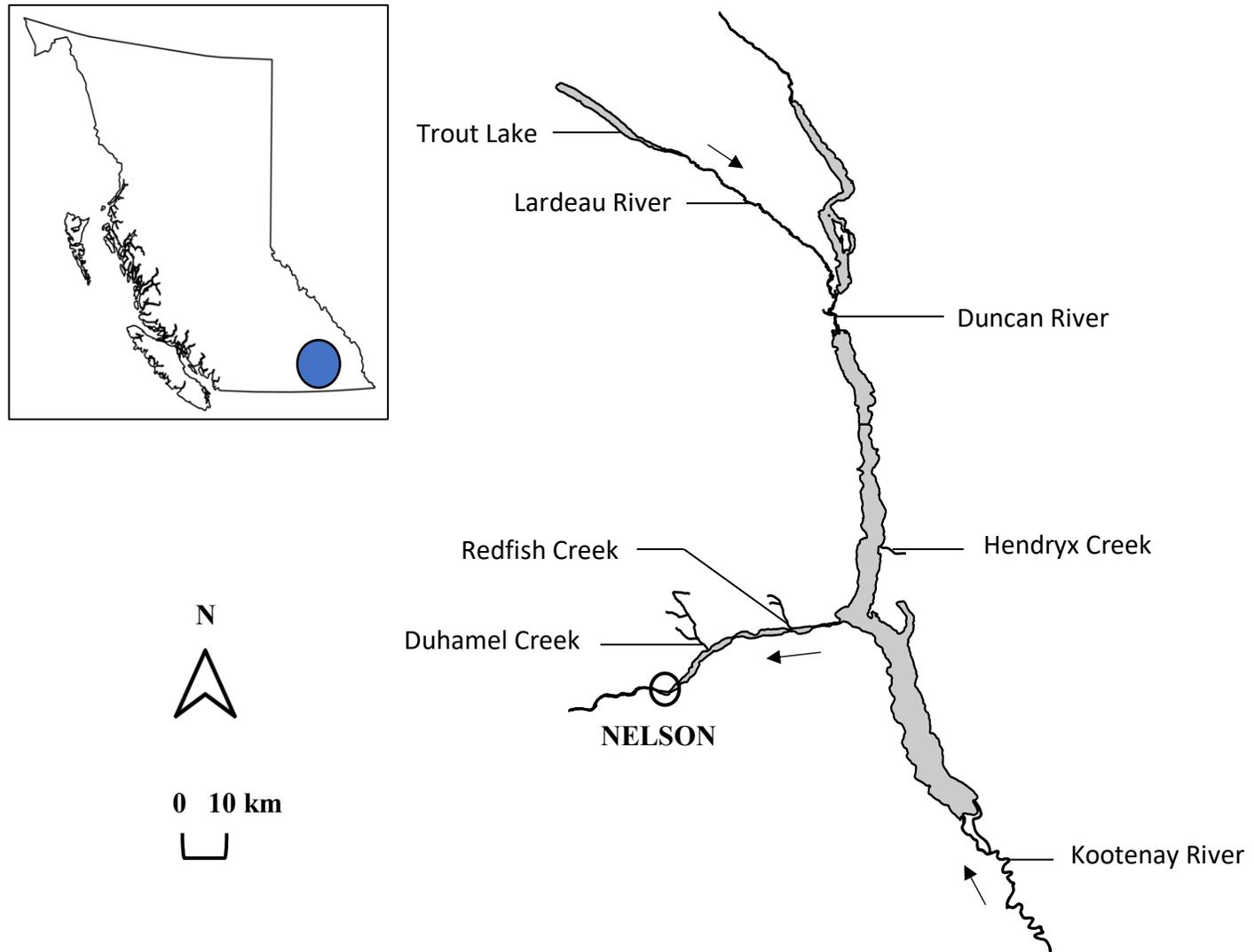


Figure A.1. Location of the piscivore (Lardeau River) and insectivore (Redfish, Duhamel and Hendryx Creeks) streams studied around Kootenay Lake (British Columbia, Canada).

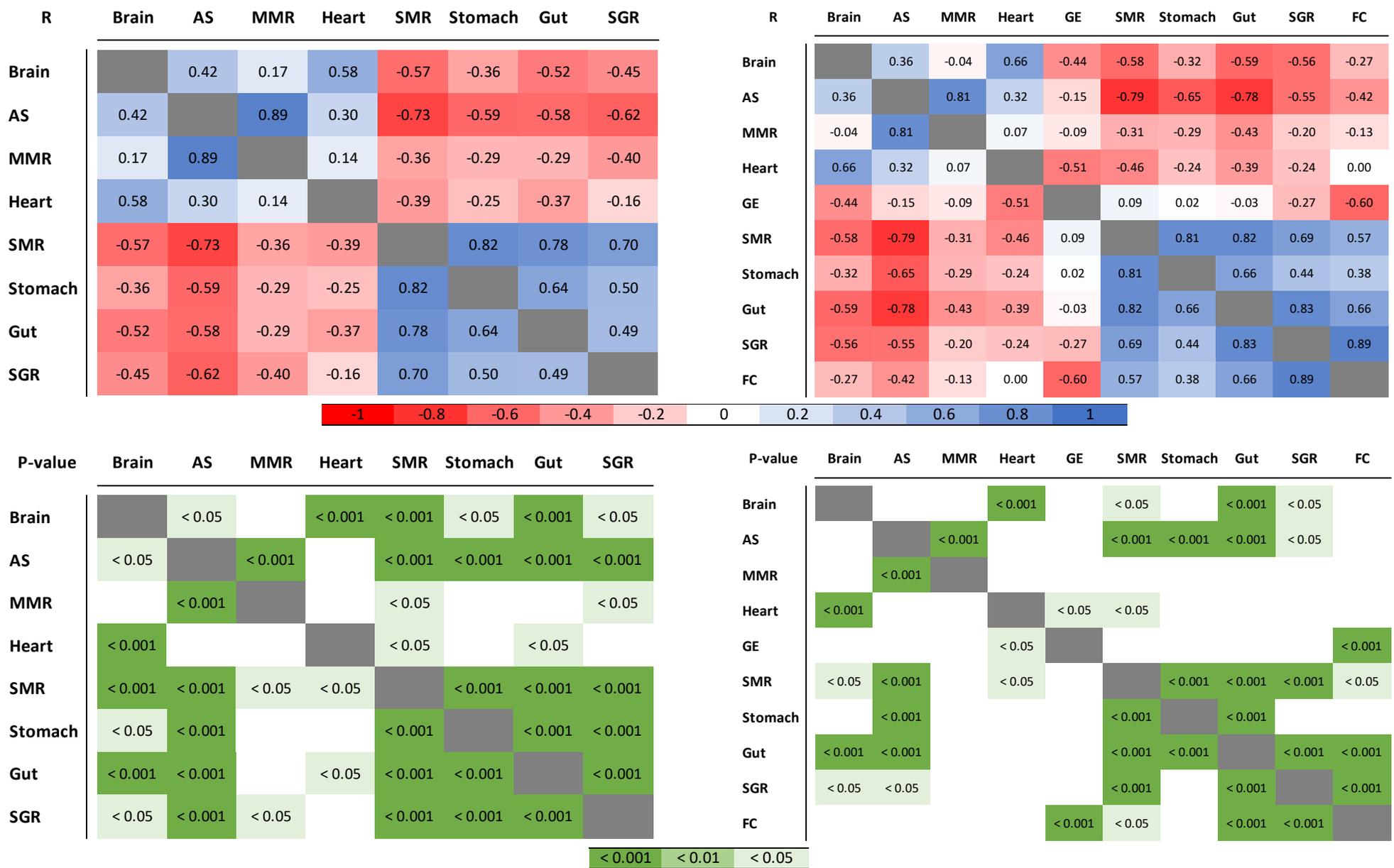


Figure A.2. Correlations (above) and associated-P-values (below) among the eight traits measured at low, mid and high ration (left), and all 10 traits measured at mid and high ration (right). P-values were corrected for false discovery rate.

Table A.1. Comparison of the analyses on mass-corrected traits (this manuscript) vs. mass-uncorrected traits, i.e. where body mass was dropped from the analysis for each measured trait.

Trait	Differences in body mass among subpopulations	Population effect (Mass-corrected data)	Population effect (Mass-uncorrected data)
SGR	Kruskal-Wallis: P = 0.36	Kruskal-Wallis: P < 0.001	Kruskal-Wallis: P < 0.001
MMR	Kruskal-Wallis: P = 0.10	Kruskal-Wallis: P = 0.41	Kruskal-Wallis: P = 0.33
CTmax	Kruskal-Wallis: P = 0.13	ANOVA: P = 0.23	ANOVA: P = 0.46
Boldness	Kruskal-Wallis: P = 0.10	Kruskal-Wallis: P = 0.24	Kruskal-Wallis: P = 0.23
Exploration	Kruskal-Wallis: P = 0.10	Kruskal-Wallis: P = 0.14	Kruskal-Wallis: P = 0.68
Sociability	ANOVA: P = 0.67	ANOVA: P = 0.58	ANOVA: P = 0.13

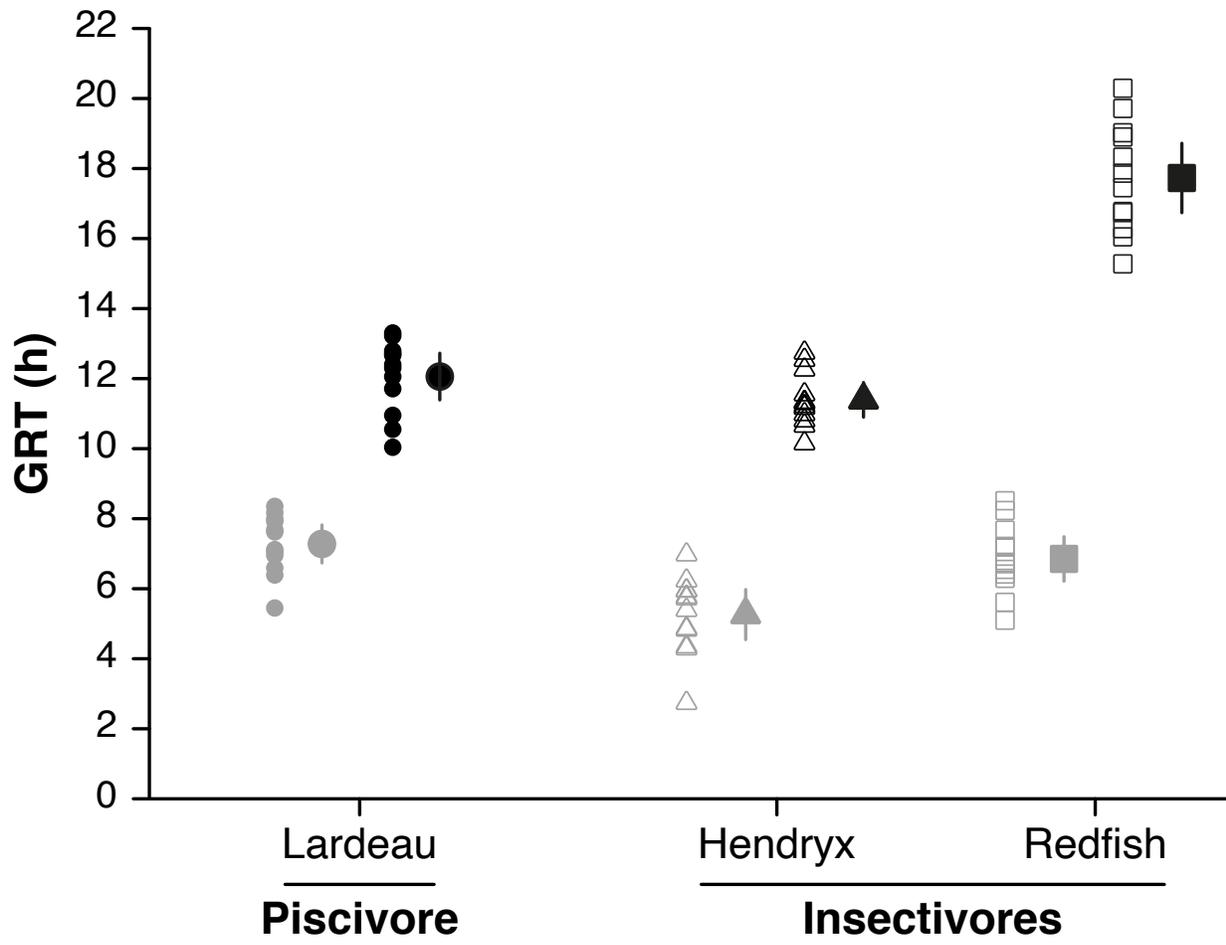


Figure A.3: Differences in GRT between piscivore fry (dots) and insectivore fry from Hendryx Creek (triangles) and Redfish Creek (squares) reared at mid ration (grey) and satiation (black). Large symbols represent population means, and vertical lines represent 95% confidence intervals.

Table A.2: Pairwise correlations among FC, SGR, SMR, and absolute SDA, SDA_{peak}, SDA_{dur}, GRT and AE. *: P < 0.05; **: P < 0.01; ***: P < 0.001. All P-values were adjusted for multiple comparisons with a Benjamini-Hochberg correction.

Trait	FC	SGR	SMR	SDA	SDA _{peak}	SDA _{dur}	GRT	AE
FC	1							
SGR	0.82 ***	1						
SMR	0.77 ***	0.73 ***	1					
SDA	-0.49 ***	-0.73 ***	-0.57 ***	1				
SDA _{peak}	0.48 **	0.04	0.39 *	0.30	1			
SDA _{dur}	-0.66 ***	-0.43 *	-0.62 ***	0.36 *	-0.67 ***	1		
GRT	-0.17	-0.44 **	-0.21	0.37 *	0.39 *	-0.34	1	
AE	0.65 ***	0.71 ***	0.49 ***	-0.60 ***	0.18	-0.56 ***	-0.11	1

Table A.3: Analyses of variance (ANOVAs) testing for differences in traits among populations (i.e., one piscivore, and two insectivores). These ANOVAs discard the effects of meal size to account for differences in slopes between each trait of interest (e.g., SDA) and meal size among populations. These results are similar to those obtained by ANCOVAs in the main text of the manuscript.

Trait	Fixed effect	F	P
SDA	Population	33.31	<0.001
SDA _{peak}	Population	9.78	<0.001
SDA _{dur}	Population	18.96	<0.001
Rel-SDA	Population	225.36	<0.001
Rel-SDA _{peak}	Population	29.54	<0.001
Rel-SDA _{dur}	Population	40.76	<0.001
GRT	Population	101.40	<0.001
AE	Population	17.40	<0.001

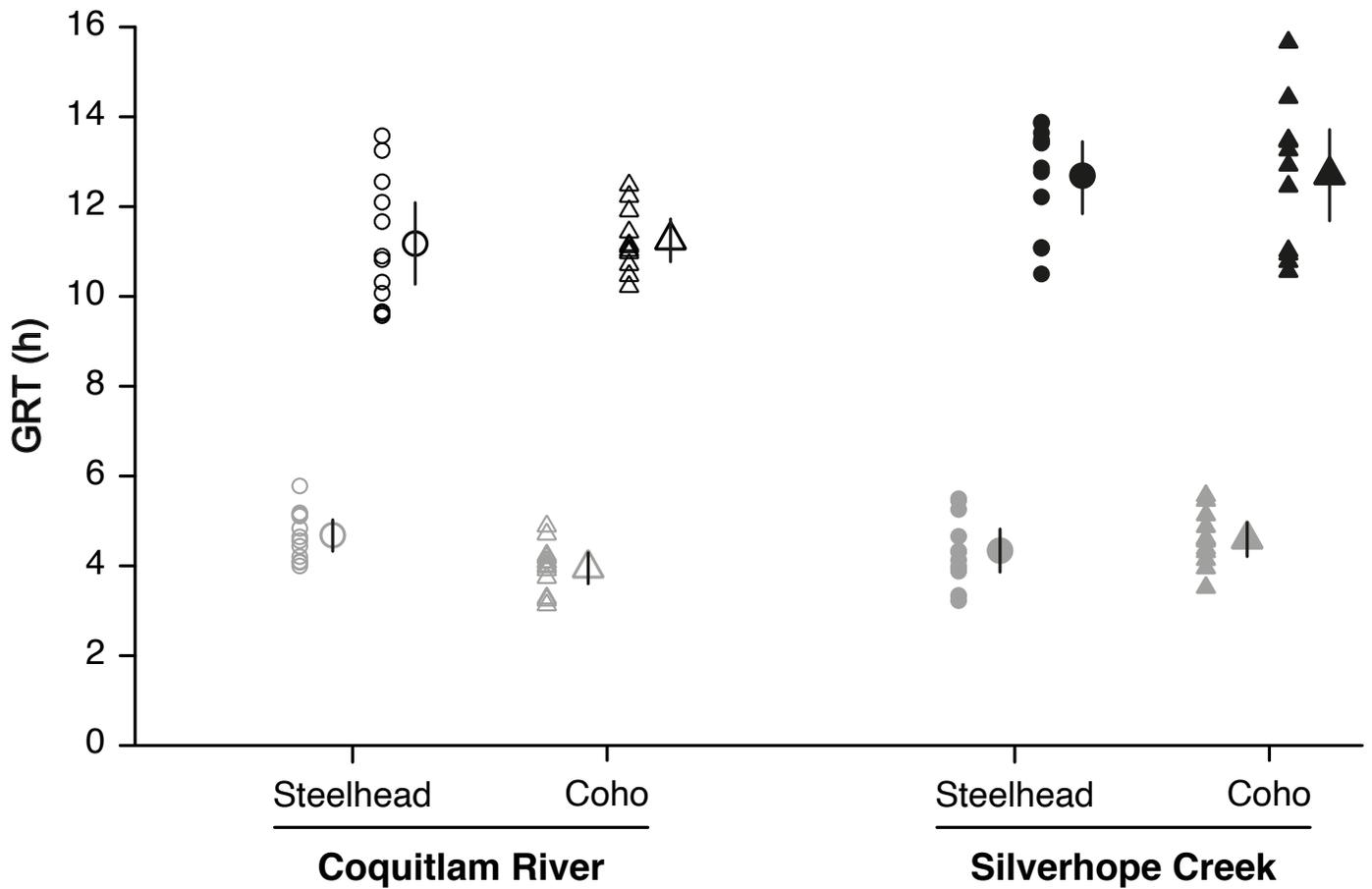


Figure A.4: Differences in GRT between steelhead trout (circles) and coho salmon juveniles (triangles) reared at mid ration (grey) and satiation (black). Black symbols represent population means, and black vertical lines represent 95% confidence intervals.

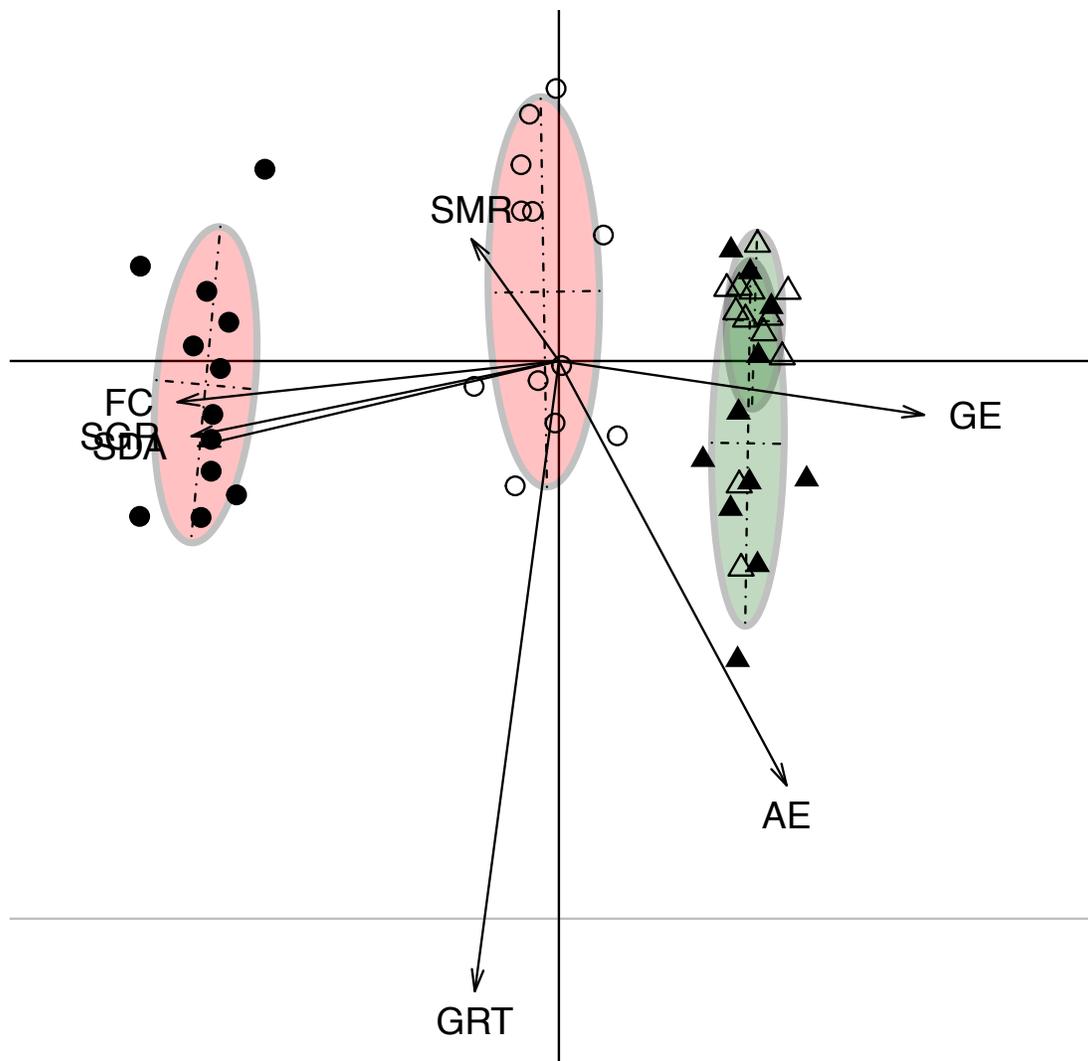


Figure A.5: Principal Component Analysis (PCA) including growth-related, metabolic and food processing-related traits, but with SDA_{peak} and SDA_{dur} excluded, using size-adjusted values for steelhead trout (circles) and coho salmon juveniles (triangles).

Table A.4: Analyses of variance (ANOVAs) testing for differences in traits between species. These ANOVAs do not include the effects of meal size to account for differences in slopes between each trait of interest (e.g., SDA) and meal size between species. Results are similar to those obtained by ANCOVAs in the main text of the manuscript. One exception is SDA_{peak} which significantly differs between species in the ANOVA model (see below), when a species effect was not retained in the ANCOVA model for SDA_{peak} .

Trait	Fixed effect	F	P
SDA	Species	76.50	<0.001
SDA_{peak}	Species	43.56	<0.001
SDA_{dur}	Species	51.70	<0.001
GRT	Species	0.02	0.88
AE	Species	19.46	<0.001