Relationships between environmental conditions, energetic strategies and performance in juvenile Atlantic salmon, *Salmo salar*

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Abstract

Energy is the fundamental currency of life that drives organismal growth and development. Energy requirements vary greatly between species but also within species due to differences in physiology, behaviour and life history. The consequence of this variation is of great interest to ecologists, as it is potentially a trait upon which natural selection can act. One of the main components of an organism's energy budget is its baseline level of metabolism, hereafter termed its standard metabolic rate (SMR). It has been shown in several species of salmonid fish that a high standard metabolic rate correlates with dominance, aggression and boldness. This competitive advantage has been shown to result in higher growth over conspecifics in simple lab environments, but the ecological consequences are less clear.

This thesis examined the performance of contrasting metabolic strategies across a range of environmental conditions to ascertain the ecological consequences of SMR variation. Experiments also investigated the relationships between SMR, food intake and absorption efficiency to help relate energetic strategies to performance. The effects of environment on the outcome of different energetic strategies were profound. Higher population densities increased intraspecific competition for preferable feeding territories, but fish with a higher SMR tended to be the best competitors and so were most likely to get a preferred territory (Chapter 2). However, for a given quality of feeding territory, whether relatively good or poor, lower SMR individuals grew best due to their lower energy requirements.

The benefit to high SMR fish of being able to secure better territories was diminished under less predictable feeding conditions, and disappeared under a structurally complex habitat, resulting in these fish having no performance advantage over fish with a lower SMR (Chapter 3). These high SMR individuals performed poorly in the presence of low densities of a heterospecific competitor, being subject to a disproportionate proportion of the aggression from a more dominant species (brown trout, Chapter 4). At higher densities of trout, intraspecific interactions appeared much more important for both species, resulting in the salmon with the highest SMR exhibiting the fastest growth. These

three chapters demonstrate that environmental conditions, both abiotic and biotic, have great consequences for the success of different energetic strategies. The consequences of metabolic strategy on physiology proved just as interesting. High SMR individuals expended more energy when digesting a given size of meal but reduced the duration of this specific dynamic action (SDA, the rise in metabolism associated with processing and digesting a meal) response (Chapter 5). This suggested that their digestion was more rapid than that of low SMR fish, but this did not lead to a higher rate of food consumption (Chapter 5) nor did they sacrifice absorption efficiency (Chapter 6).

This thesis demonstrates that the performance of fish with alternative energetic strategies is dependent on the prevailing environmental conditions, which helps explain the persistence of variation in SMR within populations.

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Candidate’s declaration

I declare that the work recorded in this thesis is entirely my own. The work described in this thesis is my own except where specifically acknowledged. No part of this thesis has been submitted for any other degree or qualification.

Donald Reid

January 2012

1. Introduction

1.1. Energy budgets and metabolism

All animals require energy to perform life processes. Energy flow enables the survival and reproduction of organisms and is the basic currency of life (Karasov & Rio 2007). Food is consumed to fuel somatic growth, reproductive investment, activity and body maintenance (Kleiber 1961). Metabolic rate (MR) is the rate at which an animal oxidizes food to produce energy and is therefore considered a fundamental measure in ecology and evolution (Brown *et al*. 2004). As some energy is lost in waste products, energy budgets are used to compare the quantity of energy entering and leaving an organism (Blaxter 1989). The energy budget is described by the general equation:

$$C = G + R + E$$

C represents the energy content of consumed food, G represents energy that is used toward growth (i.e. tissue synthesis), R represents the total energy of metabolism and E represents the energy lost in excreted waste products (Brett & Groves 1979; Jobling 1994). R can be further subdivided into R_s, R_{SDA} and R_a, where R_s is standard metabolism, R_{SDA} is specific dynamic action and R_a is energy devoted to activity. Standard metabolism is the minimal rate of metabolism of an inactive fasted animal that will sustain life at a given temperature; it is termed standard metabolic rate (SMR) in ectotherms (McNab 1988; Lucas & Priede 1992; Frappell & Butler 2004; Hulbert & Else 2004). Basal metabolic rate (BMR) is the equivalent term that refers to the minimal metabolism of endotherms in their thermoneutral zone. Both SMR and BMR thus correspond to the same trait in different taxa and for simplicity SMR will be used hereafter in this thesis as a single term to express the minimal metabolism of an animal.

Specific dynamic action is the rise in metabolism associated with digesting and processing a meal (McCue 2006). These subcomponents of R help provide a more informative description of an energy budget:

$$C = G + R_s + R_{SDA} + R_a + E$$

Energetics is of great ecological importance and managing the energy budget is an essential task for any organism. The ability to invest in growth is of great fitness consequence (Niewiarowski 2001) as body size can determine survival chances, fecundity and Darwinian fitness (Arendt 1997 and references therein). Examination of the above equation shows that growth rate can be increased by increasing energy intake (C) and/or by minimising energy expenditure (R_{SDA}, R_a or R_s) and/or by minimising energy lost in excreted waste products (E). It is thus clear that different energetic strategies could increase individual growth rate to the same extent (Metcalfe 1986). This leads to the expectation that there may be variation in metabolic rate both between and within species.

It has long been known that larger animals have relatively slower metabolic rates than smaller animals (Kleiber 1932). The relationship between mass and metabolic rate is allometric, so that metabolic rate scales with the mass of an organism (Kleiber 1947). Metabolic rate is also dependent on temperature (White, Phillips, & Seymour 2006). The metabolic theory of ecology (MTE) provides a mechanistic explanation for these relationships between metabolic rate, body mass and temperature, where metabolic rate is limited by the transport rate of energy and materials *in vivo* (West, Brown, & Enquist 1997; Brown *et al.* 2004). Metabolic rate dictates the rate of food consumption, which in turn determines growth, with higher growth rates speeding senescence and influencing life history. If the mechanisms controlling organismal metabolism are consistent and ubiquitous, metabolic rate will govern all biological processes within individuals and ultimately ecosystems (Brown *et al.* 2004; Allen & Gillooly 2007). MTE is not without its opponents, with challenges made to the value of scaling exponent to the over simplicity of linking individual metabolism to the

complexity of ecosystems (O'Connor *et al*. 2007; Killen, Atkinson, & Glazier 2010). Despite objections to MTE there is much evidence to support a link between metabolic rate and ecological processes, and it is well acknowledged that energy flow is a key component in ecology (Hannon 1973; Odum 1977; Deangelis 1980).

1.2. Standard metabolic rate and variation

In addition to SMR and BMR, resting metabolic rate (RMR) is also used to describe levels of minimal metabolism but is less rigorous as it allows individuals to be in a digestive state (Speakman, Krol, & Johnson 2004). Active metabolic rate (AMR) is the maximum aerobic metabolic rate that can be achieved by the animal. Factorial metabolic scope (AMR/SMR) is used to describe the degree to which metabolic rate can be increased (Fry 1947), but this potential increase can also be expressed as absolute metabolic scope (AMR-SMR).

SMR is usually measured by recording oxygen consumption rate (Hulbert *et al*. 2004), and is usually expressed as oxygen consumption per unit weight (or as a residual after correction for weight) since metabolic rate increases with body size (Biro & Stamps 2010). SMR is an intrinsic physiological trait within individual organisms that has been shown to be repeatable over time (Bech, Langseth, & Gabrielsen 1999; Marais & Chown 2003; Labocha *et al*. 2004; Nespolo & Franco 2007). It represents the energy upkeep for the animal that is continual and apparently unavoidable. Measures of SMR are much higher in endotherms than ectotherms, due to the costs of thermoregulation (Gillooly *et al*. 2001), but SMR nonetheless accounts for a similarly large proportion of daily energy expenditure (DEE) in both endotherms and ectotherms, generally between 25 and 45% (Hulbert & Else 2000).

Variation in SMR between species has been given much attention and has been found to reflect aspects of their physiology, ecology and life history, such as maximal metabolic rate, life span, brood size, population density and diet (Elgar & Harvey 1987; White & Seymour 2004). As such it is both theoretically

interesting and a practically useful biological trait to measure. The relationship between a species' SMR and these aforementioned traits might suggest SMR is relatively consistent between individuals; intriguingly this is not the case.

Historically the variation found within species was neglected and incorrectly considered mostly error or caused by atypical individuals around a 'true' average (Bennett 1987), overlooking the potential consequences of this variation for fitness, selection and evolution (Feder, Bennett, & Huey 2000). Furthermore, inter-individual differences in energetics may correlate with differences in other aspects of physiology (Speakman *et al*. 2004) and behaviour (Careau *et al*. 2008), highlighting its relevance for the performance of the individual and its interaction with the physical and social environment.

Intraspecific variation in SMR is now increasingly being observed across taxa even for individuals of similar size and age class (Hayes, Garland, & Dohm 1992; Kvist & Lindstrom 2001; Cruz-Neto & Bozinovic 2004; Steyermark *et al*. 2005). Some seasonal variation has been found in average SMR values for a species due to energetically demanding periods such as migration and reproduction (Kvist *et al*. 2001; Sparling, Speakman, & Fedak 2006). Where energy requirements are increased due to a higher SMR, greater food intake is necessary to meet this constraint. As SMR is discerned to be repeatable, these differences are of great ecological interest because individuals then vary in the costs of body maintenance. Understanding why there is intraspecific variation in SMR and its consequence are key areas for ecologists to explore.

The cause of intraspecific variation in SMR must be either genetic, environmental or an interaction between these two factors (including the role of maternal effects). Heritability studies have produced mixed results, showing both low (Dohm, Hayes, & Garland 2001; Nespolo, Bacigalupe, & Bozinovic 2003a) and moderate heritability estimates (Sadowska *et al*. 2005; Ronning *et al*. 2007). This suggests metabolic phenotype is also governed by an environmental component, such as maternal effects or early-life environment. The repeatability of SMR suggests that the influence of the environment on an individual's SMR may be most important in early life, after which it may be much less malleable. Regardless of the causal mechanism, if SMR becomes relatively fixed from an early age, the consequences of the resulting inter-individual

variation in SMR will persist throughout life, and are the focus of most of this thesis.

1.3. Relationships between intraspecific variation in SMR, physiology and behaviour

SMR can be thought of as the maintenance cost of the body's physiological machinery, which is mainly comprised of the internal organs. The most important of these (in terms of their energy consumption) are the heart, liver, kidney, brain and intestines (Hulbert *et al*. 2000). A higher SMR reflects higher idling costs of these organs and there is some evidence of a link between SMR and organ size (Chappell *et al*. 2007 and references therein). Possibly linked to this is the relationship between SMR and maximal metabolic rate, field metabolic rate and heart rate (White *et al*. 2004). This suggests a higher SMR may allow for a more active lifestyle.

Compelling and recurring relationships have been found between metabolic rate and behaviour. A high SMR has been linked to behavioural dominance (Bryant & Newton 1994; Metcalfe, Taylor, & Thorpe 1995) and aggression (Cutts, Metcalfe, & Taylor 1998). Social dominance can in turn lead to acquisition of preferable feeding sites or territories (Bryant *et al*. 1994; McCarthy 2001), providing the necessary food energy to support a high SMR and grow. This results in the exclusion of subordinates from the best feeding areas, resulting in their having lower feeding rates (Appleby 1980; Fausch 1984), although subordinates with lower metabolic operating costs may grow equally well without the need to acquire preferable feeding sites. Boldness, or risk-taking behaviour, is associated with SMR (Finstad *et al*. 2007b) and dominance (Adriaenssens & Johnsson 2011), and so may increase the chance of locating new resources. Recent studies have found dominance, aggression and boldness to be repeatable related behaviours (Bell, Hankison, & Laskowski 2009 and references therein). Individual behaviours that are consistent across time and situations are referred to as animal personalities, behavioural syndromes or consistent individual behavioural

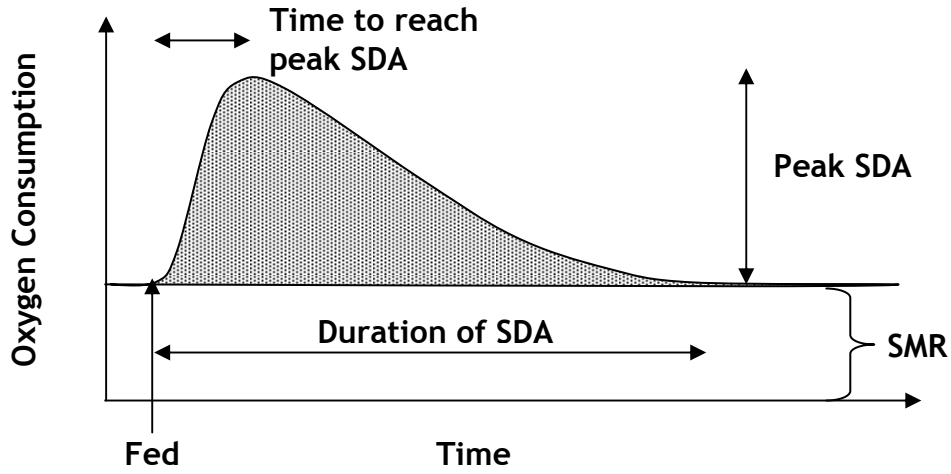
differences (Reale *et al*. 2007; Biro *et al*. 2010). From these studies we can begin to see how behavioural strategies are linked to intrinsic physiological traits such as SMR. What is less clear is how differing SMR individuals with contrasting behavioural strategies will perform in natural environments. The links between SMR and behavioural traits have been well documented in juvenile Atlantic salmon, which makes it an ideal study species in which to investigate how these traits influence performance. It is likely that different combination of behavioural-physiology strategy suit particular habitats and ecological contexts, because within-population variation in SMR persists across generations. This makes juvenile Atlantic salmon an ideal model species to examine relationships between environmental conditions, energetic strategies and performance. This thesis therefore aims to assess the performance of different metabolic-behavioural strategies of juvenile salmon across environments that reflect natural habitat heterogeneity.

1.4. Digestion and specific dynamic action

Digestion is the process by which ingested food is converted to useable energy to maintain SMR and fuel activity. Digestive processes and efficiency vary greatly across taxa, and are crucial to the interplay between physiology and ecology (Karasov & Diamond 1988). An individual's effectiveness in digestion is often summarised as its absorption efficiency, usually defined as the percentage of ingested food energy that is absorbed across the gut wall (Jobling 1994). Specific dynamic action is used to describe the rise in metabolic rate associated with processing and digesting a meal (Secor 2009). The physiological mechanisms that cause SDA are still not fully understood, although many explanations have been offered. The overall causation is the requirement of energy for catabolism, absorption and anabolism (McCue 2006). The SDA response is a part of the previously described energy budget. It can be seen as a metabolic cost, yet a process that also leads to the acquisition of essential substrates and energy.

The effect of a single meal on SDA is well documented in many animals (Guinea & Fernandez 1997; Nespolo, Bacigalupe, & Bozinovic 2003b; Pan *et al*. 2005; Fu, Xie, & Cao 2005c), and can be summarized as the response shown in Fig. 1.1 below.

Figure 1.1 Diagram illustrating the increase in oxygen consumption following a meal known as Specific Dynamic Action. The shaded area represents the magnitude of the SDA response. Modified and used with permission from Karen Millidine.



This has been extremely useful to illustrate the metabolic response of an animal to a single meal, but is not necessarily indicative of the real situation faced by the animal in its natural environment. In the wild, food items are not available at set times but are encountered sporadically. This is true of juvenile Atlantic salmon, whose potential food intake is dependent on the heterogeneous habitats they inhabit, as well as time of day (Martin-Smith & Armstrong 2002). While the idealized response shown in Fig. 1.1 assumes that the animal is able to fully digest and process one meal before consuming another, it will often be the case that the subsequent meal is consumed before metabolic rate has dropped to its baseline (SMR) level, resulting in a secondary SDA response which might peak at a higher level than the initial SDA response (Overgaard, Andersen, & Wang 2002; Fu, Xie, & Cao 2005b). Moreover, the SDA response is not fixed within an individual but varies with body size (Beaupre, Dunham, & Overall 1993; Clarke & Prothero-Thomas 1997), temperature (Secor, Wooten, & Cox 2007), meal size (Secor & Faulkner 2002; Fu *et al*. 2005c) and meal type (Secor *et al*. 2002).

In juvenile Atlantic salmon, an individual's SMR is correlated with its SDA magnitude and SDA duration: fish with a higher SMR tend to have a greater magnitude of SDA but shorter duration of SDA for a given meal size (Millidine, Armstrong, & Metcalfe 2009). This is ecologically relevant, as the duration of the SDA response is indicative of digestion speed, while the magnitude of the SDA response is indicative of the metabolic cost of digesting a meal. If individuals with a high SMR can process meals faster, they may be able to feed again sooner and exploit profitable feeding locations. Combined with the previously described SMR linked behavioural traits, this reinforces the potential for different combinations of behavioural-physiology strategy to suit alternative habitats. Therefore a further aim of this thesis is to test whether individual variation in SMR is related to the potential maximum rate of food intake and whether a rapid SDA is associated with a decrease in absorption efficiency.

1.5. Ecology and lifecycle of the Atlantic salmon

Atlantic salmon are distributed across western and northern Europe to the Eastern seaboard of Canada and North USA (MacCrimmon & Gots 1979). Populations are generally anadromous, although a minority of Atlantic salmon populations are comprised of fish that are resident in fresh water all their life (Berg 1985). This usually arises due to the population becoming landlocked, although genetically distinct resident and anadromous populations can exist in sympatry (Verspoor & Cole 1989). The majority of Atlantic salmon utilise the freshwater environment for the reproductive and nursery phases of the lifecycle, and the marine environment for greater feeding opportunity and growth (Mills 1991).

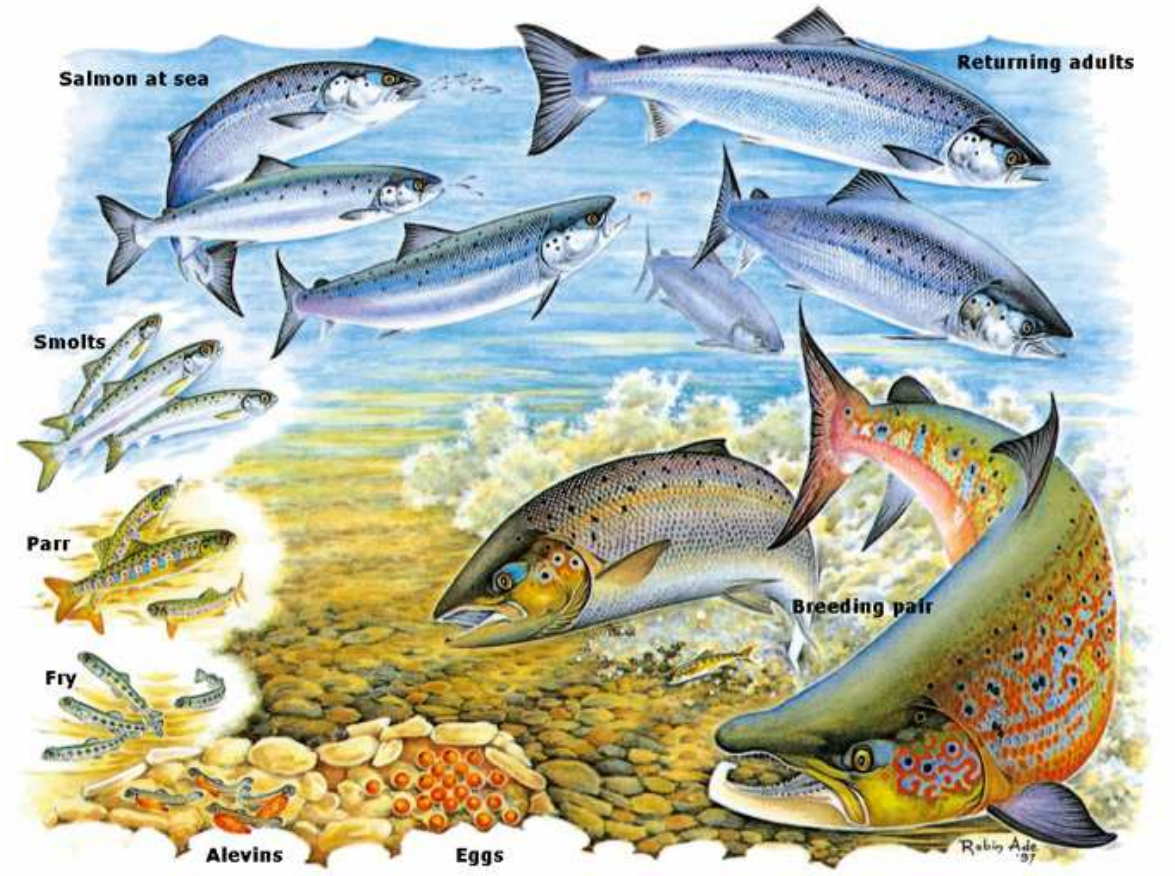
In autumn, eggs are typically buried in the gravel substrate of fast flowing headwater streams in nests known as redds. The eggs develop over winter and the young that emerge after hatching in spring are called alevins (Mills 1991). Emergence is temperature dependent and as such is later at higher latitudes, but a small proportion of the variation in emergence time can also be ascribed

to genetic differences (McCarthy 2001) and individual variation in metabolic rate (Metcalfe *et al.* 1995).

Some months later the juveniles develop vertical markings along the sides of their body distinguishing them as parr or juveniles (Keenleyside & Yamamoto 1962). The parr stage covers a body size range of around 50 to 150mm. Diet primarily consists of the larvae of aquatics insects such as blackfly, stonefly, caddisfly and chironomids; as well as invertebrates of terrestrial origin (Mills 1991; Martin-Smith *et al.* 2002). Individuals are territorial (Keenleyside *et al.* 1962) and compete for food availability and space (Kalleberg 1958). Some time later parr adopt a silver colouration and become known as smolts, which begin migrating downstream to begin the marine phase of their lifecycle. This change to smolts is known as smoltification and can occur between 1 (Nicieza, Reyes-Gavilán, & Braña 1994) and 8 (Robitaille *et al.* 1986) years of age. The time at which smoltification occurs is best predicted by body size (Metcalfe, Huntingford, & Thorpe 1988), and as such the duration of the parr lifestage is heavily dependent on the growth rate that the fish achieve in fresh water (Metcalfe & Thorpe 1990) and latitude (Hutchings & Jones 1998). Smoltification is accompanied by multiple physiological changes to prepare for life at sea.

At sea, salmon are non-territorial and feed on a range of smaller fish, such as capelin, herring, sprats, sandeels and surface dwelling crustaceans (Reddin 1988; Jacobsen & Hansen 2001). Mortality is high, with marine survival rates estimates between 5-40% mostly due to predation (Hansen & Quinn 1998). Salmon can spend 1 to 4 years at sea before returning to their native freshwater rivers and streams (Klemetsen *et al.* 2003). They home to their natal rivers with remarkable accuracy (97-99%, Stabell 1984; Quinn 1993), where they reproduce and make redds to create the next generation of salmon. Reproduction can be less straightforward due to the presence of precocious male parr, which have omitted the marine phase and mature at such a small size that they are able to fertilise eggs from adult females by a sneaking reproductive strategy (Myers, Hutchings, & Gibson 1986).

Figure 1.2 Overview of the lifecycle of the Atlantic salmon (illustration courtesy of the Atlantic Salmon Trust and Robin Ade)



Adult Atlantic salmon are prized as trophy fish in their natal streams by anglers (Wallmo & Gentner 2008) and are also a major aquaculture species (Gross 1998). The aquaculture industry produces over 1 million tonnes of Atlantic salmon every year (Ford & Myers 2008). While the Atlantic salmon has great recreational and commercial value, global populations of wild salmon have been in decline since the 1970s (Ford *et al*. 2008). Because of its interest and importance to humans, together with its fascinating ecology, the Atlantic salmon has been well studied for many years contributing to a wealth of knowledge on the species (Webb *et al*. 2007), which provides groundwork to address pertinent ecological questions.

1.6. Life history trade-offs in Atlantic salmon

Dunham et al. (1989) described an animal's life history as a heritable set of rules which determine age-specific allocations of time and energy for an individual throughout life. While these rules may be fixed, the outcomes will partly depend on the environment experienced by the fish. Atlantic salmon thus exhibit great variation in life history traits such as growth rate, maturation age, spawning time, migration age and length of marine and freshwater phase (Hansen et al. 1998). The age at which smoltification and migration occurs represents a move from a freshwater environment where density-dependent factors are most important to survival to a marine environment where survival is mostly influenced by density-independent factors (Jonsson, Jonsson, & Hansen 1998). The marine environment offers a greater food supply than is generally found in fresh waters of comparable latitude (Gross, Coleman, & Mcdowall 1988), and thus has the potential for faster growth (Klemetsen et al. 2003). As larger females lay more eggs (Thorpe, Miles, & Keay 1984; Jonsson, Jonsson, & Fleming 1996), faster growth at sea can be seen as contributing to reproductive fitness.

In light of this, the intrinsic and extrinsic factors affecting growth in Atlantic salmon parr have great ecological relevance, since body size determines the timing of smoltification and migration, which in turn influences fitness and life history.

1.7. Study system

A major underlying approach of this thesis was to examine how ecological context, specifically habitat structure, influenced the performance of metabolic strategies in juvenile Atlantic salmon. An artificial stream was used in the ecological studies (Chapters 2, 3 and 4) to allow ecological inferences to be made. The scale of the stream was sufficient to incorporate natural home ranges of the fish used (Martin-Smith *et al*. 2002) while acting as a mesocosm to which

habitat demographics could be altered and built upon to the community level, at the same time allowing direct behavioural observation.

The other main approach of this thesis was to examine physiological traits that may be related to metabolic strategies. The fine scale study of physiological processes required a setup much different from the artificial stream where variables could be controlled and as such traditional laboratory aquaria were used for these studies (Chapters 5 and 6).

1.8. Aims of thesis

The overall aim of this thesis is to examine relationships between environmental conditions, energetic strategies and performance in juvenile Atlantic salmon. Listed below are the specific questions explored and the chapter that addresses them.

- How do different metabolic strategies perform across different population densities? (Chapter 2)
- How do different metabolic strategies perform in environments where habitat complexity and food predictability varies? (Chapter 3)
- How does the presence of a competitor species affect the performance of different metabolic strategies? (Chapter 4)
- Is standard metabolic rate correlated with feeding rate?(Chapter 5)
- Does the efficiency of digestion vary with standard metabolic rate? (Chapter 6)

- The final chapter (Chapter 7) presents an overall general discussion of the issues raised by this series of interrelated experiments.

2. Estimated standard metabolic rate interacts with territory quality and density to determine growth rates of juvenile Atlantic salmon

2.1. Abstract:

Physiological traits can vary greatly within a species and consequently have a significant impact on other aspects of performance. Many species exhibit substantial variation in basal or standard metabolic rate (SMR), even after controlling for body size and age, yet the ecological consequences of this are little known.

I examined the relationships between mass-specific SMR of yearling salmon (estimated from their ventilation rate) and their feeding and growth rates across a range of natural population densities within a semi-natural stream environment.

SMR was strongly correlated with dominance rank, and higher ranking fish were more likely to acquire good feeding territories. Despite this, there was no overall relationship between SMR and growth. This can be explained because within territories of a given quality, there was a negative correlation between SMR and growth rate, presumably due to the costs of metabolism.

These effects were also influenced by density: lower densities led to reduced aggression and competition, and hence higher average feeding and growth rates. Moreover, at low densities, where availability of good feeding locations was not limiting, there was no relationship between SMR and growth.

As a result of these processes, there was a context-dependent trade-off in energy budgets: the fish achieving the greatest growth were those with the lowest SMR that was necessary to achieve dominance over conspecifics at medium and high densities (and hence acquire a good territory), but this minimum threshold SMR increased with population density. These relationships

and trade-offs can explain the persistence of variation in SMR within populations.

2.2. Introduction:

Understanding trait variation within species is of great ecological and evolutionary consequence, since it is the basis for adaptation to new or changing environments. However, studies of the impact of variation in physiological traits on fitness are unusual, and those that have been carried out have produced equivocal results (Boratynski & Koteja 2009; Boratynski & Koteja 2010). One such trait is basal or standard metabolic rate (BMR/SMR), the rate of metabolism of an inactive animal in a post-digestive state with no oxygen debt (Hulbert *et al*. 2000; Frappell *et al*. 2004) (while the term BMR strictly refers to endotherms in their thermoneutral zone and SMR is for ectotherms, hereafter for simplicity we will use the single term SMR for all taxa). SMR is increasingly being recognised across a broad range of organisms to show extensive variation even among individuals from the same life stage and population (Hayes *et al*. 1992; Kvist *et al*. 2001; Steyermark *et al*. 2005).

SMR is a basic drain on energy and on its own would be expected to correlate negatively with rate of growth. However, SMR has been shown to relate directly to metabolic scope for activity (Fry 1947; Priede 1985), the rate at which food can be digested and processed (Millidine *et al*. 2009) and the dominant and aggressive behaviour often used to sequester food (Biro *et al*. 2010). These processes may explain the possible link between SMR and growth potential (Ricklefs, Konarzewski, & Daan 1996).

SMR seems to be an intrinsic property of the individual, since a range of studies have shown that it is repeatable over time (Bech *et al*. 1999; McCarthy 2000; Labocha *et al*. 2004; Nespolo *et al*. 2007). Given that both SMR itself and the many traits with which it correlates are likely to have fitness consequences, the question arises as to how the variation in SMR is maintained in animal populations. Juvenile salmonid fish are ideal species in which to investigate this

question for a number of reasons. Individuals grow fastest over the summer, with growth rates declining towards the end of the summer (Egglishaw & Shackley 1977), allowing for significant mass changes to be measured over periods of weeks. Their SMR varies between individuals by 2-3 fold or more (Enders & Scruton 2005), and in laboratory experiments using simple tanks, juveniles with a higher SMR have been shown to be dominant (Metcalfe *et al.* 1995) and have a higher capacity for growth (Metcalfe, Wright, & Thorpe 1992). Dominant juvenile salmonids tend to acquire and control the best feeding territories and exhibit fastest growth in laboratory stream channels (Fausch 1984) and pools in natural streams (Nakano 1995a).

However, the clear positive relationships between high SMR, dominance and growth of juvenile salmonids that are found in simple laboratory tanks are often not found in the wild (Álvarez & Nicieza 2005; Harwood *et al.* 2003; Martin-Smith *et al.* 2002; Sloman *et al.* 2008), suggesting that the relation between standard metabolic rate, behaviour and performance may be habitat dependent (Finstad *et al.* 2007b). For instance, the costs of a high metabolic rate may outweigh the potential benefits if feeding rates are constrained by food availability (Bochdansky et al. 2005) (as is likely to occur in the natural environment but is rarely the case in laboratory studies). A fuller understanding of the links between SMR and performance therefore requires experimental tests under controlled settings that mimic distinct natural conditions on the effects of SMR on dominance, territory acquisition, feeding rate and growth under differing levels of competition. Here we report the first such study. We show that the estimated SMR that maximises growth rate varies with fish density and the quality of available territories, and that while fish with a high SMR are more likely to obtain a good territory, their growth is poorer on a given territory than that of a fish with a lower SMR. These relationships help explain the persistence of individual variation in SMR in natural populations.

2.3. Materials and methods

In each round of the experiment (starting on 9th June 2008) wild 1-year-old Atlantic salmon parr (*Salmo salar*, Linnaeus 1758) were caught by electro-fishing from the river Almond, and 16 fish of similar size (matched by eye, see results for weights) taken to Marine Scotland Science Almondbank field station and held in a 1 m^2 circular tank overnight. The following day, each fish was anaesthetised, weighed (to 0.1 g), measured (fork-length, to the nearest mm), and injected with a unique combination of alcian blue dye marks on the pectoral, pelvic and caudal fins. The parr were then placed in individual aerated 10L tanks, each containing an shelter (dark overhead but transparent on each side) to allow them to recover and settle for 24 hours, to ensure that guts were fully evacuated to prevent metabolism being elevated by digestion (Cutts, Metcalfe, & Taylor 2002a). The ventilation rate (VR) of each quiescent fish was then recorded as the number of opercular beats over 1 minute. This process was repeated after an hour and again a further hour later. The mean of the three VR values (repeatability=0.86 calculated as in Lessells & Boag (1987)) was used to estimate the mass-specific standard metabolic rate (SMR, mg O_2 kg^{-1} h^{-1}) of each individual, by using equations in Millidine, Metcalfe & Armstrong (2008) to relate VR to metabolic rate, with knowledge of fish weight (W, in g) and tank water temperature (T, in ºC, measured by digital thermometer). The relationship between predicted and measured metabolic rate Millidine's study is strong (r^2 = 0.91). The regression equations (from Millidine et al. (2008) used to estimate SMR from VR were:

MR = m(VR) + c

where m = 0.2773–(0.2350*log10(W))–(0.01838*T) + (0.05813*(T)*log10(W))

c = -3.4078 + (0.2958*T) + (2.1956*log10(W)) - (0.82057*(T)*log10(W)) + (0.5335*W)

The 16 fish were then allocated into groups of 2, 2, 4 and 8 fish, which would correspond to the density treatments in the final stage of the experiment (with the group size of 2 being repeated to increase the dataset for fish at low density). Each group contained an equal number of fish above and below the mean SMR of the 16 fish in the sample. Group composition was also arranged so that fish were sorted by weight to minimise the disparity between the largest and smallest individuals within each group, to limit within-group variation in performance due to body size. Each group was then placed in a separate tank (32x17x19cm, 10L) in order to assess dominance ranks, by means of a serial removal method similar to Metcalfe *et al* (1989) without the necessity of food addition to induce aggression. Juvenile salmon demonstrate aggression by making charge attacks by lunging toward each other, often biting with their mouths but rarely making contact with each other. This results in the loser swimming away, often chased by the winner. The observed winner of multiple aggressive interactions over conspecifics was considered dominant and subsequently removed and placed in a temporary holding tank. This process was repeated, allowing a 10 minute settling period between observations, until every fish had been assigned a dominance rank (from 1 to n, where n=group size).

Once dominance ranks were assigned all members of each group were re-united and placed in a section of an indoor stream for the main phase of the experiment. Each section was 7.5m long and 1.5m wide and thus measured 11.25m^2 in area. The stream was continuously fed with unfiltered water from the River Almond (i.e. the source of the fish). One side wall of the stream was of clear glass to allow behavioural observations, and was marked off in 0.3m gradations to allow recording of spatial positions. The substratum of the stream was landscaped so that each section contained an upstream and downstream riffle area (each 4.9m^2 area, 0.15-0.20m water depth) separated by a single pool (1.5m^2 area, 0.4m depth). Subtratum comprised gravel in riffles and fine sediment in pools. Water velocity was 0.29±0.1m s^{-1} in riffles and 0.07±0.1m s^{-1} in pools.

There was no barrier to colonisation of the stream substrate by the river benthic invertebrate community, leading to a natural background source of drifting food for the fish. However, a supplementary feeding system, described in more detail in Maclean, Miles & Armstrong (2003), allowed programmed randomised delivery

of defrosted bloodworms (*Chironomid* larvae) through two feeding pipes in each section (one located at the upstream end of each riffle area) between 09:00 and 17:00 each day. The rate of food input was constant among sections, being approximately 1 item every 6 minutes per feeder. This created two areas 5 to 30cm downstream of the feeding pipes in each section that were more profitable in food resources, referred to as good feeding territories relative to the rest of the stream where only the background drift food was available. Chironomids were always observed to drop to the substratum within 40cm of each feeder outlet, so food was not drifting into other downstream territories. Each section also contained 10 overhead shelters placed evenly along the viewing side of the stream. These were thin horizontal opaque plastic rectangles (12cm x 5cm) held 8cm above the substrate by rods at each corner. Parr from one of the dominance ranking tanks were introduced into each section, creating densities of 2, 2, 4 and 8 parr per section. These densities, equivalent to 0.17, 0.35 and 0.7 salmon per m^2, reflect the range of densities found locally in the wild (Egglishaw *et al*. 1977). A number of underyearling Atlantic salmon were also resident in each section of the flume throughout the experiment (3-4 m^{-2}), to reflect intercohort interactions that occur in natural populations and influence stress, aggression and foraging performance (Kaspersson *et al*. 2010).

Parr remained in the stream for 12 days. On 7 of those days (spread through the 12 day period) each fish was observed every hour that the feeder was operational (09.00-17.00), generating 56 observations per fish. Subordinate juvenile salmon develop a characteristic darker colouration, thought in part to be a signal of their status to more dominant individuals (O'Connor, Metcalfe, & Taylor 1999). During each observation stress colouration levels were therefore scored on a scale of 1 to 5 (1 = least stressed) using an index based on a combination of body and sclera colouration (Suter & Huntingford 2002; O'Connor *et al*. 1999). All individuals displayed light body colouration (1 or 2) within 24 hours of stream introduction, suggesting no detrimental stress effect from previous handling, although body colouration varied greatly over the remainder of the experiment as a consequence of social interaction (see results). The behaviour of the fish was then recorded for 2 minutes in terms of aggression, space use and foraging. For each aggressive encounter (a charge attack), the identity of the winner and loser was noted. The space use of each parr was

quantified by two measures, first by its use of the water column (estimated as 0, 33, 66 or 100% of the 2 min observation that the fish spent swimming in the water column as opposed to resting on the bottom) and second by the estimated longitudinal distance (to the nearest 0.3m) it moved over the 2 min observation. The number of feeding movements was counted, which included attacks on the bloodworms released in the good territories as well as on natural prey. Water temperature was measured every observation hour (mean water temperature was 13.3 ºC ±1.0SD, 13.9 ºC ±1.3SD, 14.8 ºC +1.4SD and 17.3 ºC ±1.0SD for each experimental round respectively).

At the end of the 12 day experimental period all fish were removed, re-weighed/measured and the experiment was repeated with a freshly caught batch of 16 parr. In total the protocol was run 4 times (i.e. 4 rounds, with stream observations starting on 12 June, 26 June, 10 July and 24 July 2008) so producing 8 replicates for the density of 2 fish and four replicates each for the densities of 4 and 8 (n=64 fish in total). The allocation of treatments to stream sections was changed between each round to avoid any possibility of systematic positional bias confounding effects of density, and the allocation of size groups to densities was similarly switched to avoid confounding density and body size effects.

For the purposes of statistical analysis (SPSS v15.0), mean values for body/eye colouration, aggression and feeding rates (events per minute) and activity (distance moved per min and % time in water column) were calculated for each fish, averaging over the 56 observations. In order to control for variation between rounds in the observed range of standard metabolic rates, estimated SMR values were converted to relative SMR (calculated as an individuals SMR minus the mean SMR of all the fish in each round of the experiment). Relative SMR was used to eliminate difference in SMR between rounds due to seasonal changes in water temperature. Feeding rates were adjusted by the same method. Growth rate during the experiment were calculated using the formula for specific growth rate ($SGR = 100\ (\ln(m_2)-\ln(m_1))/(t_2-t_1)$) (numerically equivalent to % change per unit time, in this case per day or d^{-1}), where m_1 and m_2 refer to body mass at times t_1 and t_2. SGR assumes exponential growth rate and as such is better applicable to growth of young fish (Hopkins 1992).

Linear Mixed Effect (LME) models were used for all statistical analyses, with the exception of one logistic regression (see later). Replicate (group) number was included as a random factor nested within round in LME models in all statistical analyses that evaluated the effect of density (included as a fixed factor), to control for the potential lack of independence between fish within a replicate group, while we controlled for the effect of time of season by including the Julian date on which each replicate started as a fixed factor. In order to produce minimum adequate models, non-significant terms ($P>0.05$) were removed stepwise from LME models (starting with two-way interactions, with least significant terms removed first). A logistic regression tested which factors determined the probability of individuals acquiring a high quality territory.

2.4. Results

The mean initial weight of fish (8.73g ±2.13 SD, range 4.4-14.1g) did not differ between densities but did increase through the season (LME, effect of date: $F_{3,58}=5.85$, $P=0.001$, effect of density: $F_{2,58}=1.64$, $P=0.15$). Mass-specific SMR varied up to 5-fold between individuals within replicates, but relative SMR did not differ between densities (LME, $F_{2,61}=0.35$, $P=0.707$).

We examined the link between SMR and dominance status by comparing the relative SMR of dominant and subordinate fish within the same group. Across all densities, relative SMR was negatively correlated with dominance rank (analysis 1 in Table 2-1). These dominance ranks mirrored in-stream spatial patterns: the most dominant fish within each group, as assessed by the serial removal method, always obtained a good feeding territory (n = 16 out of 16) as did all second-ranking fish except in one case where the third most dominant individual obtained the remaining good feeding territory. A logistic regression across all densities showed that the probability of acquiring a good territory was significantly influenced by relative SMR (Fig. 2.1, Wald = 3.868, 1 d.f., $P=0.049$) after controlling for the effect of density (Wald = 13.902, 1 d.f, $P<0.001$). Body mass and both measures of activity were also considered as explanatory candidates in this logistic regression analysis but were dropped due to being non-

significant (P>0.05). However, the importance of relative SMR increased with density (relative SMR*density interaction, Wald = 4.073, 1 d.f., P=0.044), since at the lowest tested density there were as many good territories as fish and so all fish obtained good feeding territories irrespective of their SMR, whereas at high densities the competition for good territories was intense and a higher relative SMR became important (Fig. 2.1).

Figure 2.1 Plotted logistic regression equation showing probability of acquiring a good feeding territory is dependent on population density, standard metabolic rate, and the interaction between them (Equation: $1/(1+e^{-Z})$ where $Z = (-3.1325+0.0094x+0.5518y-0.005339xy)$, x = relative SMR and y = density; p=0.044). Relative SMR was calculated as SMR – mean SMR, across the fish in each round of the experiment and then pooled across rounds.

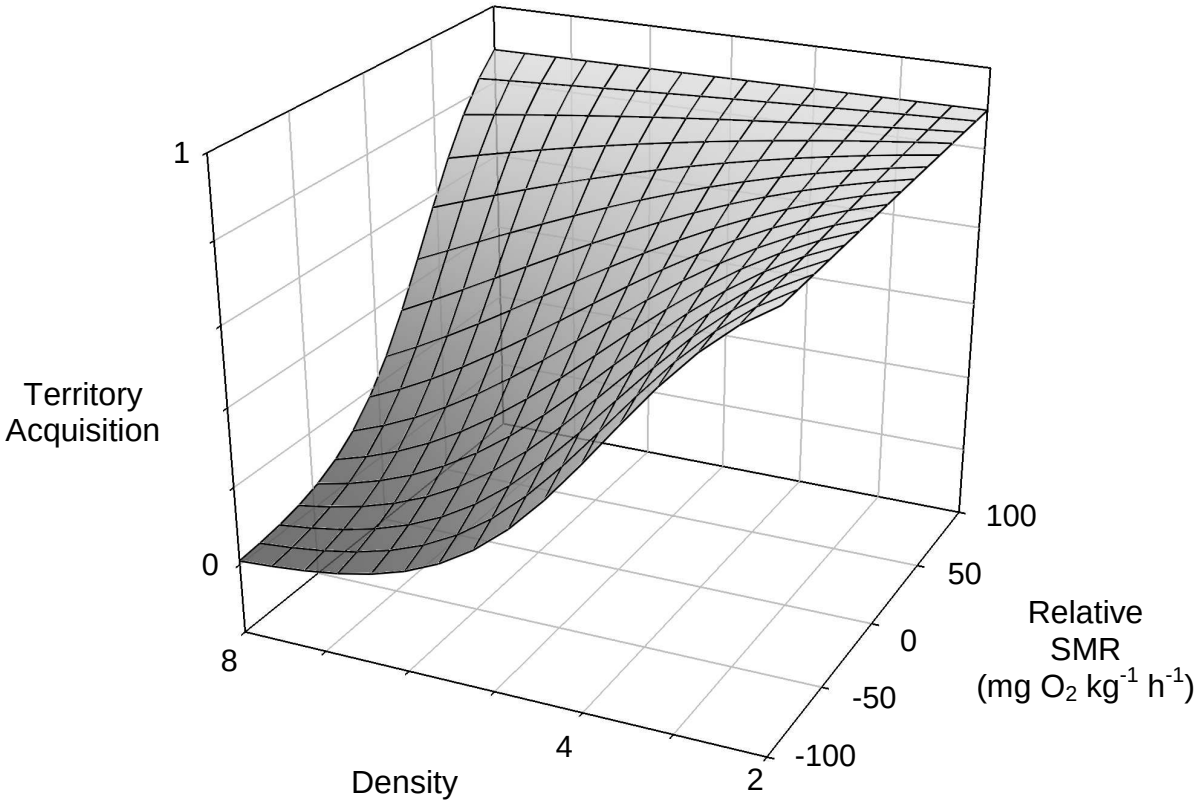
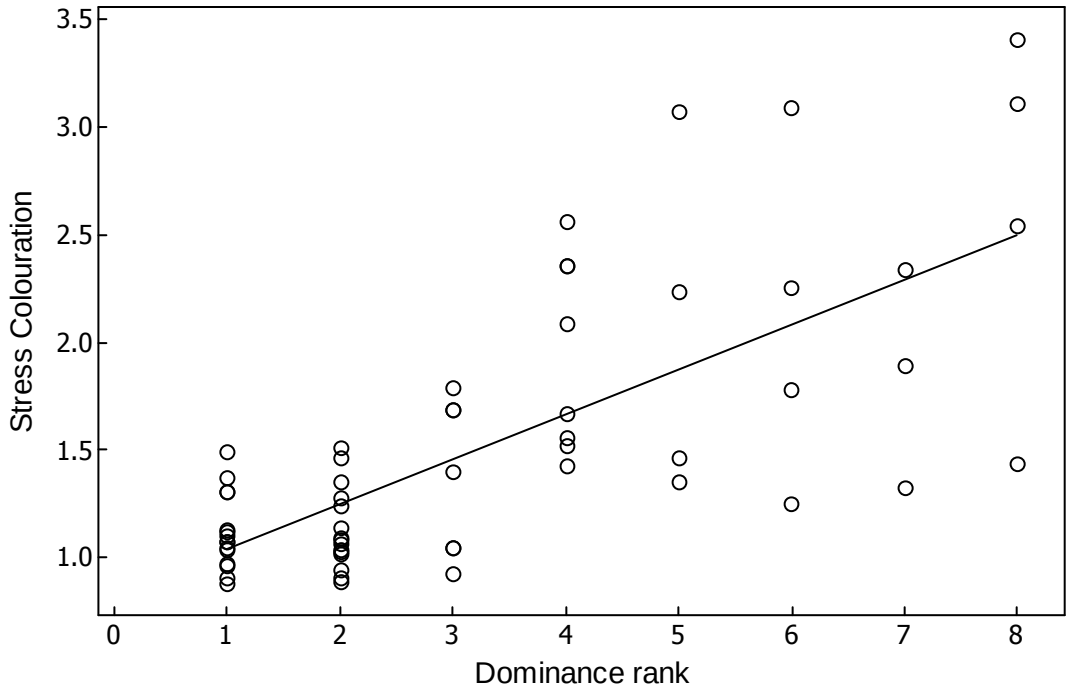


Figure 2.2 The relationship between dominance rank (1 = most dominant fish in group) and mean stress colouration score, where higher scores indicate more stressed fish (values have been offset in the figure to reveal otherwise superimposed data points, p<0.001).



Per capita aggression rates increased significantly with density (0.008 min^{-1}±0.0004SE, 0.0313 min^{-1}±0.011SE, 0.1155 min^{-1}±0.0016SE for low, medium and high densities respectively; analysis 2 in Table 2-1), but did not influence stress colouration levels; instead the mean stress colouration score of a fish was related to dominance rank such that more subordinate fish were darker in colour (Fig. 2.2, Table 2-1, analysis 3).

When considered without reference to the quality of territory it acquired, the feeding rate of a fish was found to be positively related to its relative SMR (Table 2-1, analysis 4b). However, the importance of SMR for feeding rate appears to be indirect, due to its influence on territory acquisition, as in the full analysis feeding rate was found to be influenced solely by whether or not a fish obtained a good feeding territory (analysis 4a in Table 2-1). To test whether this was a consequence of multicollinearity between SMR and the other candidate explanatory variables, Variance Inflation Factor (VIF) analysis was carried out on all potential candidate explanatory variables. VIF measures how much the variance of an estimated regression coefficient is increased because of collinearity. Since VIF analysis indicated that levels of multicollinearity were

relatively low (<4, where values >10 indicate significant potential problems of multicollinearity (O'Brien 2007); values were similarly low for all other statistical tests). Neither the mean distance a fish covered per min nor the percentage of time it spent in the water column were predicted by its relative SMR (Table 2-1, analyses 5 and 6), nor by any of the other candidate variables.

Taking a 'population level' approach by analysing the data on growth rates without reference to information on individual differences in rank, metabolic rate or territory quality showed the expected pattern of growth rates declining through the summer and being lower at higher densities (Fig. 2.3, analysis 7a). A superficial assessment might suggest that growth rate was not related to metabolic rate (Fig. 2.4). However, when rank, SMR and territory quality were considered as candidate explanatory variables in addition to density, growth rate was found to relate significantly to SMR, territory quality and date, while the effect of density was no longer significant (Table 2-1, analysis 7b). After controlling for date, the strongest effect was quality of territory (fish with good territories growing fast, with no difference in growth between good territory holders at different densities. Within fish holding a particular quality of territory, the relationship between relative SMR and growth rate was actually negative, with fish with lower metabolic rates growing faster on a given quality of territory (Fig. 2.4). Exclusion of low density data (i.e. 2 fish per group) had no effect on this result, although there was no relationship between SMR and growth at this density (LME, $F_{1,14}$=0.04, P=0.836; relative SMR sole considered explanatory variable).

Figure 2.3 The effect of population density (2, 4 or 8 fish per stream section, p=0.001) and Julian date on growth rate (% change in body mass per day, p<0.001). Individual data plotted with interval bars +/- one S.E.

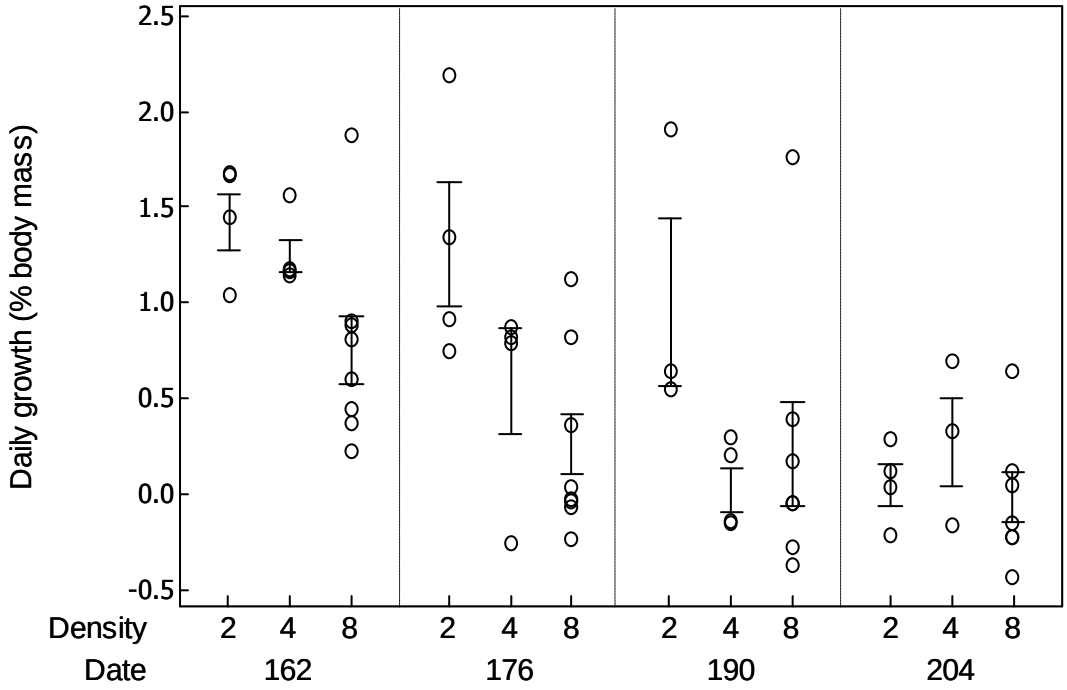


Figure 2.4 The negative relationships between relative SMR and relative daily growth rate (% body mass change per day, expressed as relative growth calculated within rounds, p=0.01) for those fish that acquired a good feeding territory (squares/fine broken line) and for those that did not (circles/solid line). The coarse broken line is the regression for the combined data, irrespective of feeding territory quality, demonstrating the lack of an overall relationship between relative SMR and growth when territory quality is ignored (r^2 <0.001, 1 d.f., P=0.784). (Data from the lowest density treatment (2 fish) have been omitted since all fish in such trials obtained good feeding territories irrespective of relative SMR, however inclusion of these data does not affect the significance of the results).

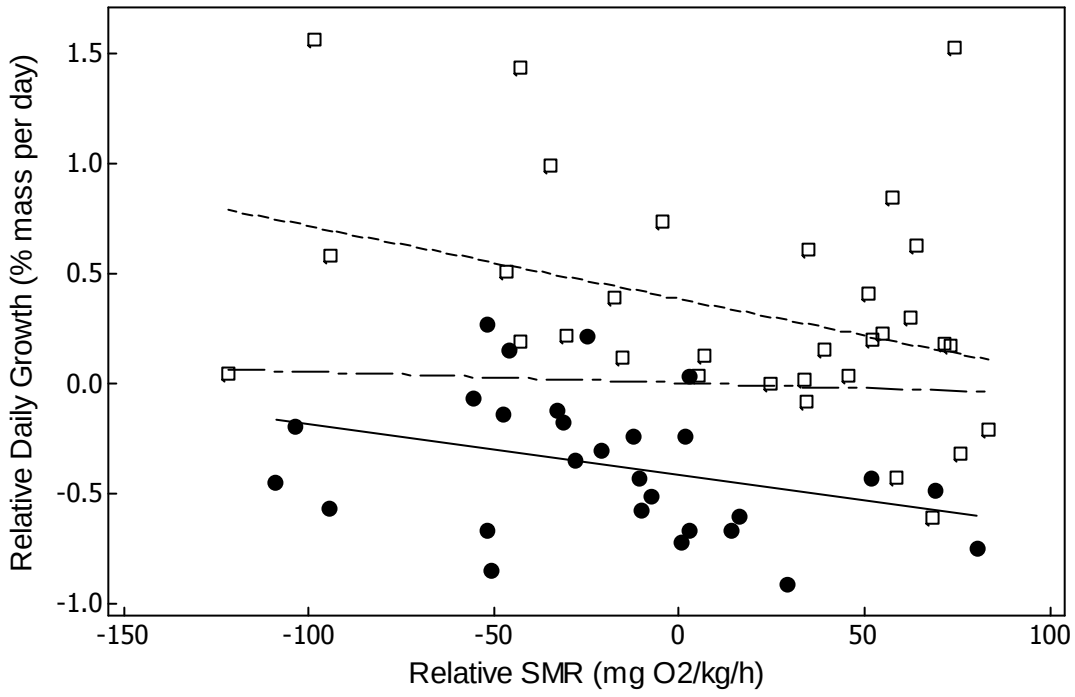


Table 2-1 Results from all linear mixed effect (LME) model analysis, detailing the dependent, considered explanatory and significant explanatory variables (with parameter estimates) in each final model. The following candidate explanatory variables were initially included in all models (except when used as dependent variable): relative SMR, dominance rank and body mass (as covariates), density and Julian date (as fixed factors, with levels of each categorical listed in parentheses), and replicate as a random nested factor. Stress colouration and feeding rate (as covariates) and territory quality (as a fixed factor) were included as candidate explanatory variables where stated. Where a potential explanatory variable has been *a priori* excluded from an analysis it is stated below the dependent variable. Non-significant variables were removed from models in a stepwise fashion to produce minimum adequate models.

	Dependent variable	Additional explanatory variables considered in model	Significant explanatory variables	F	df	Estimated parameter values	Significance
1	Dominance (Density excluded)		Relative SMR	18.95	1,62	-0.020	<0.001
2	Aggression rate		Density (2) (4)	15.87	2,61	-6.267 -4.867	<0.001
3	Stress colouration		Dominance rank	56.88	1,62	0.061	<0.001
4a	Feeding rate	Stress colouration Territory quality	Territory quality	34.52	1,62	9.584	<0.001
4b	Feeding rate (Territory excluded)	Stress colouration	SMR	4.46	1,62	0.031	0.04
5	Activity (in distance)	Stress colouration Feeding rate Territory quality	None				
6	Activity (in the water column)	Stress colouration Feeding rate Territory quality	None				
7a	Growth (Territory and relative SMR excluded)	Stress colouration Feeding rate	Density (2) (4) Date (1) (2) (3)	7.99 10.52	2,58 3,58	0.637 0.224 0.993 0.557 0.292	0.001 <0.001
7b	Growth	Stress colouration Feeding rate Territory quality	Territory quality Relative SMR Date (1) (2) (3)	47.39 7.10 15.00	1,58 1,58 3,58	0.790 -0.002 0.985 0.599 0.319	<0.001 0.010 <0.001

2.5. Discussion

The use of ventilation rate as an indirect estimate of mass-specific SMR (Millidine, Metcalfe, & Armstrong 2008) provides a useful tool to approximate the metabolism of freely moving juvenile salmon living in groups in a semi-natural environment, but is not without its limitations. Error associated with calculated estimates can be up to ±25% (Millidine et al. 2008), a not insignificant range, and for increased accuracy individual calibration would be desirable (Millidine *et al*. 2008). However this error is small in comparison with the 5-fold (equivalent to 500%) variation in SMR found in the present study, so will merely add noise to the analyses in the present study that compared the performance of similar-sized fish of differing metabolic rate.

While virtually all fish grew over the course of the trials, average growth rates declined over the period of experimentation, mirroring the growth rates of wild juvenile salmon from early to late summer (Egglishaw *et al*. 1977). Density-dependent growth was observed (Table 2-1, analysis 7a) which, although contrary to Elliot's (1984a; 1984b) study in high density brown trout, mirrors more recent studies (Bohlin *et al*. 2002; Imre, Grant, & Cunjak 2005; Lobón-Cerviá 2007). However, these broad-scale ecological factors do not explain the variation in growth rates within each group of fish (with the fastest-growing salmon in each group typically growing 5 to 20 times faster than the slowest). Instead we need to look at individual variation in physiology and behaviour and how they interact with good feeding locations. As in previous studies of Atlantic salmon, fish with higher standard metabolic rates were more likely to be socially dominant (Metcalfe *et al*. 1995). At low population densities the social status of a fish was of little importance, since all fish were able to secure good feeding territories. Under these benevolent conditions there was no difference in the growth rates of higher and lower SMR fish.

However, at medium to high population densities good feeding locations became limiting and it was the high SMR (dominant) individuals that monopolised the better feeding territories. Fish that held good territories showed the highest

growth rates due to increased food intake, yet there was no overall relationship between SMR and growth rate. This paradox arose because we show that among the fish obtaining a good feeding territory, individuals with a lower SMR had highest growth rates, although not at low densities. Salmon that obtained a good feeding territory at the cost of a very high SMR actually exhibited lower growth rates than some low SMR individuals feeding in relatively poorer territories. This reflects the"principle of allocation" theory where, for a given level of food intake, higher metabolic rates lead to slower growth, as also found in correlative studies of snapping turtles by Steyermark (2002), sage brush lizards by Sears (2005) and brown trout by Álvarez & Nicieza (2005). The growth of holders of good territories was relatively unaffected by density, but higher population densities led to more individuals occupying poor feeding territories, resulting in lower average growth rates. The increased competition at higher densities also led to an increase in the minimum SMR needed to be within the dominant fraction of the population that would obtain a good territory.

These novel results indicate a trade-off in energy budgets. A low SMR requires less energy expenditure in upkeep and minimises the energy costs of maintenance (Steyermark *et al.* 2005), but usually results in subordination to conspecifics, whereas a high SMR is energetically costly to sustain but usually results in a higher social status (Metcalfe *et al.* 1995). This dominance can lead to acquisition and defence of preferred territories, leading to higher food intake (Cutts *et al.* 1999). Therefore where good feeding territories are available and defendable but scarce, high SMR fish gain advantages because they can outcompete other individuals to obtain a higher energy intake and growth rate. A higher SMR is economical if it results in an increase in food capture that more than offsets its extra ‘running cost’. Otherwise it is energetically detrimental, although it may confer other advantages such as acquisition of shelter resulting in a reduced risk of predation (Valdimarsson & Metcalfe 1998).

For a given level of territory quality, fish with a low estimated SMR in the present experiment grew faster than those with a higher estimated SMR (presumed to be more dominant), indicating that the incurred metabolic costs of subordination (Sloman *et al.* 2000) were not significant. Moreover, the negative correlation between SMR and growth disappeared at low densities, suggesting that the difference in growth of high and low SMR fish with the same access to food was not only due to their baseline metabolic costs. It may have been the

case that higher metabolic rate individuals were also more actively aggressive in defending their territory. Since territorial defence is known to be energetically expensive in salmonids (Puckett & Dill 1985), more aggressive fish would incur greater energy costs of defence at high densities, contributing to the negative relationship between SMR and growth in such situations.

This experiment has shown that the SMR that produced the highest growth was context- (and primarily density-) dependent. High growth rates tended to be observed in individuals that had a SMR that was just sufficient to make them dominant over conspecifics. However, as future population densities (and the distribution of metabolic rates within populations) are unpredictable, the optimal metabolic rate will vary spatially and over time.

The negative relationship between SMR and growth is only apparent when effects of dominance and subsequent territory acquisition are taken into account. This may help explain why there have been divergent and somewhat paradoxical results from laboratory and field studies investigating a link between growth and SMR or dominance in stream-living fish (Martin-Smith *et al*. 2002; Harwood *et al*. 2003; Álvarez *et al*. 2005), since laboratory studies have typically involved very artificial social structures or feeding conditions (with food often being supplied *ad lib*.), while field studies are often unable to include the visual observations of the behaviour of the fish which (as shown by this study) provide valuable insights into the processes linking individual traits with performance (see Nakano (1995a) and Sloman et al. (2008) for rare field studies that achieve this). The methodology of size-matching fish was useful in revealing the performance effects of SMR while ultimately negating the effect of body size. However in the wild, cohorts will vary to a greater degree in size and body mass will also be a significant predictor of dominance. The present study was able to reveal these processes by using a stream tank that allowed observations of the behaviour of the fish in semi-natural conditions; it showed that the benefits of a given SMR will depend on the environmental context (thus possibly maintaining intraspecific variation in metabolic rate within the population). However, further studies are needed to explore how spatial and temporal variation in food supply or competition influences the costs and benefits of a given metabolic rate, as population densities are influenced heavily by habitat demographics (Armstrong *et al*. 2003).

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3. The performance advantage of a high resting metabolic rate in juvenile salmon is habitat dependent

3.1. Abstract:

Basal levels of metabolism vary significantly among individuals in many taxa, but the effects of this on fitness are generally unknown. Resting metabolic rate (RMR) in juvenile salmon and trout is positively related to dominance status and ability to obtain a feeding territory, but it is not clear how this translates into performance in natural conditions.

The relationships between RMR, dominance, territoriality and growth rates of yearling Atlantic salmon *Salmo salar* were examined in relation to predictability in food supply and habitat complexity, using replicate sections of a large-scale controlled semi-natural stream.

Estimated RMR was a strong predictor of dominance, and under conditions of a predictable food supply in a structurally simple habitat high estimated RMR fish obtained the best feeding territories and grew faster.

When the spatial distribution of food was made less predictable, dominant (high estimated RMR) fish were still able to occupy the most profitable feeding locations by periodically moving location to track the changes in food availability, but RMR was no longer a predictor of growth rate. Moreover, when a less predictable food supply was combined with a visually more complex (and realistic) habitat, fish were unable to track changes in food availability, grew more slowly and exhibited greater site fidelity, and there were no relationships between estimated RMR and quality of occupied territory or growth rate.

The relative benefit of RMR is thus context-dependent, depending on both habitat complexity and the predictability of the food supply. Higher habitat

complexity and lower food predictability decrease the performance advantages associated with a high RMR.

3.2. Introduction:

Inter-individual differences in physiology are widespread within species (Chown, Gaston, & Robinson 2004), yet until recently their effects on organism performance have seldom been studied. The importance of this individual variation in physiology is likely to vary spatially, since different environments may favour different physiological phenotypes. For instance, western terrestrial garter snakes *Thamnophis elegans* in populations experiencing higher resource availability and lower predation pressure have evolved greater cellular defences against oxidative damage and so senesce more slowly (Robert & Bronikowski 2010). One fundamental physiological trait in all organisms is their rate of energy usage, since somatic growth and reproductive investment depend on surplus energy. Standard metabolic rate (SMR) is the minimal energy expenditure for upkeep in an ectothermic animal (Hulbert *et al*. 2000; Frappell *et al*. 2004). This is often measured as resting metabolic rate (RMR), which is also used to describe levels of minimal metabolism but is less rigorous as it allows individuals to be in a digestive state. We will use the single term RMR hereafter for brevity). RMR is a repeatable physiological trait (Bech *et al*. 1999; Marais *et al*. 2003; Labocha *et al*. 2004; Nespolo *et al*. 2007; Norin & Malte 2011) which may vary significantly among individuals of the same species, age and size (Cruz-Neto *et al*. 2004; Speakman *et al*. 2004; Labocha *et al*. 2004; Steyermark *et al*. 2005). RMR governs the pace but also the energy cost of life (Brown *et al*. 2004), so that a low RMR (i.e. a low energy upkeep) might appear advantageous. However, RMR may also correlate positively within species with physiological traits such as metabolic scope (Priede 1985) and growth/performance potential (Ricklefs *et al*. 1996) and also with behavioural traits such as activity (Sears 2005), dominance and aggression (Bryant *et al*. 1994; Biro *et al*. 2010). High levels of such traits can be advantageous but accrued only in environments in which resources can be

monopolised via dominance rank (Appleby 1980). This leads to the possibility that physiological optima vary with habitat.

Juvenile salmonids are a good species for examining the ecological consequences of RMR variation), since RMR varies 2-3 fold among individuals (Enders *et al*. 2005) and correlates directly with dominance and aggression (Metcalfe *et al*. 1995; Cutts *et al*. 1998). Since these fish compete for feeding territories (Keenleyside *et al*. 1962), individuals with higher size-specific metabolic rates are able to displace conspecifics and so obtain preferential feeding positions (Metcalfe *et al*. 1995). Laboratory studies have shown that when groups of fish are held in simple tanks with a very predictable food supply, such dominance-mediated resource acquisition leads to faster growth (Metcalfe *et al*. 1990; Thorpe, Metcalfe, & Huntingford 1992; Yamamoto, Ueda, & Higashi 1998).

When examined in the much more complex environment of natural streams, this relationship between RMR (or dominance status) and growth has been found to be inconsistent, with the reported trend being positive (Nakano 1995b; Höjesjö, Johnsson, & Bohlin 2002), negative (Álvarez *et al*. 2005) or nonexistent (Martin-Smith *et al*. 2002; Harwood *et al*. 2003). This suggests that the nature of the physical or social environment may influence the relationships between metabolic rate, dominance status and growth. Using a large near-natural stream tank, we have recently shown that, while high RMR fish are better able to obtain territories, the extra energy cost that they incur can result in impaired growth, so that growth is highest in those individuals with metabolic rates just high enough to allow acquisition of a good territory (Reid, Armstrong, & Metcalfe 2011). However, the success of high RMR/dominant individuals in that experiment was possibly due to their experiencing a highly predictable food supply; in contrast, in the natural stream environment the relative quality of different food patches can fluctuate markedly over short time periods (Martin-Smith & Armstrong 2002). Moreover, the social and physical structure of the stream may prevent fish from tracking such temporal changes in the spatial distribution of food (MacLean et al. 2005), but potentially increase shelter availability to reduce metabolic cost (Millidine, Armstrong, & Metcalfe 2006) and so facilitate growth (Finstad *et al*. 2007a).

Here we test whether the relationship between an individual's metabolic rate and its growth performance is dependent on the habitat, using stream-living juvenile salmon as a model system.

3.3. Methods:

In each round of the experiment (commencing 21st May 2009) a sample of 24 year-old wild salmon parr was caught from the river Almond (56.46° N, 3.80° W) by electrofishing and taken to Marine Scotland's Almondbank field station, where they were held overnight in a 1 m^2 circular tank supplied with water from the Almond. The following day the fish were anaesthetised (Benzocaine, 7.5ml/L), weighed (to the nearest 0.1g), measured (fork-length, to the nearest mm) and uniquely fin-marked with alcian blue dye applied by panjet. After full recovery, parr were size-matched into groups of four and each group was allocated randomly to one of six sections along the length of an indoor artificial stream.

Each stream section was 7.5m long and 1.5m wide and thus measured 11.25m^2 in area. The stream was continuously fed with unfiltered water from the river Almond. One side wall of the stream was made of clear glass to allow behavioural observations, and was marked off in 0.3m gradations along its length to allow recording of spatial positions. The substratum of the stream was landscaped so that each section contained an upstream and downstream gravel substrate riffle area (each 4.9m^2 area, 0.15-0.20m water depth) separated by a single pool (1.5m^2 area, 0.4m depth). Each section included 10 equally spaced overhead shelters near the viewing side of the stream. These consisted of opaque plastic rectangular roofs (12 x 5cm) held 8cm above the substrate by rods supporting each corner.

The natural invertebrate drifting food of the stream was supplemented by an automated feeding system (as described in Maclean *et al* (2003)) which allowed

input of food (*Chironomid* larvae) via four outlets in each section of stream. All feeders operated from 09:00 to 17:00 daily. Two feeder outlets were positioned in each riffle section, one at the upstream end and the second at the midpoint of the riffle. The feeder outlets could be programmed independently so as to have different levels of food output, so creating four feeding territories of specified quality (with controlled temporal patterns of food delivery) within each stream section. Three different experimental treatments were used (with two replicates of each treatment being run in each round of the experiment). In the first treatment (Simple Predictable, or SP), each feeder had a different but constant food output (1 item per 3, 6, 12 or 24 minutes throughout the daily period of feeding) over the duration of the experiment. The second treatment (Simple Unpredictable, or SU) had the same four levels of food output, but the output of each feeder changed unpredictably every 2hr 40min to one of the other food outputs (so providing food at each of the four rates over the course of each daily feeding period). This ensured the total food availability per stream section was the same as in the SP treatment, but the output at a given feeder was unpredictable at any given time. The randomisation of food output over time was such that each feeder had identical productivity when averaged over the experimental period. The third treatment (Complex Unpredictable, or CU) followed the same unpredictable feeding regime as the SU treatment but had the additional feature of lines of boulders (8-10cm in height) being placed on the gravel substrate across the width of the stream at the midpoint between feeders. This greater habitat complexity created visual barriers between adjacent feeding territories, so that a fish's evaluation of the quality of a neighboring territory was possible only by active exploration rather than visual observation from its own current territory. In all three treatments, the instantaneous quality of a feeding territory at any given time was ranked 1 to 4, with rank 1 the most profitable at that moment; this scoring system was used to calculate a mean territory quality occupied by each individual (so that an individual that always moved to the best feeding territory available would score an average of 1.0, whereas fish occupying territories at random would score an average of 2.5).

Visual observations were made between 09:00 and 17:00 each day, when the feeder system was in operation, with each fish being observed continuously for 2

minutes every 1hr 20min. At the start of each observation social stress levels were scored on a scale of 1 to 5 (1 = least stressed) by a combination of body and eye sclera coloration, where darker coloration corresponds to a more stressed and subordinate fish (O'Connor *et al*. 1999; Suter *et al*. 2002). The behaviour of the fish was then recorded for 2 minutes in terms of aggression, space use and foraging. Aggression was quantified as the number of aggressive encounters the fish was involved in during the 2 min observation, with a record being taken of the identity of the winner. Juvenile salmon demonstrate aggression by making charge attacks by lunging toward each other, often biting with their mouths but rarely making contact with each other. This results in the loser swimming away, often chased by the winner. This was used to determine a dominance rank within each cohort of 4 individuals (see results for analysis). The space use of each fish was quantified by two measures, first by its use of the water column (estimated as 0, 33, 66 or 100% of the 2 min observation that the fish spent swimming in the water column as opposed to static on the substrate), and second by the estimated longitudinal distance along the stream (to the nearest 0.3m) it moved during the observation. The number of food items captured per 2 mins was counted, and a note made if the fish used overhead shelter (including boulders) at any point during the 2 min observation period.

Ventilation rate (opercular beats per minute) was counted over 1 minute of each observation (i.e. during half of the 2 minute observation time) and this, together with information on water temperature (T, in ºC, measured by digital thermometer) and fish weight (W, in g), was used to estimate metabolic rate (MR, mg O_2 kg^{-1} h^{-1}) using equations derived by Millidine *et al*. (2008). Water temperature was measured at the start of every observation round (i.e. every 1 hour 20 mins). The regression equations used to predict MR from VR were:

$$MR = m(VR) + c$$

Where $m = 0.2773 - (0.2350 * \log10(W)) - (0.01838 * T) + (0.05813 * (T) * \log10(W))$,

$$c = -3.4078 + (0.2958 * T) + (2.1956 * \log10(W)) - (0.82057 * (T) * \log10(W)) + (0.5335 * W)$$

Fish spent a total duration of 13 days in the stream, after which they were removed, anaesthetised, reweighed and measured (fork length) for calculation of growth rates. The 6 observations per fish per day resulted in a total of 42 observations per fish over 7 days in each trial.

The experiment was repeated a further 3 times during the period May – July 2009 to provide a total of 8 replicates for each treatment (overall n = 32 fish per treatment, 96 in total). The sections of stream used for each treatment were randomised between replicates. Growth was calculated using the formula for specific growth rate ($SGR = 100\ (\ln(m_2)-\ln(m_1))/(t_2-t_1)$), where m = mass, t = time). This allowed the weight of each fish to be estimated by interpolation for each observation day, to be used in MR calculations.

The lowest 10th percentile of MR values (Chabot & Claireaux 2008; Killen *et al.* 2011) was used as an estimate of RMR for each fish, rather than the absolute minimal value to eliminate transient low MR that may be related to reflex bradycardia. In order to control for variation between rounds in the observed range of standard metabolic rates, estimated RMR values were converted to relative RMR (calculated as an individuals RMR minus the mean RMR of all the fish in each section). Relative RMR was used to eliminate difference in RMR between rounds due to seasonal changes in water temperature and body size. As relative RMR could differ between treatment groups of four individuals and as it is the relative value of a fish's RMR compared to its opponents (rather than its absolute value) that is linked to dominance (Metcalfe *et al.* 1995), each individual in a group was given a RMR rank (1-4, with 1 being the fish within the group with the highest relative RMR). Feeding rates (events per min) were adjusted by the same method as relative RMR.

Statistical analyses were based on mean values for body/eye colouration, aggression and activity (distance moved per min and % time in water column) for each fish, averaged over the 42 observations. Replicate (cohorts of four) was included as a random factor in all Linear Mixed Effect (LME) models to control for possible non-independence of fish within each cohort. To control for slight seasonal changes between replicates, Julian date (start of each round of experiment) was included as a fixed factor in each statistical model. Non-significant terms ($P>0.05$) were dropped stepwise from models (least significant

first, and starting with interactions) to ensure minimum adequate models. Analyses were performed using SPSS (v15.0).

3.4. Results:

The mean initial weight of fish at the start of each trial (for each round respectively: 3.7g±0.1, 4.7g±0.2, 5.2g±0.2, 5.9g±0.2; overall range 2.8-7.7g) did not differ between treatments but showed the typical seasonal increase (Table 3-1, analysis 1). Antagonistic interactions were sufficiently frequent that dominance ranks could be assigned to each fish during the first 48 hours (settling period) of each trial. These assigned ranks were robust since on average 92.5% of all observed interactions over the course of each trial (n = 529) were won by the higher-ranked individual of the pair and the percentage of aggressive interactions won declined linearly with dominance rank (Table 3-1, analysis 2).

In all treatments, both relative RMR and RMR rank were significant predictors of dominance (Fig 3.1 and Table 3-1, analyses 3a and 3b); hereafter RMR rank is used as the measure of dominance potential. There was no discernable effect of the habitat treatment on either measure of activity or on stress (Table 3-1, analysis 5, 6 and 7). However, treatment did affect aggression rates, which were much higher in the SP treatment than in either the SU or CU conditions (Fig 3.2 and Table 3-1, analysis 8).

Across all treatments, estimated feeding rate was not predicted by the quality of territory that a fish was occupying at the time (Table 3-1, analysis 9) but this was likely due to insufficient total observation time and/or the necessity of pooling different prey types (bloodworms and other, smaller, food items) in the same measure. Individual growth rates varied from 0.87 to 4.89% of body mass per day.

Figure 3.1 Interval plot showing the relationship between dominance rank (1 = most dominant fish in a group) and relative RMR across all treatments (p<0.001).

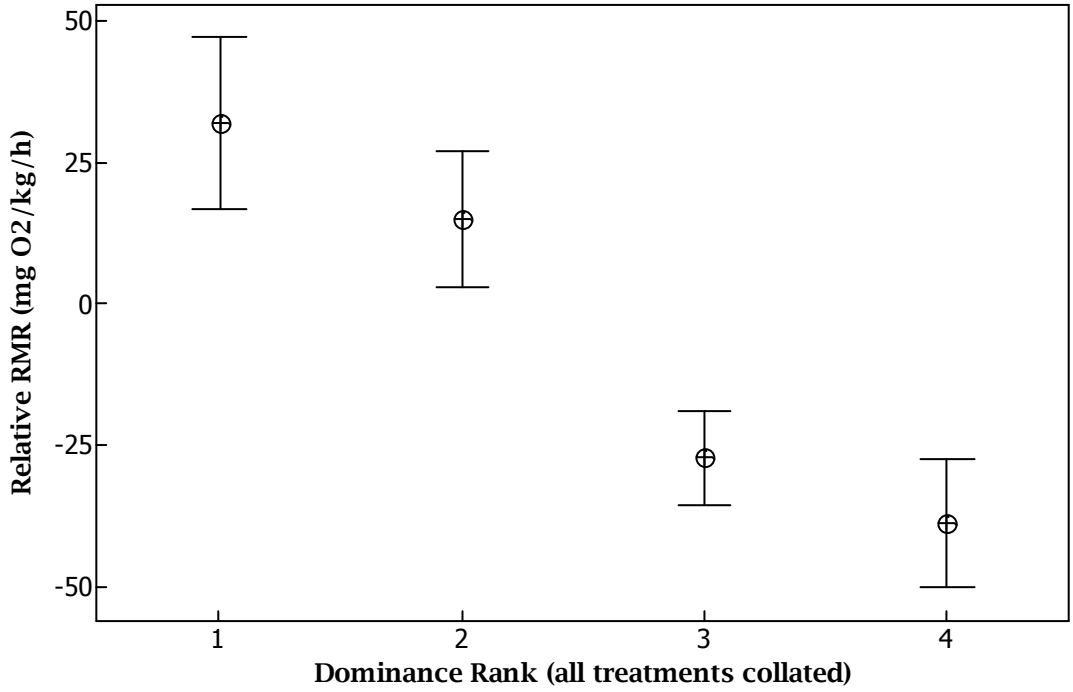
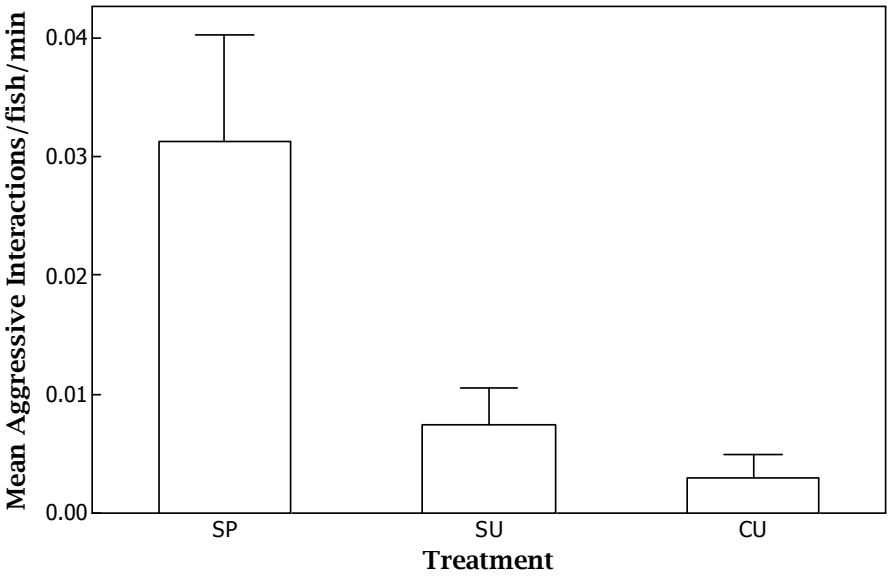


Figure 3.2 Mean aggression rates (aggressive encounters per fish per minute) between salmon for each treatment ±SE (p=0.04).



A fish's RMR rank correlated with the mean rank of territory quality that it occupied in the SP and SU treatments, although the relationship was much stronger in the former (Fig 3.3 and Table 3-1, analysis 10). No relationship between RMR rank and mean rank of territory quality was found in the CU treatment, leading to an interaction between rank and treatment (Fig. 3.3 and Table 3-1, analysis 10). Site fidelity, defined as the percentage of time each

individual spent at their most frequently used feeder, was found to be highest in CU (Fig. 3.4 and Table 3-1, analysis 11).

Figure 3.3 The effect of RMR rank on mean territory quality held by a fish in each treatment. RMR rank correlated with mean territory quality rank in the Simple Predictable (SP, p=0.002) treatment but not in the Simple Unpredictable (SU, p=0.048) or Complex Unpredictable (CU, p=0.612) treatments (see Table 3-1 for statistical analyses).

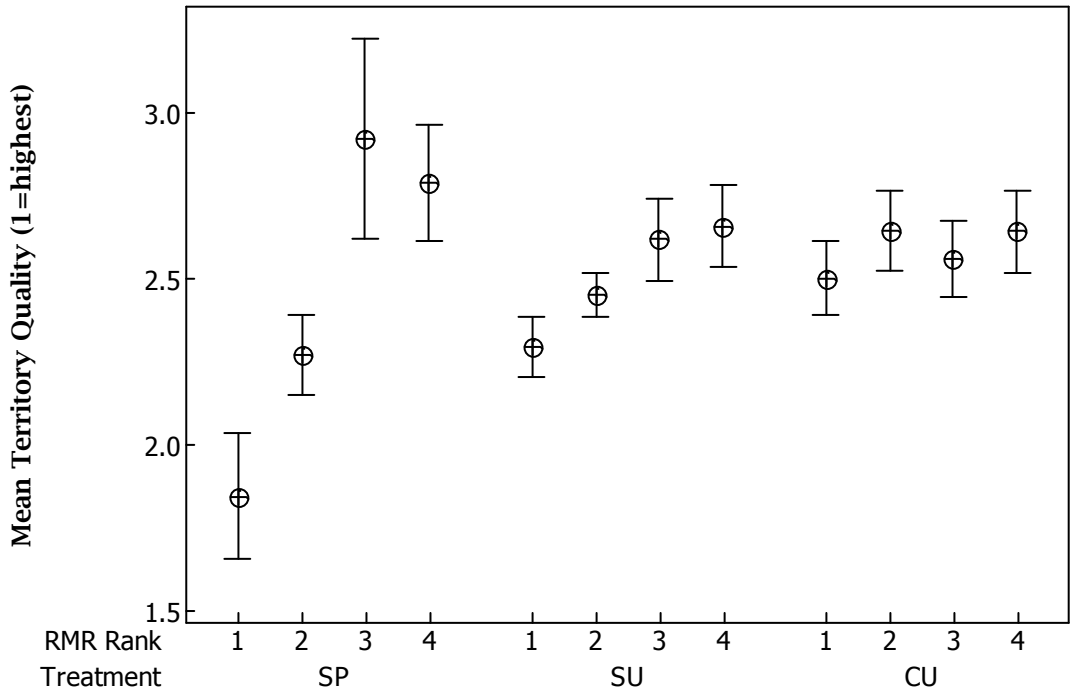
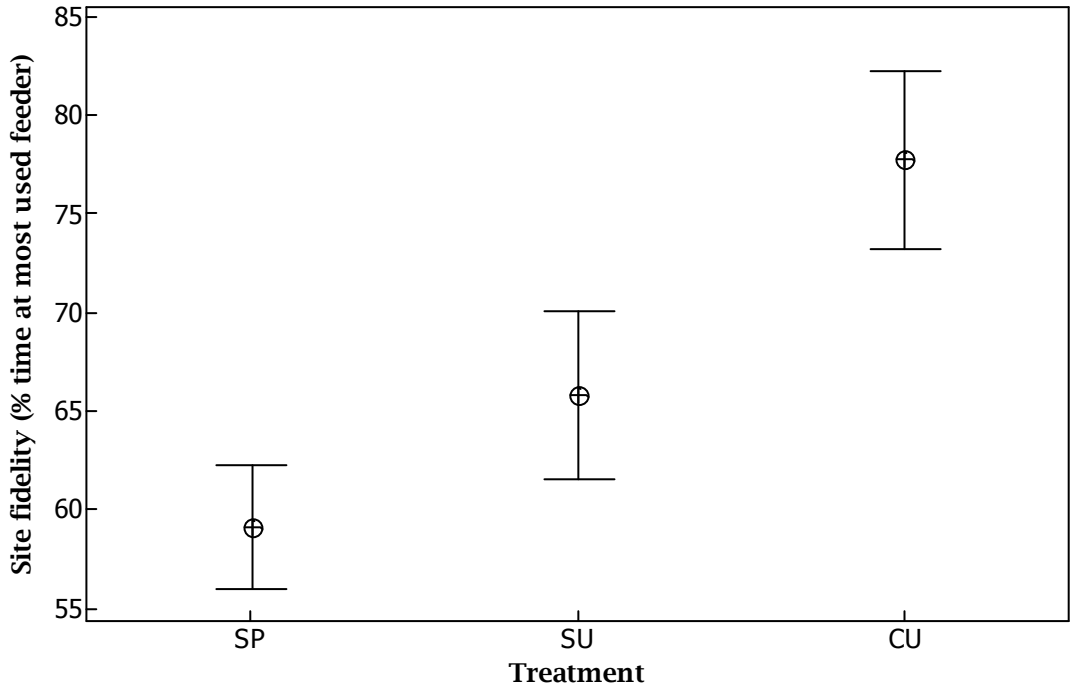


Figure 3.4 The effect of treatment on site fidelity (calculated as the percentage of time each individual spent at their most frequently used feeder, p=0.002)



RMR rank was negatively related to growth in the SP treatment but not in either the SU or CU treatments (Fig 3.5 and Table 3-1, analysis 12). There was also an effect of treatment, with average growth rates being significantly higher in the SU than either the SP or CU treatments, which were very similar to each other (Fig 3.6 and Table 3-1, analysis 12).

Figure 3.5 The relationship between RMR rank and mean daily growth of fish in each treatment (SP (p=0.001), SU (p=0.364), CU (p=0.411), relative growth within replicates).

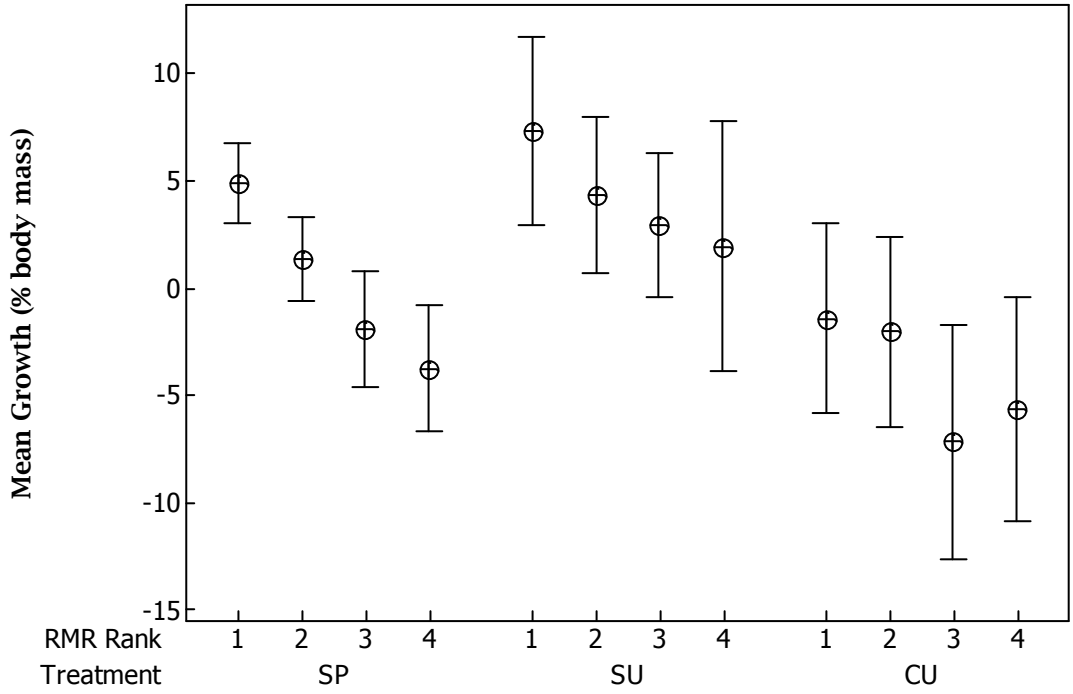


Figure 3.6 The effect of treatment on fish specific growth rate (per day, p=0.004), (NB growth data are absolute, interval bars are derived from data points for each individual).

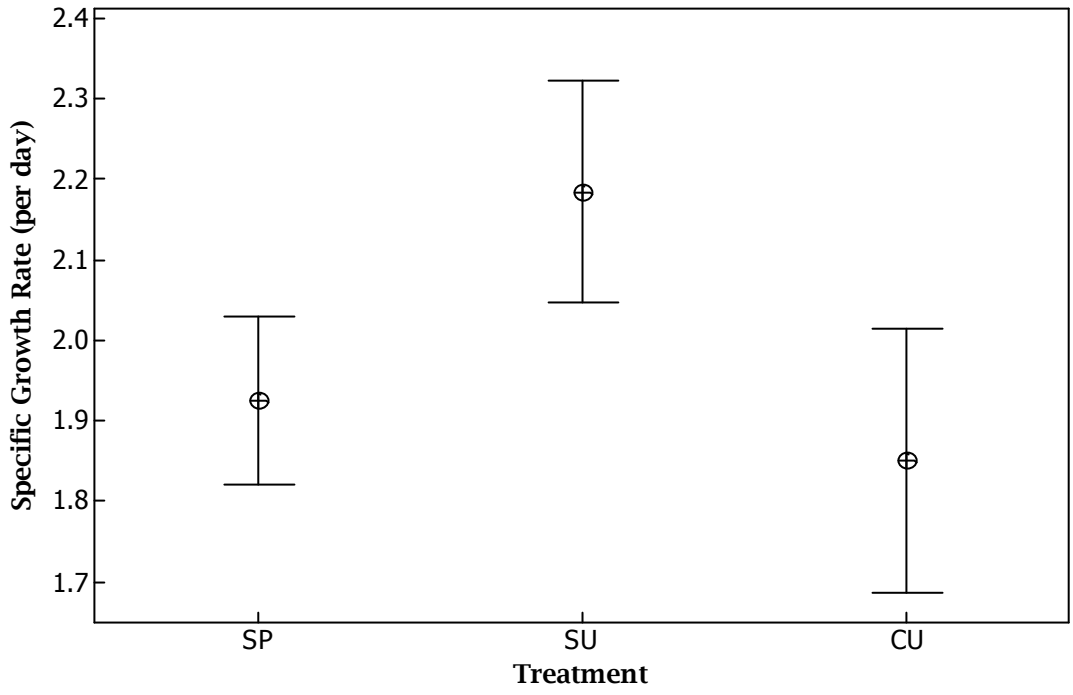


Table 3-1 Results from all LME model analysis, detailing the dependent, considered explanatory and significant explanatory variables (with parameter estimates) in each final model. Julian date (as a fixed factor), and replicate as a random factor were initially included as candidate explanatory variables in all models. Stress colouration, feeding rate, dominance rank, relative RMR, RMR rank, mean territory quality and body mass (as covariates) and treatment (as a fixed factor) were included as candidate explanatory variables where stated. Non-significant variables were removed from models in a stepwise fashion to produce minimum adequate models. Post hoc (least significant difference or LSD) tests were applied to treatment to differentiate significance between experimental treatments.

Dependent variable	Additional explanatory variables considered in model	Significant explanatory variables	F	df	Estimated parameter values	Significance
1. Initial weight	Treatment	Date (1) (2) (3)	22.24	3,92	-1.89 -1.13 -0.51	<0.001
2. Aggressive interactions won (%)	Dominance rank	Dominance rank	327.5	1,94	-29.0	<0.001
3a. Dominance	Relative RMR Body mass	Relative RMR	21.69	1,94	-0.01	<0.001
3b. Dominance	RMR rank Body mass	RMR rank	37.30	1,94	0.56	<0.001
4. Shelter use	Treatment RMR rank Body mass	Treatment (SP) (SU)	2.51	2,93	-0.85 -0.21	0.087
5. Activity (time in water column)	Treatment RMR rank Body mass	None				NS
6. Activity (distance moved)	Treatment RMR rank Body mass	None				NS
7. Stress colouration	Treatment	None				NS
8. Aggression rate	Treatment	Treatment (SP) (SU) Post hoc LSD: SP v SU SP v CU SU v CU	7.35	2,93	0.03 0.01	0.04 0.007 0.002 0.579
9. Feeding rate	Territory quality Body mass Treatment	None				NS
10. Territory quality	RMR Rank Treatment	RMR rank Treatment (SP) (SU) RMR rank* Treatment (SP) (SU) (CU)	17.84 6.31 10.21	1,90 2,90 2,90	0.18 -0.52 0.11 0.18 -0.05 0.00	<0.001 0.003 0.002 0.048 0.612
11. Site fidelity	Treatment Dominance Rank	Treatment (SP) (SU)	6.87	2,93	-18.67 -11.92	0.002
12. Specific growth rate	RMR rank Treatment Body mass	RMR rank Treatment (SP) (SU)	20.91 5.98	1,92 2,92	-4.07 4.00 8.47	<0.001 0.004

	Stress colouration	RMR rank* Treatment (SP)	-0.24	0.001
	Feeding Rate	(SU)	-0.01	0.364
	Territory quality	(CU)	0.00	0.411

3.5. Discussion:

There was substantial variation in growth rates between fish in the same environment, which was partially explained by individual variation in RMR. The estimation of metabolic rate from ventilation rate, while indirect and so associated with some error (Millidine *et al*. 2008), allows insight into the energetics of juvenile salmonids in more varied habitats than would be possible with the use of respirometry methodology. In the present study the technique reveals that the link between a juvenile salmon's ranking in resting metabolic rate (relative to neighbouring fish) and its growth performance is highly dependent on the nature of the environment, and is linked to its ability to track the spatial changes in food distribution over small spatial scales.

The correlation between RMR and dominance was consistent with previous studies (Metcalfe *et al*. 1995). When occupying a topographically simple habitat in which the food supply was spatially and temporally predictable, high RMR (thus dominant) individuals were able to outcompete conspecifics for the best feeding territories and achieve the highest growth rates. The approximately linear relationship between social rank and growth rate supports previous studies of salmonid fish using similar habitats (Nakano 1995b; Sloman *et al*. 2008), with subordinates being forced into poorer feeding locations and subject to higher rates of aggression due to food patch predictability (Grand & Grant 1994; Ryer & Olla 1995).

When feeding territories were unpredictable in terms of their relative profitability at any one time, high RMR individuals in the simple habitat

demonstrated the ability to track changing resources, such that they still spent more time in better feeding territories than low RMR individuals (this is not to imply that low RMR individuals could not track changing resources, simply that they were unable to displace conspecifics). However, this greater time in the best feeding locations did not lead to significantly faster growth for high RMR fish. The relationship between RMR and performance in the SU treatment was thus not as strong as in the simple predictable habitat, which may be due to the higher energy costs needed to track changing resources and displace conspecifics, and to the greater routine energy costs of high RMR fish. Dominance-mediated territorial acquisition in this environment thus generated insufficient foraging benefits to offset higher metabolic costs. Unpredictable food resources reduced aggression (Goldberg, Grant, & Lefebvre 2001), possibly reducing metabolic costs for dominants instigating and subordinates fleeing aggressive encounters.

An unpredictable feeding environment coupled with complex habitat created a situation where it was difficult for individual fish to track resources, as they were unable to see either the food or the foraging activity of neighbours in adjacent territories, effectively decreasing territory size (Imre, Grant, & Keeley 2002). Habitat complexity was observed to reduce both aggression (Baird, Patullo, & MacMillan 2006) and food monopolisation (Basquill & Grant 1998). As a result, each individual typically spent more of its time within a single feeding territory (i.e. greater site fidelity), even though the ranking of that territory (in terms of quality) varied over time. This led to no significant relationship between relative RMR and either quality of territory occupied or growth rate, as all individuals performed similarly irrespective of their metabolic rate.

This study demonstrates that the potentially advantageous traits associated with a high RMR that can lead to higher growth performance are lessened when individuals face a more unpredictable environment and nullified with increased habitat complexity. This potentially explains the conflicting results regarding the relationship between RMR or dominance and growth rate in the wild (Martin-Smith *et al*. 2002; Harwood *et al*. 2003; Álvarez *et al*. 2005; Sloman *et al*. 2008; Adriaenssens *et al*. 2011). The variability in RMR-mediated performance between habitats may help to maintain intraspecific variation in resting metabolic rate, as slight spatial and temporal variation in habitat characteristics and foraging

profitability will result in no one single metabolic rate being favoured. Stream invertebrate abundance, and whether terrestrial or aquatic in origin, exhibits considerable variation within a day (Martin-Smith *et al*. 2002), therefore territory quality will likely vary over short time intervals. The temporal shifting of feeding location by salmonids of this age class has been observed in the wild (Bachman 1984), and in this study as a consequence of food unpredictability in a topographically simple environment, although this was not found by Maclean *et al*. (2005) possibly due to a more complex habitat treatment in that study more representative of our CU treatment.

At a population level, the greatest increase in biomass was observed in the treatment with a simple habitat combined with unpredictable food, presumably due to increased foraging efficiency (Kemp, Armstrong, & Gilvear 2005). However, a structurally more complex habitat will likely provide other benefits, such as providing shelter that act as refuges from predators and that may also lower baseline metabolic costs (Millidine *et al*. 2006; Finstad *et al*. 2007a).

This study has highlighted the benefits of measuring the performance of individuals differing in behaviour and physiology traits under a range of environments. The consequence of a given metabolic rate also vary with neighbour density (Chapter 2, Reid *et al*. 2011). Further study would be interesting to determine how metabolic rate relates to fitness under environmental conditions that incorporate features such as interspecific, inter-cohort and predator interactions, and across more challenging habitat complexities that match the varied nature of salmonid environments (Armstrong *et al*. 2003).

Across taxa, resting levels of metabolism are associated with behavioural traits which influence performance (Biro *et al*. 2010). As habitat is subject to change (Sousa 1984) and animals often move between habitats (Wegner & Merriam 1979; Selonen, Hanski, & Desrochers 2010), the fitness consequences of inter-individual traits will also be variable. The results of this study suggest that research on trait-mediated performance should, where possible, incorporate the natural habitat heterogeneity in order to increase its ecological relevance.

A version of this chapter has been submitted as a manuscript and accepted by the Journal of Animal Ecology (in press).

4. Effect of heterospecific competitors on the link between estimated standard metabolic rate and growth performance in stream-living juvenile salmon

4.1. Abstract:

A range of laboratory experiments have suggested that inter-individual variation in energy budgets impacts greatly on performance, but this influence becomes less clear when examined in systems that better reflect natural conditions. Standard metabolic rate (SMR) in Atlantic salmon (*Salmo salar*) is positively related to dominance status and ability to obtain a territory. However, it is not apparent how this is affected by competition from heterospecifics that would be expected to occur in the wild, given that salmon are generally less dominant than species such as the brown trout, *Salmo trutta*.

The relationships between estimated SMR, dominance and growth rates of yearling Atlantic salmon were examined under different trout densities using replicate sections of a large scale controlled experimental stream. The SMR of salmon was strongly correlated with their dominance rank, but was not correlated with growth rate when in the absence of trout. Moreover, at low trout densities salmon demonstrated a negative relationship between SMR and growth, possibly because the trout had a disproportionate effect on higher ranking salmon, disrupting their territorial status and allowing low SMR (subordinate) individuals to perform better. However, trout interacted amongst themselves more at a higher density, leading to a positive SMR-growth relationship in the salmon. The relative performance benefit of a high SMR is thus conditional on the presence and density of heterospecific populations.

4.2. Introduction:

The fitness impact of inter-individual variation in physiological traits has been increasingly of interest in recent years (Williams 2008; Boratynski *et al.* 2009), but investigations of its importance have tended to focus on single-species systems. While this is an essential first step, traits should really be evaluated in more natural situations incorporating other species, since competition from heterospecific competitors that exhibit niche overlap can have significant effects on performance (Persson 1983; Griffiths, Edgar, & Wong 1991; Grosholz 1992) and subsequent life-history strategies (Connell 1980). Here we consider the effect of interspecific competition on the link between metabolism and growth.

Standard, or basal, metabolic rate (SMR/BMR) denotes the minimal energy requirement of an organism in a quiescent state (Hulbert *et al.* 2000; Frappell *et al.* 2004) (BMR strictly refers to endotherms and SMR for ectotherms; hereafter for simplicity for all taxa we will use the single term SMR). SMR has been found to be a repeatable physiological trait within individuals, yet often varies significantly among individuals of the same species and life stage (Hayes *et al.* 1992; Kvist *et al.* 2001; Steyermark *et al.* 2005). These inter-individual differences in physiology are also linked to behavioural traits. High SMR individuals are more likely to be socially dominant, aggressive and bold, thus outcompeting low SMR individuals for food (Biro *et al.* 2010). While this strategy has the potential to confer an obvious competitive advantage, it however requires a higher food intake to maintain.

Atlantic salmon *Salmo salar* is an ideal species in which to examine effects of SMR because individuals can vary over 3-fold in their minimal energy requirements (Enders *et al.* 2005). High SMR salmon have been found to be more dominant (Metcalfe *et al.* 1995), aggressive (Cutts *et al.* 1998) and bold (Finstad *et al.* 2007b) than conspecifics with a lower SMR. This results in their being more able to acquire good territories and/or profitable feeding areas, potentially leading to faster growth rates (Reid *et al.* 2011). The growth performance advantages of high dominance status are most pronounced when investigated in

simple predictable environments (Fausch 1984, Reid et al. in prep.), which do not necessarily reflect the complexity of natural systems where little relationship between SMR or dominance and growth has been found (Martin-Smith *et al*. 2002; Harwood *et al*. 2003; Álvarez *et al*. 2005; Sloman *et al*. 2008).

Throughout most of their range, the biggest competitor of juvenile Atlantic salmon is the closely-related brown trout (*Salmo trutta*), juveniles of which are found in similar riverine habitats and exhibit extensive niche overlap in terms of diet, space use, refuge requirements and foraging mode (Heggenes, Bagliniere, & Cunjak 1999; Armstrong *et al*. 2003). Both species are territorial, but brown trout are generally more aggressive than, and dominant over, Atlantic salmon of the same size class (Heggenes, Bagliniere, & Cunjak 1995; Harwood *et al*. 2002; Höjesjö *et al*. 2005). Thus trout provide an appropriate species in which to examine whether the performance benefits of a high SMR are affected by the presence of more competitive heterospecifics, as would often coexist with salmon in the wild. We hypothesise that trout, through their more dominant behaviour, will disrupt the social hierarchies that form in a homogeneous salmon population and as a result will reduce the competitive advantage of high SMR/dominant salmon. As a result, low SMR/subordinate salmon are predicted to experience less aggression and exhibit higher growth rates in a sympatric population than in an allopatric population, even if the trout are in addition to the salmon such that the total population density of salmonids has increased.

4.3. Methods

36, second-summer juvenile Atlantic salmon parr and 6 second-summer juvenile Brown trout were caught by electrofishing from the River Almond, Central Scotland on 17th May 2010, and size-matched by eye to obtain individuals of roughly similar size and age. They were taken to Marine Scotland's Almondbank field station and held in 1m diameter circular stock tanks. The following day fish were anesthetised (Benzocaine 7.5ml/L), weighed (to nearest 0.1g), measured (fork length to nearest mm) and uniquely fin marked with alcian blue dye via

Panjet. Each salmon recovered in an individual aerated 10L tank (32x17x19cm, darkened with a shelter) and the trout were returned to a separate stock tank.

After 24 hours, each quiescent salmon had its ventilation rate (VR) measured as the number of opercular beats observed in one minute. This was repeated a further two times with at least 1 hour between measurements. Mean VR (repeatability = 0.88, calculated as in Lessells & Boag (1987)) was combined with knowledge of water temperature (T, in °C, measured by digital thermometer) and body mass (W, in g) to estimate each fish's metabolic rate (mg O_2 kg^{-1} h^{-1}) using calculations derived by Millidine *et al*. (2008). As individuals were in a quiescent post-prandial state, this metabolic rate was used as a value for SMR. The regression equations (taken from Millidine et al. (2008)) used to predict SMR from VR were:

MR = m(VR) + c

Where m = 0.2773–(0.2350*log10(W))–(0.01838*T) + (0.05813*(T)*log10(W))

c = -3.4078 + (0.2958*T) + (2.1956*log10(W)) - (0.82057*(T)*log10(W)) + (0.5335*W)

This approach of using VR to estimate metabolic rate has been shown to be very reliable with this population of salmon (Chapter 5, Fig 5.2). Salmon were then size-matched within cohorts of 6 individuals, with each cohort being introduced into a separate section of a large indoor stream. A single trout parr was placed in each of two of these stream sections, and two trout parr in a further two sections. This resulted in two simultaneous replicates of three treatments: one of 6 salmon, another of 6 salmon with one trout and a third of 6 salmon with two trout (for a total of 6 stream sections). For brevity, each treatment will be referred to by its component population of trout (T) as 0T, 1T and 2T. This experimental design thus maintained the salmon density at 6 fish per section regardless of treatment, so that the trout were in addition to (rather than in place of) salmon; this additive design (*sensu* Fausch 1998) was thus a conservative test of the effect of the trout, since the predicted improved performance of low SMR salmon in the 2T condition would be in spite of the overall increase in fish density.

Each stream section was 7.5m long and 1.5m wide and thus measured 11.25m^2 in area. The stream was unoccupied by fish for 6 months prior to experiments and continuously fed with unfiltered water from the river Almond (source of the fish), which allowed the inflow of small (<4mm diameter) invertebrates through the upstream mesh screens; these colonised the indoor stream to create a natural resident invertebrate fauna and source of drifting prey for the fish. No supplementary food was added to the stream. One side wall of the stream comprised clear glass to allow behavioural observations, and was marked off in 0.3m gradations to facilitate recording of spatial positions. The substratum of the stream was landscaped so that each section contained an upstream and downstream gravel substrate riffle area (each 4.9m^2 area, 0.15-0.20m water depth) separated by a single pool (1.5m^2 area, 0.4m depth). Each section included 10 equally-spaced overhead shelters near the viewing side of the stream. These were opaque plastic rectangular roofs (12 x 5cm) held 8cm above the substrate by rods supporting each corner.

Fish remained in the stream for 13 days, 7 of which were observation days. During these days, each fish (both salmon and trout) was observed (in random order) in turn for 2 minutes, once every 80 minutes, providing six 2-minute observations over each observation day. Subordinate juvenile salmonids develop a characteristic darker colouration to both their body and eye sclera, as a signal of their status to more dominant individuals (O'Connor *et al*. 1999); this coloration is thus an indication of the degree of social stress that they experience. In each observation, a combination of body and sclera colouration was used to determine a stress value (1 to 5, 1 being lightest and hence least stressed) (O'Connor *et al*. 1999; Suter *et al*. 2002). Aggressive interactions were recorded, with a note of the winner and loser. Juvenile salmonids demonstrate aggression by making charge attacks by lunging toward each other, often biting with their mouths but rarely making contact with each other. This results in the loser swimming away, often chased by the winner. The longitudinal distance covered by each focal fish within the 2 min observation period were recorded using the aforementioned 0.3m gradations for upstream/downstream movements to give a activity measure of distance covered for each observation. Activity was also measured by time spent in the water column (estimated as 0, 33, 66 or 100%

of each 2 min observation time). It was also noted whether the fish occupied pool or riffle areas (or both).

This resulted in 42 observations for each fish. After the experimental stream period (13 days) was over, all fish were removed from the stream, anaesthetised, re-weighed and measured (fork length). A new group of salmon and trout were then collected from the river and the procedure repeated. In total the protocol was run four times (i.e. 4 rounds, with stream observations starting on 20th May, 3rd June, 17th June and 1st July 2010), producing 8 replicates for each of the three treatments (total n = 144 salmon and 24 trout, with each fish being used once). Treatment allocation to stream sections was changed between each round to avoid any possibility of confounding systematic positional bias.

For statistical analyses (conducted using SPSS v15.0), mean values for stress coloration, spatial activity, water column activity, aggression, pool/riffle use and shelter use were calculated for each individual. Growth rate during the experiment was converted to % gain in mass per day, using the formula for specific growth rate (SGR = 100 $(\ln(m_2)-\ln(m_1))/t_2-t_1$). In order to control for variation between rounds in the observed range of standard metabolic rates, estimated SMR values were converted to relative SMR (calculated as a salmon's SMR minus the mean SMR of all the salmon in each round of the experiment). Relative SMR was used to eliminate difference in SMR between rounds due to seasonal changes in water temperature and body size. Feeding rates were adjusted by the same method. Linear Mixed Effect (LME) models were used for statistical analysis. Non-significant terms ($P<0.05$) were sequentially dropped from the models (least significant first) unless stated otherwise. Further details can be found in Table 4-1.

4.4. Results:

Mean salmon initial weight was 3.32g (±1.06 SD, range 1.6-6.2g), mean trout initial weight was 7.37g (±2.36 SD, range 3.5-12g), with the trout always being

heavier than the salmon with which they were grouped. As expected, the mean initial mass of both species increased throughout the season, but importantly did not vary among treatments (Table 4-1, analysis 1).

Presumably in part because of their slight weight advantage, all trout were observed to win aggressive encounters with salmon (n = 24 of 24 interactions) and were therefore considered the more dominant species. Trout were involved in 22% of aggressive encounters observed in 1T treatments and 44% in 2T despite comprising only 14 and 25% of the fish population respectively. Treatment had no detectable effect on the rate of aggressive interactions per min between salmon (Table 4-1, analysis 2). Dominance was assigned to salmon by constructing linear hierarchies derived from aggressive interactions during the first 48 hours of the experiment, which mirrored the percentage of aggressive interactions each individual won overall (Fig 4.1, Table 4-1 analysis 3). In the T1 treatment there was an approximately linear relationship between a salmon's dominance rank and the rate at which it was involved in aggressive interactions with the trout (which salmon initiated on 38% of occasions, but never won), with more dominant salmon being more frequently involved in (and hence losing) aggressive interactions with trout (Fig. 4.2). This relationship was curvilinear in the T2 treatment, suggesting a quadratic relationship (Fig. 4.2); testing this by inclusion of the square of dominance rank resulted in a significant treatment x rank^2 interaction, with middle-ranking salmon being involved in fewest interactions with the trout (Table 4-1, analysis 4). Relative SMR was a predictor of dominance rank in salmon in all treatments (Table 4-1, analysis 5).

Figure 4.1 The relationship between assigned dominance rank and the percentage of intraspecific aggressive interactions won by juvenile salmon, all treatments combined (ignoring interactions with trout; values plotted are means +1SE bars, p<0.001)

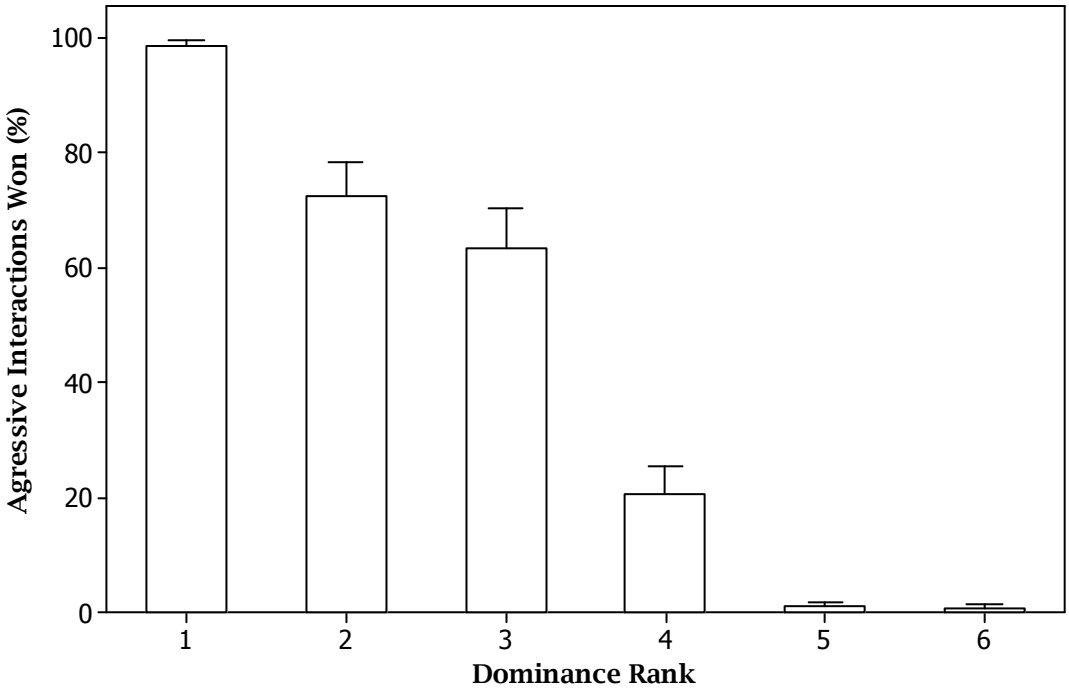
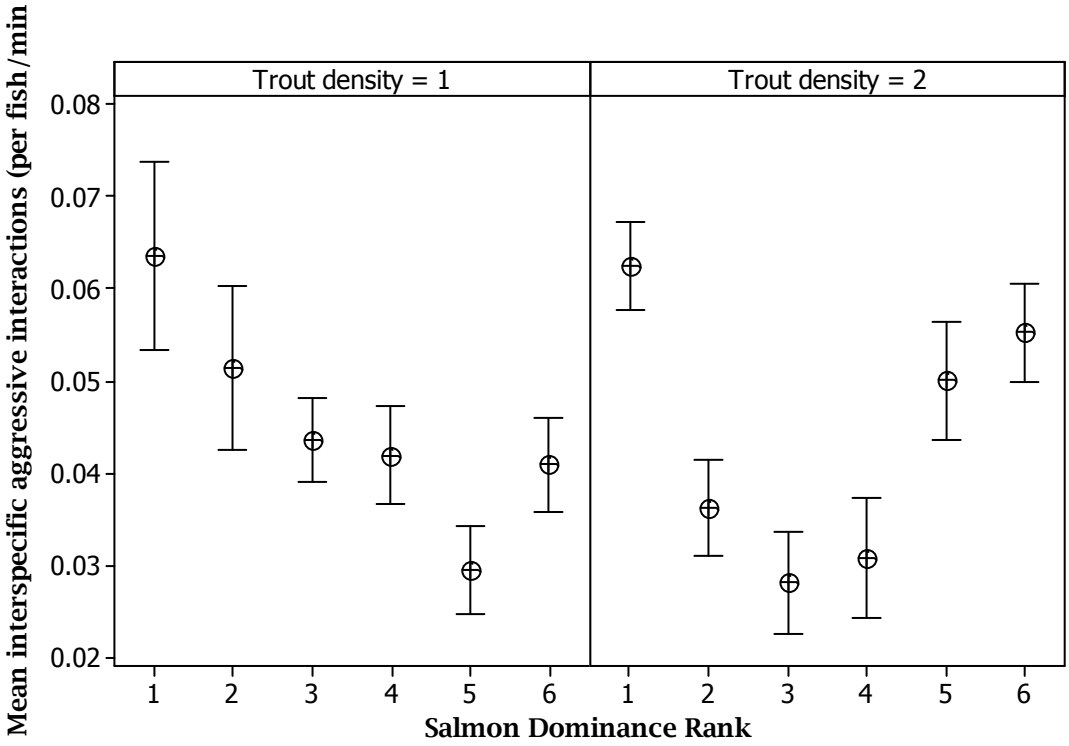


Figure 4.2 The relationship between salmon dominance rank and the frequency with which they were involved in (and lost) aggressive interactions with trout for both T1 and T2 treatments, (p=0.03).



Trout spent more time in pools (and less in riffles) than salmon but this did not significantly differ between treatments (Fig 4.3, Table 4-1, analysis 6a). Across all treatments, salmon with a higher relative SMR used the riffle habitat more than the pools (Fig 4.4, Table 4-1, analysis 6b). An increasing trout density led to salmon spending more time on average in the water column (Table 4-1, analysis 7a), whilst no such effect was found for trout (Table 4-1, analysis 7b). The horizontal distance moved per observation period did not vary with treatment or between species (Table 4-1, analysis 7c, 7d). Salmon stress colouration was not observed to be dependent on relative SMR or trout density (Table 4-1, analysis 8).

Figure 4.3 Mean riffle use by salmon and trout (i.e. percentage of observation time spent in riffle habitat, p=0.022)

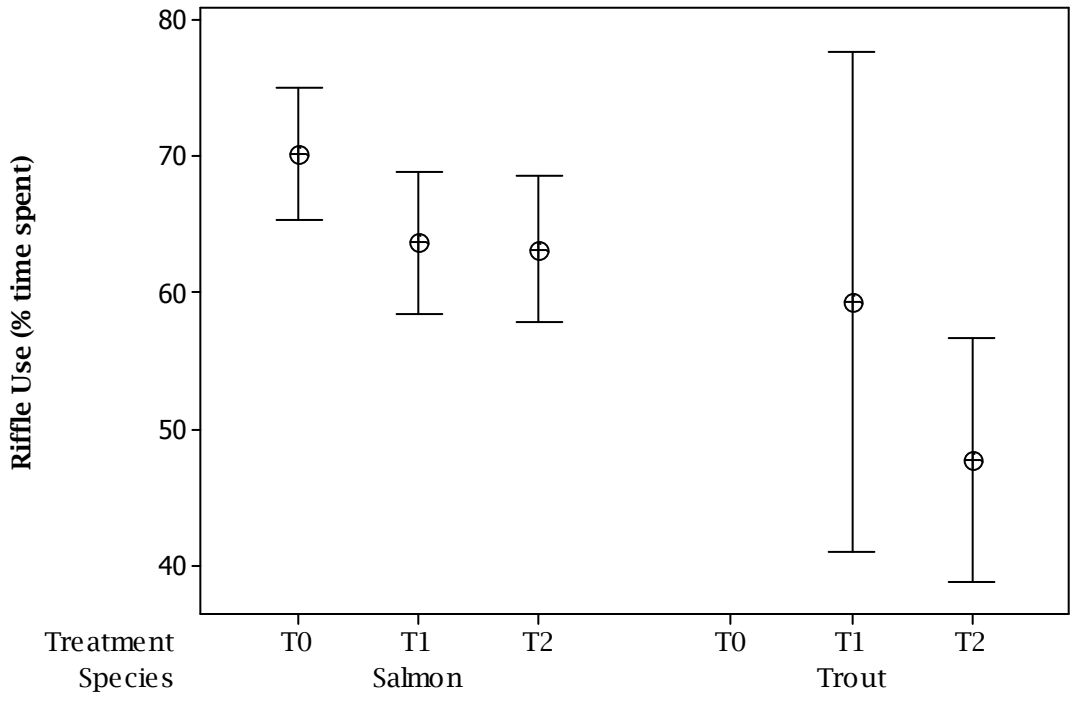
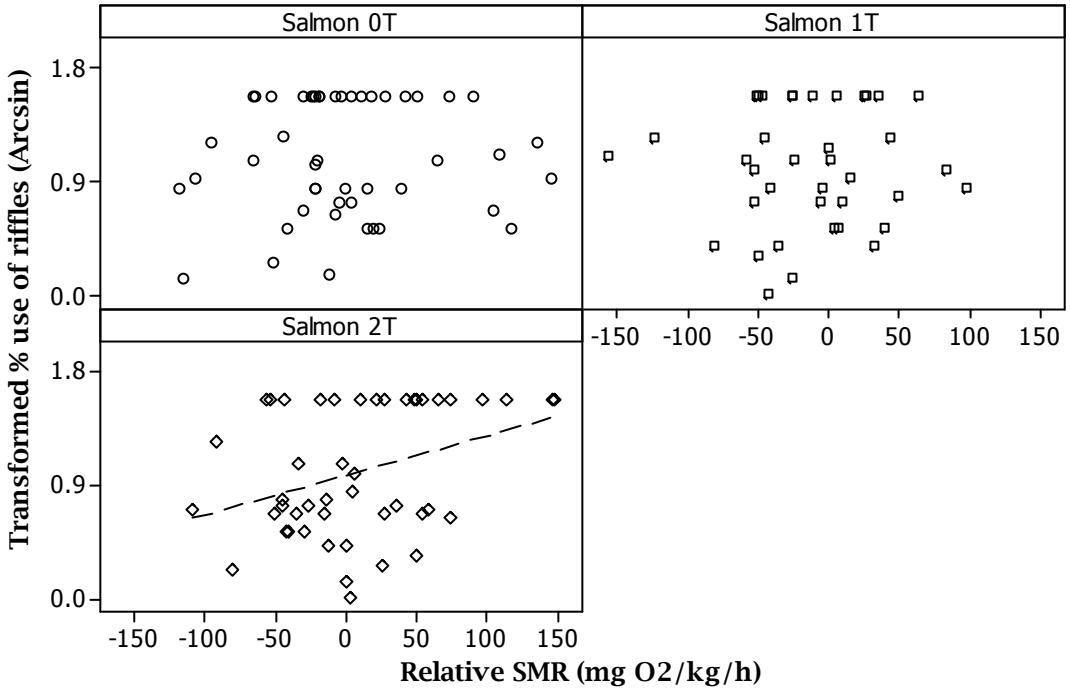


Figure 4.4 The relationship between relative standard metabolic rate of salmon and the percentage of time spent in riffle habitat (0T represents salmon from allopatric cohort, 1T represents salmon sympatric with one trout and 2T (p=0.046) represents salmon sympatric with two trout).



Observed feeding rates were not indicative of growth rates for either trout or salmon (Table 4-1, analysis 9), likely due to the impossibility of identifying drift/prey items which could differ greatly in size and calorific content.

There was no significant difference in mean salmon or trout growth between treatments, although growth did decline with season (Fig 4.5, Table 4-1, analysis 10a and 10b). Only 1 trout (out of 36) and 1 salmon (out of 144) lost weight during the experiment (growth increment for salmon ranged from -0.19 to 4.76%, trout: -0.16 to 2.82%). The effect of SMR on individual growth rates of salmon was dependent on trout density (Fig 4.6, Table 4-1, analysis 10c). Thus in the absence of trout (0T) there was no relationship between a salmon's SMR and growth. In the presence of a single trout a negative relationship between SMR and growth was observed, whilst with two trout this trend was reversed.

Figure 4.5 The relationship between species, treatment and growth (percentage weight change from initial mass, interval bars showing means ±SE are present for each species in each treatment, p=0.244)

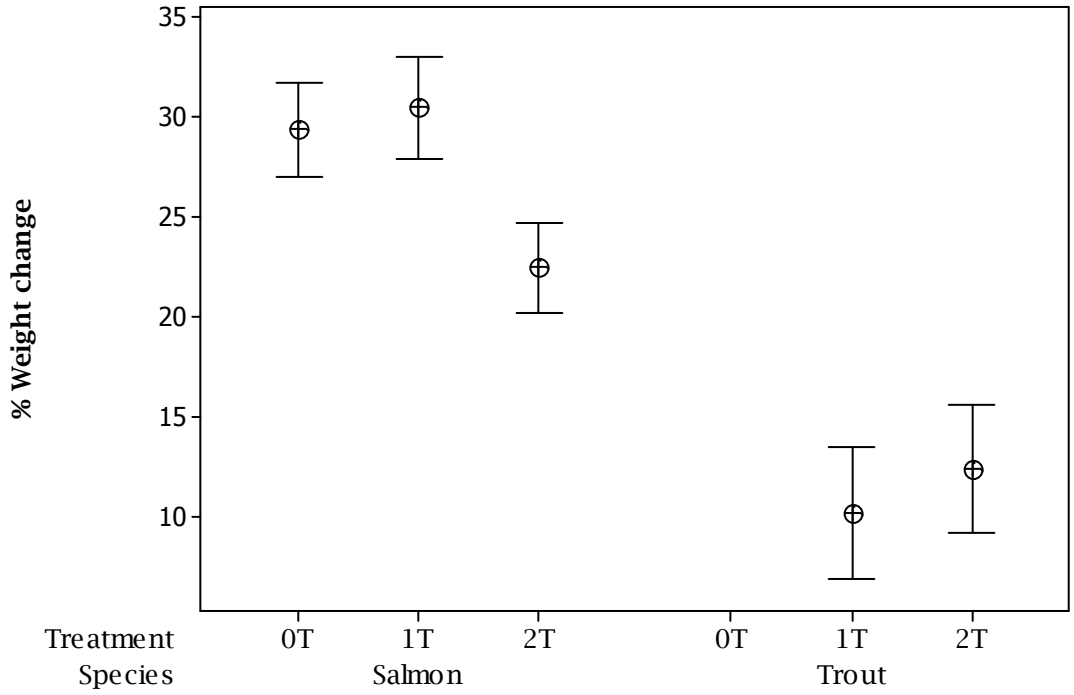


Figure 4.6 The relationship between relative standard metabolic rate and growth (percentage weight change from initial mass) for salmon in each treatment (0T represents salmon from allopatric cohort (p=0.983), 1T represents salmon sympatric with one trout (p=0.029) and 2T represents salmon sympatric with two trout (0=0.015)).

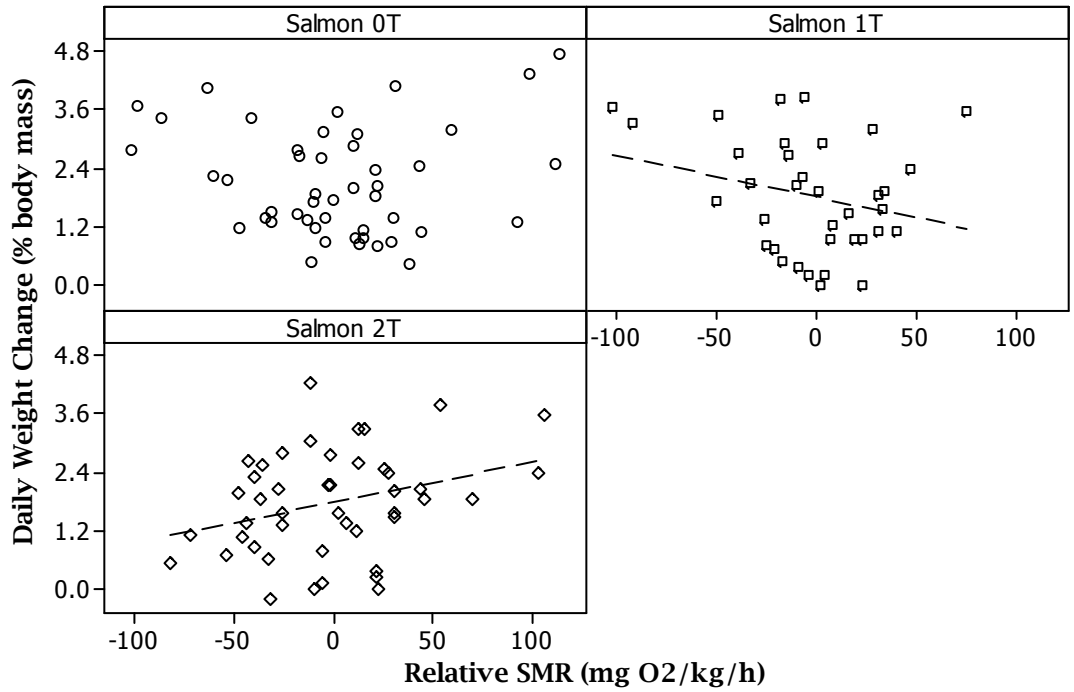


Table 4-1 Results from all LME model analyses, detailing the dependent, considered explanatory and significant explanatory variables (with parameter estimates) in each final model. Julian date and treatment (as fixed factors), and replicate as a random factor were initially included as candidate explanatory variables in all models. Stress colouration, feeding rate, dominance rank, relative SMR, and body mass (as covariates) and treatment (as a fixed factor) were included as candidate explanatory variables where stated. Non-significant variables were removed from models in a stepwise fashion to produce minimum adequate models. Post hoc (least significant difference or LSD) and pairwise comparison tests were applied to treatment to differentiate significance between experimental treatments. (S) refers to analyses only performed on salmon or (where species is a significant explanatory variable) to parameter estimates for salmon, where estimate for trout = 0, (T) refers to the same for trout.

Dependent variable	Additional explanatory variables considered in model	Significant explanatory variables	F	df	Estimated parameter values	Significance
1. Initial weight	Species	Species (S)	302.18	1,175	-2.03	<0.001
		Date (1)	32.08	3,175	-0.94	<0.001
		(2)			-0.48	
		(3)			0.23	
		(4)			0	
2. Aggressive interaction rate		None				NS
3. Aggressive interactions (% won) (S)	Dominance rank	Dominance rank	1023	1,142	1.34	<0.001
4. Aggressive interactions between S and T (0T data excluded)	Dominance rank	Dominance rank	23.66	1,91	-0.254	0.03
		Treatment	6.16	1,91	0.014	0.015
		Dominance rank^2	20.13	1,91	0.004	0.025
		Dominance rank^2 *Treatment	8.38	1,91	-0.001	0.005
5. Dominance rank (S)	Relative SMR Body mass	Relative SMR	4.10	1,142	-0.02	0.004
6a. Pool use	Species	Species (S)	5.33	1,178	-0.15	0.022
6b. Pool use (S)	Relative SMR	Relative SMR	4.05	1,142	0.01	0.046
7a. Activity – time in water column (S)	Relative SMR	Treatment	5.24	1,142	0.22	0.024
		(T0)			-0.34	
		(T1)			-0.20	
		(T2)			0.00	
		Post-hoc (LSD):				
		T0 v T1				0.261
		T0 v T2				0.003
		T1 v T2				0.072
7b Activity – time in water column (T)		None				NS
7c. Activity - distance moved (S)	Relative SMR	None				NS
7d. Activity - distance moved (T)		None				NS
8. Stress colouration (S)	Relative SMR	None				NS

9. Feeding rate	Species Body mass	None				NS
10a. Mean salmon growth (S)		Date (1) (2) (3) (4)	14.69	3,140	1.46 1.07 0.38 0	<0.001
10b. Mean trout growth (T)		Date (1) (2) (3) (4)	19.18	3,32	11.59 2.35 -0.56 0	<0.001
10c. Individual salmon growth (S) (Date excluded)	Relative SMR Body mass Stress colouration Feeding rate	Relative SMR Treatment Treatment* Relative SMR Post-hoc (LSD): Relative SMR* (T0) (T1) (T2)	0.00 1.43 3.38	1,138 2,138 2,138	0.000 0.000 0.000 -0.008 0.008	0.983 0.244 0.037 0.983 0.029 0.015

4.5. Discussion:

Growth rates of both species demonstrated that the established artificial stream used in this study could provide sufficient natural drift prey items to support fish of this age class and density. The growth rates also showed that salmon could feed and grow effectively in the presence of more dominant, trout populations (Höjesjö *et al*. 2005), possibly by utilising riffle habitat. Variation in salmon SMR was closely related to dominance status, as in previous studies (Metcalfe *et al*. 1995, Chapter 2), but despite high SMR fish appearing to obtain their preferred feeding positions through successful aggression, in the absence of trout this did not lead to their achieving higher growth than fish with a lower SMR. The lack of relationship between SMR or dominance and growth mirrors some studies of salmon in the wild (Harwood *et al*. 2003; Seppanen *et al*. 2009). As the present study did not use highly localized feeders (unlike Chapters 2 and 3), territory quality would vary little spatially and the dispersed nature of the food could not

therefore be easily monopolised by dominant individuals, as demonstrated in the dark eyed junco, *Junco hyemalis* (Theimer 1987).

When present, trout were found to be the more aggressive and dominant of the two species, mirroring previous studies (Harwood *et al*. 2002; Heggenes *et al*. 1995; Höjesjö *et al*. 2005). The presence of low densities of a dominant competitor disrupted the social hierarchy (Carrete *et al*. 2010), and resulted in a significant level of aggressive interactions between dominant salmon and trout (Blanchet *et al*. 2007). The negative effect of a single trout may thus have been felt disproportionately by high-ranking salmon. While not detected in this study, the presence of a superior heterospecific competitor might have led to a reduction in the feeding rates of the most dominant salmon, as shown in some avian species (Millikan, Gaddis, & Pulliam 1985; Alatalo & Moreno 1987), and/or led to greater energy expenditure due to attempted territorial defence (Marler *et al*. 1995). The combination of a greater energetic cost of a high SMR together with no benefits from increased intake may explain the observed negative relationship between SMR and growth in this situation. Interestingly, these trout densities had no effect on mean salmon growth, suggesting no detrimental density or interspecific effects at the population level, while facilitating growth in subordinates as we hypothesised.

However, at the higher trout density the relationship between interspecific aggression and salmon dominance became more complex: salmon in the middle of the hierarchy experienced least aggression from trout, while both the highest ranking and the most subordinate salmon incurred frequent aggression. A possible mechanism for this is related to the habitat preferences of the two species. Juvenile trout show a preference for slower velocity water in stream systems than salmon, and consequently are more commonly found in pools while salmon occupy riffle areas (Heggenes *et al*. 1999). In the present study a higher trout density may have led to the trout moving less within the stream, and becoming even more concentrated in the pools, leading to a greater contact rate with low-ranking salmon (which also tended to be found in the pools, presumably because they were excluded from the more favoured riffles by higher-ranking salmon). The only other salmon to incur appreciable aggression from trout was then the most dominant individual, possibly because it was most prominent and posed most threat to the trout. The greater aggression

experienced by low ranking salmon in 2T (Fig 4.2) may explain their poor growth rate at the higher trout density, which resulted in a positive relationship between salmon SMR and growth in this situation. The foraging shift by subordinates due to presence of competitors, and an intraspecific social hierarchy, may mirror that found in birds (Alatalo 1981).

Temporal discharge changes can affect water height and subsequently open up or decrease the area of habitat available for juvenile salmonids, leading to changes in the intensity of competition (Stradmeyer *et al*. 2008). Given that the two species normally select differing microhabitats, but that the relative availability of these microhabitats is dependent on water levels (causing shifts of fish from riffle into pool at low water levels), the intensity of interspecific competition between trout and dominant salmon will therefore also be dependent on discharge. However, increased habitat complexity, as would be found in the wild, would likely decrease both intraspecific (Chapter 3) and interspecific competition (Hasegawa & Maekawa 2008), possibly mitigating the trends seen in this study, although this requires future study.

This study shows that the links between dominance and performance can be affected by the presence of heterospecifics, as shown earlier (Blanchet *et al*. 2007). However, the direction of this trend was found not to be linear with increasing heterospecific density, with the status of individual salmon most affected itself changing with trout density. This should be taken into consideration when examining relationships between SMR or dominance status and growth in wild salmonids, and may help account for divergent results from field studies where the level of heterospecific density and interaction may not always have been measured (Álvarez *et al*. 2005; Harwood *et al*. 2003). This study also demonstrates effects of heterospecific competitors on habitat use (Höjesjö *et al*. 2010). Across taxa, dominant behaviour is seen as a competitive advantage over conspecifics (Appleby 1980; Tokarz 1985; Stahl *et al*. 2001), but these relationships are seldom studied in the presence of heterospecifics (Sih *et al*. 2004). For further investigation in the wild, knowledge of competitor species should be incorporated to better inform and explain relationships between individual physiology, dominance and performance.

5. The relationships between estimated standard metabolic rate, specific dynamic action and feeding rate in juvenile Atlantic salmon

5.1. Abstract:

The magnitude of basal metabolism can influence other aspects of physiology, behaviour and performance and may vary within species independently of body size and temperature. High standard metabolic rates (SMR) have been linked to a higher capacity for growth. A possible mechanism by which this is achieved is that higher SMR is correlated with faster digestion and assimilation of food and therefore an ability to re-feed sooner after a meal.

The relationship between SMR, specific dynamic action (SDA) and food intake was tested using young of the year Atlantic salmon (*Salmo salar*). On consuming a given size of meal, high SMR salmon were found to have a shorter but heightened SDA response, in addition to a greater overall magnitude of SDA.

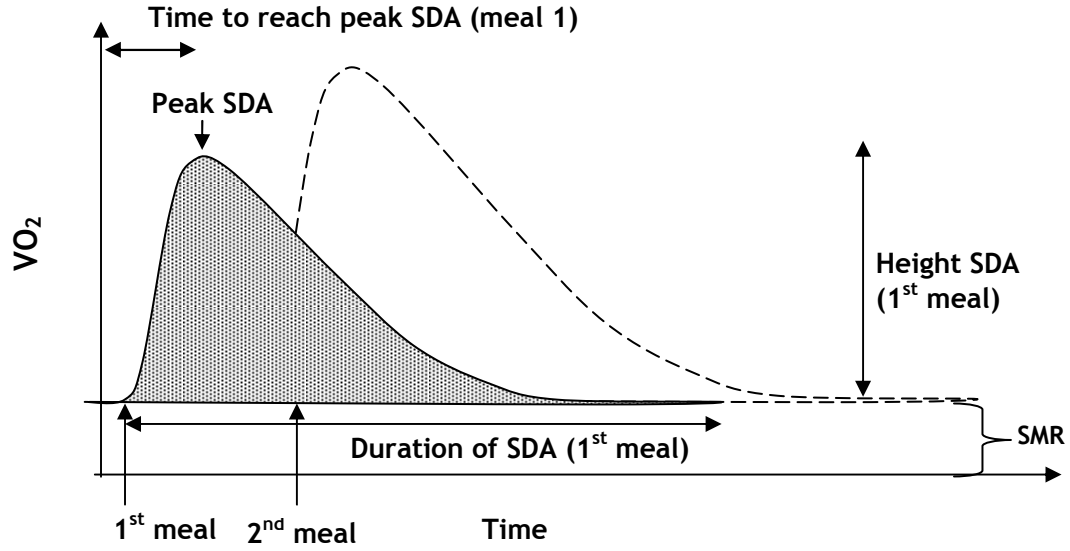
Monitoring the cumulative metabolic response to a sequence of meals demonstrated that an individual's SMR correlated with its absolute SDA scope (i.e. the highest recorded elevation of metabolic rate during digestion, minus SMR). This potentially provides a mechanism by which high SMR fish have a shorter duration of SDA, by allowing a greater metabolic capacity for digestive processes. However this did not result in high SMR fish having more meals per day or a larger maximal daily food intake compared to low SMR conspecifics over the experimental period. These results are not consistent with the relationship between SMR and growth potential being due to variation in SDA response influencing re-feeding.

5.2. Introduction:

Energy availability is of fundamental importance to an organism for provision of both somatic maintenance and investment in growth and reproduction (Lika & Kooijman 2003). The availability of energy for this investment is directly influenced by an organism's other metabolic demands, but may also be dependent on its cellular metabolic machinery. Standard metabolic rate (SMR) is considered the baseline energy requirement of an organism in a post-absorptive quiescent state with no oxygen debt (McNab 1988; Hulbert *et al*. 2000). SMR shows considerable variation between members of the same species of the same age and body size in both endotherms (Steyermark *et al*. 2005) and ectotherms (Hayes *et al*. 1992). Having a relatively high SMR has been linked to increased aggression, dominance and ability to gain access to preferred territories in a range of taxa (Bryant *et al*. 1994; Cutts, Adams, & Campbell 2001; Brown *et al*. 2003). This relationship between an individual's SMR and its social status has also been found in juvenile salmon (Metcalfe *et al*. 1995; Cutts *et al*. 1998), which exhibit up to 3-fold variation in SMR (Enders *et al*. 2005)). There are links between SMR, dominance and growth (Yamamoto *et al*. 1998), but due to *ad libitum* feeding in such experiments the trait may more aptly be considered as growth potential.

One mechanism by which an animal's SMR might influence its growth potential is through its speed in processing food, thus allowing re-feeding sooner and greater energy intake. Feeding and digestion are associated with metabolic costs due to gut motility, catabolism and absorption (McCue 2006; Secor 2009). The term specific dynamic action (SDA) is used to describe the increase in metabolic rate associated with the preabsorptive, absorptive and postabsorptive processes associated with ingesting a meal (Beamish 1974; McCue 2006). After ingestion, the rate of oxygen consumption rises quickly to a peak and subsequently decreases slowly throughout digestion (Jobling 1981, see Fig 5.1). During this time SDA can account for a significant portion of the energy budget (McCue 2006) and hence some animals try to minimise this cost (Radford, Marsden, & Davison 2004; Fu, Xie, & Cao 2005a; Jordan & Steffensen 2007) and divert energy toward growth (Kalarani & Davies 1994; Alsop & Wood 1997).

Figure 5.1 Hypothetical graph illustrating the typical rise and fall of metabolism after ingestion of a meal categorised as specific dynamic action or SDA. The shaded area represents the magnitude of SDA. The second line (dashed) illustrates the effect of a second meal before the SDA response to meal 1 has been completed, resulting in a longer heightened SDA of greater magnitude. Diagram is modified from original by Karen Millidine and used with permission.



Analyses of an individual's SMR and its SDA response show that juvenile salmon with a high SMR expend more energy in total on digesting a given size of meal (with a higher peak energy demand), but return to the baseline (SMR) level of energy expenditure sooner (Millidine *et al*. 2009). This suggests that digestion speed correlates positively with SMR. If so, individuals with a high SMR should be able to process meals faster and resume feeding sooner after consumption of a meal than can conspecifics with a lower SMR. The ecological advantage of this becomes obvious when food is abundant, since a high SMR would allow individuals to increase their food intake and so fuel a higher growth rate. However, this would cease to be beneficial if food was more limiting, as the extra energy cost of digesting a given amount of food would then be a disadvantage. If rapid digestion is fuelled by a higher peak energy demand, the degree to which individuals can increase metabolism (SDA scope) may facilitate this. Scope, of metabolism, can be defined in both absolute (peak MR minus SMR) and factorial (peak MR divided by SMR) terms. High SMR individuals have been found to have lower factorial aerobic scopes (Cutts, Metcalfe, & Taylor 2002b), which if also true for SDA scope could be detrimental as a high SMR may limit scope for increasing metabolic rate at peak food times. As food availability

varies both spatially and temporally, this may explain the divergent relationships found between SMR or dominance and capacity for growth/physiological scope for growth (Nakano 1995b; Harwood *et al*. 2003; Álvarez *et al*. 2005).

However, as yet there has been no investigation of whether SMR influences food intake. Here we investigate the possible links between SMR, SDA and food intake in juvenile salmon, using an experimental approach in which both the oxygen consumption rate and the food intake of fish are measured over time. We find similar relationships between SMR and the SDA response as (Millidine *et al*. 2009), but no relationship between SMR and food intake.

5.3. Methods:

Atlantic salmon parr (0+) were obtained from the Marine Scotland Science field station at Almondbank and transferred to the University of Glasgow in early November 2007. Although hatchery reared, all fish were offspring of wild parents caught from the river Almond. They were held in a square tank (1m diameter, 0.5m depth) at 10ºC in re-circulated, fully aerated copper-free water. The tank contained multiple small sections of plastic piping and artificial plants to provide ample shelter. The fish experienced an ambient photoperiod and were fed *ad libitum* daily on defrosted frozen bloodworms (Chironomid larvae). Bloodworms were the sole prey item used throughout the experiment as meal type can influence the SDA response (Pan *et al*. 2005; Secor 2009). At the start of the experiment, 10 randomly selected salmon, unfed for 24hrs, were weighed and subsequently placed singly into separate sections (25x20x20cm) of a flow-through tank that was supplied with recirculated fully oxygenated water. Each section of the tank contained a shelter. The fish were left for c.24 hours to settle, to reduce post-handling stress (Cutts *et al*. 1998). The water flow was kept minimal, so that it was sufficient to provide a continuous supply of oxygenated water but was not strong enough to force the fish to swim to maintain position. The temperature was kept constant (9.9 ºC ±0.4SD) throughout the experiment by using a recirculating water supply and by running the experiment in a constant temperature room.

120 individual defrosted bloodworms, taken from six different frozen packs, were blotted dry and weighed in order to calculate the mean wet weight of a bloodworm and so accurately determine the weight of food in any meal. Broken bloodworms were neither weighed nor fed to fish during the experiment.

Each fish was offered a small meal of defrosted bloodworms on the first experimental day of the protocol. This was done using a plastic pipette to place bloodworm into the tank, one at a time, and familiarise the fish with this new feeding method. The meal was restricted in size (judged to be significantly less than would be consumed in a single meal) and was given early in the day to allow complete gut evacuation by the following day (Cutts *et al*. 2002a). No data on feeding behaviour or metabolic rate (estimated through opercular ventilation rate (Millidine et al. 2008)) were recorded on this day. The next morning (experimental day 2), prior to feeding, three measurements at least 30 minutes apart were made of each fish's opercular ventilation rate (VR). VR was recorded as the number of opercular beats in a 20 second period; these values were later averaged and converted to an estimate of standard metabolic rate using equations from Millidine et al. (2008) – see below.

Individuals were then offered a sequence of single bloodworms, dropped every 60 seconds for a maximum of 30 minutes. Satiation was defined as being achieved when two consecutive bloodworms were refused, at which point feeding for that individual ceased. The total number of bloodworms eaten in that meal was noted. VR was recorded again immediately after each meal and subsequently every 15 minutes until ambient photoperiod dusk. A further three meals were offered during the day as described previously, with an interval of 90 minutes between the end of the previous meal and start of the next meal. Four meals were offered the following day (day 3 of the experiment) with repeated VR observations, exactly as the previous day except that only a single measurement of VR was made prior to the first meal. On the fourth day a single meal was given and VR monitored before and after it as before. During each VR observation stress levels were scored on a scale of 1 to 5 (1 = least stressed) by a combination of body and eye sclera coloration, where darker coloration corresponds to a more stressed and subordinate fish (O'Connor *et al*. 1999; Suter *et al*. 2002).

Opercular ventilation frequency together with information on water temperature (T, in ºC, measured by digital thermometer) and initial fish weight (W, in g), was used as a predictor of metabolic rate (MR), as described in Millidine *et al* (2008). Water temperature was measured at the start of every observation round (i.e. every 1 hour 20 mins). The regression equations used to predict MR from VR were:

MR = m(VR) + c

Where m = 0.2773–(0.2350*log10(W))–(0.01838*T) + (0.05813*(T)*log10(W)),

c = -3.4078 + (0.2958*T) + (2.1956*log10(W)) - (0.82057*(T)*log10(W)) + (0.5335*W)

To assess the accuracy and validity of this method, two experimental fish (in addition to the 10 previously mentioned) were placed individually in respirometer chambers (approx 1.6l volume) for the duration of the experiment and were subject to the same protocol of feeding and measurement of VR as other individuals. However, for these two fish oxygen consumption was measured directly, at the same time as the VR measurements, using a Strathkelvin S1130 microcathode oxygen electrode connected to a SI 928 oxygen meter. The meter was calibrated with fully oxygenated aerated water from a header tank (100% saturation) and sodium sulphite in 0.01M di-sodium tetraborate (0% saturation). The respirometer used open-closed (intermittent) respirometry to calculate oxygen concentrations in the water, as described in Millidine *et al*. (2006). In the 'open' position, water entered and exited the chamber via a large aerated water bath. When switched to the 'closed' position, water re-circulated in the enclosed chamber and the drop in O_2 concentration due to respiration by the fish was recorded by the electrode. Tests were conducted to confirm that O_2 levels did not decrease when the system was open containing a fish, or when closed without a fish present.

Each measure of fish oxygen consumption was achieved by closing the system for 6 minutes; the system was then opened for at least 10 minutes prior to any subsequent measurements to flush the system with fully aerated water. The drop in oxygen (%) while the system was closed was used to calculate O_2

consumption per hour. The total oxygen (mg) dissolved in the closed system was calculated from knowledge of oxygen solubility at the experimental temperature and pressure, multiplied by the volume of water present in the chamber and connecting tubing. The quantity of O_2 consumed per hour could then be determined. The simultaneous measurement of VR was used to calculate predicted MR by using the equations in Millidine *et al*. (2008), and the two MR measurements were then compared.

At the end of the 4th day of the experiment the fish were removed from their respirometry chambers or experimental tank sections, and placed in a separate holding tank. The entire protocol was then repeated a further two times, selecting new fish each time, so producing data from 36 individuals, including 6 who underwent respirometry.

Statistical analysis was carried out using SPSS v15.0. The measurement of SDA parameters (see Fig. 5.1 for definitions) was carried out by plotting MR measurements in MATLAB v7.5. The monitoring of MR during the investigation allowed the duration, height and magnitude of the SDA associated with each feeding event to be calculated. SDA duration was measured as the length of time it took metabolism to return to SMR, as measured before feeding. SDA height was calculated as peak SDA minus SMR. SDA magnitude was measured as the area under the SDA curve. For the single meal on day 4, the maximum post-prandial elevation in estimated metabolic rate was termed 'peak SDA' and the maximum elevation in metabolic rate over the experimental period, i.e. including SDA induced by multiple meals, was termed 'maximum peak SDA'. The degree to which an individual could increase its oxygen consumption to cope with digestion was calculated as both its absolute aerobic scope (peak SDA-SMR) and its factorial aerobic scope (peak SDA-SMR/SMR) (Secor 2009). Replicate (i.e. whether the fish was part of the 1st, 2nd or 3rd group of fish tested in the experiment) was included in all linear mixed effect (LME) models as a random factor and initially retained; other independent variables were dropped sequentially if non significant to produce minimum adequate models. Exclusion of the 6 individuals used in the respirometry calibration trials had no effect on the outcome of statistical models, hence these data were retained in all analyses. Meal size, i.e. mass of bloodworm consumed in a single meal, was converted to percentage of the body mass of the fish for use in analysis and

termed ‘relative meal mass’. The same transformation was applied to the absolute total mass of bloodworm consumed over the experimental period (days 2-4) and termed ‘relative total food mass’. As each fish likely had an empty stomach at the start of days 2 and 3, differences in the extent to which fish consumed multiple meals are potentially detectable in the total food mass eaten in meals 2 to 4 on each of these days, this total food mass was termed ‘subsequent meal mass’ for either day 2 or 3. SMR was converted to relative SMR (calculated as an individuals SMR minus the mean SMR of all the fish in its replicate of the experiment) to remove error in SMR estimation by slight water temperature fluctuations between experimental rounds, and the transformed values were termed ‘relative SMR’.

5.4. Results:

The range of initial fish weights was 5.41 -9.17g (mean 7.02g ± 0.82SD, 5.41-7.57g for respirometry chamber fish and 5.93-9.17g for flume fish) and lengths ranged from 83-98mm (mean 89.1mm ±3.5). The mean weight of a bloodworm was 0.006g ±0.0018.

The values for MR derived from direct measurements of oxygen consumption correlated closely with the values predicted by the VR measurements (mean Pearson’s correlation coefficient of 6 fish = 0.965, all $P<0.001$, range 0.942-0.984) (Fig. 5.2), so the VR data were taken to provide realistic estimates of MR. All fish exhibited low stress colouration (1 or 2 on scale) throughout the experiment, and so oxygen consumption should not have been elevated by any physiological stress.

All meals ended in satiation before the 30 minute limit, i.e. two consecutive food items were uneaten, with the maximum meal size being 12 bloodworms. Meals were associated with a postprandial rise in MR, characteristic of a SDA response. On experimental days 2 and 3, when fish were offered more than one meal, their metabolic rate had usually not returned to the baseline SMR level before they consumed more food. This prevented measurement of the

magnitude and duration of the SDA response except on the fourth day when only a single meal was offered. Analysis of this single feeding event showed that there was a positive relationship between the quantity of food eaten in the meal and the duration (LME, $F_{1,34}$=4.99, P=0.036; replicate NS) and magnitude of the resulting SDA response (LME, $F_{1,34}$=4.88, P=0.038; replicate NS). The factorial SDA scope (SDA peak/SMR) was found to be significantly higher in low SMR individuals (Fig 5.3, LME, $F_{1,33}$=32.65, P<0.001; relative meal mass and replicate NS). The opposite trend was found for the relationship between SMR and absolute SDA scope (Fig 5.3, LME, $F_{1,33}$=6.41, P=0.016; relative meal mass: $F_{1,33}$=6.00, P=0.02; replicate NS), showing that fish with a larger SMR increased their metabolic rate more in absolute (but not relative) terms when consuming a meal. There was no significant relationship between relative SMR and relative meal mass (Fig 5.4, LME, $F_{1,34}$=0.79, P=0.52; replicate NS). However, SMR was positively correlated with peak SDA (Fig 5.5, LME, $F_{1,34}$=89.59, P<0.001; replicate NS) and oxygen expended per gram of food ingested (magnitude/meal mass, LME, $F_{1,34}$=4.55, P=0.04; replicate NS), but negatively with SDA duration (LME, $F_{1,34}$=10.07, P=0.003; replicate NS).

Figure 5.2 Plotted values for 6 fish of predicted metabolic rate (estimated by ventilation rate via equations from Millidine *et al*. (2008)) and observed metabolic rate derived from respirometry. Data points for each fish are represented by different symbols as shown in the graph legend box (all p<0.001).

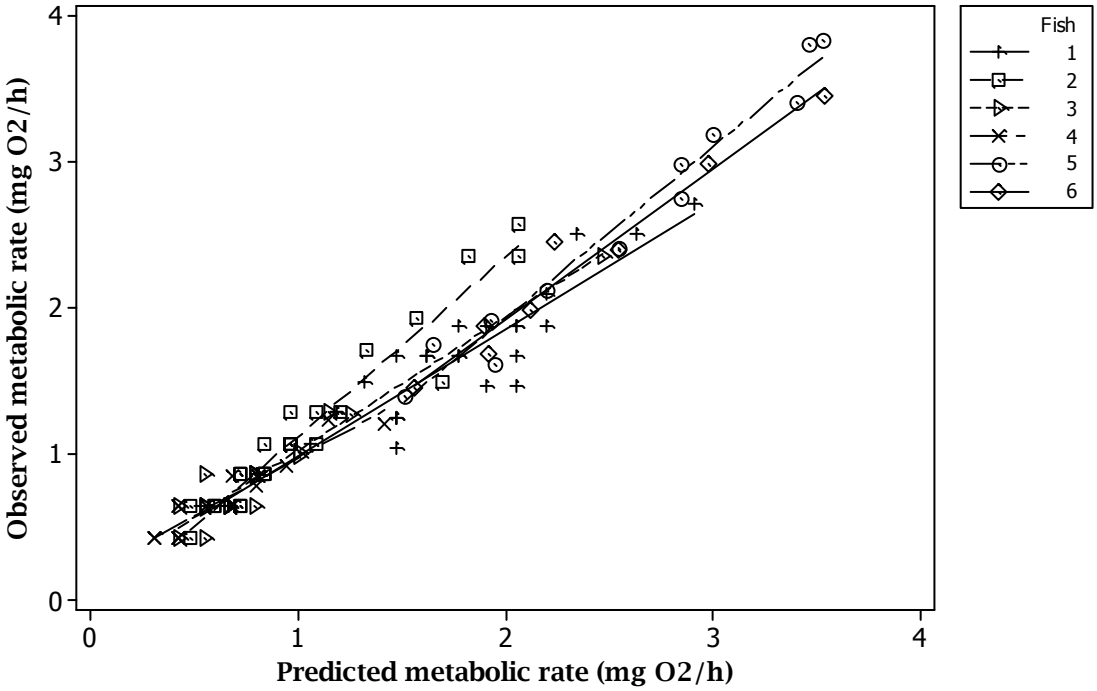


Figure 5.3 The divergent relationships between (a) relative SMR and absolute SDA scope (peak SDA-SMR, p=0.016), and (b) relative SMR and factorial SDA scope (peak SDA/SMR, p<0.001) after a single meal

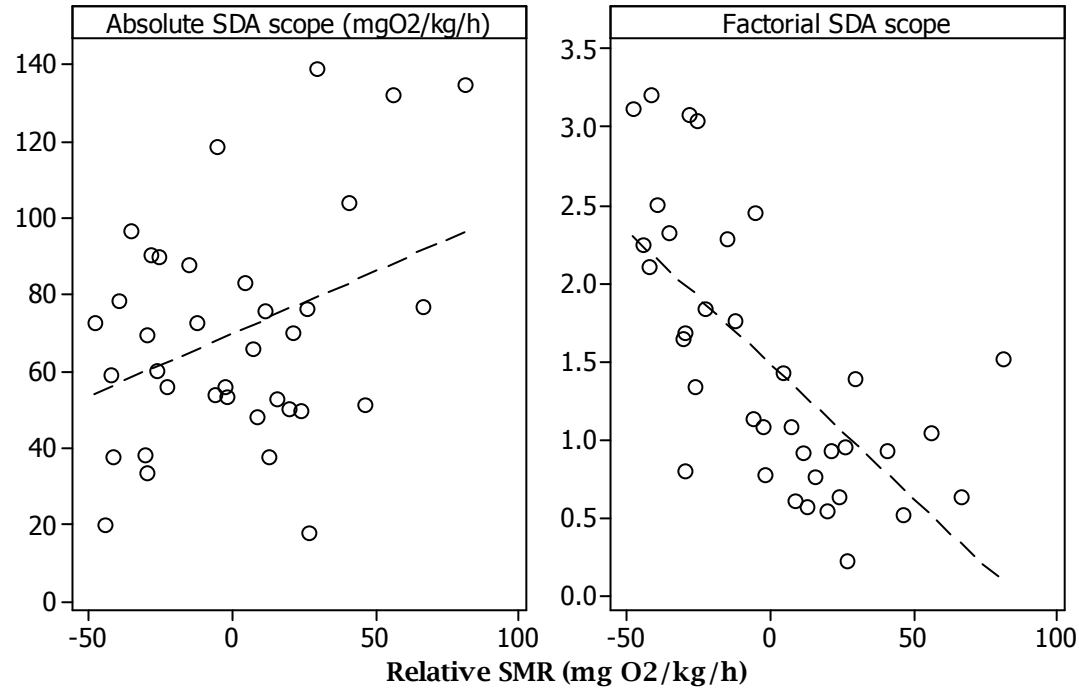
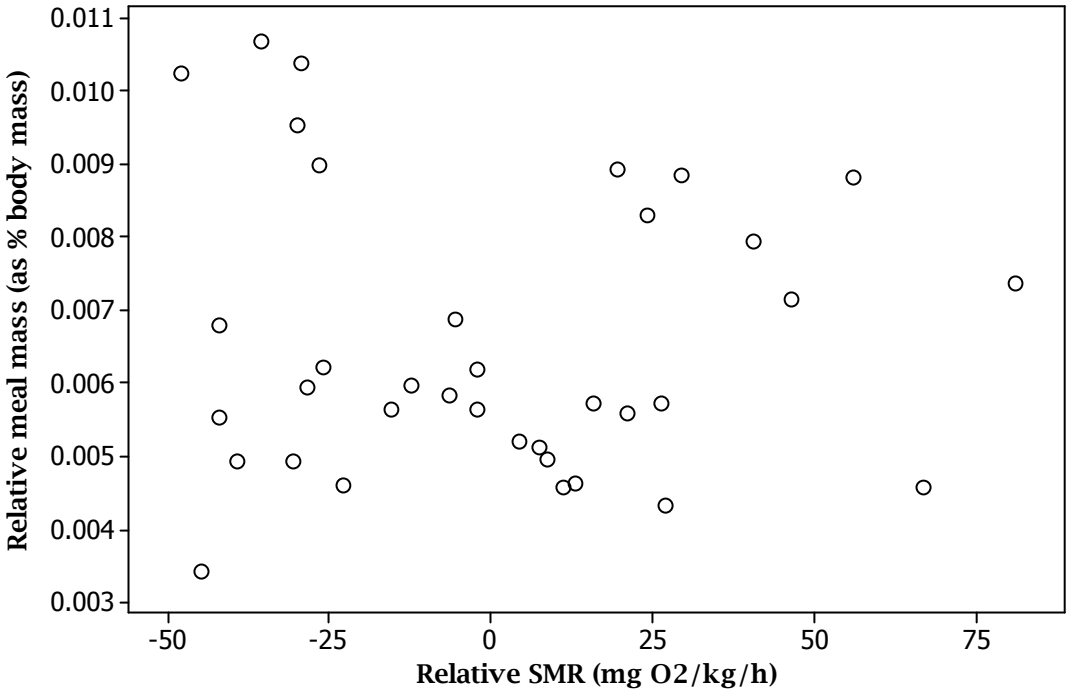


Figure 5.4 Graph showing that relative SMR has no effect on satiety meal size (expressed as a percentage of body mass, p=0.52)



Multiple feeding events resulted in higher subsequent SDA peaks, showing an additive effect on MR when a second meal was ingested before digestion of the first had apparently been completed. The maximum MR recorded for each fish

during the experiment was thus always reached on the days when it consumed more than one meal. The peak SDA after a fourth meal had been consumed was significantly higher than the corresponding peak SDA after the first meal that day (LME, $F_{1,34}$=35.82, P<0.001; replicate NS) but no higher than after the third meal (LME, $F_{1,34}$=0.92, P=0.856; replicate NS), suggesting that a maximum peak SDA was being reached. SMR correlated with maximum peak SDA (Fig 5.5, LME, $F_{1,34}$=13.62, P=0.001; replicate NS).

The absolute mass of food eaten by each individual over the course of the 3 days of observations was strongly correlated with body mass but not predicted by relative SMR (LME, body mass: $F_{1,33}$=11.54, P=0.002; relative SMR: $F_{1,33}$=1.97, P=0.17; replicate NS) (Fig. 5.6). Subsequent meal mass on day 2 was not predicted by relative SMR after controlling for the significant effect of body mass (LME, body mass: $F_{1,33}$=5.47, P=0.026; relative SMR: $F_{1,33}$=1.57, P=0.218; replicate NS). The same was true of subsequent meal mass on day 3 (LME, body mass: $F_{1,33}$=7.47, P=0.01; relative SMR: $F_{1,33}$=1.79, P=0.19; replicate NS). Similarly the number of meals eaten by an individual (over the entire experiment) was unrelated to relative SMR (LME, $F_{1,34}$=0.11, P=0.739; replicate NS).

Figure 5.5 The relationships between relative SMR and both maximum peak SDA (after multiple meals, p=0.001) and peak SDA after a single meal (p<0.001).

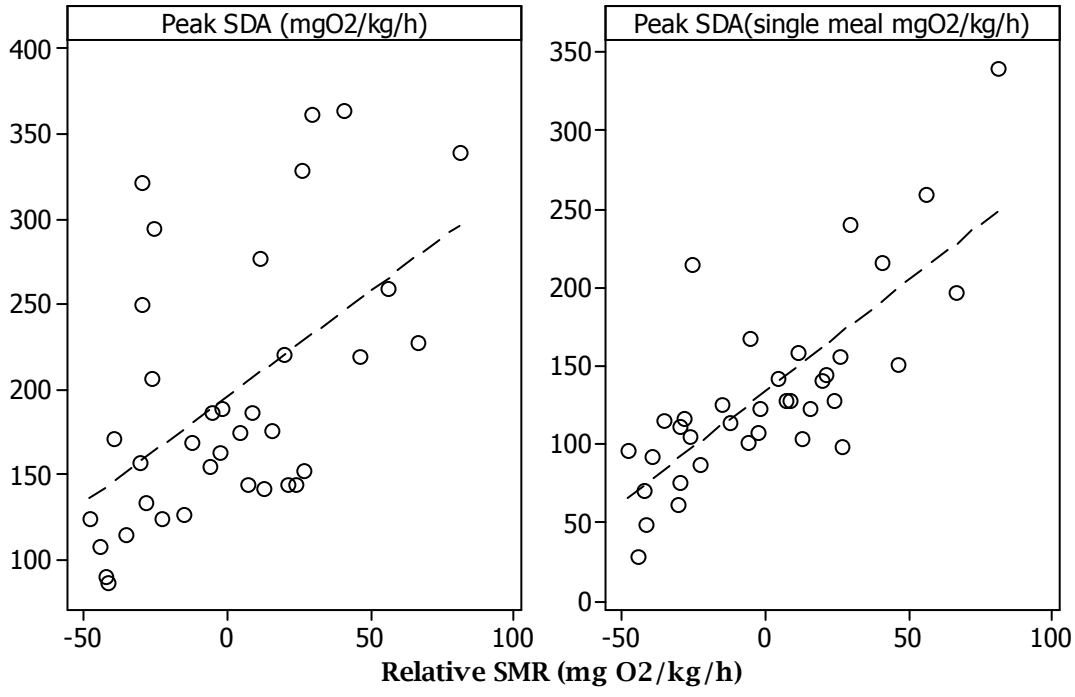
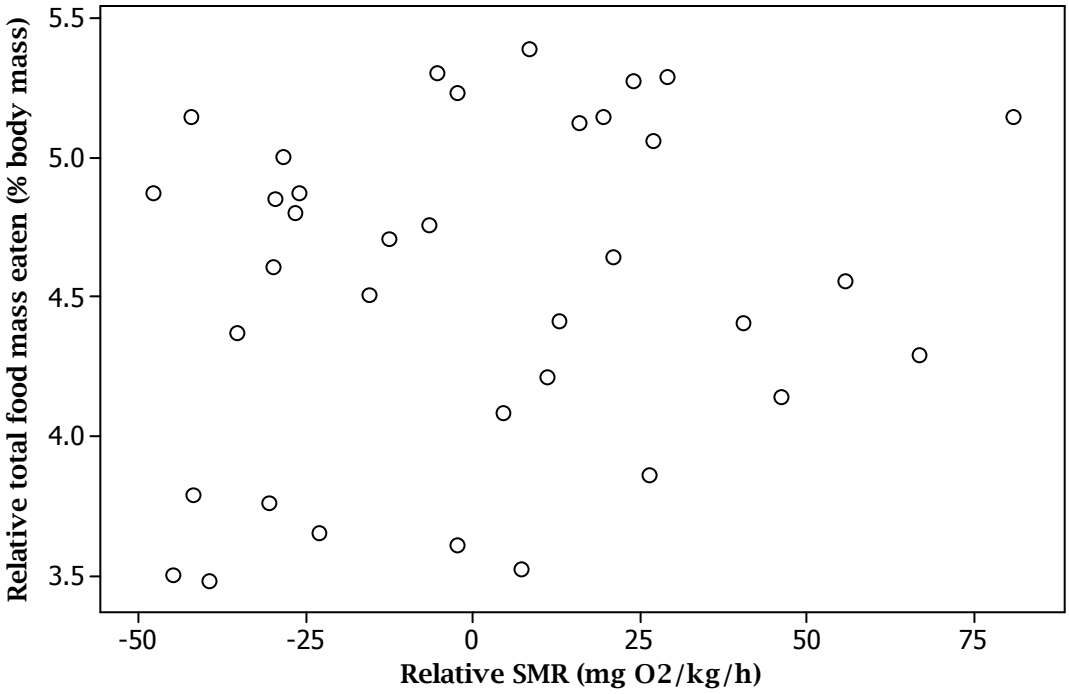


Figure 5.6 Graph showing that, despite a positive trend, relative SMR has no effect on the total food eaten over the experimental protocol (expressed as a percentage of body mass, p=0.17)



5.5. Discussion:

Ventilation rate proved to be an accurate proxy for oxygen consumption on the individuals whose metabolic rate was estimated by both measures. The error noted by Millidine *et* al. (2008) of around ±25% associated with the calculation does not appear detrimental as the highest SMR fish had values for SMR up to 5 times that of lowest SMR fish in this study, clearly differentiating individuals with high or low levels of resting metabolism.

The lack of relationship between SMR and individual meal size suggests SMR was unrelated to gastric capacity; therefore any difference in food intake over the experiment due to SMR would be due to food processing speed. As individuals did differ in the size of meals that they consumed, a clear relationship between meal size and SDA response was observed as in previous studies (Pan *et al*. 2005; Fu *et al*. 2005c).

SMR was found to correlate positively with the size of the peak of the SDA response but negatively with its duration; similar results were obtained by Millidine *et* al. (2008), suggesting that these relationships are robust for juvenile Atlantic salmon. Despite having a shorter SDA duration, high SMR individuals did not exhibit any tendency to re-feed sooner or ingest a greater mass of food over the experimental period. The absence of any relationship between SMR and food intake was observed in spite of individual salmon differing in total food intake by up to 50%. This suggests that the measurable rise in metabolism due to the SDA response is not solely responsible for the delay until re-feeding can occur. However, there may be subcomponents of the SDA response that cause little change in metabolism yet are still necessary before re-feeding can occur, e.g. evacuation of the gut. From our stress colouration data, it is unlikely that stress differences between individuals accounted for variation in feeding. Nonetheless, our methodology may have affected the findings since food was only available during the day and not overnight, so constraining the overnight pattern of feeding and digestion. In European sea bass, a faster SDA response led to higher compensatory growth; while the SDA response was not to linked to SMR or energetic efficiency, it was assumed that a fast SDA would result in quicker re-feeding (Dupont-Prinet *et al*. 2010).

Populations can vary in metabolic parameters such as their resting levels of metabolism and intestinal glucose absorption, possibly due to habitat productivity, despite similar diets and gut morphology (Mueller & Diamond 2001). The scale of the SDA response may also vary with nutritive organ sizes (i.e. larger stomach, liver, intestines and kidneys) (Secor *et al*. 2002). It has also been linked to baseline metabolism (Konarzewski & Diamond 1995), yet such links are often weak (Chappell *et al*. 2007) or absent (Koteja 1996; Secor *et al*. 2002) and may be influenced by environmental conditions, as suggested by the food habits hypothesis (McNab 1986; Bozinovic & Sabat 2010). As experimental salmon in the present study were hatchery reared and exposed to little environmental variability, this may reduce differences in physiology that may otherwise differentiate between individuals in terms of food intake.

After multiple meals, peak SDA was seen to plateau, suggesting an upper limit to the energy available for digestion (Priede 1985; Fu *et al*. 2005c), unless some factor such as surface area of the gut or activity of enzymes associated with

assimilation, or capacity of cell walls to pass chemicals is limiting the speed of the processesing of food. The factorial increase in metabolic rate from SMR to peak SDA (i.e. peak SDA/SMR) was inversely related to SMR, showing low SMR individuals have increased factorial SDA scope, similar to the pattern observed for aerobic scope (i.e. active metabolic rate/SMR; (Cutts *et al*. 2002b). Absolute SDA scope (i.e. peak SDA-SMR/SMR) showed a positive relationship with SMR, suggesting no limitation on the metabolic scope available for digestion over the range of observed SMR values. With individuals of similar size (as in this study), absolute measures of scope are arguably a more appropriate biological measure for comparison than factorial scopes (Killen *et al*. 2007), but it is useful to include both plots for information. This suggests that high SMR individuals can allocate more energy to digestion than can low SMR fish, a possible mechanism by which they can shorten the duration of SDA. However, energy devoted to digestion results in less energy available for other activities (Alsop *et al*. 1997; Jordan *et al*. 2007) and thus there is a trade-off between the two (Owen 2001). A faster SDA response may impair short-term activity but may allow the fish to regain full aerobic scope sooner than can conspecifics with a slower SDA profile. This does suggest that individuals with a faster SDA response may be more vulnerable during digestion, e.g. through a reduced ability to escape predators. However this may confer its own ecological advantages if a quick return to a baseline metabolic rate allows a rapid return to exploratory, foraging or territorial behaviour. As faster SDA responses are associated with a greater absolute SDA scope, any short-term activity impairment is likely minimised.

In conclusion, despite finding no relationship between SMR and food intake, this study demonstrated distinct SDA responses for differing metabolic strategies, with high SMR individuals exhibiting a greater physiological capacity for SDA.

6. Estimated standard metabolic rate and absorption efficiency are not related in juvenile Atlantic salmon (*Salmo salar*)

6.1. Abstract:

Across taxa, there is some evidence of a link between basal metabolism and absorption efficiency. Juvenile Atlantic salmon vary in the dynamics and extent of energy allocated to digestive processes after consumption of a meal, and this individual variation is related to variation in standard metabolic rate (SMR): high SMR individuals use up more energy in digestion, but digest meals faster than do low SMR fish, suggesting they can feed more frequently especially if resources are abundant.

However it is unknown what effect this accelerated digestion has on assimilation efficiency, as efficiency may be sacrificed to speed digestion. Here we investigate if there is a potential trade-off between absorption efficiency and SMR (and hence speed of digestion).

Individual salmon were given a single meal (1% body mass) of known calorific value. Solid waste products were collected after gut evacuation and their calorie content determined through CHN analysis to calculate absorption efficiency.

Significant inter-individual variation in absorption efficiency was observed, but no relationship was found between SMR and absorption efficiency. This suggests that high SMR individuals do not sacrifice absorption efficiency when digesting meals quickly.

6.2. Introduction:

All animals utilise energy from the environment to fuel body maintenance, activity, growth and reproduction. This energy is acquired through food ingestion by catabolic digestive processes, which releases energy for metabolism and somatic growth. The effectiveness by which an animal can obtain energy from its food is termed absorption efficiency, or digestive efficiency, used to describe the proportion of food energy that is absorbed across the gut wall (Kleiber 1961; Jobling 1994).

Absorption efficiency (AE) is not a fixed constant as it can be influenced by extrinsic parameters such as food composition i.e. protein or cellulose content (Pritchard & Robbins 1990; Spencer, Thompson, & Hume 1998), as well as by environmental temperature in the case of ectotherms (Avery *et al*. 1993; Xu & Ji 2006). The consistency of AE within individuals is a much neglected study area, although evidence is growing that it is repeatable in domesticated livestock species (Kelly *et al*. 2010 and references therein). As a physiological trait, absorption efficiency exhibits phenotypic flexibility as a result of diet variability between habitats (Olsson *et al*. 2007; Bozinovic *et al*. 2010) and therefore can be considered a resource polymorphism (Olsson *et al*. 2007). However, while it is consistent and repeatable within individual animals consuming the same diet (Kelly *et al*. 2010), it varies between conspecifics of similar age and life history state (Afik & Karasov 1995; Johnson, Ferrell, & Jenkins 2003). Organ size is thought to be responsible for some of this intraspecific variation, with larger or longer digestive organs leading to increased efficiency (Magnan & Stevens 1993).

The potential ecological consequence of intraspecific variation in absorption efficiency is great, since greater efficiency could fuel faster growth and/or sustain higher resting levels of metabolism. Standard, or basal, metabolic rate (SMR/BMR) has been shown to vary greatly between individuals of the same species (Blaxter 1989; Speakman *et al*. 2004) and is considered a repeatable trait (Nespolo *et al*. 2007). There is some evidence that SMR is linked to organ size (Chappell *et al*. 2007 and references therein), and if this were true of digestive organs it would provide a link between SMR and absorption efficiency.

Despite its likely importance, the effect of metabolic rate on absorption efficiency has received little attention (Cox & Secor 2007). Derting (1989) suggested that absorption efficiency was negatively correlated with BMR in juvenile cotton rats. The same relationship was seen in production efficiency (proportion of food energy used toward tissue synthesis) in juvenile Burmese pythons (Cox *et al*. 2007), although this was as a consequence of high SMR individuals having a higher energetic upkeep rather than their having a lower absorption efficiency. However SMR is frequently seen to correlate with behavioural dominance and access to food (Biro *et al*. 2010 and references therein), leading to a possible growth advantage for high SMR animals. Therefore a cost-benefit trade-off may exist between SMR and energy intake.

Juvenile Atlantic salmon have previously been shown to vary in the energy they devote to digestion in parallel with variation in their metabolic rate. High SMR individuals spend more energy in processing a given size of meal but appear to digest it faster, possibly allowing then to begin feeding again sooner (Millidine *et al*. 2009). It is unknown if this faster digestion affects AE. If AE is maintained, e.g. if variation in digestion speed is due to organ morphology or size rather than simply speed of gut passage, then those individuals with a higher SMR would have a foraging advantage (this may not translate into a growth advantage due to spending more energy processing the meal). However, if AE is sacrificed to speed digestion, then any potential foraging advantage is dependent on resource abundance. Therefore the nature of the relationship between SMR and absorption efficiency is of ecological interest as it will influence performance.

In this study we examine the relationship between SMR and absorption efficiency, using juvenile Atlantic salmon obtained from the same population as those used in the previous study of the links between metabolic rate and SDA profiles in salmon (Millidine *et al*. 2009).

To investigate this we examined the relationship between SMR and absorption efficiency and protein absorption efficiency, by direct measurement of faeces (as described in Jobling 1994). AE was measured as the percentage of food energy not excreted through faeces. Protein absorption efficiency (PAE, measured as the percentage mass of ingested protein not excreted through faeces) was used as an alternative measure of AE. Higher PAE represents a

greater retention of protein from the diet, which would result in a greater relative availability of amino acids for tissue synthesis and therefore body growth (Jobling 1994). AE and PAE were determined via elemental CHN analysis using methods from Gnaiger and Bitterlich (1984).

6.3. Methods:

Experimental procedures

Atlantic salmon parr (1+) were obtained from Marine Scotland Science Almondbank field station and transferred to the University of Glasgow aquaria facilities in November 2008. They were held in a large stock tank ($1m^2$) containing recirculated, aerated copper-free water at 10±0.2ºC. The tank included numerous plastic shelters and artificial flora to provide refugia. The fish were subject to an ambient photoperiod and were fed bloodworm (Chironomid larvae) ad libitum daily.

12 fish, unfed for 24 hours to allow complete gut evacuation (Cutts *et al*. 2002b), were randomly selected from the stock tank then anaesthetised, weighed and placed in individual tanks (0.32 x 0.19 x 0.17m) containing exactly 8L of water. Each tank was aerated via an airstone and contained a shelter, to reduce stress levels on the fish and so lower their metabolic rate (Millidine et al. 2006); tanks were held at 10°C in a temperature-controlled room. Each fish was allowed to settle for 24 hours before having its ventilation rate (opercular beats per minute) recorded. The recording of ventilation rate was repeated after 1 hour and again after a further hour. Ventilation rate (VR) was used to estimate metabolic rate (mg O_2 kg^{-1} h^{-1}) using the equations from Millidine et al. (2008) and information on fish body weight (W) and water temperature (T, measured °C). The regression equations used were:

$MR = m(VR) + c$

where $m = 0.2773–(0.2350*\log10(W))–(0.01838*T) + (0.05813*(T)*\log10(W))$

$c = -3.4078 + (0.2958*T) + (2.1956*\log10(W)) - (0.82057*(T)*\log10(W)) + (0.5335*W)$

A single meal of bloodworms (1% body weight) was then given to each fish, added to each tank from above. All fish ate the entire meal. 24 hours later, to ensure gut evacuation at 10°C (Higgins & Talbot 1985), the fish were removed, reweighed and placed in a new stock tank. The water from each of the 12 tanks in which the experimental fish had been held for 24h was filtered once (11µm pore filter paper with a Büchner funnel and flask) to remove faecal material, which was dried (4 hrs at 60ºC), weighed and stored in a seal 5ml eppendorf tube. This protocol was repeated a total of three times, so obtaining paired SMR data and faecal samples from a total of 36 individuals.

CHN analysis

Elemental CHN analysis was carried out as described in detail by Gnaiger and Bitterlich (1984); here we provide only a summary out of the protocol. A 2mg dried sample (dried for 4 hrs at 60ºC) and a 5mg ashed sample (8hrs at 450 ºC) of faeces from each tank underwent CHN analysis, using a 440 Elemental Analyser (Exeter Analytical CE). The difference in C, H and N percentages between the dried and ashed samples was assumed to be of organic origin; these values were then converted to the mass of organic carbon, hydrogen and protein present in the dried faecal sample. Using the relevant equations (from Table 2 of Gnaiger & Bitterlich 1984), we then estimated the protein, lipid and carbohydrate mass of each faecal sample:

Protein = organic Nitrogen mass * 5.78

Carbohydrate = $2.337*(1- W_{H2O}) - (3.012*W_C) - (4.3*X_{PN}*W_N)$

Lipid = $-1.337*(1- W_{H2O}) + (3.012*W_C) - (1.48* X_{PN}*W_N)$

$W_{H2O} = 0.0697 + 1.4483*W_C + 0.284*X_{PN}*W_N + 9.2471*W_H$

Where W_C is the fraction by mass of organic carbon in the dried sample (i.e. grams of organic carbon in the sample divided by its total ash-free dry mass), X_{PN} is the fraction that protein-based nitrogen makes up of the total organic nitrogen in the dried sample (assumed to be approximately 1.0) and W_N is the fraction by mass of organic nitrogen in the dried sample. W_H is fraction by mass of hydrogen in the dried sample (organic and bound to water) and W_{H2O} the fraction of residual water in the dried sample.

Energy content of each sample was estimated using the standard physiological fuel values of 4 kcal/g for protein and carbohydrate and 9 kcal/g for lipid.

CHN analysis was also carried out on ten samples of dried and ashed bloodworm (each 2mg) in order to calculate the protein, lipid, carbohydrate and energy content per unit weight of the food, using the same method as previously described.

With knowledge of energy input (single bloodworm meal of 1% body mass) and energy output (collected faeces), an estimation of the energy assimilated by the fish was then calculated. The energy assimilated as a percentage of total energy input was calculated for each fish and is referred to as absorption efficiency (AE). Protein absorption efficiency (PAE) was also measured as a secondary measure of absorption efficiency (calculated as the percentage of ingested protein mass not found in faeces) (Jobling 1994).

Statistical analysis was carried out using SPSS v15.0. Replicate (i.e. whether the fish was part of the 1st, 2nd or 3rd group of fish tested in the experiment) was initially included in all linear mixed effect (LME) models as a random factor; all independent variables were dropped sequentially if nonsignificant to produce minimum adequate models.

6.4. Results:

The mean initial weight of fish was 8.92±0.23g (SE) (range 5.60-11.43g) and mean mass specific SMR was 117.91±5.18 mg O_2 kg^{-1} h^{-1} (SE) (range 65.18 – 182.99 mg O_2 kg^{-1} h^{-1}). Prior to feeding there was no faecal material present in the experimental tanks, and each fish ate the entire meal (1% body mass) provided. Each individual produced sufficient faecal material for CHN analysis (i.e. a minimum of 2mg dry matter). The mean calorific content of the provided meal was 0.36± 0.01 kcal (range 0.22-0.45) and the mean absorbed energy assimilated by the fish was 0.31± 0.01 (range 0.19-0.40). Mean AE was 86.82±0.32% (range 80.72-89.23), mean PAE was 93.50± 0.15 (range 90.88-94.47).

AE was not predicted by SMR (Fig. 6.1, LME, $F_{1,34}$=0.54, P=0.469; body mass and replicate NS). There was no relationship between SMR and PAE (Fig. 6.2, LME, $F_{1,34}$=0.46, P=0.502; body mass and replicate NS).

Figure 6.1 The relationship between standard metabolic rate and absorption efficiency (calculated as the percentage of ingested food energy not lost in faeces, p=0.489)

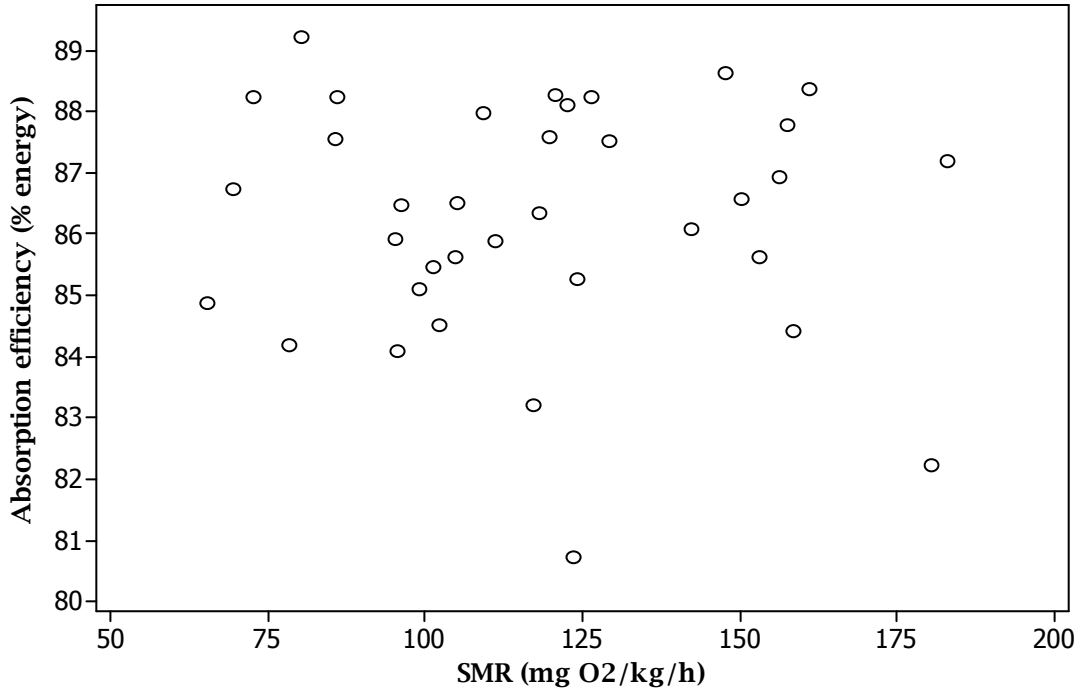
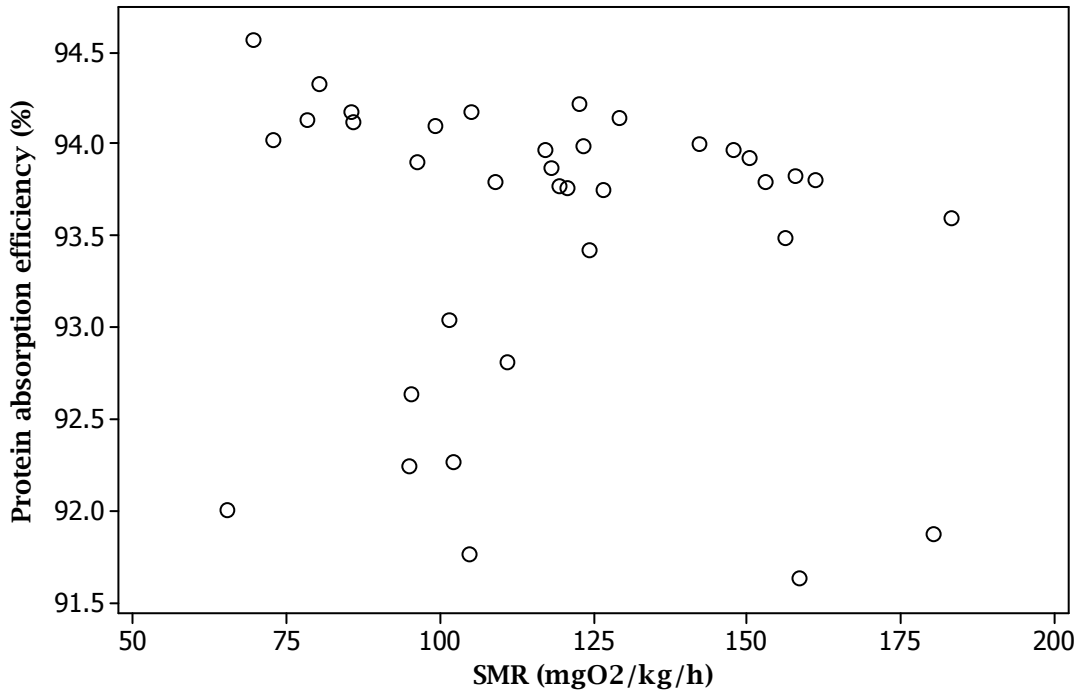


Figure 6.2 The relationship between standard metabolic rate and protein absorption efficiency (calculated as the percentage of ingested protein mass not lost in faeces, p=0.502)



6.5. Discussion:

No relationship between SMR and measures of absorption efficiency were found in juvenile Atlantic salmon, although significant variation existed among individuals, up to 10.5% for AE and 3.1% for PAE. The use of Gnaiger and Bitterlich's (1984) equations included their conversion value of 5.78 to multiply organic nitrogen to estimate protein content as well as their generally accepted values for the average energy content of carbohydrate, lipid and protein. The precise values used in calorific calculations like this are often debated in the literature (Sosulski & Imafidon 1990; Sriperm, Pesti, & Tillman 2011), but the exact values used are of less importance in the present study since we are applying the same equations to all individuals in order to compare individual variation in absorption efficiencies.

AE values are likely relatively high for all individuals due to the brief period of starvation prior to feeding (Elliott 1976), although necessary for the experiment. As such, data are of greater value for comparative analysis between individuals and should not be treated as absolute values for absorption efficiency. This may explain why the results are a little higher than the typical 70-85% range of absorption efficiencies found by Jobling (1994) for a range of species, including salmonids, feeding on oligochaetes.

Across taxa, gut morphology and digestive enzymes show intraspecific variation due to diet quality and quantity (Naya *et al*. 2009; Bozinovic *et al*. 2010; Karasov, del Rio, & Caviedes-Vidal 2011). Juvenile fish such as those used in this study are undergoing physiological development and so their gut morphology may still be subject to change due to the environmental conditions that they experience. As the juvenile fish used in this study were hatchery reared on an identical and constant ideal diet, with a high degree of temporal and spatial predictability, this may have minimised differences in gut morphology and absorption efficiency that might otherwise arise due to habitat variability and phenotypic plasticity. It has also recently come to light that hatchery juvenile salmonids exhibit less SMR variation than their wild counterparts (Van Leeuwen, Rosenfeld, & Richards 2011). As SMR is correlated with dominance and the

acquisition of preferable territories (Chapter 2), in nature high SMR individuals are likely to inhabit productive territories that may facilitate plastic changes in gut morphology to increase their energy intake (Olsson *et al*. 2007; Bozinovic *et al*. 2010). However it should be reiterated that these fish were obtained from the same hatchery and reared under the same conditions as those in Millidine *et al*. (2009), so were suitable to test whether high SMR salmon sacrifice AE to shorten digestion time.

Despite these possible aforementioned limitations that we have addressed, our study shows that there is no indication of any intrinsic relationship between SMR and absorption efficiency in juvenile Atlantic salmon. This suggests that high SMR salmon do not sacrifice AE to achieve a shorter SDA (Millidine *et al*. 2009). Therefore these individuals can return to baseline metabolism sooner after consuming a meal than can conspecifics with a lower SMR, without compromising on the energy extracted from their food. Nonetheless, there was no evidence that such high SMR fish consumed more meals per unit time (Chapter 5).

The individual variation in AE, if not attributable to SMR, must therefore be explained by other mechanisms. It is unlikely that habitat heterogeneity could have influenced the digestive plasticity (Olsson *et al*. 2007; Bozinovic *et al*. 2010) of these fish, given that they were reared in a relatively uniform hatchery environment under ad libitum feeding. However, variation in food intake rate cannot be discounted, and this can influence gut morphology (Brugger 1991). Moreover, the stability of the environment may have reduced the level of resource polymorphism, despite the intrinsic genetic physiological differences between individuals (Hori 1993; Smith 1993). It is therefore possible that these Atlantic salmon do not posses the same degree of digestive plasticity as other animals whose life history can lead to greater variation in habitat use e.g. littoral and pelagic lake perch (Olsson *et al*. 2007).

Nonetheless it is clear that any fitness advantage of higher absorption efficiency within juvenile Atlantic salmon is not a consequence of, or correlated with, SMR. Future studies may shed more light on this by relating variation in absorption efficiency to the individual's capacity for food intake and possibly growth.

A version of this chapter has been submitted as a manuscript.

7. General Discussion

7.1. Summary

The thesis set out to examine the relationships between environmental conditions, energy budget strategies and growth in juvenile Atlantic salmon. This was addressed broadly by two complementary themes. First, the consequences of SMR in different environments (Chapters 2, 3 and 4) and second the consequences of SMR on other aspects of physiology (Chapters 5 and 6). When combined, these approaches can shed light on the ecological consequences of SMR variation.

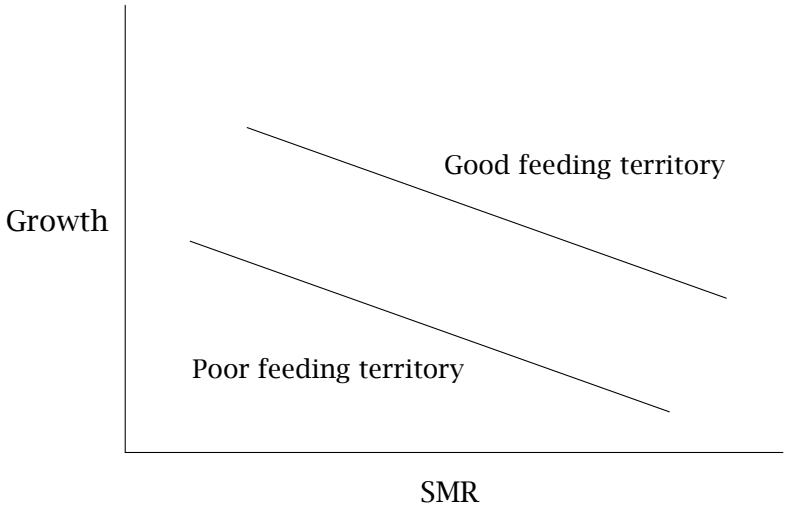
In salmonids, a high SMR, although costly, has often been seen as advantageous as it is linked to social dominance (Metcalfe *et al*. 1995), aggression (Cutts *et al*. 1998) and control of preferred feeding territories (McCarthy 2001) through the exclusion of subordinates. These relationships derived from studies of fish in small aquarium tanks held true in the stream setting used in the present study (Reid *et al*. 2011, Chapter 2). In simple settings this led to high SMR individuals growing fastest as in previous laboratory studies (Metcalfe *et al*. 1995; Yamamoto *et al*. 1998). However the links between SMR or dominance and growth appear to vary in the wild (Martin-Smith *et al*. 2002; Harwood *et al*. 2003; Álvarez *et al*. 2005; Sloman *et al*. 2008), which suggests that habitat features or biotic interactions may have an important role to play. If simpler habitats favoured high SMR individuals, their performance would likely be reduced by increasing habitat complexity (e.g. structure, food distribution, presence heterospecific or predators). This was found to be the case in Chapters 3 and 4, providing evidence for why SMR-performance associations in the wild are inconsistent. Interestingly, individuals showed an ability to track changing food resources (Chapter 3) provided that the environment was not too complex. This appears contrary to results obtained by Maclean *et al*. (2005), but their protocol included rows of boulders separating feeding patches, akin to my complex habitat where resource tracking was not observed. A high SMR can therefore be seen as potentially advantageous as it allows fish to obtain preferable feeding locations, but this is dependent on the local environment. Access to feeding locations by high SMR individuals provides greater feeding

opportunity but not increased feeding capacity in the sense of more rapid digestion and re-feeding (Chapter 5) as hypothesised by Millidine *et al*. (2009), nor reduced digestive efficiency (Chapter 6). Conversely, a low SMR should not be seen as disadvantageous, since some individuals employing this metabolic strategy did equally well, if not better, than high SMR fish under conditions of high population density (Chapter 2), high habitat complexity (Chapter 3) and/or low heterospecific competitor density (Chapter 4). In light of these findings, a trade-off among alternate metabolic strategies can be seen where a low SMR fish that has low idling costs can perform equally well as a high SMR fish with a greater feeding potential depending on context. Contrasting energy budget strategies thus persist and selection is context dependent on local habitat conditions. Broadly, simpler habitats where agonistic encounters are more frequent and resource predictability is high (or can be easily tracked) favour a higher SMR.

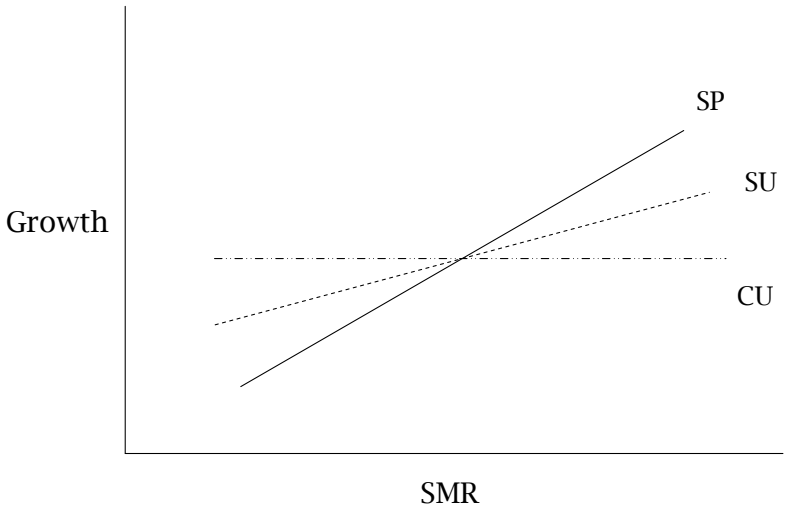
What is the evidence that these conditions are found in the natural environment of juvenile salmon? Food abundance and origin has been shown to vary temporally and spatially for juvenile Atlantic salmon (Martin-Smith *et al*. 2002). Given the range of physical complexity and heterogeneity found in salmonid habitats (Bardonnet & Bagliniere 2000; Armstrong *et al*. 2003), it is likely that a range of different metabolic strategies perform equally well if fish can utilise appropriate different microhabitats on a local scale. An example of how microhabitat variation might interact with SMR is in microhabitats containing a wide range of water velocities, favouring low SMR growth, but containing areas of high food availability, favouring high SMR growth (Armstrong, Millidine, & Metcalfe 2011). Specific habitat features can play a central role in accounting for life history variation among species (Rice 2005). As growth determines some life history decisions in Atlantic salmon (Klemetsen *et al*. 2003), this role of habitat appears equally likely at the intraspecific level as well. The habitat-dependent success of metabolic strategies observed in these studies helps explain why variation in SMR is maintained and persists through evolutionary time.

Figure 7.1 Summary figures indicative of the relationships found between Atlantic salmon SMR and growth in environments where: a) feeding territories differ in quality (Chapter 2); b) food supply is predictable in a simple habitat (SP), food supply is unpredictable in a simple habitat (SU) or food supply is unpredictable in a complex habitat (CU) (Chapter 3); c) the density of trout (T) heterospecifics varies (either 0, 1 or 2 trout; Chapter 4)

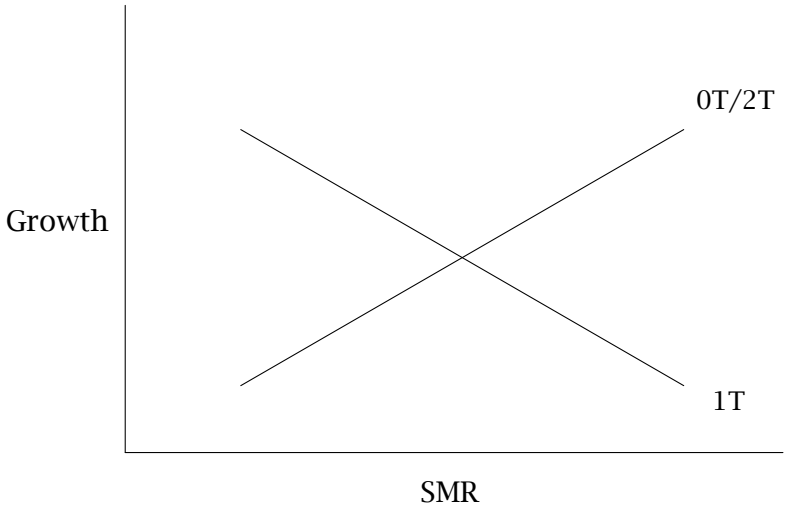
a)



b)



c)



7.2. Justification of methodology and possible limitations

Different approaches were used for physiological and environmental questions to address the aims of this thesis (Introduction, 1.8). To test the relationship between SMR, SDA, feeding rate and digestive efficiency it was necessary to do so under very controlled conditions. The use of simple aquaria allowed me to isolate the effects of individual variables by controlling environmental parameters so that potential differences between individuals could be appropriately identified and compared. For example, observed individual variation in SMR could not have been due to temperature as it was kept constant, or by presence of conspecifics as fish were housed individually. The experimental set-up also isolated SDA parameters from other causes of energy expenditure as individuals had no need to raise their metabolic rate for activity or aggression.

In contrast, exploring the consequences of SMR variation across different environments required a different approach. Attempting to reflect natural conditions was necessary to facilitate behaviours representative of the wild to increase the ecological relevance of the results (Price 1999). However, it was also necessary to simultaneously allow observations of fish at any time. The use of an artificial stream as a mesocosm met these criteria. It was of sufficient scale as to allow the home ranges that these fish would exhibit in the wild (Martin-Smith et al. 2002). It was fed by the same river that fish were caught from and had been operational for over 10 years, giving rise to established invertebrate fauna as potential prey items. It also allowed for manipulation of habitat demography that was crucial for the questions asked in this thesis. For example, without knowledge of territory quality, no relationship between metabolic strategy and growth would have been observed (Chapter 2). This may be why some studies have found no such relationship in the wild (Martin-Smith et al. 2002; Sloman et al. 2008) and supports the value of using such methodology.

The 'environmental conditions' experiments (Chapters 2, 3 and 4) used wild caught fish while the physiology experiments used hatchery reared offspring from wild caught parents. Wild individuals were chosen for the former experiments so that their behaviour would best reflect that occurring in the wild. Hatchery reared salmon may exhibit less variation in SMR than their wild counterparts (Van Leeuwen *et al*. 2011), although it is unknown if this study used first or multiple generation hatchery reared fish. As this thesis is ecological in its focus, this could be considered a limitation. However, the individuals used in the physiology experiments (described in Chapters 5 and 6) still exhibited marked differences in their baseline metabolism and could be categorised clearly into contrasting metabolic strategies. Nonetheless, it may be prudent in future ecophysiological studies to use wild salmon where possible, since the ecological relevance of the results is then more assured.

The method by which SMR was measured from ventilation rate was consistent across all studies in this thesis, apart from Chapter 3. Here all VR measurements were made from in-stream observations. In this study, calculations of relative SMR could have been derived from cohorts of 4 individuals, where each individual's relative SMR was dependent on 3 other conspecifics. Testing this approach led to relative SMR values being distorted by the presence of fish with

extreme metabolic rates in some cohorts. As a consequence, I ranked SMR within each group of four fish and used these SMR ranks to avoid this problem. While it would have been preferable to avoid any inconsistency in the method used to measure SMR across chapters, use of SMR rank in this particular case was a more appropriate and robust measure.

7.3. Implications and applications

Among humans, individual differences have long been recognised. Areas of expertise, such as healthcare, often account for physiological differences, predispositions and risks to great beneficial effect. In ecology as we continue to move away from the 'tyranny of the golden mean' (Bennett 1987) and quantify variation within populations and species, management and conservation methods can attempt to cater for this variation. For instance, anthropogenic impacts can often lead to simpler, less diverse habitats (Almany 2004; Sondergaard & Jeppesen 2007). Urbanisation, agricultural practises, deforestation and damming can all destroy habitat or reduce habitat variation in streams and rivers (Malmqvist & Rundle 2002). The results of this thesis would suggest that in simple habitats high SMR individuals would grow better (Chapter 2), and likely migrate sooner than conspecifics (Metcalfe *et al.* 1988). However if habitats were managed to be more complex and diverse, fish with a greater range of metabolic strategies would perform well (Chapter 3), so helping to maintain the physiological diversity within the population. Therefore decisions of management can have great influence on populations and life histories but can also provide inside into overall production. In predictable feeding habitats high SMR individuals performed best, yet unpredictable feeding habitats led to higher mean growth rates (Chapter 3). This illustrates that habitat alteration can influence population biomass productivity as well as individual performance.

When linking energetic strategies to performance, it is sometimes implied that it is consistently the underlying physiology that is driving the behaviour and performance, especially when examined in the context of the metabolic theory of ecology (Brown et al. 2004). However there is evidence to show that behaviour influences physiology (Piersma & Lindstrom 1997), and so we need to be cautious when ascribing causal mechanisms to these relationships as there is literature to support both viewpoints (Careau et al. 2008, and references therein). The relationships between metabolism and behaviours, such as dominance, that form such groundwork for these results have been documented across taxa (Hogstad 1987; Røskaft et al. 1986; Lahti et al. 2002). Whether these behaviours are cause or consequence of metabolism, or both, the common presence of these relationships suggest a common mechanism. If so, the relationships between SMR, physiology, behaviour and environment found here may well translate to other species that possess dominance hierarchies and maintain feeding territories. At the least, the results in this thesis can provide for better informed hypotheses to examine relationships between environmental conditions, energetic strategies and performance in other species.

This thesis provides data for how metabolic strategies perform under varying habitat demographics. Combined with the recent literature this provides opportunity for individual based modelling of how metabolic strategies perform in the wild. This can aid freshwater habitat management to promote growth in salmonids both at an individual and at population level (Guensch, Hardy, & Addley 2001). As many habitats are currently changing or under threat of change by human activity, it is becoming increasingly important to be able to predict how diverse populations will respond to habitat change (Hardy 1998; Chevin, Lande, & Mace 2010). This is especially relevant for species such as Atlantic salmon that are in decline (Ford *et al*. 2008).

This study has shown that the success of varying metabolic strategies differed greatly with manipulation of relatively few habitat demographics (density, territory quality, food predictability, habitat complexity, interspecific competition). If a general metabolic theory of ecology (MTE; Allen *et al*. 2007) is to prevail it has to make accurate predictions at different levels of organisation, i.e. both within and across species. The theory asserts that metabolic rate sets resource uptake from the environment and allocation of those resources (Brown

et al. 2004). From this thesis, being able to characterise an individual's metabolic rate without knowledge of its environment has little bearing on its realized food intake and growth. MTE also uses a fixed scaling exponent that predicts metabolic rate from body mass. This approach negates the marked size-independent variation in SMR found in salmon (Chapters 2-6, Enders *et al*. 2005) and other taxa (Hayes *et al*. 1992; Kvist *et al*. 2001; Steyermark *et al*. 2005). This variation is often the basis for behavioural (Biro *et al*. 2010), physiological (Speakman *et al*. 2004) and life-history (White *et al*. 2004) differences that are both fascinating and ecologically relevant. These problems suggest that MTE, in its current form, may be too simplistic to provide useful predictions or information at the organismal level.

This work adds to a significant and growing body of scientific literature that relates metabolic strategies to other aspects of physiology, behaviour and ecology (White et al. 2004; Careau et al. 2008). One importance of metabolic strategies is that they may start to represent a suite of behavioural and physiological traits, so it becomes less useful to think of social rank in isolation but to think of the suite of traits that are likely to be found in individuals based on their social rank (Sih et al. 2004; Sih, Bell, & Johnson 2004). This helps build a more representative picture of the individual incorporating both needs (e.g. metabolic requirements) and abilities/predispositions (e.g. aggression). The value of linking behavioural and physiological strategies in this way gives a more complete picture of the how a particular individual will interact and perform across contexts and environments. It also allows future studies to focus on measuring a few traits which themselves are indicators of many more traits, provided the literature evidently supports such links between traits. Caution should be used when applying this approach to small cohorts and relative trait measures (i.e. where ranking in a particular trait can be influenced by one or two individuals that possess an extreme value of a particular trait). This proved important in chapter 3 where cohorts of 4 individuals were used and SMR rank proved more informative than relative SMR. As these repeatable linked traits incorporate aspects of both physiology and behaviour, terms such as consistent individual differences (CIDs; Dall, Houston, & McNamara 2004) are more representative than animal personality or repeatable behaviours.

7.4. Recommendations for future research

Metabolic strategies clearly have wide ranging implications for performance, growth and life history. These implications are gradually being added to a significant body of literature. As understanding of the consequences of SMR and its variation improves, determining the causal mechanisms of the variation will become essential. Although likely hereditary in part, early environment or maternal effects may well contribute. Maternally derived hormones are transferred to eggs during oogenesis (Tagawa, Suzuki, & Specker 2000), which can influence offspring phenotype (Groothuis et al. 2005; Eriksen et al. 2007) and possibly metabolism (McCormick & Nechaev 2002). Mothers may influence egg hormone content to produce a wide variety of metabolic strategy offspring as a bet-hedging tactic for an unpredictable future environment and facilitate higher overall biomass production. If both cause and consequence are understood, useful predictions can be made about the likely performance of organisms, especially in light of a rapidly changing world.

The lack of relationship between SMR and absorption efficiency (AE, Chapter 5) was interesting and may merit further investigation. AE is known to vary within individuals due to an array of factors (Pritchard *et al*. 1990; Avery *et al*. 1993; Xu *et al*. 2006) including meal size (Elliott 1976). It is unknown if the decrease in AE associated with increasing food intake is uniform amongst fish of differing metabolic strategy, and so an experiment across multiple feeding regimes is really necessary to refute any relationship between AE and SMR.

There remain many habitat parameters that affect salmon performance (Armstrong et al. 2003) and may well influence the performance of alternative metabolic strategies, e.g. presence of predators or anthropogenic impacts, which have not been addressed in this thesis. Research in these areas may provide further explanations for the persistence of varying metabolic strategies in the wild and aid the ability to model the effect of changing environments on different individuals within species

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