Within- and among-individual variation in metabolic rate in juvenile Atlantic salmon, *Salmo salar*

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Abstract

Animals may adopt a range of energy strategies to persist and perhaps prosper under various prevailing environmental conditions. For example, they may achieve similar growth by investing heavily in maintaining a high capacity food capture and processing system or by using a lower capacity system that is cheap to run but which is less effective at accruing further resources. The way that individuals within species allocate energy resources is an intriguing issue that has implications for understanding competition, population structuring and the response of populations to environmental change. Energy budgets account quantitatively for the pathways by which food energy results in variation in somatic resources and constitute an important basis for evaluating links between behaviour and aspects of performance. Atlantic salmon have been an important model for exploring relationships between growth performance, metabolic strategies and individual behaviour. Juvenile salmonid fish have been a particularly useful subject for exploring individual variation in metabolism because it has been possible to relate standard metabolic rate (SMR) to behavioural traits and lifestyle within a species. SMR correlates with dominance status, which is reflected in the ability of fish to access high value food patches and may promote faster growth under some conditions. This thesis focuses on the within- and among-individual variation in SMR and looks at how a fish's external environment and social interactions can influence its SMR, and whether this variation in SMR can affect other aspects of metabolism such as feeding. Experiments were carried out to determine: (A) whether the presence of a shelter reduces SMR; this may provide an additional

reason for the extent to which these fish will compete aggressively for shelters when these are limiting (Chapter 2); (B) the extent of intraspecific variation in SDA (i.e. differences in the measured parameters that are used to define SDA), and to test whether the SDA for a given meal varies as a function of individual traits such as SMR. If SMR is directly related to scope for growth, then the speed and size of the SDA response should also correlate with SMR (Chapter 3); (C) whether ventilation rate is sufficiently accurately related to metabolic rate (MR) at a range of temperatures and activities so as to allow energy expenditure to be predicted outside a respirometer (Chapter 4); and (D) how visual isolation and the presence of a conspecific can affect SMR, using the method developed in the previous chapter (Chapter 5). Access to shelter was shown to have a significant impact on SMR, producing on average a 30% increase in metabolic costs in the absence of shelter. Therefore, the presence of appropriate shelter not only reduces the risk of predation but also provides a metabolic benefit to fish that is likely to have implications for growth performance and activity budgets. Variation in SMR was shown to affect the profile of energy consumption during digestion of a meal. The peak in post-prandial oxygen consumption, the duration of elevated metabolism and the overall magnitude of SDA all increased with the size of meal consumed. However, for a given meal size, fish with a higher SMR also had a higher peak and greater magnitude of SDA, yet experienced a shorter duration over which metabolism was elevated following the meal. Intraspecific variation in SMR is thus linked to variation in digestive strategies, although the costs and benefits of a given SMR are likely to vary with resource availability and predictability. Ventilation frequency was shown to be a good correlate of

MR, since MR was found to correlate strongly with VR in all fish tested, at all temperatures and the relationship was independent of causal factor. The relationship was linear, and both the slope and corresponding intercept of the regression equation were strongly dependent on the fish's body weight and the test temperature. Visual measurements of VR may therefore provide a highly accurate, cheap and non-invasive method of measuring the energy consumption of fish engaged in natural behaviours. Comparing SMR of individual fish when alone with response to presence and absence of physical shelters showed that all fish exhibited a reduction in SMR when provided with an appropriate shelter. However, when grouped, there were both positive and negative group effects on SMR depending on the relative size of fish. The consequences are that, although the group effect on SMR may be small on average, at the population level it is large, of variable sign and profoundly important in terms of the overall energy budget at the individual level. This thesis therefore shows that metabolic rates can vary extensively within as well as between individuals, depending on the context, and this variation will have profound effects on overall energy budgets.

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Declaration

The candidate has composed this thesis in its entirety. Except where specifically acknowledged, the work described in this thesis has been conducted independently and has not been submitted for any other degree; chapters 2 and 4 are based on manuscripts with two co-authors who helped design the studies and advised on the analysis and writing, but I conducted all the experimental work and statistical analyses. I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan.

Signature of the candidate ______________________________

(Karen Millidine)

Date: 14th November 2008

Chapter 1 – General Introduction

1.1 Energy budgets

A fundamental property of all organisms is their metabolism, which constitutes the collective biochemical processes by which they transform energy and materials to support various life functions and structures (Glazier 2003). Energy ingested by animals as food (I) is used in the synthesis of tissues (G) and as fuel (usually in the form of adenosine triphosphate or ATP) in the metabolic processes that power this synthesis and in other physiological processes which allow an organism to maintain their structures and respond to their environment. The overall rate of these processes, the MR, sets the pace of life (Brown 2004). The metabolic theory of ecology (MTE) has been formulated based on the premise that the structure and dynamics of ecological communities are inextricably linked to individual metabolism (Allen & Gillooly 2007). Physiologists have long known that there are three primary factors that control MR: body size, body temperature, and resource availability (Allen & Gillooly 2007). The MTE builds on this earlier work by providing a quantitative framework to better understand how these three variables combine to affect MR (Allen & Gillooly 2007), and links the performance of individual organisms to the ecology of populations, communities, and ecosystems (Brown 2004). However, the MTE is still very controversial (Whitfield 2004; Martínez del Rio 2008). It cannot explain all of the variation in MR between and within species, and is therefore not a “theory of everything” (Brown 2004), which makes measurements of MR and its variation particularly important.

For a poikilotherm, a term referring to an organism whose internal temperature varies and often corresponds to the ambient temperature of its immediate environment, metabolic energy can be divided into three categories 1) standard metabolism (R_s), which is the minimum required energy to maintain basic body function whilst at rest, 2) active metabolism (R_a), which is the energy loss associated with locomotory activity, and 3) specific dynamic action (R_{SDA}), which is the energy expenditure associated with feeding and includes excited locomotor activities, the mastication, digestion and absorption of food in the gut, and the biochemical transformation of the absorbed material (see below for a more detailed description of R_s, R_a and R_{SDA}). Energy is also lost in faeces and urea (E) and the balance of these components is reflected as growth of soma and gonads or loss of tissue energy according to the energy budget (Winberg 1956):

$$G = I - (R_s + R_{SDA} + R_a + E)$$

1.2 Standard metabolism

The basal metabolic rate (BMR) of homeothermic animals is the level of energy consumption when inactive, not assimilating a meal and not paying off any oxygen debt associated with previous anaerobic activity (McNab 1988; Hulbert & Else 2000; Frappell & Butler 2004). BMR is thought to represent the minimum energetic cost of maintaining cells and organs in readiness for higher levels of activity (Ricklefs, Konarzewski & Daan 1996) and is therefore, in effect, the summed energy expenditure of all tissues and organs working together under the influence of the regulatory systems in the living animal (Kvist & Lindström 2001). An equivalent term for poikilotherms is standard metabolic rate (SMR) (Frappell & Butler 2004), that is, R_s. The BMR of homeotherms, which accounts for a large (30 – 80%) and unavoidable component of daily energy expenditure (Blaxter 1989), is 5 – 10 times higher than the SMR of poikilotherms of similar size (Boily 2002). This is a result of homeotherms using metabolic heat production to maintain a relatively high and constant body temperature (Boily 2002), unlike poikilotherms which rely on the external environment for temperature control. However, since the two terms are so closely related, from now onwards the single term SMR will be taken to also include BMR.

Over several decades, the SMRs of many animal species have been measured and catalogued (reviewed by Hulbert & Else 2000). Differences in standard metabolism within lineages exist, often associated with ecology or lifestyle (Clarke & Fraser 2004). Thus in fish, more active species tend to have higher SMRs (Morris & North 1984; Zimmerman & Hubold 1998) and in insects, winged forms have a higher SMR than flightless species (Addo-Bediako, Chown & Gaston 2002). Although a higher SMR is costly, it may be associated with benefits, in that a higher minimal MR is thought to allow the animal to achieve greater maximal MR's (i.e. higher absolute aerobic scope), which, together with improved response times for convective oxygen transport (Krosniunas & Gerstner 2003), will result in increased performance (Frappell & Butler 2004). SMR, independent of body size, is also correlated with a variety of variables (such as field MR, heart rate, population density, number of offspring), which therefore makes it a potentially useful predictor of ecological and physiological processes (White & Seymour 2004). Changes in SMR could also reflect physiological adaptations to local food sources or food supply, climate or other local factors through individual acclimation to the ecological conditions which exist (Kvist & Lindström 2001).

The SMR of a poikilothermic animal is also influenced by temperature (Clarke & Fraser 2004; Frappell & Butler 2004). Temperature plays an important role in various aspects of the life history, ecology, and physiology of poikilotherms (Angilletta, Niewiarowski & Navas 2002). Physiological processes, such as MR, generally increase with temperature (Beaupre, Dunham & Overall 1993; McNab 2002), and the temperature dependence of SMR in animals is generally described by an exponential relationship (Ohlberger, Staaks & Hölker 2007).

1.3 Active metabolism

Maximum metabolic rate (MMR) is the maximum sustained aerobic metabolism measured in non-limiting conditions, i.e. not under hypoxic conditions (Fry 1971; Priede 1985; Neill *et al.* 1994). Metabolic scope, measured in temperature–acclimated animals, is defined as the difference in energy expenditure between R_a and SMR and is thought to represent the energy available to the organism for energy demanding processes such as locomotor activity, feeding / digesting, reproduction etc (Jobling 1994). Metabolic scope can be expressed either in absolute terms (as the difference between R_a and SMR) or as factorial scope (by dividing absolute scope by SMR), which indicates the ratio by which the animal can elevate its metabolism above the baseline.

1.4 Specific dynamic action

Postprandial increases in metabolism were first documented in animals over two hundred years ago (reviewed by McCue 2006). Many animals show a substantial increase in their MR while they are digesting and processing meals (McCue 2006), and this rise in metabolism following feeding is known as the heat increment or specific dynamic action (SDA). The effect, usually measured in terms of oxygen consumption following a feeding event, is characterized by a rise in metabolism to a peak and then a more gradual decline back to the resting level (Jobling 1981). The energy costs associated with SDA are the result of numerous pre-absorptive, absorptive and post-absorptive physiological processes (McCue 2006) such as, but not limited to, intestinal absorption (Secor 2003), intestinal remodelling (Secor & Diamond 1995) and general costs of growth (Vahl 1984). Thus, the physiological mechanisms that underlie the SDA response may vary among different animals depending on feeding habits, food composition, temperature, and other factors (Wang, Hung & Randall 2006).

SDA can be characterized by three parameters: the maximum or peak level to which MR rises during the SDA response, the duration of the effect (until MR returns to pre-prandial levels), and its magnitude (the overall metabolic cost of processing the meal) (Jobling 1981). The magnitude of the SDA response is determined by plotting the whole of the curve of oxygen consumption against time until the effect has subsided, then integrating the area beneath the curve which lies above the ‘resting’ or ‘low routine’ level of oxygen consumption (Jobling 1981) (see figure 1.1). A fourth parameter, the time taken to reach the peak SDA, may also be useful as an indicator of the speed with which the animal can commence processing a meal.

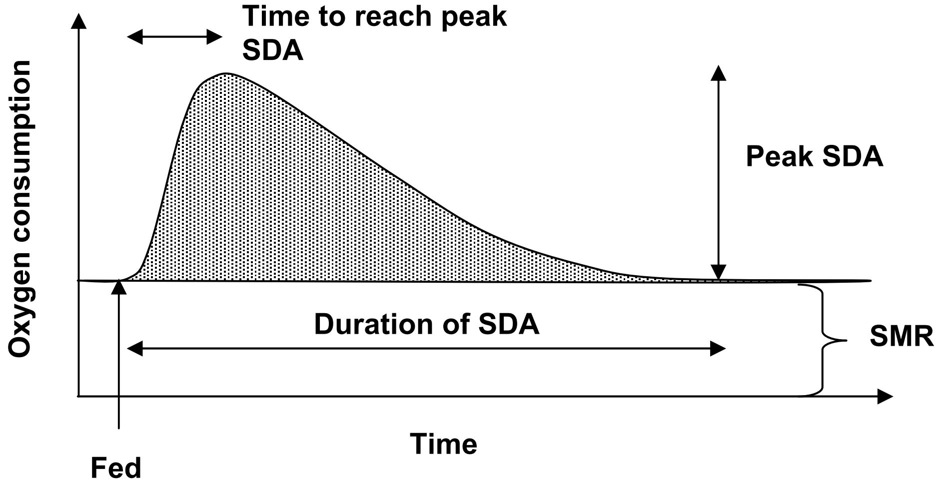


Figure 1.1 Diagram of the measured parameters of specific dynamic action (SDA). The shaded area indicates the magnitude of SDA, which is expressed as the integrated increase in oxygen consumption above maintenance (SMR) after feeding.

SDA responses, like many other physiological variables, are known to vary allometrically with an animal’s body mass (Boyce & Clarke 1997; Secor & Faulkner 2002; Toledo, Abe & Andrade 2003). Other recognized determinants of the SDA response include meal size (Zaidan & Beaupre 2003), meal type (Secor & Faulkner 2002), body and environmental temperature (Jobling & Davies 1980; Secor, Wooten & Cox 2007). A universal phenomenon of SDA is that larger meals generate greater magnitudes of post-feeding responses (Secor & Faulkner 2002). Peak oxygen consumption, the duration of the SDA response and the overall SDA all increase as a function of meal size, and this has been observed in invertebrates (Carefoot 1990), fishes (Jobling 1981), amphibians (Powell, Mansfield-Jones & Gatten 1999), reptiles (Zaidan & Beaupre 2003), birds (Janes & Chappell 1995) and mammals (LeBlanc & Diamond 1986; Rosen & Trites 1997). For example, increasing the ration size from 1 – 8% of body mass increases the time course of the SDA effect by as much as 12 hours in the Adele penguin (Janes & Chappell 1995). The size of a meal can also affect the magnitude of the SDA response (Andrade, Cruz-Neto & Abe 1997; Secor & Diamond 1997).

The duration of the SDA response varies widely among taxa (McCue 2006). In birds and mammals it typically lasts only a few hours (LeBlanc & Diamond 1986; Janes & Chappell 1995; Rosen & Trites 1997). However, animals that have either very low SMR or live in extremely cold environments may show SDA responses lasting at least several days (Boyce & Clarke 1997; McCue & Lillywhite 2002). In the common starfish, it was reported that the postprandial metabolism lasted up to 42 days (Vahl 1984). The effects of feeding and the SDA duration may vary greatly among species with differing feeding strategies and ecological specializations (Robert & Thompson 2000).

In homeotherms, such as birds (Janes & Chappell 1995) and sealions (Rosen & Trites 1997), MR's can increase by 1.2 – 2.5 times the pre-feeding values within 1.2 – 3.7 hours of feeding (Robert & Thompson 2000). A similar range of increase in SDA (but a slower response) is seen in poikilotherms such as fish, where the peak of SDA in response to feeding ranges from 1.7 – 2.5 times pre-feeding values within 12 hours (depending on variables such as temperature, food type, meal size etc) (Jobling 1981). An exception to this has been observed in large sit-and-wait foraging reptiles: the oxygen consumption rates of the Burmese Python when digesting a meal can be up to 44 times (depending on meal size) the pre-feeding values (Secor & Diamond 1997). The postprandial peak in oxygen consumption has been found to plateau with larger meal sizes for fishes (Jobling & Davies 1980), amphibians (Secor & Boehm 2006) and reptiles (Roe *et al.* 2004). However in other studies, peak oxygen consumption progressively increased from the smallest to the largest size meals and exhibited no levelling in 9 species of anurans (Secor *et al.* 2007).

1.5 Individual variation in metabolism between and within populations

Over the course of a year, animals may have to adjust their physiology, morphology and metabolic functions to varying ecological conditions (i.e. seasonal changes, migration to warmer/cooler climates) and demands (Kvist & Lindström 2001). Therefore, the energy budget is highly variable and can be affected by both biotic and abiotic factors, resulting in some populations of the same species having better / poorer growth compared with others. As well as this variation in metabolism between populations, many animal species show consistent variation between conspecific individuals within the same population in basic physiological processes (Spicer & Gaston 1999), and it is important to understand the mechanisms that underlie such variation and their consequences for fitness (Taylor *et al.* 2002).

Individual variation in SMR and its link with life-style has been examined in many taxa. Since SMR represents a substantial component of the overall energy budget of animals (about 25 – 40% of the total daily energy demands of free-living individuals; Congdon, Dunham & Tinkle 1982; Hulbert & Else 2000; Speakman *et al.* 2003), it is of fundamental importance to understand the functional relevance of this wide intra-specific variation. One well-documented example is that of juvenile Atlantic salmon which, from first feeding, have widely differing rates of resting oxygen consumption (Metcalfe, Taylor & Thorpe 1995), and this variation remains consistent over time (McCarthy 2000). Recent studies have highlighted the link between SMR and relative social status in salmonid fish (McCarthy 2000); individuals with higher than average SMRs (controlling for body size) at first feeding are more aggressive and tend to be dominant over conspecifics with lower relative SMRs (Metcalfe *et al.* 1995; Cutts, Metcalfe & Taylor 1998, 1999). Studies of a variety of animals have shown that the most competitive or dominant individuals in a population may have a considerable influence on the feeding ecology of those of lesser status (Metcalfe 1986). This can result in the subordinates being excluded from the best feeding stations and therefore suffering a reduced feeding rate compared with that of more dominant individuals (Appleby 1980; Fausch 1984; Monaghan & Metcalfe 1985). There are therefore potentially strong links between behaviour and respiratory physiology, with animals of differing MR having contrasting behavioural strategies. However, the links between behaviour and the different components of the energy budget have been little studied – nor has there been much investigation of individual variation in aspects of the energy budget apart from SMR.

This PhD has therefore focused on intraspecific variation in different components of metabolism, and the links between individual variation in SMR and SDA and whether these are linked to behavioural life-styles, using juvenile Atlantic salmon as a study organism.

1.6 Atlantic salmon

Atlantic salmon are among the most well studied of fish (Marschall *et al.* 1998). Much of this research is attributed to their great commercial and recreational importance on two continents (Europe and North America), a very large Atlantic salmon aquaculture industry, and their suitability as models for studies of behaviour, ecology, and evolution, among other disciplines (Marschall *et al.* 1998). Recreational inland fisheries are recognised to have considerable socio-economic benefits for the participating individuals and for other local and wider communities (Peirson *et al.* 2001). The net economic value of the salmon rod and net fisheries in England and Wales in 1996 was estimated to be broadly £108 million and £2.5 million respectively (MAFF 2000). Anglers are willing to pay large sums for the right to fish, and the total annual expenditure by anglers on coarse and game fishing trips in England and Wales alone was recently estimated at £3.15 billion (Hughes & Morley 2000). Scotland supports one of the largest and most diverse of the Atlantic salmon resources in Europe, with nearly 400 salmon rivers supporting many hundreds of populations (The Scottish Executive 2000). As a consequence Scotland has some of the most important commercial and recreational salmon fisheries in the world, with the angling fishery producing a net economic value of around £270 million to £430 million in 1995 (The Scottish Executive 2000).

1.7 Historical and current distribution of Atlantic salmon

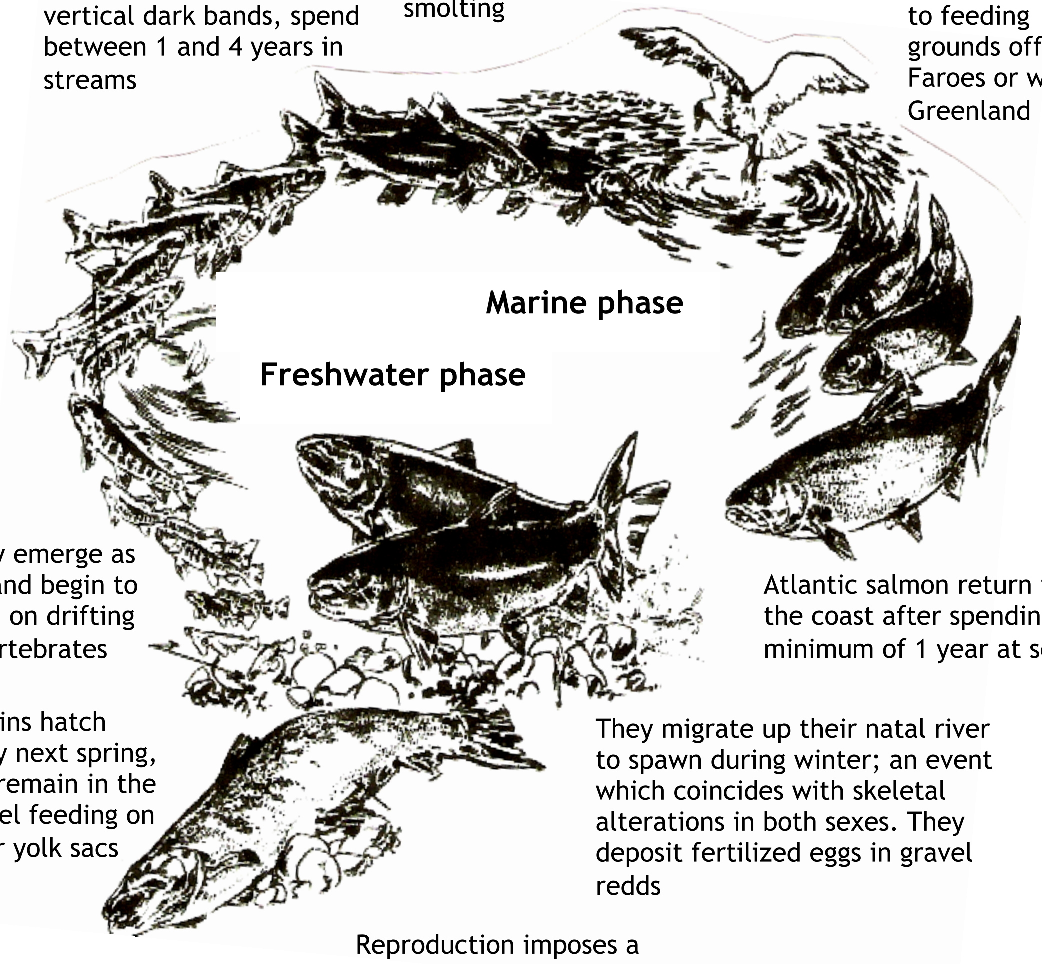
Atlantic salmon are found within the waters of countries bordering on the North Atlantic Ocean and Baltic Sea (Shearer 1992) and in the costal waters of the Barents and White Seas (Kazakov & Veselov 1998). The Atlantic salmon is a species that re-colonized north European waters following the last ice age (Tonteri *et al*. 2005). The last glacial event occurred around 22 000 – 18 000 years ago (MacCrimmon & Gots 1979). In Europe, the species' main glacial refugia are thought to have been in the Iberian Peninsula, the North Sea and the lakes east of the Baltic Sea. Following deglaciation and the re-establishment of the North Atlantic Drift, some 11,500 years ago (Adams, Maslin & Thomas 1999; Lynch-Stieglitz, Curry & Slowey 1999) these fish would have been able to radiate northwards and colonize the British Isles, Iceland, Scandinavia and the Baltic (Consuegra *et al*. 2002).

The present distribution of the species stretches from Massachusetts in the United States, through the eastern seaboard of Canada, across to Greenland, to Iceland, the Faroes, Scandinavia, eastward to Russia, westward again to the Baltic Sea and the rivers of France which flow into the Bay of Biscay and the English Channel, and then to the British Isles (including the United Kingdom and the Republic of Ireland) (Shearer 1992). The species reaches its southern limit on the Atlantic coast of Spain and Portugal (Shearer 1992).

1.8 Life cycle and alternative life-history strategies of salmon

The Atlantic salmon is an anadromous fish that moves between fresh water and the sea during the course of its life (see Figure 1.2). Although salmon spawn in fresh water and the young fish spend the first part of their life there, they grow most rapidly in the sea. As with other salmonids, their life-history patterns are characteristically flexible both between and within populations (Thorpe 1989; McCarthy, Sánchez & Blanco 2003). That flexibility depends on two principal physiological decisions made by juveniles in any given year: firstly, whether or not to emigrate to sea, and secondly, whether or not to mature sexually (Thorpe, Metcalfe & Huntingford 1992). There is also much variation in the length of the freshwater and marine phases of the lifecycle and in the age at first maturity (McCarthy *et al*. 2003).

Life for the Atlantic salmon begins in fresh water. Adult salmon migrate up stream at varying speeds (depending on the time of year, water temperature and stream flow) to spawn (Mills 1989). The spawning time varies between rivers and may be influenced by the water temperature and amount of daylight; while spawning will usually occur during the period November-December in upland and northern areas of their range and may be confined to a period of only 2 or 3 weeks, it may extend from November until March in rivers with less severe conditions and extend over 4 – 7 months depending on temperature (Crisp 1993; Bardonnet & Bagliniere 2000). Like other anadromous salmonids (Hasler & Scholz 1983), Atlantic salmon show high fidelity to the river where they grew as juveniles, with a homing accuracy that can average between 97% and 99% (Stabell 1984).



When a parr is ready to migrate downstream to sea, they undergo a series of changes – a process termed smolting

Parr, recognized by their vertical dark bands, spend between 1 and 4 years in streams

Smolts migrate to feeding grounds off the Faroes or west Greenland

They emerge as fry and begin to feed on drifting invertebrates

Atlantic salmon return to the coast after spending a minimum of 1 year at sea

Alevins hatch early next spring, but remain in the gravel feeding on their yolk sacs

They migrate up their natal river to spawn during winter; an event which coincides with skeletal alterations in both sexes. They deposit fertilized eggs in gravel redds

Reproduction imposes a huge energetic cost, and most fish die after spawning. However, a few do survive and return to the sea as kelts to recover and potentially spawn another year

Figure 1.2 The life-cycle of the Atlantic salmon (adapted from Mills 1971).

Salmonid fishes deposit fertilized eggs in gravel redds and the surviving fish, known as alevins, hatch early the following spring. The alevins remain in the gravel for a short period, feeding on their yolk sacs, and then emerge as fry to begin feeding exogenously on drifting invertebrates (Milner *et al.* 2003). The early post-emergence phase is a crucial one in which fry develop the swimming behaviour that allows them to maintain position and feed in flowing water; this is also the period during which dispersion from the redds occurs (Milner *et al.* 2003). This period of transition to independent feeding and dispersal can be a time of substantial density-dependent mortality due to intense competition for feeding territories (Nislow, Einum & Folt 2004; Armstrong & Nislow 2006).

After this period the fish are termed parr (Armstrong & Nislow 2006). The surviving parr, easily recognized by their vertical dark bands on their sides, spend between 1 and 4 years in streams in Britain (Milner *et al.* 2003), but this freshwater phase may last up to 8 years in colder more northerly rivers (Metcalfe & Thorpe 1990). The age at which fish turn into smolts and migrate to the sea is largely determined by growth rates during the preceding summer (Metcalfe & Thorpe 1992). In mid- to late summer the growth trajectories of potential emigrant and potential resident individuals in a population diverge (Thorpe *et al.* 1980; Nicieza, Braña & Toledo 1991). By November, this results in a bimodal length frequency distribution, with the upper modal group (UMG) representing individuals which have maintained relatively high metabolic and growth rates and which will emigrate as smolts the following spring (Thorpe 1987; Nicieza *et al.* 1991), and the lower modal group (LMG) representing individuals which have reduced metabolic and growth rates and which will remain in the freshwater system for at least another year (Thorpe *et al*. 1992). These LMG individuals show a decline in appetite in late July and August (Thorpe *et al*. 1998), which has been shown to be independent of food availability and temperature (Higgins 1985; Higgins & Talbot 1985; Metcalfe, Huntingford & Thorpe 1986).

When a parr is ready to migrate downstream to sea, it undergoes a series of physiological and morphological changes in preparation for movement into seawater, a process termed smolting or smoltification (McCormick *et al*. 1998). Whilst at sea, most European Atlantic salmon post-smolts make long migrations to feeding grounds off the Faroes or west Greenland (Hansen & Quinn 1998), before maturing and returning to natal rivers to spawn after 1 to 4 years (Milner *et al*. 2003). If the salmon has spent only one winter at sea before returning it is termed a 'grilse', whereas those that have spent at least two winters at sea before returning to fresh water are known as 'salmon' or multi-sea-winter salmon (Mills 1971). The return of Atlantic salmon to their home river for spawning coincides with skeletal alterations in both sexes, the most prominent being the development of a kype (hook) at the tip of the lower jaw in males (Witten & Hall 2003). On approaching fresh water, the salmon stops feeding and does not feed again until after spawning, if it survives. Salmon that survive spawning are termed kelts as they recover condition. Reproduction imposes a huge energetic cost and both males and females can lose over 50% of their energy reserves over the course of spawning (Jonsson, Jonsson & Hansen 1997). Therefore, despite being iteroparous, the consequence of this energetic cost is the low post-spawning rate of survival, and only a small fraction (approximately 10% on many rivers) will breed again in subsequent years (Mills 1989; Shearer 1992).

1.9 Aims of the thesis

This study investigates aspects of metabolism in individual juvenile Atlantic salmon, and in particular the extent of individual variation in various metabolic parameters. It focuses on SMR and SDA and the potential links between them. The thesis explores the potential for measuring MR by observation of opercular beat rates, so allowing near-instantaneous measurements of metabolism of fish in semi-natural conditions.

The objectives of this study include:

1) To evaluate whether the presence of a shelter reduces SMR; this may provide an additional reason for the extent to which these fish will compete aggressively for shelters when these are limiting (Chapter 2).

2) To examine the intraspecific variation in SDA, and to test whether the SDA for a given meal varies as a function of SMR. If SMR is directly related to scope for growth, then the speed and size of the SDA response should also correlate with SMR (Chapter 3).

3) To determine whether ventilation rate is sufficiently accurately related to MR at a range of temperatures and activities so as to allow energy

expenditure to be predicted outside the confines of a respirometer (Chapter 4).

4) To use the model developed in chapter 4 to compare the MRs of fish when paired and under a range of simple shelter habitats. Establishing the effect of social habitat and its interaction with physical habitat on SMR is of fundamental importance for understanding the energy budgets that link feeding, growth and activity of animals (Chapter 5).

The thesis concludes with a general discussion in which the findings of these experimental studies are related to previous research and potential future directions are identified (Chapter 6).

Chapter 2 – Presence of shelter reduces maintenance metabolism of juvenile Atlantic salmon

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2.1 Summary

Shelter is of major importance to many animals in providing protection both against the physical environment and potential predators.

I hypothesised that animals without shelter suffer metabolic costs associated with a need for increased vigilance and preparedness to escape attacks from predators or competitors. This possibility was tested by comparing the SMR's of inactive post-digestive juvenile Atlantic salmon held either with or without a shelter, which took the form of a semi-circular ledge under which the fish could fit comfortably.

The ledges were semi-transparent (so did not substantially reduce light levels) and provided no protection against the minimal water velocities in the testing arena. Nonetheless, absence of ledge shelter resulted in a 30% higher rate of oxygen consumption.

Fish without a ledge shelter typically positioned themselves against vertical walls of the observation arena, which presumably afforded the best available sheltering option, and adopted a significantly darker coloration

(indicative of greater stress) than those under ledges. Fish with ledges rested outside and adjacent to rather than beneath the shelter. Therefore, it seems that awareness that a shelter is readily available, rather than the act of sheltering, results in reduced metabolism.

I conclude that the presence of appropriate shelter not only reduces the risk of predation but also provides a metabolic benefit to fish that is likely to have implications for growth performance and activity budgets. SMR rate can be a function of habitat structure.

2.2 Introduction

The availability of shelter has been shown to be of extreme importance to the survival of animals. The reasons for using shelter are numerous, but they fall into two broad categories: protection against the physical environment, and protection against other animals. The physical environment in which animals live can sometimes be very hostile. For example, many marine intertidal organisms have to tolerate periods of air exposure. Crabs are able to survive these dry periods by moving to suitable moist cracks, crevices or burrows that significantly reduce the risk of water loss (Nybakken 1993). Seebacher & Alford (2002) showed that it was essential for cane toads to find suitable diurnal shelters in order to prevent dehydration and temperature stress during the dry season. In the Brazilian lizard, the abundance of microhabitats suitable for egg development influences the length of the breeding season since low levels of moisture negatively affect egg survival and reduce embryos sizes (Wiederhecker, Pinto & Colli 2002). Significant energy economies may be accrued by sheltering from the environment. For example, sheltering can potentially reduce water loss by as much as 81% in the Hoopoe lark (Williams, Tieleman & Shobrak 1999), and can cut locomotory costs of fish holding station in flowing waters (Rimmer, Paim & Saunders 1984). Such energy savings can also have indirect benefits for reproduction: the use of nest boxes during the night by Great and Blue Tits during the egg-laying period has been suggested to reduce the birds' thermoregulatory costs (Pendlebury & Bryant 2005), and therefore allow an enhanced allocation of resources to egg production.

Many animals use shelters to hide from predators (Kotler 1984; Godin 1997; Valdimarsson & Metcalfe 1998; Steele 1999), but the benefits may not be restricted to the obvious increase in safety. Shelters may replace the need for other mechanisms for avoiding predators, such as camouflage or vigilance, which may have associated costs, for example in producing appropriately coloured compounds and maintaining acutely active sensory systems. The absence of a refuge might be expected to result in an overall elevation in metabolism of the animal even when quiescent. However, I am not aware of any other study regarding the occurrence or magnitude of such additional energy costs in the absence of shelter.

The objective of this experiment was to evaluate whether the presence of a shelter reduces the standard metabolic costs. SMR can be measured as the oxygen consumption of non-feeding, post-absorptive and inactive fish with no anaerobic activity debt (Brett & Grove 1979), and is synonymous with basal metabolism and costs of tissue maintenance. The study was carried out on juvenile Atlantic salmon, which like other species of freshwater fish are known to use crevices under stones on the substrate as shelters (Cunjak 1988). Salmon compete for shelter (Kemp, Armstrong & Gilvear 2005), which suggests that they are a highly valued resource (Armstrong & Griffiths 2001). Suggested benefits of sheltering for salmon have included protection from harsh environmental conditions (Valdimarsson & Metcalfe 1998), seeking refuge from the water current (Rimmer *et al.* 1984; Pickering & Pottinger 1988; Heggenes *et al.* 1993), avoidance of displacement by floods or ice (Hartmann 1965; Heggenes *et al.* 1993; Whalen, Parrish & Mather 1999) and/or sheltering from the light at cold temperatures (Cunjak 1988; Contor & Griffith 1995). However, direct avoidance of predation is now thought to be the most likely primary function of this sheltering behaviour (Valdimarsson & Metcalfe 1998; Greenwood & Metcalfe 1998). I now suggest that the use of these shelters could have further benefits if they allow a reduction in metabolic costs.

I test this idea by measuring the SMR of salmon parr in relation to whether or not they have access to a refuge. I also assess body coloration, since this is thought to be associated with levels of physiological stress in other contexts (Abbott, Dunbrack & Orr 1985; O'Connor, Metcalfe & Taylor 1999; Höglund, Balm & Winberg 2002; Suter & Huntingford 2002).

2.3 Materials and methods

Atlantic salmon parr in their first year post-hatching were caught in mid January 2005 from the River Almond (Perthshire, Scotland) and transferred to Glasgow University. Prior to the experiment being carried out the fish were held in circular tanks (1m^2) at 8.5ºC in aerated, re-circulated, copper-free water under an ambient photoperiod. They were fed to satiation on dry pelleted food once a day. Whilst in the holding tank, the fish had access to shelters in the form of large stones and lengths of semi-circular piping (approximately 120mm in diameter). They were allowed to settle in the holding tank for 2 months before the first respirometry experiments took place. During the first month the water temperature was increased to 10°C, but was then kept constant (± 0.5°C) throughout the remaining settling period to allow acclimation. This increase in water temperature was carried out in order to reflect the mean ambient temperature found in their natal river over the course of the next month. In order to measure SMR, two fish were selected each day and placed without food in individual respirometer chambers to settle and evacuate their gut contents overnight (a minimum of 21 h in chamber and 48 h from last feeding before the first measurements were taken); this time period is sufficient to allow their MR to drop to resting levels (McCarthy 2000). The same sized chambers were used for each fish tested to control for any effect of chamber size on MR. The respirometry chambers (150mm by 100mm by 100mm and made of clear perspex) were placed inside two aerated water baths and each received a continual pumped input of aerated water. One chamber contained a shelter, whilst the other one had no internal structures. The shelter (placed in the centre of the chamber) was made from clear, semicircular perspex (radius 20mm, 120mm in length) so that light from the overhead illumination remained relatively similar between the two treatments. Light intensity within the chambers was measured using a Skye Instrument SKL 300 display meter connected to a SKL 310 lux sensor and was recorded as 5.50 lx, dropping to 4.31 lux under the shelter. The apparatus was screened with black plastic sheeting along the front and sides to minimize disturbance. The temperature of the room where the respirometry measurements took place was recorded as 10ºC (± 0.5) throughout the entire experimental period.

SMR was measured as the reduction in oxygen concentration over time, using intermittent flow (or open-closed) respirometry. This method is described in greater detail by Steffensen, Johansen & Bushnell (1984) and Herskin (1999), and so only summary details are given here. Oxygen concentrations in the water exiting from the respirometry chamber were measured using a Strathkelvin instrument S1130 oxygen electrode contained within an SI EH100 electrode holder, linked to a SI 928 6-channel meter. To make a measurement of oxygen consumption, the oxygen content of the water was measured initially (t_0) when the system was in the open position (i.e. the chamber was receiving a continual pumped supply of aerated water from the water bath). Then the system was closed for 30 minutes by means of valves so that the water within the chamber was continually recirculated around a closed loop (volume including pump, chamber and tubes was ≈ 1.6 l). At the end of the period of closure (t_1) the valves were returned to their original positions so allowing fully aerated water to again be pumped through the chamber. The oxygen concentration was recorded continually from 10 min before the system was closed until 10 min after it was re-opened. A plastic screen was placed between the chamber and valves so that the valves could be adjusted out of sight of the fish; moreover, the arrangement of pumps and valves was such that the rate of flow of water through the chamber did not alter noticeably when the system was switched from the open to the closed position. At no point did oxygen concentrations drop below 90% saturation (Appendix 2).

Recordings of oxygen consumption for each fish were first taken at 1100 h, 21 hours after being put into the chamber, and then again at 1230 h. After the second recording the fish were removed, weighed and then placed into a separate holding tank. The water bath was drained, refilled with fresh water and the electrodes recalibrated using a solution containing an oxygen saturation of zero (a pinch of sodium sulfite anhydrous dissolved in a small amount of water). Two new fish were then put into the chambers and the entire procedure was repeated until 14 pairs of fish had provided suitable data (i.e. both fish were inactive throughout the period of measurement – see below). The shelter was switched between chambers after the 7th pair to control for any possible effects of chamber location. Oxygen consumption by the fish was calculated from the rate of decline of oxygen in the closed respirometer. The two measurements of MR on each fish were very consistent (repeatability = 0.77, Lessells & Boag 1987), and so the mean of the two SMR values for each fish was used for subsequent analysis. The values for MR for each pair of fish tested on a given day were then compared using a paired t-test.

Continuous behavioural observations were taken by watching the fish during the entire closed period of 30 minutes; the fish's activity, position in the chamber and colour were noted. As this experiment was carried out to measure SMR, only fish resting on the bottom of the chamber during the experimental period were used; these were the majority as, after the initial settling period, fish spent virtually all their time resting motionless on the bottom of the respirometry chamber (since the water current was not strong enough to induce swimming against the flow). Any fish that did not settle during the experimental period ($n = 5$) were removed and replaced by another fish. The fish within the other chamber was also removed and replaced by another fish to prevent any acclimation differences between the two treatments. The position of the resting fish in the chamber (either with or without a shelter) was also recorded. Fish positioned in the centre of the chamber (either in or just outside the shelter, if provided) were given a score of 0 whilst those that remained around the edges of the chamber received a score of 1. The overall score of the position of fish (at the edge or centre of chamber) from each treatment group was compared using a chi-square test.

Juvenile salmon normally have a cryptic pattern of coloration on their back and sides, and are capable of adjusting their skin coloration to some degree to blend in with their surroundings. However, skin and eye (sclera) patterning can also darken rapidly, notably in losers of aggressive encounters (O'Connor *et al.* 1999). In order to ascertain whether dorsal and lateral skin and eye pigmentation varied depending on shelter presence, each pair of fish (one with and one without a shelter) was compared during the two periods the system was closed. The lighter coloured fish of the pair was given a score of 0 while the darker was scored 1. A colour difference was clearly evident in all tested pairs of fish, and was consistent between the two periods of measurement. The relative frequency with which the fish with a shelter was darker or lighter than its corresponding fish without a shelter was compared using a binomial test.

2.4 Results

The weight of the fish without shelter ranged from 3.19g – 7.76g (mean weight 4.36g ± 1.51 s.d.) whilst the weight of fish with shelter ranged from 3.01g – 6.93g (mean 4.68g ± 1.16). There was no difference between the weight of fish used in the two treatment groups (paired t-test, t = 0.70, 13 d.f., P = 0.496). The fork length of each fish was also measured and ranged from 66 – 90mm (mean length 72.71mm ± 7.65) in the fish without shelter and 66 – 84mm (mean 76.29mmm ± 6.97) in those with shelter. Again there was no significant difference between the length of fish used in the two treatment groups (paired t-test, t = 1.30, 13 d.f., P = 0.216).

Given the lack of size difference between the treatment groups and the small range of body sizes used in the experiment, MR was expressed as oxygen consumption per unit bodyweight per h. Fish with shelter had a mean SMR of 197.44mgO_2 kg^{-1} h^{-1} ± 39.34 s.d. (1st measurement) and 191.62mgO_2 kg^{-1} h^{-1} ± 46.89 (2nd measurement). Fish without access to shelter had a mean SMR of 264.78mgO_2 kg^{-1} h^{-1} ± 81.19 s.d. (1st measurement) and 262.30mgO_2 kg^{-1} h^{-1} ± 86.54 (2nd measurement). Comparison of the two treatments showed that the presence of a shelter led to a significant reduction in the mean SMR's of the fish (paired t-test comparing fish with/without shelter on a given testing day: t=3.52, 13 d.f., P=0.004; Figure 2.1).

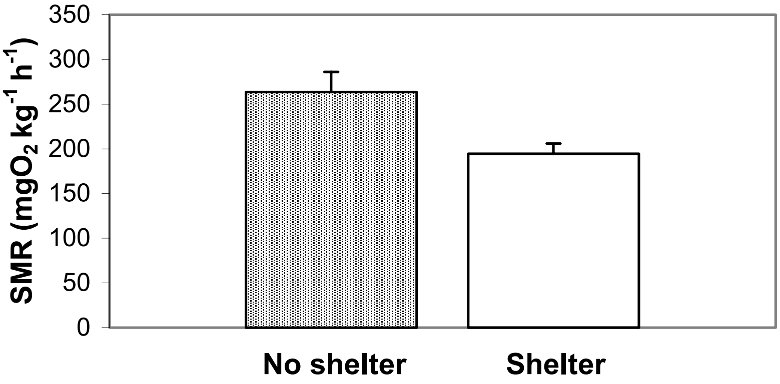

Figure 2.1 Comparison of SMR of Atlantic salmon parr in the absence and presence of a single shelter. The data are presented as means + s.e.m. The difference between the two groups was significant (paired t-test: t=3.52, 13 d.f., P=0.004).

Behavioural observations revealed that the fish with a shelter appeared to rest inside it only when they were first put into the chamber. When observed the following morning they were usually lying on the bottom of the chamber against the outside edge of the shelter. Comparison of the two treatments in relation to their position, categorised as the number of fish positioned at the edge versus the centre, showed that fish with a shelter preferred resting in the centre of the chamber near to the shelter whilst those without a shelter preferred to wedge themselves at the edge of the chamber. In total 86% of the fish with a shelter preferred to rest at the centre of the chamber compared with only 43% of the fish without a shelter (Chi-squared test, P=0.046). Moreover, the fish that had access to the shelter was lighter in both skin and sclera coloration than the corresponding fish without a shelter in 13 of the 14 pairs, which is significantly more frequent than expected from a random association (binomial test, P=0.0017). The fish with the lighter coloration also had the lower MR in all of the 14 pairs of fish used.

2.5 Discussion

The fish without access to a shelter had the higher SMR in all but one of the 14 pairs of fish. The magnitude of this difference was substantial, averaging a 30% increase in metabolic costs in the absence of shelter. In all cases the fish in a test pair (shelter/no shelter) with the higher MR also had the darker coloration. However, while the salmon hid under the shelter when first introduced into the respirometry chamber, once they had settled (and during readings of MR) they rested immediately adjacent to the shelter rather than inside it. Therefore, the reduction in MR appears to be associated with the fish being aware of the availability of a nearby shelter rather than their current use of that shelter.

There was no suggestion that the between-treatment variation in MR was due to differences in activity, since in all 14 pairs the salmon were resting immobile on the floor of the chamber and were experiencing a water flow that was too low to require active resistance. There are several factors that might explain, at least in part, the metabolic costs of shelter absence. First, it is well established that ventilation rate is elevated in Atlantic salmon fry when exposed to the immediate threat of predation, even in the absence of the ‘flight’ response (Hawkins, Armstrong & Magurran 2004). This response was suggested to show a preparation for possible subsequent activity, and this indicates that predation threat can be metabolically costly even in the absence of activity, particularly when 25% of the total MR of a fish consists of gill ventilation (Hughes & Shelton 1962). This ‘non-lethal’ effect of predation (reviewed by Lima & Dill 1990) occurs when predators alter the physiology or behaviour of the prey in such a way as to reduce growth rates, increase energetic demand, and ultimately decrease fitness (Cooke *et al.* 2003). Increase in outputs of the opercular and cardiac pumps may result from release of vagal tone and changes in the adrenergic function associated with stress (Mazeaud & Mazeaud 1981). This adrenergic response results in the catecholamine hormones adrenaline and noradrenaline being released into the circulation from the chromaffin cells when exposed to a wide range of internal and environmental stressors (Reid, Bernier & Perry 1998). One of the primary roles of plasma catecholamines is to modulate cardiovascular and respiratory function in order to maintain adequate levels of oxygen in the blood and, therefore, a sufficient supply to the tissues (Reid *et al.* 1998). It is possible that those fish with shelter nearby can reduce their resting metabolism because a short anaerobic burst is sufficient to reach cover should it become necessary to flee from a predator. However, in the absence of shelter, escape may require prolonged swimming activity and fish may have to be in a sustained state of physiological readiness for such activity. This would entail a greater sustained metabolic investment in fuelling cardiac and respiratory pumps and possibly other associated changes, such as osmoregulation.

A second factor that may influence metabolism is the state of mental alertness. It is well established that vigilance can have time costs in the sense of lost feeding opportunities (Carrascal & Moreno 1992; Fritz, Guillemain & Durant 2002; Fortin *et al.* 2004). However, mental activity is metabolically demanding and so heightened vigilance may increase energy costs with various knock-on consequences (Roulin 2001). Persons *et al.* (2002) reported that antipredator behaviour of the wolf spider increased energy costs and decreased foraging efficiency, which resulted in both fewer and lighter egg sacs being produced compared with spiders with reduced predator vigilance. In other studies, the ability to process information effectively has been shown to reduce during long periods of vigilance (Nuechterlein, Parasuraman & Jiang 1983; Warm 1984; Parasuraman & Mouloua 1987). The nervous and immune systems are tightly linked (Maier, Watkins & Fleshner 1994) and so a requirement for high-level vigilance may also trade-off against the efficiency of the immune system (Roulin 2001). Therefore the use of refuges (especially during periods when predation risk is high) may reduce the physiological costs associated with increased vigilance, so providing indirect benefits as well as the more evident protection from predators. However, an additional cost may be incurred by the fundamental demands of maintaining sensory apparatus at a sustained elevated state of acuity. Such may be the case for those fish without shelters for which early detection would be most important.

This study showed that 13 out of 14 fish without a shelter were significantly darker than the corresponding fish with a shelter. Changes in skin coloration have been associated with the signalling of social status in salmonids, since social subordination results in rapid darkening of the skin and sclera (Abbott *et al.* 1985; O'Connor *et al.* 1999; Höglund, Balm & Winberg 2000) and communication by means of rapid, reversible visual signals mediated through differential colour patterns plays an important role in the control of aggressive behaviour in fish (Huntingford & Turner 1987). However, in the present experiment all fish were in individual chambers and had no contact with others, and so changes in coloration are unlikely to be linked to social signalling. Moreover, on each occasion the darker fish had the higher MR, suggesting that these two factors are linked in some way. While it is theoretically possible that being dark (for whatever reason) is energetically expensive, it is more plausible to suggest that the darker coloration and elevation in physiological metabolic state are linked via the stress response since skin darkening in fish appears to be related to stress (Höglund *et al.* 2000; 2002). Stress responses in teleosts are mediated via the hypothalamic-pituitary-interrenal (HPI) axis, which regulates the level of cortisol production (Donaldson 1981). It has recently been shown that the degree to which fish are conspicuous against their background can affect cortisol responses (Rotllant *et al.* 2003). Therefore, prolonged elevation in MR in the absence of shelter (and possibly because of a perceived greater vulnerability to predation) may be sustained by cortisol, which in turn leads to darkening of the skin. Such darkening may have a role in camouflage under natural conditions.

It has been shown that juvenile Atlantic salmon prefer to use refuges that allow them to hide (i.e. are dark and opaque) rather than those that shield them from the water current (Valdimarsson & Metcalfe 1998). Simulated ice cover (darkness) was shown to reduce the resting metabolism of juvenile Atlantic salmon by an average of 30% compared with fish kept without ice cover (6h light per day) (Finstad *et al.* 2004a). While this might appear to be a similar effect to the one I report here, there are in fact several factors that could contribute to the lower metabolism of fish in darkness. Wendelaar Bonga (1993) showed that there is an increase in metabolism caused by the effect of light on melatonin secretion mediated by the pineal gland. This alone may result in the higher SMR in fish without simulated ice cover. Alternatively, there may have been differences in activity levels between the fish held at different light levels, since Finstad *et al.* (2004a) did not monitor activity. Moreover, while the removal of ice cover may increase the risk of predation particularly by homeothermic animals, the present study highlights the fact that the availability of shelter affects metabolism even when both light and activity levels remain unchanged.

An increase in SMR in the absence of shelter may have significant implications for growth rate, especially since a state of heightened anti-predator vigilance can also result in lost foraging opportunities (Metcalfe, Huntingford & Thorpe 1987). Metcalfe *et al.* (1987) showed that increased anti-predator vigilance resulted in recognition errors of food items and hence a decrease in energy intake. It has been suggested that the depletion of energy stores is the major source of winter mortality in juvenile temperate freshwater fishes, including Atlantic salmon (Gardiner & Geddes 1980; Post & Evans 1989; Miranda & Hubbard 1994; Finstad *et al.* 2004b; Biro *et al.* 2004). Fish with greater energy-loss rates would be more susceptible to mortality than those with lower energy-loss rates, especially during periods when feeding is energetically less profitable. Simpkins *et al.* (2003) demonstrated that an increase in metabolic demands associated with swimming activity resulted in a 20% reduction in lipid mass and a 40% reduction in triacylglyceride concentrations, compared to those of sedentary fish. However, an increased metabolism due to the lack of appropriate refuges may also indirectly influence winter survival since this would ultimately increase the rate at which energy is lost. Therefore, if this heightened rate of metabolism was maintained over long periods, it could result in poorer growth or survival of low-energy fish in habitats where refuges are scarce, and might help explain the higher overwinter survival of trout in sites with shelters compared to sites without, even when predators were excluded (Smith & Griffith 1994). It also provides another reason for the extent to which fish will compete aggressively for shelters when these are limiting (Gregory & Griffith 1996; Harwood *et al.* 2002). More generally, the study shows that resting metabolism may vary, independently of activity levels or nutritional state, simply because of changes in the physical structure of the external environment.

Chapter 3 – Relationships between standard metabolic rate and digestive strategies in juvenile Atlantic salmon

3.1 Summary

Basal or Standard Metabolic Rate has been found to exhibit substantial intraspecific variation in a range of taxa, but the consequences of this variation are little understood. Here I explore how SMR is related to the speed and energetic cost of digestion.

In poikilotherms, the elevation of metabolism following ingestion of a meal is termed the apparent Specific Dynamic Action (SDA). Using juvenile Atlantic salmon, I test the hypothesis that the nature of the SDA response is influenced by an individual’s SMR.

The peak in post-prandial oxygen consumption, the duration of elevated metabolism and the overall magnitude of SDA all increased with the size of meal consumed. However, for a given meal size, fish with a higher SMR also had a higher peak and greater magnitude of SDA. Despite this, they experienced a shorter duration over which metabolism was elevated following the meal.

These relationships are likely to have implications for overall growth rates since when resources are abundant, fish with a higher SMR may be able to resume feeding more quickly after a meal and so increase their overall rate of energy intake. The relationship between SMR and the magnitude of SDA

may also indicate that SMR is related to assimilation efficiency. Intraspecific variation in SMR is thus linked to variation in digestive strategies, although the costs and benefits of a given SMR are likely to vary with resource availability and predictability.

3.2 Introduction

The BMR and SMR of homeothermic and poikilothermic animals is the level of energy consumption when inactive, not assimilating a meal and not paying off any oxygen debt associated with previous anaerobic activity (McNab 1988; Hulbert & Else 2000; Frappell & Butler 2004). It is well established that SMR varies substantially among species (Blaxter 1989), tending to be highest in those animals that have the most active lifestyles (White & Seymour 2004). However, there can also be substantial (up to five fold) variation in SMR within species, even under controlled conditions (Metcalfe *et al.* 1995; Steyermark, Miamen & Feghahati 2005). Since SMR represents a large component of the overall energy budgets of animals (Congdon *et al.* 1982; Hulbert & Else 2000; Speakman *et al.* 2003), it is of fundamental importance to understand the functional relevance of this wide intraspecific variation.

Based on variation among species, two distinct, but not mutually exclusive, hypotheses have been advanced to seek to explain variation in SMR. First, it has been hypothesised that SMR may relate to the cost of maintaining a scope for activity that is adapted to the lifestyle of the species (Hammond & Diamond 1997; Meerlo *et al.* 1997; Speakman *et al.* 2003). Scope for activity is the difference between standard and maximum MR (Fry 1947; Fry

1971). A high performance capacity could be expected to require a large mass of aerobically active tissue, such as heart and lungs, which are relatively expensive to maintain (Steyermark *et al.* 2005) and would lead to high basal costs. Such a relationship is observed when comparing across inter-specific trends in life style, such as are illustrated in fish by relatively sessile pike (Armstrong, Priede & Lucas 1992) and flatfish (Duthie 1982), compared with more active salmonids (Brett 1965) and tunas (Dewar & Graham 1994).

The second hypothesis, derived from changes in metabolism during ontogeny, is that SMR relates to the capacity for growth. Within a poikilothermic species, smaller individuals tend to have a higher SMR per unit weight (Schmidt-Nielsen 1984), and this correlates with having relatively high maximum weight-specific growth rates and associated costs of processing food (Goolish & Adelman 1987).

Juvenile salmonid fish have been a particularly useful subject for exploring individual variation in metabolism because it has been possible to relate SMR to behavioural traits and therefore lifestyle within a species (Metcalfe *et al.* 1995; McCarthy 2000). SMR correlates with dominance status, which is reflected in the ability of fish to access high value food patches (Metcalfe *et al.* 1989; Gotceitas & Godin 1992). Dominant individuals with high SMR tend to exhibit active aggressive behaviours, whereas subordinate fish are more passive, more cryptic and feed in more marginal areas (Janson 1985; Höglund *et al.* 2000; Höjesjö, Armstrong & Griffiths 2005). Therefore, high SMR in dominant salmon is consistent with the first hypothesis linking SMR to scope for activity. However, in direct tests, Cutts, Metcalfe & Taylor

(2002) found that juvenile Atlantic salmon with a higher SMR actually had a lower scope for activity.

Dominance, and hence SMR, is also linked to growth rate in habitats where spatial distributions of food patch qualities are predictable and can be defended, such as pools in rivers (Nakano 1995). This relationship is consistent with the second hypothesis that within-individual variation in SMR relates to a physiological scope for growth. This hypothesis has not previously been tested and is the subject of the present study. Scope for growth depends on the rate at which tissues can be accreted. Growth of tissues involves expenditure of energy in mechanical and chemical processing of ingested food. The chemical component is termed specific dynamic action which, when combined with mechanical activity (such as swallowing), is apparent specific dynamic action (SDA) (Beamish 1974). SDA largely represents the costs of synthesising protein and so reflects the instantaneous rate of tissue accretion. SDA increases rapidly to a peak or plateau after food has been ingested, and then decreases more slowly as digestion of the meal proceeds (Jobling 1981). Potential growth rate may therefore be related to the size of the SDA response, since a rapid and pronounced peak in SDA would reflect faster accretion of tissues and digestion of food, and would enable more rapid re-feeding and greater through-put of food.

It is well established that the height and magnitude of SDA relate directly to meal size (Jobling & Davies 1980; Secor & Diamond 1997; Zaidan & Beaupre 2003), but there is no information on whether SDA for a given meal varies within species as a function of individual traits such as SMR. Here I explore this area, in particular testing the hypothesis that if SMR is directly related to scope for growth, then the speed and size of the SDA response should also correlate directly with SMR. By measuring changes in the rate of oxygen consumption by Atlantic salmon of differing SMR that eat standard meal sizes, I show for the first time that there are strong links between SMR and the profile of energy expenditure during digestion, indicating the existence of intraspecific variation in digestive strategies.

3.3 Materials and Methods

The experiments were carried out on underyearling Atlantic salmon parr derived from wild parents; these were reared at the Fisheries Research Services (FRS) Almondbank field station, Perthshire and transferred to Glasgow University in early August 2006 where they were held in a circular tank (1m^2) at 13.5ºC in aerated, re-circulated, copper-free water under an ambient photoperiod. They were fed to satiation on defrosted bloodworms (Chironomid larvae) once a day. While in the holding tank, the fish had access to shelters in the form of large stones and lengths of semicircular cut piping (approximately 120mm in diameter). They were allowed to settle in the holding tank for 1 month before the first respirometry experiments took place. At the start of each round of experiments, four of the fish were selected at random and weighed / measured. They were then placed without food in individual respirometer chambers, maintained at the same temperature of 13.5ºC (± 0.5) throughout the whole experimental period, and allowed to settle for two days before the first respirometry measurements commenced. Each of the separate respirometer chambers contained a clear perspex shelter, since fish have a higher resting rate of metabolism when denied access to any shelter (Millidine, Armstrong & Metcalfe 2006). The chambers were kept in dim light to further reduce stress and activity levels; the water flow through the chambers was insufficient to prompt swimming, and so the fish remained resting on the substrate. The apparatus was similar to that described in chapter 2 (Appendix 2), and allowed measurements of oxygen consumption to be made using intermittent flow respirometry based on a Strathkelvin Instruments SI130 oxygen electrode linked to a SI928 6-channel meter.

In order to measure SDA, it was first necessary to record the resting level of metabolism of the fish in a post-absorptive and quiescent state (i.e. their SMR). This was carried out between 08.00 and 09.00 h, two days after the fish had been placed in the respirometers. Measurements of oxygen consumption by fish were taken by closing the respirometer valves, so preventing any water exchange, for a period of 10 minutes. After this time the system was re-opened to allow the water within the respirometer to completely flush with 100% saturated water.

Food, in the form of defrosted bloodworms, was then given to the fish whilst inside the respirometers. Two of the 4 fish were given a single meal of bloodworms weighing 0.15% of their body mass whilst the other two were given a meal weighing 0.30% of body mass. The bloodworms were individually introduced in to the respirometer chamber via the water inlet valve when the system was fully open. These were usually eaten immediately, and as soon as all the bloodworms had been consumed, the system was closed for a period of 5 minutes and the rate of oxygen consumption was measured. Then the system was re-opened, and when the water within the respirometer was completely flushed with 100% saturated water (approximately 3 minutes), the system was again closed for a further 5 minutes to obtain another reading. This continued for approximately the first 2 hours until the peak in oxygen consumption rate had passed. After this point oxygen consumption was recorded every half hour, extending to every hour over the last two hours of the SDA response when decline in oxygen consumption rate was minimal (<10% change). Once the rate of oxygen consumption had dropped to the initial rate (prior to the introduction of food) the fish were removed and replaced with another 4 fish of known weights. This procedure was then repeated until all fish had been tested. Any fish that either refused to eat or became active whilst within the respirometer was removed and replaced. The oxygen consumption of the fish was calculated from the rate of decline of oxygen in the closed respirometer, and expressed as oxygen consumption per unit bodyweight per h.

Over a 5-week period a total of 26 fish provided useable data (i.e. they consumed the correct amount of bloodworm introduced to the respirometer and were inactive throughout the feeding day). The weight of the fish given the 0.15% body weight meal ranged from 3.37 – 11.34g (mean = 6.42g ± 2.55 s.d., n=12) whilst that of fish given the 0.30% body weight meal ranged from 3.99 – 11.37g (mean = 7.36g ± 1.78, n=14). There was no difference between the weights of fish used in the two meal groups (independent samples t-test, t_{24} = 1.14, P = 0.27; combined mean body mass 6.78g ± 1.88).

A range of parameters quantifying SDA was then calculated for each fish's response: peak SDA (defined as the maximum postprandial MR – SMR), time taken to reach peak SDA, duration of the SDA response (until the MR had returned to the preprandial level) and the magnitude of SDA (defined as the overall oxygen consumption due to digestion, and equivalent to the area under the curve – see Figure 1.1, Chapter 1). The magnitude was determined by using Matlab version 6 to plot each SDA response and then calculate the area under the curve. The corresponding data from all 26 fish were then analysed using a stepwise regression approach in which these different measures of SDA were used as the dependent variables in successive analyses, with SMR, body weight, water temperature at the time of testing (which varied very slightly between trials) and relative size of meal as candidate independent variables.

3.4 Results

Shortly after feeding the rate of oxygen consumption increased to a peak, and then gradually declined to the pre-feeding level over a period of 2.5-9.5 hrs. The duration of this SDA response, the size of the peak SDA, the time to reach that peak and the overall magnitude of SDA were all significantly greater in fish consuming the larger meal (Table 3.1). Figure 3.1 illustrates the general pattern of postprandial oxygen consumption for the two meal sizes. These effects of meal size were not confounded by SMR, since SMR did not differ between fish in the two meal size treatments (Mean SMR = 179.06mg O_2 h^{-1} ± 57.81 SD for fish given the meal equivalent to 0.15% of body mass and 179.21 ± 48.06 for those given 0.30%, independent samples t-test, $t_{24} = 0.007$, $P = 0.994$).

Meal size (% of body weight)	No. of fish	Peak rate of O_2 consumption (mg O_2 kg^{-1} h^{-1}) above resting level	Time to reach peak (mins)	Duration of SDA effect (mins)	Magnitude of SDA, (mg O_2 kg^{-1})
0.15	12	92.91 ± 23.47	35.08 ± 7.35	199.08 ± 51.58	871.95 ± 200.22
0.30	14	131.34 ± 41.56	50.42 ± 14.06	447.71 ± 57.57	1940 ± 551.11
Independent samples t -test		$t_{24} = 2.39$, $P = 0.024$	$t_{24} = 3.42$, $P = 0.002$	$t_{24} = 11.56$, $P < 0.001$	$t_{24} = 6.75$, $P < 0.001$

Table 3.1 The effects of meal size on the measured parameters of apparent Specific Dynamic Action (the energy cost of digesting a meal) in juvenile Atlantic salmon. Data are presented as means ± S.D.

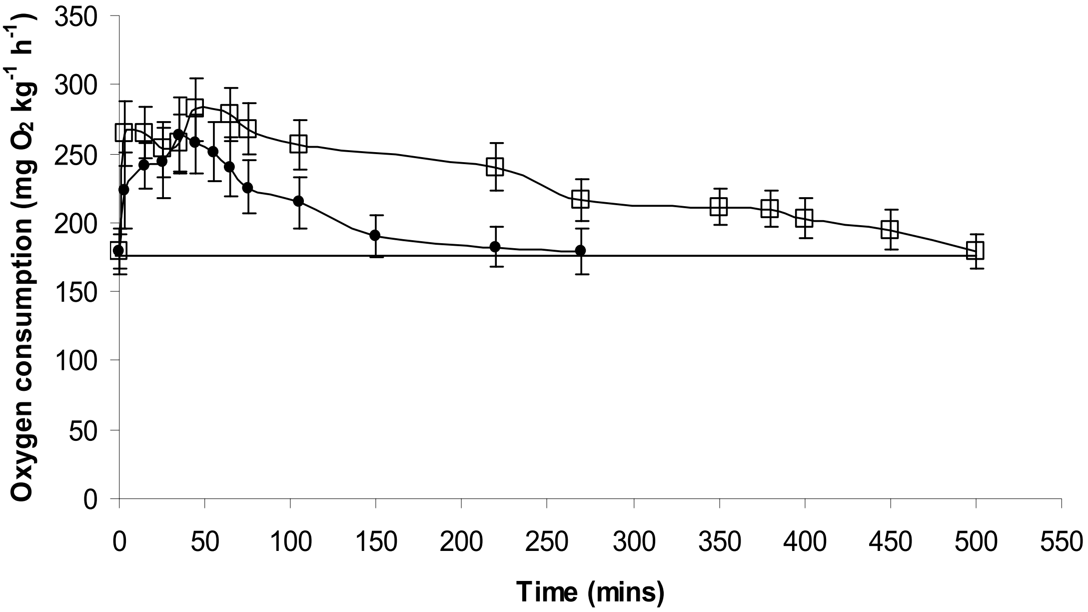

Figure 3.1 The general shape of the SDA response for fish eating different meal sizes (● 0.15% of fish body mass; □ 0.30% of body mass). The diagram shows the mean ± S.D based on data from 12 fish and 14 fish consuming meals at 0.15 and 0.30% of body mass respectively. The horizontal line indicates the average SMR of the fish.

However, many of the dimensions of apparent SDA were significantly related to inter-individual variation in SMR (Table 3.2). In order to determine the relationships between SDA and SMR, separate stepwise regression analyses were carried out using peak SDA, time to reach peak SDA, duration of SDA and magnitude of SDA as the dependent variables and SMR, body weight and temperature as the independent variables. The general profile of the SDA response for fish of higher and lower SMR is shown in figure 3.2 (a) and (b) respectively.

Dependent variable	**Significant independent variables**	**Coefficient**	**Significance**
(a) Peak SDA (mg O_2 kg^{-1} h^{-1})	Meal size (% bodywt)	196.31	P=0.002
	SMR (mg O_2 kg^{-1} h^{-1})	0.32	P<0.001
	Constant	7.60	P=0.599
(b) Time to reach peak SDA (mins)	Meal size (% bodywt)	92.83	P=0.002
	Constant	21.43	P=0.005
(c) Duration of SDA (mins)	Meal size (% bodywt)	1525.74	P<0.001
	SMR (mg O_2 kg^{-1} h^{-1})	-0.61	P=0.009
	Constant	90.67	P=0.062
(d) Magnitude of SDA (mg O_2 kg^{-1})	Meal size (% bodywt)	6344.0	P<0.001
	SMR (mg O_2 kg^{-1} h^{-1})	3.77	P<0.001
	Constant	-777.3	P=0.001

Table 3.2 Results of stepwise regression analyses to test whether the observed SDA responses from 26 juvenile salmon (see Table 3.1) were significantly related to ration size, SMR, body weight (g) and/or temperature (ºC). Separate analyses were run using **(a)** peak SDA, **(b)** time to reach peak SDA after food consumption, **(c)** duration of SDA effect and **(d)** magnitude of SDA as the dependent variable; significant predictor variables are listed with their unstandardized regression coefficients (body weight and temperature were not significant and were removed from the model in all cases).

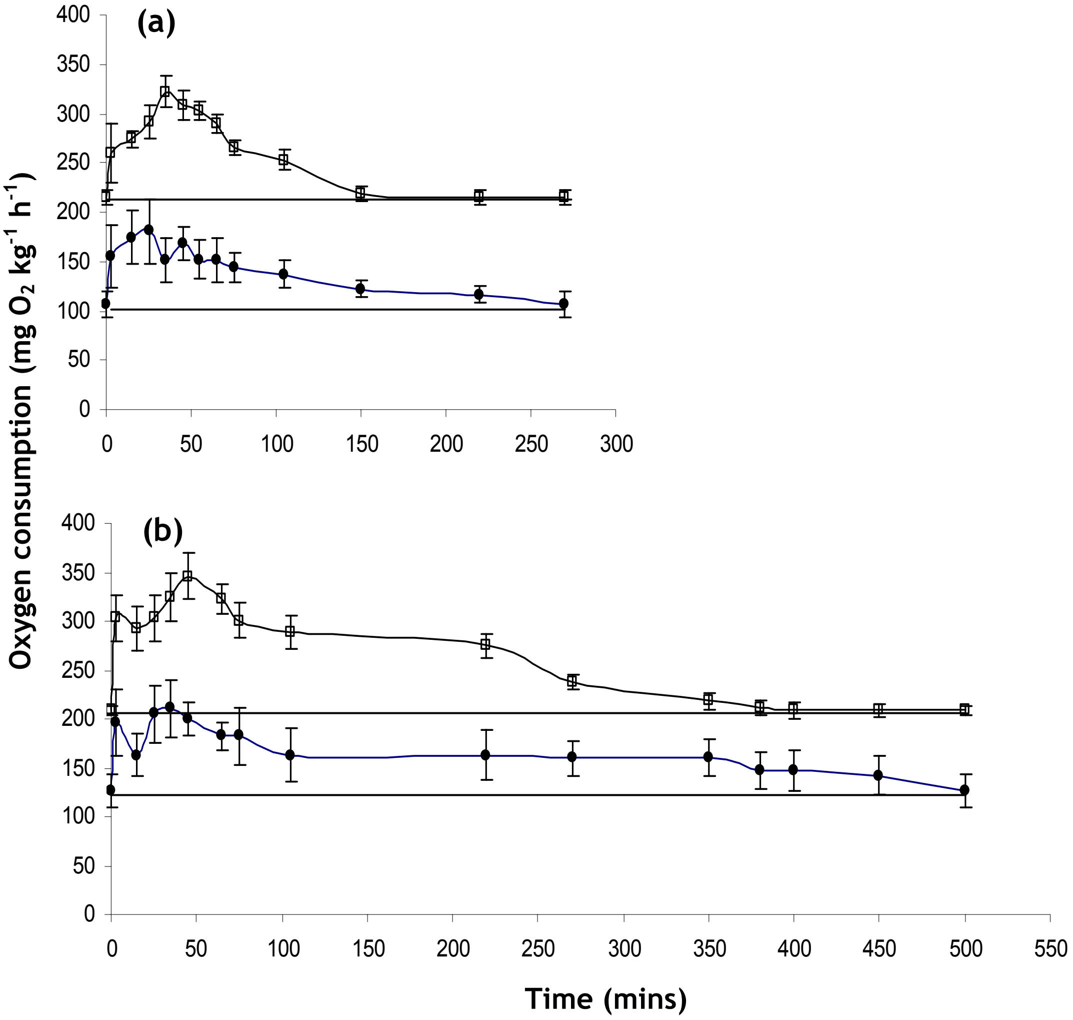

Figure 3.2 The postprandial oxygen consumption following feeding by fish with high (□) and low (•) SMR whilst eating meal sizes of **(a)** 0.15% of fish body mass and **(b)** 0.30% of body mass. All fish with SMR values above the mean for a given meal treatment were classed as having a high SMR, and the remaining fish were classed as low SMR; the data are plotted as means ± S.D. Horizontal lines indicate the mean SMR for fish in the high and low SMR categories.

As a fish's SMR increased, both the peak SDA and the overall magnitude of SDA increased for a given meal size, whereas the duration of the SDA response decreased (Figure 3.3). In contrast, SMR did not influence the time that elapsed from when a meal was ingested until the peak SDA was reached (Table 3.2, Figure 3.3).

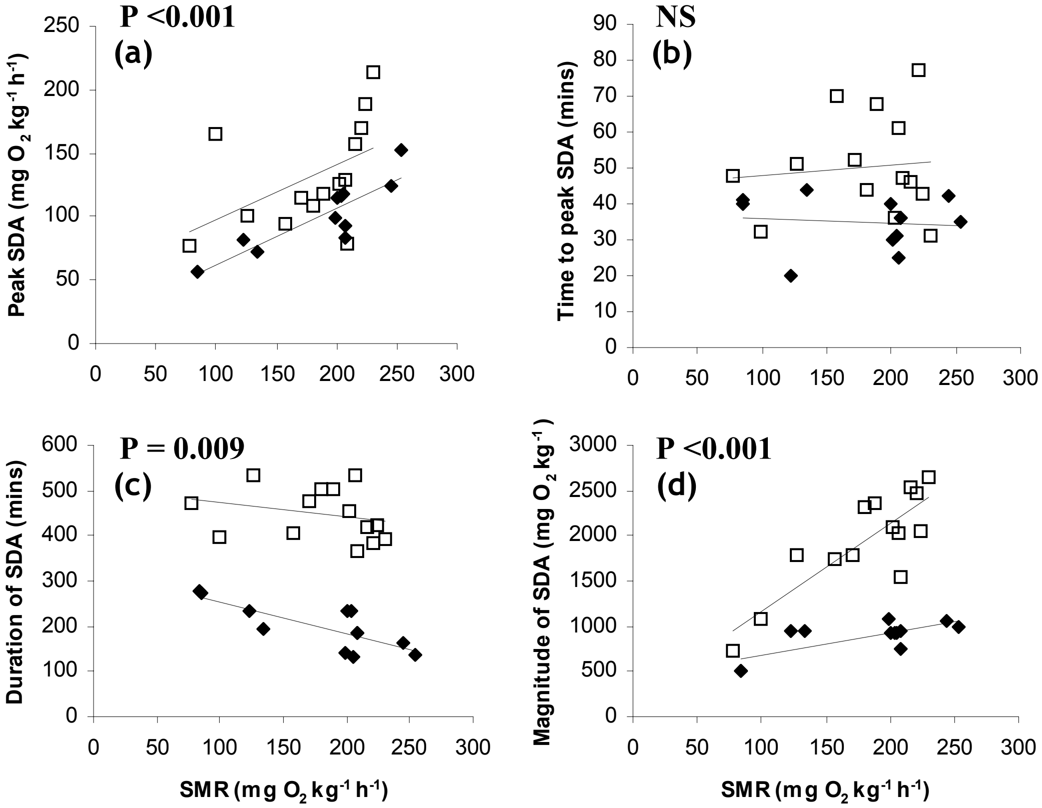

Figure 3.3 The effect of variation in individual SMR on **(a)** peak SDA, **(b)** time taken to reach peak SDA after ingesting a meal, **(c)** duration of SDA and **(d)** magnitude of SDA. Open squares and closed diamonds represent fish fed a single large or small meal (0.30% or 0.15% of body mass) respectively. See Table 3.2 for statistical analyses.

3.5 Discussion

The effect of meal size on subsequent oxygen consumption in the present study was similar to that often observed in other animals. Larger meals correlated directly with SDA magnitude, duration, peak elevation and time to reach the peak. Such trends have been observed in other species of fish (Beamish 1974; Jobling & Davies 1980; Boyce & Clarke 1997) and other poikilotherms including snakes (Zaidan & Beaupre 2003; Toledo *et al*. 2003) and salamanders (Secor & Boehm 2006).

More interesting, however, were the differences in energy expenditure among individuals, in particular the relationship between the SMR of a salmon and its profile of energy consumption while digesting a meal. This finding suggests that SMR is linked to assimilation strategy. There were positive relationships between a fish's SMR and the increase in oxygen consumption following feeding, both in terms of the peak level of oxygen consumption and the overall magnitude of the SDA response. Fish with higher SMR used more oxygen in processing a meal of a given size, and had a higher peak demand, but the time taken for the rate of oxygen consumption to drop to pre-prandial levels was shorter.

SDA magnitude is often directly related to ration size and protein content of the meal (Jobling & Davies 1980; Secor & Diamond 1997; Zaidan & Beaupre 2003). However, in the present case, the magnitude of SDA also varied as a function of SMR at constant meal size and energy content. The greatest post-prandial increase in MR (i.e. the highest peak SDA) tends to be found in fish that are growing most rapidly (Jobling 1981). The common starfish exhibits a similar correlation between the magnitude of SDA and growth (Vahl 1984). This suggests that those fish with a higher SMR and associated high SDA may be extracting more nutrients from a given meal and so may be able to grow relatively fast when food is abundant.

The high energetic costs during digestion incurred by salmon with high SMR were accommodated by a relatively high peak metabolic rate but short SDA duration. This phenomenon suggests that not only might they be extracting more energy from the food, but also the rate at which they could process and assimilate meals would be higher than in fish with a lower SMR. Such a link between SMR and food processing exists among species of snake (Secor & Diamond (1998) showed that the Burmese python is able to consume meals relatively more frequently than species of snake with a lower SMR) but has not previously been shown to apply within species.

The results of the present study therefore suggest that a high SMR in salmon enables a high physiological potential for growth. Dominant salmonid fishes with high SMR at high densities in a stable heterogeneous patchy environment can aggressively monopolise high quality food patches and grow most quickly (Ward, Webster & Hart 2006). In this case, subordinate fish would benefit by saving energy through having a low SMR and economising on their physiological potential for food processing (Metcalfe 1986). However, the high food processing potential of dominant salmonids does not always result in high growth, especially in the wild (Martin-Smith & Armstrong 2002; Harwood *et al*. 2003; Álvarez & Nicieza 2005), despite the relationship between SMR and social status in these fish (Metcalfe *et al*. 1995; McCarthy 2000). This lack of positive correlation between MR and growth may be explained at least in part by the spatio-temporal variability in resources (Martin-Smith & Armstrong 2002), interspecific interactions (Harwood *et al.* 2003), or the presence of predators (Reinhardt 1999) (Sloman & Armstrong, review 2002), which can reduce the capacity for fish to dominate high quality resource patches. A heightened rate of food intake and extraction of energy from each unit of food must outweigh any additional energy costs, including the elevated SMR, for the potential growth advantages to be realised. Hence, a low SMR is likely to be favoured when food is particularly scarce or unpredictable. High SMR individuals are able to reduce their MR when food is scarce (O'Connor, Taylor & Metcalfe 2000), but there is a lag in the process and it is not clear whether they can do so to the same extent as those individuals with low SMR at the outset.

The way in which alternative metabolic, foraging, assimilation and growth strategies are linked, and the trade-offs that underlie them, is an area that is open to much further research. This study provides the first evidence that intraspecific variation in MR may be linked to alternative digestive strategies, and that high MR's enable a high potential for growth when food is abundant, through their influence on the assimilation process as reflected in the SDA response.

Chapter 4 – The use of ventilation frequency as an accurate indicator of metabolic rate in juvenile Atlantic salmon

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4.1 Summary

Bioenergetics studies of free-living animals have long been hampered by limitations on our abilities to measure the energy costs of different activities. Here I evaluate whether it is possible to use the opercular ventilatory beat rate of a fish to estimate its rate of energy expenditure.

Changes in MR and ventilation rate (VR) were recorded in yearling Atlantic salmon (weight range 1.76 – 12.64g) engaged in different activities whilst within a respirometer. These activities, which included settling after initial handling, feeding (and consequent digestion and assimilation) and response to a disturbance, were chosen to allow the method to be evaluated over a broad range of oxygen consumption rates; trials were repeated at different temperatures (8, 13 and 16°C) to check for generality.

MR was found to correlate strongly with VR in all fish tested and at all temperatures. The relationship was linear, and both the slope and corresponding intercept of the regression equation were strongly

dependent on the fish's body weight and the test temperature. From these relationships, a general equation was generated to predict MR at a range of temperatures from knowledge of a fish's weight and its VR; this proved to be highly accurate (correlation between predicted and observed MR's: r = 0.95).

Visual measurements of VR may therefore provide a highly accurate, cheap and non-invasive method of measuring the energy consumption of fish engaged in natural behaviours in more natural settings.

4.2 Introduction

All animal activity incurs an energy cost. There is significant variation (both between and within species) in the extent of such costs, and this variation has great ecological significance. For instance, there are links between energy efficiency and life history patterns (Hayes, Garland & Dohm 1992), and metabolic costs can drive evolution (Mueller & Diamond 2001). However, limitations in our abilities to measure MR are still a methodological constraint in studies of ecological energetics. Various techniques have been used to estimate the energy expenditure of free-living animals (Grantner & Taborsky 1998). The use of doubly labelled water, for example, is suitable for measuring medium term averages of energy expenditure of terrestrial animals (Speakman 1997). Treadmill experiments have also been used to estimate aspects of MR including the locomotor capacity and costs of foraging behaviour of lizards (Huey *et al*. 1984; Garland *et al*. 1987) and thermoregulation during fasting in king penguins (Fahlman *et al*. 2005).

Estimates of the metabolism of free-ranging fish are difficult to obtain. While measuring MR by the oxygen consumption rate of single fish in a static or swimming respirometer is straightforward (Cech 1990), this method, with rare exceptions (Grantner & Taborsky 1998), does not allow measurements to be made on individual fish engaged in social behaviours on a scale of those living in more natural environments. Moreover, the doubly labelled water technique usually used to study free-living terrestrial animals is unsuitable due to the high water turnover rates of fish (Brodeur, Dixon & McKinley 2001): the radioisotope tritium has a half-life of just 15 minutes since it is quickly flushed out of the body via the gills (cited in Speakman 1997). Other approaches, such as bioenergetic modelling (Boisclair & Leggett 1989; Boisclair & Tang 1993; Rennie *et al*. 2005) can provide averaged values for energy costs over timescales of weeks or days, but are rarely possible over shorter timescales.

At present, one of the few ways of making estimates of MR in fish over timescales of minutes in the field is through telemetry of physiological correlates of MR such as ventilation rate (VR) (Oswald 1978; Rogers & Weatherley 1983; Dalla Valle, Rivas-Diaz & Claireaux 2003), tailbeat frequency (Young *et al*. 1972; Stasko & Horrall 1976), locomotor muscle electromyograms (EMG's) (Briggs & Post 1997; Økland *et al*. 1997; Thorstad *et al*. 2000) and heart rate (Priede & Young 1977; Armstrong 1986; Lucas *et al*. 1991). While these techniques can work well, their use in small fish is limited, since they rely on the use of potentially invasive monitoring devices (which may impede small fish and so affect their energetic costs); they are also expensive and can be difficult to calibrate (Thorarensen, Gallaugher & Farrell 1996). Furthermore, only some components of MR may be monitored. For instance, EMG telemetry can be used to estimate the direct cost of some activities, but cannot measure the energy costs of feeding and assimilation of food or environmental stress. Therefore, in order to gain a fuller picture of the energy expenditure of fish in their natural environment, there is a need to develop new methods for estimating MR of free-living fish. One possibility is to use measurements of VR directly as a possible predictor of MR; this approach has been explored in several studies in the past (e.g. Van Rooij & Videler 1996; Grantner & Taborsky 1998; Dalla Valle *et al.* 2003) but these have not evaluated its reliability under a broad range of situations.

The objective of the present study was therefore to determine whether VR is sufficiently accurately related to MR in juvenile Atlantic salmon at a range of temperatures within their normal environmental range and over a range of different activities so as to allow energy expenditure to be predicted from observations of VR.

4.3 Materials and methods

The experiments were carried out on yearling hatchery-reared Atlantic salmon parr derived from wild parents; these were reared at the Fisheries Research Services (FRS) Almondbank field station, Perthshire and transferred to Glasgow University at the end of April 2006 where they were held in a circular tank (1m^2) at 8ºC in aerated, re-circulated, copper-free water under an ambient photoperiod. They were fed to satiation on defrosted bloodworms (Chironomid larvae) once a day. While in the holding tank, the fish had access to shelters in the form of large stones and lengths of semicircular cut piping (approximately 120mm in diameter). On the 7th May 2006, 12 of these fish were selected at random (mean body mass 3.04g ±0.83 SD, range 1.76 – 4.50g) and transferred to individual holding tanks under the same environmental conditions as in the holding tanks. They were allowed to settle for two weeks before the first respirometry experiments commenced. These fish were used throughout the experiment, which involved simultaneous measurements of MR and VR during different activities at a range of temperatures (8ºC, 13ºC and 16ºC); use of the same fish at different temperatures allowed us a more accurate analysis of the effect of temperature than if separate fish were used.

In each run, four fish were placed individually without food in four separate respirometer chambers, each of which contained a clear perspex shelter since fish have a higher resting rate of metabolism when denied access to any shelter (Chapter 2). The apparatus was similar to that described in chapter 2 (Appendix 2), and allowed measurements of oxygen consumption to be made using intermittent flow respirometry based on a Strathkelvin Instruments SI130 oxygen electrode linked to a SI928 6-channel meter. VR was recorded as the number of opercular beats per 20 seconds (but expressed as beats min^{-1} in analyses), with the fish being observed from the side through a slit in the screens that otherwise enclosed the tank.

Each fish was retained within the respirometer for 5 days in order to measure both MR and VR over a range of activities (initial settling, resting immobile, feeding/digestion, responding to disturbance) that would generate significant variation in MR. Measurements were made more frequently at times when MR was changing more rapidly (e.g. when settling, after disturbance and immediately after having been fed) so that a roughly equal distribution of measurements was obtained across the spectrum of MR. The basic protocol was as follows (further details of each stage being given further below). The first two days constituted the settling period, during which the MR of the fish dropped from an initially high level (due to the stress of being handled and placed in a new environment) down to a stable, resting level. This was also a period of initial exploration, after which fish were generally inactive and resting on the bottom of the chamber (either under or adjacent to the shelter) for the remainder of the trial. Each fish was then fed on the 3rd day and its apparent specific dynamic action (SDA) was recorded. On the 5th day the fish were deliberately disturbed, before being removed from the respirometer and returned to their individual flow-through tanks. This procedure was then repeated in the following run using the next group of 4 fish. Once all 12 fish had been tested at the first temperature (8ºC), the temperature in holding and experimental tanks was increased to 13ºC. The fish were allowed to acclimatize to the new temperature for 2 weeks before the trials were repeated so that each fish experienced settling, feeding and disturbance. When the trials had finished at this temperature, the procedure was repeated at 8ºC again (to check for sequence or temporal effects, and to allow cross-validation of the predictive equations – see below) and then gradually to 16ºC (at a rate allowing for acclimatisation; Wright *et al.* 2001). The fish grew over the time period of the experiment (140 days; mean gain in mass 183 % ± 166 SD, range 44 – 618 %), so providing a robust test of the effects of fish size on the VR – MR relationship. Blank runs were also carried out after each week (before new fish were put back in) in order to measure the biological oxygen demand (BOD): the system was closed for a period of one hour and the drop in percentage saturation of oxygen was recorded (BOD < 0.038 ml 0_2 h^{-1} in all cases, and therefore assumed to be negligible).

4.3.1 Settling period

Fish were placed into the respirometer chambers between 08.00-09.00 on day 1. Both MR and VR were measured as soon as each fish was introduced to the respirometer, and continued at regular intervals for a further 8 hours that same day and again between 09.00 – 17.00 the following day. By this time the MR of all fish had dropped to a stable level, taken to be SMR (after the initial period of introduction, fish spent virtually all their time resting motionless on the bottom of the respirometry chamber either in or just next to the shelter). MR was measured from the reduction in water oxygen concentration over a period of 6 minutes, when the water flow-through system was temporarily closed, and this procedure was repeated at intervals of 15 minutes for the first hour after introduction of the fish to the respirometer and then every 30 - 35 minutes throughout the rest of day 1. On day 2 the MR was measured a further 3 – 4 times. VR was recorded for 20 seconds every minute throughout the period of measurement of MR and then averaged.

4.3.2 Feeding and Specific Dynamic Action

Food, in the form of defrosted bloodworms, was given to the fish whilst inside the respirometers. Since our aim was to ascertain whether the relationship between MR and VR still existed whilst the fish were obtaining and digesting food, the exact amount of food given to each individual was not important, provided it gave a measurable increase in SDA. As soon as the bloodworms were introduced into the respirometer the system was closed for a period of 6 minutes and the MR and VR were measured. Then the system was re-opened, and when the water within the respirometer was completely flushed with 100% saturated water (approximately 3 minutes), the system was again closed for a further 6 minutes. This process was continued until the change in MR from one period of closure to the next was much reduced (<10% change). When this point had been reached, the system was closed for 6 minutes every 30 – 35 minutes for the remainder of the day and for a further 3 – 4 times through the following day (09.00 – 17.00), by which time the MR in all cases had dropped to a level very similar to the original SMR (mean % difference = 8.65± 0.07 s.e.).

4.3.3 Disturbance

To create a disturbance, the lid of the respirometer was removed and a blunt instrument was inserted into the water and gently moved around to elicit a swimming response by the fish. After about 10 seconds the lid was replaced and the oxygen consumption and ventilation rate were measured for periods of 6 minutes immediately after disturbance and 36 minutes later. This disturbance procedure was carried out 3 separate times on each fish at each temperature. The fish were then removed from the respirometers and returned to their individual holding tanks, where they remained until they were tested again at the next temperature.

4.3.4 Analysis

MR was plotted against VR for each fish and temperature, combining all the data from a single 5-day trial (i.e. settling, feeding and disturbance measurements) so as to determine the relationship between VR and MR over the broadest possible range of MR's for that individual at that body size and temperature. Log-normal and power fits were tested but rejected in favour of normal linear fits, which explained the greatest proportion of variation. Since the relationships were linear, it was then possible to test whether both the slope and the intercept of the VR-MR regression lines were dependent on (and so could be predicted from) fish size and temperature. I ran two General Linear Mixed Model analyses, with the observed values for either slope or intercept (taken from all available fish at both 13°C and 16°C, but a randomly-selected half of the fish from the first 8°C trial and the other half from the second 8°C) being the dependent variable, and temperature, body weight and the interaction between weight and temperature as candidate independent variables; both untransformed and logarithmic values of body weight were used. Fish identity was included as a random factor to control for the non-independence of data points from the same individual. The analyses were run by repeatedly eliminating the least significant term until only significant terms remained.

4.4 Results

There was a significant linear relationship between VR and MR in all tested cases. However, due to a small number of mortalities during the 5-month experimental period, not all fish were tested on each of the four occasions (overall, 8 fish were tested four times, so each generated 3 regression plots after elimination of one 8°C trial, 2 fish were not tested at 16°C and 2 fish were only tested at a single temperature, producing a total of 30 regression plots; n = 18 - 27 data points per plot; in all cases r > 0.80, P<0.001, significant after sequential Bonferroni correction (Rice 1989). Example plots are given for two fish in Figure 4.1 showing the relationship between MR and VR over the ranges of (a) temperatures and (b) activities.

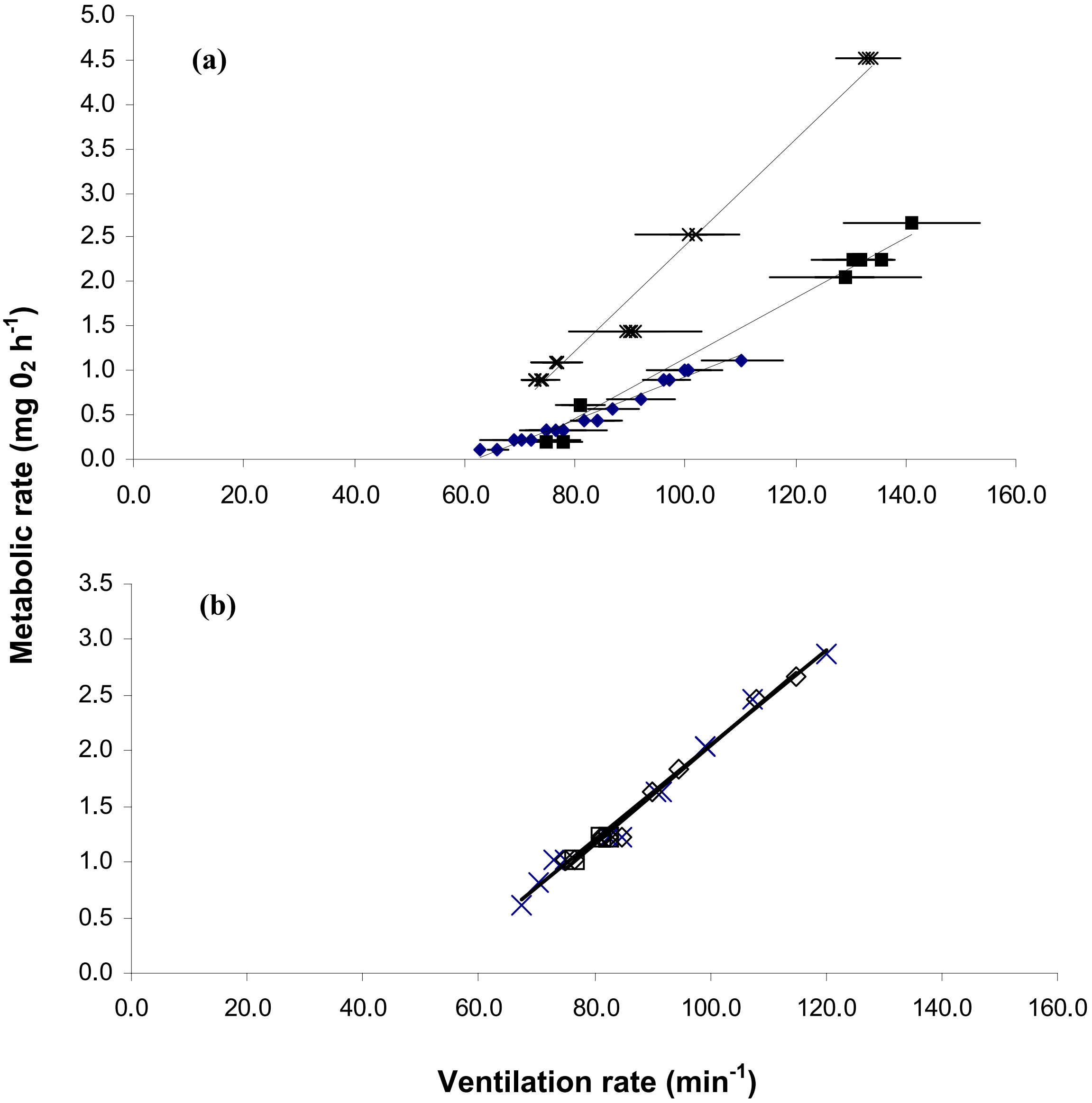

Figure 4.1 Examples of the relationship between metabolic rate and ventilation rate.

(a) The effect of temperature (diamonds = 8°C, squares = 13°C, crosses = 16°C). The same salmon was used at all temperatures, and weighed 2.15g when tested at 8°C (r^2 = 0.97), 2.98g at 13°C (r^2 = 0.99) and 5.66g at 16°C (r^2 = 0.98). Each data point shows the mean VR ± s.d.

(b) The effect of activity at a single temperature (13°C; crosses = settling (r^2 = 0.99), diamonds = feeding (r^2 = 0.99), squares = disturbance (r^2 = 0.94). The salmon weighed 5.94g (combined r^2 across all activities = 0.99).

The General Linear Mixed Model analysis showed that the slope of the regression predicting MR from VR could be predicted from knowledge of fish weight (W, in g), temperature (T, in ºC), and the interaction between these two explanatory variables (Table 4.1(a) and Equation (1); Akiake's Information Criterion = -149.56):

Slope $= 0.2773-(0.2350 * \mathrm{log10}\ (W))-(0.01838 * T) + (0.05813 * (T) * \mathrm{log10}\ (W)) \qquad - (1)$

The intercept of the MR – VR regression could similarly be predicted from these variables, with the addition of the (untransformed) body weight (Table 4.1(b) and Equation (2); AIC = 35.68):

Intercept $= -3.4078 + (0.2958 * T) + (2.1956 * \mathrm{log10}\ (W)) - (0.82057 * (T) * \mathrm{log10}\ (W)) + (0.5335 * W) \qquad -(2)$

Equations 1 and 2 were then used to formulate a general relationship to predict MR from VR (MR = m(VR) + c), in which VR is expressed as beats min^{-1}, m is derived from equation (1) and c from equation (2).

	Independent variables	Unstandardized regression coefficients	t	P
(a)	(Constant)	.2773	3.700	0.001
	log10(weight)	-.2350	-2.109	0.045
	log10(weight)*temperature	.0581	6.678	<0.001
	temperature	-.0184	-2.843	0.009
(b)	(Constant)	-3.408	-3.618	0.001
	temperature	.296	3.036	0.006
	log10(weight)	2.196	1.615	0.119
	log10(weight)*temperature	-0.821	-5.853	<0.001
	weight	.534	3.730	0.001

Table 4.1 Results of General Linear Mixed Model analysis to test whether the slope and intercept of the regression of metabolic rate on ventilation rate could be predicted from knowledge of the weight of fish and the temperature. Observed values for **(a)** slopes and **(b)** intercepts used as the dependent variable; significant predictor variables are listed below (in **(a)** the untransformed body weight and its interaction with temperature were not significant and were removed from the model; the log weight was also not significant in **(b)** but was retained in the model since the interaction of log weight and temperature was significant).

The accuracy of this equation was evaluated by cross validation, comparing the values for MR observed to those predicted using the remaining half of the 8ºC results (those not used to generate the equations). This was carried out by calculating the mean observed and predicted MR in each activity (settling, feeding and disturbance) for each fish, and then running a repeated measures ANOVA with activity (3 levels) and the measurement type (observed or predicted MR; 2 levels) as the within-subjects factors and fish weight as a covariate. The observed values were not significantly different from those predicted (measurement type: $F_{1,8} = 0.38$, $P = 0.56$), and showed a close general agreement across all rates of energy expenditure (Figure 4.2).

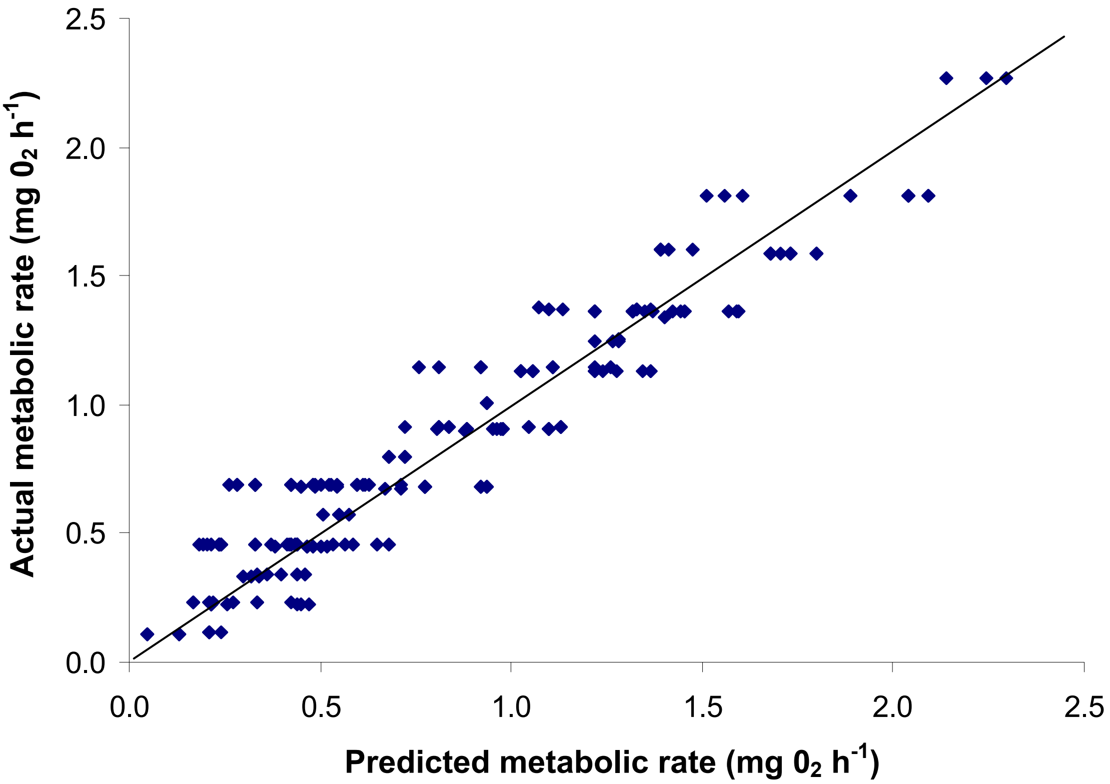

Figure 4.2 The relationship between metabolic rate ($mgO_2\ h^{-1}$) as predicted using the regression equations and the fish's actual metabolic rate ($mgO_2\ h^{-1}$). The data shown here were not used to generate the predictive equations. Overall $r^2 = 0.91$; the slope of a regression line (0.89 + 0.11 ± 0.018 S.E.) is not significantly different from a line of equality (shown here), indicating no systematic departure between predicted and observed values over the documented range of rates of metabolism. See text for full analysis.

There was no effect of activity type or fish body weight on the discrepancy between observed and predicted MR (measurement type by activity interaction: $F_{2,16} = 1.03$, $P = 0.38$; measurement type by weight interaction: $F_{1,8} = 0.48$, $P = 0.51$), indicating that the predictive power of the equations was not influenced by the type of activity occurring at the time of the measurements (i.e. whether the fish was settling, digesting food or disturbed) nor by the size of the fish. However, deviation from unity varied among fish. When fish identity was included as a factor in a GLM that analysed the data in figure 4.2, it explained some of the variation in observed MR (effect of fish identity: $F_{9,10} = 57.07$, $P < 0.001$; effect of predicted MR: $F_{1,10} = 3531.86$, $P < 0.001$; fish identity by predicted MR interaction: $F_{9,19} = 1.53$; $P=0.14$). Thus the predicted-observed regression line differed slightly in elevation among fish, although there was no significant difference in the slopes of those lines.

4.5 Discussion

The objective of this study was to determine whether VR of juvenile Atlantic salmon was a good correlate of MR during settling, feeding and disturbance periods, across a range of temperatures. If VR is to be a reliable predictor of MR outside the respirometer, it needs to satisfy certain criteria. First, it must have a close mathematical relationship with MR over a range of both activities and temperatures. Secondly, it must be possible to use any such relationship to create a general equation that can accurately predict the MR of fish in a range of environmental conditions, and so provide a valuable tool to investigate the bioenergetics of fish in more natural settings than the solitary confinement of small respirometers. Thirdly, ideally a common relationship between VR and MR should exist at the population level so that there is no need for extensive calibration of fish. This study showed that in salmon, the relationship between VR and MR was well described by a very tight linear fit across a range of activities at any given temperature; fish naturally had much higher rates of oxygen consumption in some contexts than others, but the cause of an elevation in metabolism (e.g. whether due to recently being disturbed, or to eating and assimilating a meal) did not affect the manner in which MR was related to

VR. Moreover, while the position of the regression lines varied with both the body weight of the fish and the temperature, in both cases the patterns were statistically predictable. Thus the slope of the regression line increased with increasing temperature and body mass (i.e. a given VR corresponded to a higher MR at higher temperatures and in larger fish), whereas its corresponding intercept tended to decrease with increasing temperature and body mass. There was a high correlation between the MR predicted from these variables and the actual MR as measured by the respirometer ($r = 0.95$). However, there was nevertheless sufficient unexplained among-individual variation to generate significant scatter in predictions of MR. Therefore, although the general equations are good for estimating absolute MR at the population level and change in MR within individual salmon, individual calibration may be required for comparison of individuals within populations.

In principle, changes in MR can be accommodated by change in VR, or buccal volume (volume of water pumped over the gills), or an increased efficiency of oxygen extraction from water passing the gills; to an extent alterations in MR may be independent of the ventilatory system if cutaneous uptake is important. The percentage contribution of ventilation rate to change in oxygen consumption (%conVR) at a given weight and temperature is given by $100(\log VR_2 - \log VR_1)/(\log MR_2 - \log MR_1)$, where subscripts 1 and 2 denote minimum and maximum values for the parameter respectively across a measurement range (Scharold & Gruber 1991; Armstrong & Smith 1992). The %conVR for salmon parr was 27.7% (8ºC), 31.6% (13ºC) and 32.4% (16ºC) and therefore there is clearly a substantial contribution of buccal volume (e.g. Kiceniuk & Jones 1977; Altimiras & Larsen 2000; Dalla Valle *et al*. 2003) and/or variation in other respiratory parameters as MR varies. However, the good approximation of a linear relationship between VR and MR and small variation around the relationship indicates that changes in VR are closely coupled and proportional to overall changes in gill stroke volume and other potential variables. Change in buccal volume and other respiratory parameters as fish grow and temperature varies would be expected to influence the patterns of variation in MR-VR relationships across fish weight and thermal regimes. Similarly, variations in these parameters may account for some of the residual among-individual variation in MR-VR relationships beyond that attributed to body mass and temperature.

Several previous studies have found close relationships between VR and MR of fish, but such studies have each considered only a limited range of temperatures, behaviours or timescales and so the broad applicability of the method has been unknown. Thus while Van Rooij & Videler (1996) found that MR was closely related to VR across a range of body sizes in the reef fish, they did not evaluate the effect of water temperature nor varying activity patterns. Grantner & Taborsky (1998) looked at VR and MR in the cichlid fish. Mean VR was shown to correlate significantly with routine MR (r^2 = 0.32) and with MR when more active behaviours were exhibited (r^2 = 0.66); the relatively low variance in MR explained by VR in their study in comparison with our own may be due to the fact that their analysis did not control tightly for temperature or body weight. Dalla Valle *et al*.'s (2003) study of VR in flounders is similarly restrictive, since they made measurements only over a two day period as the fish were recovering from surgery. To our knowledge, our study is the first to evaluate the method over a sufficiently broad range of environmental and behavioural situations to reveal its true potential, and is the first to produce equations that permit the prediction of MR from opercular beat rates.

An advantage of using visual recording of VR in preference to some other approaches is that it is non-invasive. Although radio and acoustic telemetry-based measurements of opercular or heart muscle contractions potentially allows the automated monitoring of metabolism in wild fish, in practice the method is restricted to relatively large individuals and is limited by the duration of the transmitter (Rogers *et al.* 1981; Weatherley *et al.* 1982; Lucas *et al.* 1991). The value of heart rate as a measure of MR varies among species of fish (Armstrong *et al.* 1992) and the relationship between these variables may differ as a function of environment and with time after disturbance (Thorarensen *et al.* 1996; Webber, Boutilier & Kerr 1998). For salmon parr, the relationship between VR and MR identified in the present study appears to be substantially more stable than that recorded between heart rate and metabolism in small salmonids (Priede & Tytler 1977; Thorarensen *et al.* 1996). However, further calibration would be wise should VR be applied as a measure of metabolism under unusual conditions, for example in severely hypoxic and polluted waters.

The results obtained here open up the possibilities of estimating MR in contexts in which it has been very difficult to obtain such information in the past, such as fish engaging in social interactions in aggregations, or performing activities more complex than simply swimming in straight lines or eating a single meal. Although the method relies on being able to observe the fish without disturbing it, the development of both large-scale controlled environment streams (MacLean *et al*. 2005) and underwater remotely-operated video equipment should permit the energy expenditure of salmon parr across a broad range of activities to be measured for the first time in natural and large scale near-natural conditions.

Chapter 5 – Presence of a conspecific causes standard metabolism to either decrease or increase in juvenile salmon, depending on relative size

5.1 Summary

The phenomenon of a change in the MR of fish depending on whether they are held singly or in groups has been recorded for many species in studies since the early 1900s. This group effect has usually been recorded as a reduction in metabolism but its mechanistic basis is obscure. In part, it may reflect basic changes in the resting (standard) MR associated perhaps with psychological stress. Additionally, there can be changes in activity of fish related to group size within metabolism measuring chambers. Hitherto, investigation has focussed on average changes in metabolism within groups, which may mask important variation at the individual level.

In the present study, the group effect was explored further by directly observing the individual SMR of young Atlantic salmon, estimated from gill ventilation frequency. All fish exhibited a reduction in SMR when provided with physical shelter, the effect being strongest in relatively small fish. The presence of a conspecific had divergent effects on SMR, depending on their relative body size: the larger fish in a pair had a reduction in SMR of c.40% whereas the SMR of the smaller approximately doubled. The consequences are that although the group effect on SMR may be small on average at the population level, it is large, of variable sign and profoundly important in terms of the overall energy budget at the individual level. Variations in SMR

were mirrored by changes in the shade of the sclera of the eye, which have been associated previously with change in stress but not compared to MR.

5.2 Introduction

The SMR of an ectotherm reflects its rate of energy consumption when at rest; major components of SMR are the costs of continual protein turnover and repair. Such costs can vary substantially (up to five fold) among individuals within species under constant conditions (Metcalfe *et al.* 1995; Steyermark *et al.* 2005). This variation has been linked to differences in behaviour, notably aggressiveness and position in dominance hierarchies (Cutts, Adams & Campbell 2001; McCarthy 2001). SMR also varies within an individual depending on its nutritional state (O'Connor *et al.* 2000) and the environmental temperature (Zari 1996; Claireaux & Lagardère 1999; Litzgus & Hopkins 2003). More interestingly, it has recently been determined that absence of suitable shelter can induce a substantial increase in SMR, perhaps due to a heightened physiological state in preparation for fast reaction escape movements (Fischer 2000; Chapter 2).

Other features of the local habitat apart from the physical elements such as shelter could also affect an individual's metabolism. The sight of a predator can cause an elevation in MR, especially if no refuge is present (Woodley & Peterson 2003), but the presence of conspecifics has more complex effects. They may be beneficial through conferring advantages of shoaling, principally reduced predation risk, but may also be disadvantageous through increased competition for resources (Krause & Ruxton 2002). Hence, conspecifics may effectively provide shelter (*sensu*

Hamilton's (1971) selfish herd, Orpwood *et al*. 2008) but also the aggressors from which flight may be required. Confinement in the presence of a more dominant individual can lead to elevated stress levels, causing an increase in MR (Sloman *et al*. 2000). It is therefore unclear whether the presence of conspecifics would be expected to cause a reduction or an increase in MR. The phenomenon of a "group effect" on metabolic rate, whereby the mean MR decreases when animals are in groups, has been recorded in many species of fish, including both shoaling and non-shoaling species (Parker 1973). A negative group effect (i.e. an increase in MR in the presence of a conspecific) occurs in the common blenny, which is highly territorial (Wirtz & Davenport 1976). However, this study (like most others) measured routine MR and therefore could not determine whether the group effect occurred through a change in SMR or variations in activity levels. The two studies that have examined fish at zero activity and metabolic minima have found evidence both for (Schleuter *et al*. 2007) and against (Herskin 1999) a group effect on SMR.

One of the reasons for the discrepancies among studies is that individual fish may differ in their response to the presence of conspecifics, depending on their relative size: being in the proximity of smaller individuals may confer anti-predator benefits, whereas larger fish may be perceived as a competitive or predatory threat. No information is available regarding among-individual variation in group effects on metabolism because studies hitherto have used respirometers that are only able to measure the total oxygen consumption of a group rather than the individuals within it. However, Chapter 4 established that the rate at which a fish's operculae (gill covers) oscillate during ventilation of the gills correlates closely with the SMR of juvenile Atlantic salmon, providing a means of determining the metabolism of each individual within a group. A further available indicator of the behavioural and physiological state of individual salmon is the colour of the eye sclera. In salmonids, social subordination results in darkening of the skin and eyes (Abbott *et al.* 1985; O'Connor *et al.* 1999; Suter & Huntingford 2002). It has been suggested that this darkening may be associated with stress (O'Connor *et al.* 1999), so providing a second non-invasive means of monitoring the physiology of individual animals within a group.

Establishing the effect of both physical and social environment on MR is of fundamental importance for understanding both the energy budgets that link feeding, growth and activity of animals, and the costs and benefits of grouping. Using juvenile Atlantic salmon as a subject and the opercular method for the measurement of MR in individuals within a group, I test for the first time whether any change in SMR when animals associate with conspecifics is dependent on their relative size. I also test whether such changes are dependent on either physical interactions between individuals or the availability of habitat refuges, and relate changes in metabolism to levels of physiological stress as indicated by eye colouration.

5.3 Material and methods

The experiments were carried out on yearling hatchery-reared Atlantic salmon parr derived from wild parents. They were reared at the Fisheries Research Services (FRS) Almondbank field station, Perthshire and transferred to Glasgow University at the beginning of April 2007 where they were held in a circular tank ($1m^2$) at 9ºC in aerated, re-circulated, copper-free water under an ambient photoperiod. They were fed to satiation on defrosted bloodworms (Chironomid larvae) once a day. While in the holding tank, the fish had access to shelters in the form of large stones and lengths of semicircular cut piping (approximately 120mm in diameter). The experiment was conducted using replicate compartments in a series of glass-sided observation channels. Each compartment was 12.5l x 20w x 20h cm and contained 3cm of levelled natural coloured standard aquarium gravel; the water depth was 12cm and the flow rate through the compartments was $20\ l\ min^{-1}$. On 20 April 2007 18 pairs of fish were selected, such that one fish was noticeably larger than the other (mean initial body mass of smaller fish = 2.05g ± 0.41 SD, range 1.17-2.63g; larger fish = 3.63g ± 0.63, range 2.35-4.57g; mean weight difference between pairs = 1.59g ± 0.66, range 0.27-2.63g). Pairs were allocated randomly to the 1st (of 6) experimental treatments in the observation channels (see description below): each pair experienced each of the 6 treatments in turn during the course of the experiment.

The basic protocol was as follows. The first two days (after introduction into the new environment) constituted the settling period, during which the MR of the fish dropped from an initially high level (due to the stress of being handled and placed in a new environment) down to a stable, resting level. This was also a period of initial exploration, after which fish were generally inactive and resting on the bottom of the experimental tank. None of the fish used in this experiment were active after the initial settling period. Ventilation rates and sclera colour was then recorded for each fish at three times on day 3 (08.00, 11.00 and 14.00 h). On each of these occasions, the number of ventilatory movements of the operculum per 20 seconds was recorded at three separate times (within 5 minutes of each other); a mean value for the day was then calculated from the 9 individual measurements. Activity was monitored throughout the day of observations and, since all the fish remained inactive, suggests that the values taken corresponded to SMR. At the end of the observations, the fish were weighed and measured and returned to the same experimental treatment tank. The equation used to convert VR into MR (Millidine, Metcalfe & Armstrong 2008) was then used to work out an individual fish's mean SMR each day, taking into account the water temperature that day and the fish's weight.

Sclera (i.e. eye iris) colour of each fish was assessed at the above times using the method described by Suter & Huntingford (2002). A score ranging from 1 (non-stressed) to 5 (very stressed) was assigned on each occasion, and the mean of these values was used in subsequent analyses.

On days 4 and 5 the fish were fed *ad lib.* with defrosted bloodworms once per day. On day 6 each pair of fish was moved to the adjoining experimental compartment, which was configured to be a different treatment. The following day (classed as day 1 of the protocol) constituted the settling period again, and this procedure (from day 1 through to day 6) was then followed repeatedly until all fish had been observed in each of the 6 experimental compartments (i.e. had experienced all six treatments). If one member of the pair died during the experiment, then its corresponding fish was removed from the experiment and none of the data from this pair were used in subsequent analyses. At the end of this period, the fish were removed and replaced (18 May 2007) with another 18 pairs of fish (mean initial body mass of smaller fish = 2.81g ±0.68, range 1.66-4.51g; larger = 4.25g ±0.65, range 3.31-5.74g; mean weight difference between pairs = 1.44g ± 0.53, range 0.58-2.12g) and the same protocol was repeated. There was a significant difference between the initial weights of the first and second groups (independent samples t-test, $t_{64} = 2.88$, $P = 0.005$), as a consequence of growth whilst in the holding tanks.

The experiment was designed to test how the physical and social environment experienced by a fish affected its resting rate of metabolism. The 6 experimental treatments therefore provided a range of environments as follows (Figure 5.1): -

C1) on own with shelter (unable to see another fish)

C2) on own without shelter (unable to see another fish)

C3) on own without shelter (able to see another fish that has access to shelter)

C4) on own with shelter (able to see another fish that does not have access to shelter)

C5) on own with shelter (able to see another fish that has access to shelter)

C6) paired with other fish (two shelters provided)

In all treatments the fish density was the same (achieved by doubling the area of the compartment in treatment 6), and if another fish was visible it was always its pair member (and so of the same relative size in each treatment).

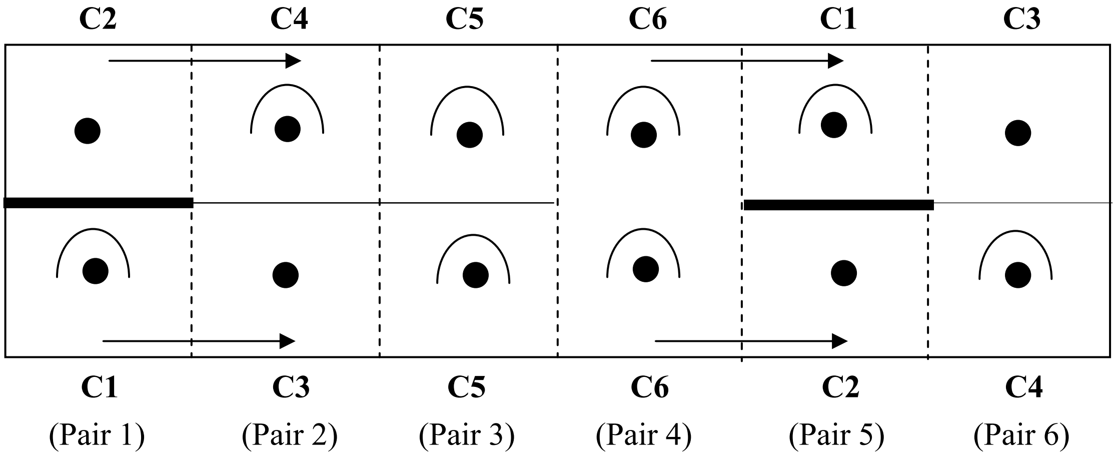

Figure 5.1 A schematic representation of a group of experimental compartments that together provided the 6 possible treatments (● = fish, ∩ = shelter). Thick solid lines indicate opaque partitions, thin lines indicate transparent partitions, and dashed lines indicate opaque white mesh. Arrows indicate direction of water flow. Note that in some cases the two members of a pair were experiencing different treatments (e.g. in pair 1 only one fish had a shelter), while in other cases they were experiencing the same treatment (e.g. in pair 3 both had access to a shelter). This group of compartments was triplicated to allow 18 pairs of fish to be observed simultaneously. However, the order of treatments was different to prevent any sequence effects.

5.3.1 Analysis

The effect of treatments on MR and sclera colouration was analysed using repeated measures ANOVAs, with the individual fish as a subject. Each fish was classified as being the larger or smaller member of its pair (on the basis of body mass), and relative size (larger or smaller) was then used in the analysis as a between-subjects factor. A fish's absolute size (calculated as the mean of the body mass measurements taken after each observational day during the six treatments) was used as a covariate.

5.4 Results

There was a significant effect of treatment on mean SMR (treatment: $F_{5,59} = 6.39$, $P < 0.001$) (Figure 5.2).

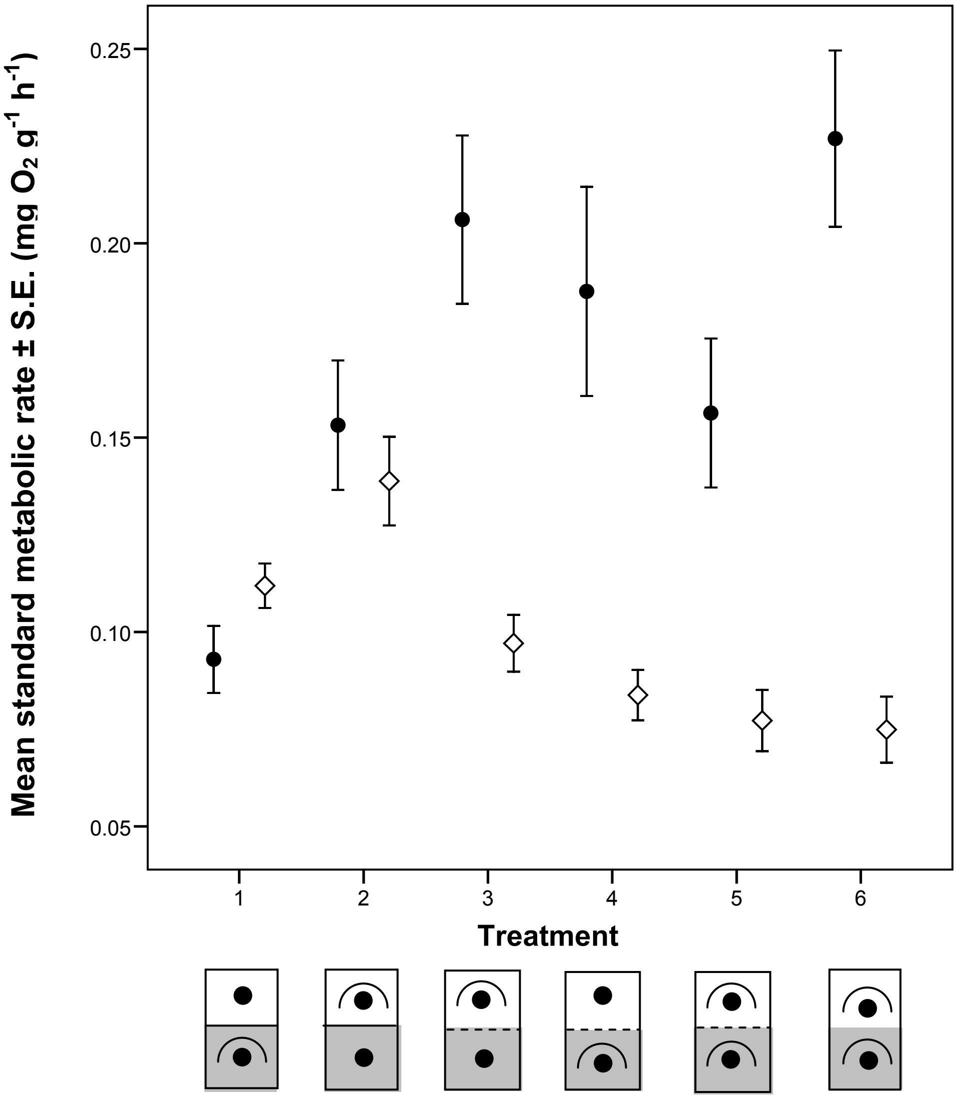

Figure 5.2 The effects of treatment on mean SMR (± S.E.). Open diamonds and closed circles represent the larger and smaller member of a pair respectively. The x-axis also shows a schematic representation of each treatment (the focal fish’s compartment is shaded); solid and dashed lines indicate opaque and transparent partitions respectively. *N*=33 fish for each size category of fish.

However, there was also an effect of fish size (in absolute terms: $F_{1,63}$ = 11.68, P = 0.001), and interactions between both measures of size (absolute and relative fish size) and treatment (treatment*relative size of fish (larger or smaller): $F_{5,59}$ = 2.73, P = 0.028; treatment*absolute size of fish: $F_{5,59}$ = 4.05, P = 0.003), indicating that the effect of the treatment on MR differed for fish of different relative and absolute size. These complex interactions were therefore teased apart in a series of repeated measures ANOVA comparisons of specific treatment pairs.

Comparing treatments 1 and 2 (visually isolated fish with/without shelter respectively), both size categories of fish had higher SMR's when shelter was absent, although the effect was marginally stronger for fish of lighter body mass (effect of treatment: $F_{1,63}$ = 2.12, P = 0.004; treatment*relative size of fish: $F_{1,63}$ = 0.02, P = 0.900; treatment*absolute size of fish: $F_{1,63}$ = 3.43, P = 0.069; absolute size of fish: $F_{1,63}$ = 3.20, P = 0.079; relative size of fish: $F_{1,63}$ = 2.08, P = 0.154).

The sight of another nearby fish (even behind a transparent barrier) caused a marked change in MR, but in divergent directions depending on its relative size. Thus comparing treatments 2 and 3 (i.e. a focal fish without a shelter, that was either isolated or able to see another fish with a shelter) there was no overall effect of treatment ($F_{1,63}$ = 2.12, P = 0.151) or relative size of fish ($F_{1,63}$ = 0.03, P = 0.864) but a significant interaction between treatment and relative size of fish ($F_{1,63}$ = 6.00, P = 0.017): the focal fish's MR increased in the presence of a larger fish, but decreased if the other fish was smaller. The absolute size of the fish also had a significant effect ($F_{1,63}$ = 7.52, P = 0.008), with larger fish having a lower MR. However, the interaction between absolute size and treatment was nonsignificant ($F_{1,63}$ = 1.84, P = 0.179). If the situation was the same except that the focal fish had the shelter (comparison of treatments 1 (isolated) and 4 (able to see other fish without shelter), then there was an overall effect of treatment (with MRs being higher in treatment 4 when the focal fish is smaller; $F_{1,63}$ = 9.08, P = 0.004) as well as the same interaction between treatment and relative size of fish ($F_{1,63}$ = 6.89, P= 0.011). There was also an interaction between treatment and absolute size ($F_{1,63}$ = 5.80, P = 0.019), the sight of another fish caused a bigger change in MR if the focal fish was small. Although absolute size alone had a marginal effect ($F_{1,63}$ = 4.14, P = 0.046), with larger fish having a lower MR, there was no effect of relative size on MR ($F_{1,63}$ = 0.17, P = 0.683). The extent of these changes depended on whether the other fish was actually in the same compartment or was simply visible through a transparent partition: a comparison of treatments 5 and 6 showed that the relatively smaller fish had a higher MR when actually in the presence of a larger fish, whereas in larger fish MR was unaffected by whether the smaller fish was in the same compartment or was only visible through a partition (effect of treatment: $F_{1,63}$ = 4.55, P = 0.037; treatment*relative size of fish: $F_{1,63}$ = 12.76, P = 0.001). There were also significant effects of relative size (the smaller fish in the pair having a higher MR overall; $F_{1,63}$ = 6.40, P = 0.014) and absolute size (small fish having higher MR’s; $F_{1,63}$ = 5.68, P = 0.020).There was no significant effect of treatment by absolute size: $F_{1,63}$ = 1.02, P = 0.316.

Changes in the sclera colour of the fish showed similar trends to the changes in MR (Figure 5.3).

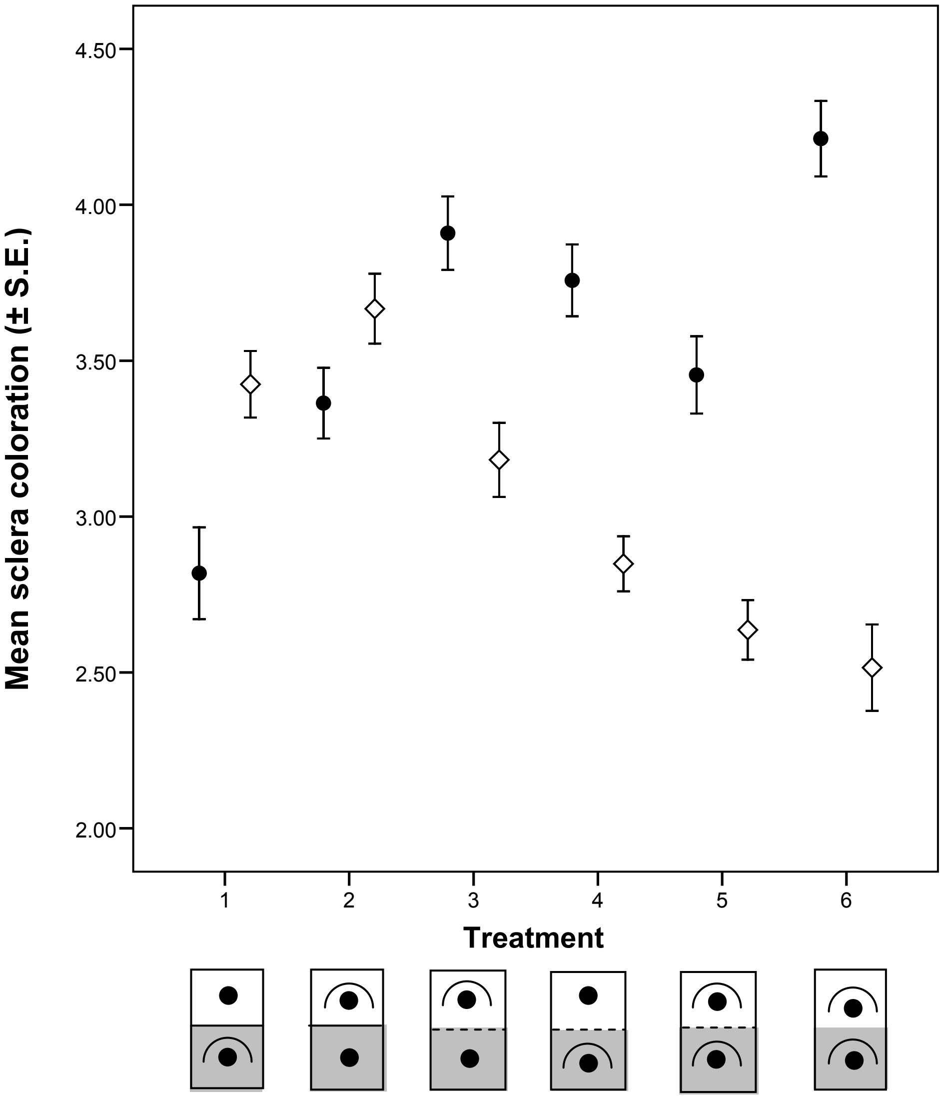

Figure 5.3 The effects of treatment on mean sclera coloration (± S.E). Open diamonds and closed circles represent a larger and smaller conspecific respectively. The x-axis also shows the schematic representation of treatments as in Figure 5.2; *N*=33 fish per size category.

Over all treatments there was a significant effect of treatment ($F_{5,59}$ = 4.15, P = 0.003) and of relative size ($F_{1,63}$ = 11.13, P = 0.001) but not of absolute size ($F_{1,63}$ = 0.16, P = 0.69), with the larger fish in a pair having a paler sclera on average (mean sclera in smaller fish over all treatments = 3.58 ± 0.06 S.E. and in larger fish = 3.04 ± 0.05). However, the effects of both relative size and absolute size depended on the treatment (treatment*relative size of fish: $F_{5,59}$ = 6.92, P = <0.001; treatment*absolute size: $F_{5,59}$ = 3.42, P = 0.009).

Conducting targeted pairwise tests as before revealed that the sclera were darker when a shelter was absent in both categories of fish (comparison of treatments 1 and 2: effect of treatment: $F_{1,63}$ = 9.01, P = 0.004; treatment*relative size of fish: $F_{1,63}$ = 0.56, P = 0.46). However, this effect was more pronounced in smaller fish (treatment*absolute size of fish: $F_{1,63}$ = 5.22, P = 0.026). There was no effect of either absolute size ($F_{1,63}$ = 3.91, P = 0.052) or relative size ($F_{1,63}$ = 0.21, P = 0.39). The sight of another fish caused the eyes to darken if that focal fish was larger, but lighten if it was smaller (comparison of treatments 2 and 3: effect of treatment: $F_{1,63}$ = 1.08, P = 0.30; treatment*relative size of fish: $F_{1,63}$ = 7.22, P = 0.009). There was no significant effects of absolute size ($F_{1,63}$ = 0.44, P = 0.51) or relative size ($F_{1,63}$ = 0.36, P = 0.55), nor a treatment*absolute size interaction ($F_{1,63}$ = 0.003, P = 0.32). As with the MR trends, the removal of a transparent partition between the two fish caused a further darkening in the sclera of the smaller fish but no change in that of the larger (comparison of treatments 5 and 6: effect of treatment: $F_{1,63}$ = 0.002, P = 0.97; relative size of fish: $F_{1,63}$ = 22.96, P <0.001; treatment*relative size of fish: $F_{1,63}$ = 12.88, P = 0.001). There was no significant effects of absolute size of fish ($F_{1,63}$ = 0.81, P = 0.12 or treatment*absolute size ($F_{1,63}$ = 0.44, P = 0.51).

Sclera colour score was clearly related to MR, as is evident from the positive relationship between them (Figure 5.4). To investigate this, a General Linear Mixed Model analysis of sclera colour was run with relative fish size as a fixed factor, SMR as a covariate and fish identity as a random factor to control for the non-independence of the 6 data points contributed by each individual (one per treatment).

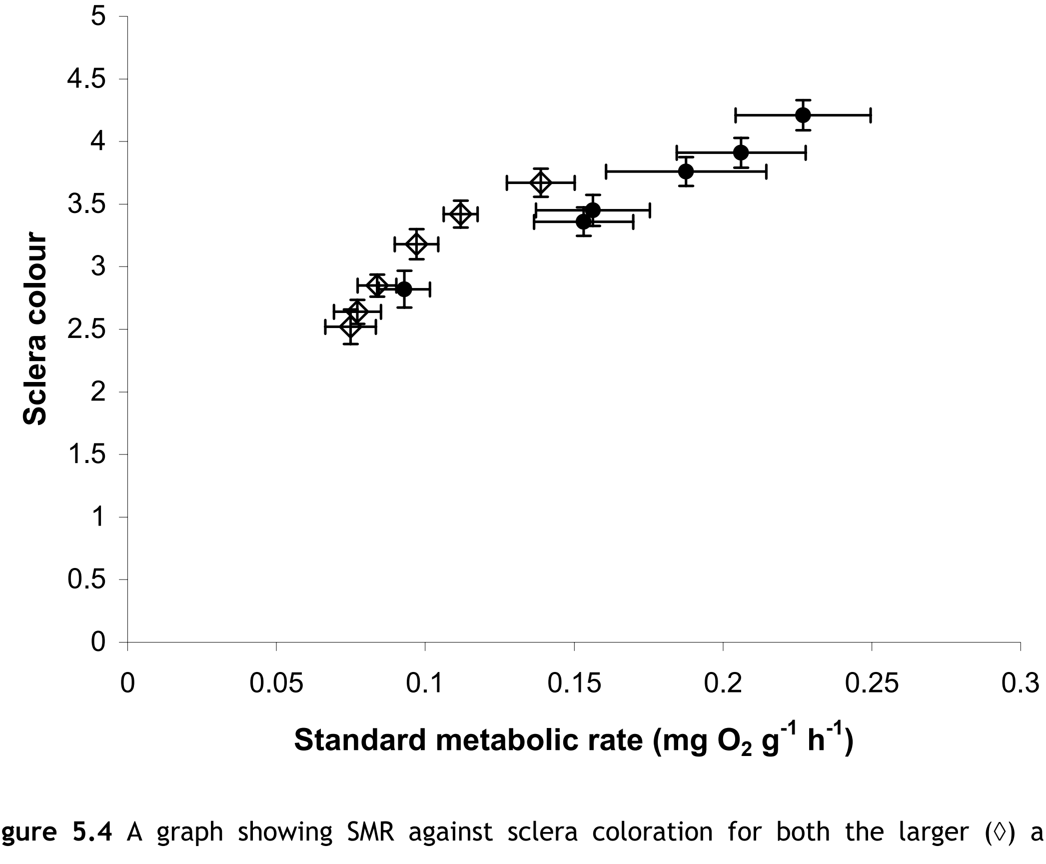

Figure 5.4 A graph showing SMR against sclera coloration for both the larger (◊) and smaller (●) fish in a pair. The data points show the mean values ± S.E. for each of the 6 treatments that the 33 pairs of fish experienced. Sclera colour was significantly related to MR (P <0.001).

Sclera colour was related to MR ($F_{1,391.4} = 345.3$, $P < 0.001$) and to relative size of fish, being darker for a given MR in the larger member of the pair ($F_{1,146.8} = 20.71$, $P < 0.001$), but the rate at which sclera colour darkened with an increasing MR was greater in the relatively larger fish ($F_{1,391.4} = 38.92$, $P < 0.001$).

5.5 Discussion

This study demonstrated a group effect on the metabolic rate of fishes, with the rate of a quiescent single fish altering when it was able to see a conspecific. More importantly, it showed that the sign of the effect varied among individuals depending on their relative size, and that the magnitude of the effect varied with the degree of exposure to conspecifics. The availability of physical shelter had an additional influence on metabolism.

Juvenile Atlantic salmon can be territorial in riverine habitats (Kalleberg 1958; Vehanen 2003) and the negative group effects (i.e. increased MR when with others) observed in some contexts might be interpreted as reflecting aggressive behaviour, as in territorial blennies (Wirtz & Davenport 1976). However, this seems unlikely since the elevation of metabolism when in the presence of another fish was only seen in the smaller of the two individuals, which also showed coloration indicative of a subordinate (O'Connor *et al.* 1999; Suter & Huntingford 2002). In contrast, the larger dominant fish actually showed a decrease in metabolic costs when in the presence of a smaller 'rival'. Moreover, a negative group effect has also been observed in night-time groups of perch, a species considered to be shoaling rather than territorial (Schleuter *et al.* 2007). It may be misleading to consider fish as either shoaling or territorial, since the behaviours adopted may be flexible and reflect costs and benefits experienced in a particular context. While shoaling provides anti-predator benefits of increased vigilance and/or dilution of the risk of capture (Hamilton 1971; Pitcher & Parrish 1993; Queiroz & Magurran 2005), it also exposes individuals to close competition for resources with their neighbours. We might therefore expect flexibility in social structure, and indeed salmon shoal in exposed river pools and marine habitats (McCormick *et al.* 1998; Crisp 1999). This pattern of switching between distribution modes may be widespread since the European minnow, which is commonly considered to be a shoaling species, tends to space out when there is sufficient physical cover in its environment (Orpwood *et al.* 2008).

The precise costs and benefits of aggregation will vary between individuals. For many species, the larger members of a group may gain greater benefits, since they can enjoy the advantages of shoaling whilst also experiencing less competition for food resources due to their greater competitive ability or dominance status. They are also much less likely to face aggression than the smaller members of the group. In the present study, the reduction in SMR of the larger salmon when in company was similar to their response to the presence of physical shelter and may result from a similar physiological relaxation, or calming effect, associated with reduced predation risk (Millidine *et al.* 2006). The situation is different for the smaller fish in a pair of salmon, which may enjoy some shoaling advantage of aggregation, but is also at serious risk of attack from their larger conspecific. For these fish, the larger salmon are effectively both a form of shelter and an aggressor. The metabolic response to the presence of a larger salmon was much greater than that to the absence of a physical shelter. This effect suggests that the fish were either extremely stressed (Sloman *et al*. 2000), or in a heightened state of physiological readiness to respond to an attack. An elevation in SMR resulted from only the sight of a larger fish, but was highest when the fish also had direct physical access to one another. This difference suggests that the fish are aware of variation in level of risk.

Most previous observations of group effects have not differentiated among variations in SMR, in activity level or in swimming efficiency (e.g. through use of vortices from other fish when swimming in a cohesive school), and were not able to explore individual variation in metabolic response. This study used pairs of fish to tease out effects, but the same approach could be used to investigate the still more complex trends that may occur in larger groups. This could explain some of the apparent discrepancies seen in previous studies – for instance, if similar individual variations exist in perch then these could underpin the variation in sign and magnitude of mean group effects observed under different physical and social habitats observed in perch observed by Schleuter *et al*. (2007).

The group effect resulted in a two-fold variation in SMR within the same fish due to changes in the social environment. Energy budget calculations for free-living teleost fishes suggest that a two-fold variation in SMR can result in up to 100% variation in growth performance (Armstrong & Hawkins 2008). Such large metabolic costs will have substantial effects on growth rates across a range of energy intakes, as reflected perhaps in widely reported group effects on growth of fish (Marchand & Boisclair 1998; Jenkins *et al*. 1999). For salmon parr, the group effect accentuates the difference between high and low status fish, increasing the growth potential of dominant individuals and reducing growth potential of subordinates, which may explain why dominance rank has such a pronounced effect on growth rate of similar-sized fish eating identical amounts of food (Abbott & Dill 1989).

Chapter 6 – General Discussion

The overall aims of this thesis were to examine aspects of metabolism in individual juvenile Atlantic salmon, and in particular the extent of individual variation in different metabolic parameters. It particularly focused on SMR and SDA and demonstrated a potential link between them. In this chapter I will assess firstly how the findings of these experimental studies are related to previous research and then discuss the potential future directions leading from this work.

The availability of shelter has been shown to be of extreme importance to the survival of animals, providing protection against the physical environment (Nybakken 1993; Seebacher & Alford 2002; Wiederhecker *et al.* 2002) and protection against other animals (Kotler 1984; Godin 1997; Valdimarsson & Metcalfe 1998). In juvenile Atlantic salmon, shelters are actively competed for (Kemp *et al.* 2005), suggesting that they are a highly valued resource (Armstrong & Griffiths 2001). For such fish, the primary function of shelter, usually comprised of crevices under stones on the substrate (Cunjak 1988), is thought to be that of predator avoidance (Valdimarsson & Metcalfe 1998). However, I hypothesized that the presence of appropriate shelter would provide further benefits by allowing a reduction in their metabolic costs. By measuring the SMR of individual fish it was found that when a shelter was present the oxygen consumption rate was much lower, in the order of a 30% reduction (Chapter 2). An increased availability of shelter has been shown to significantly influence the changes in body mass of overwintering juvenile Atlantic salmon (Finstad *et al.* 2007). By manipulating shelter availability (interstitial spaces in the substrate), Finstad *et al.* (2007) were able to measure the direct effect of habitat complexity on the body mass of 200 juvenile Atlantic salmon during winter. Although all fish lost mass during the experiment, this loss was reduced with increasing numbers of measured shelters. The metabolic benefit of shelter may have been one of the mechanisms responsible for this effect. The effect of shelter on SMR may also be important, and provide similar benefits, to other species of freshwater fish including the European minnow (Greenwood & Metcalfe 1998), steelhead trout (Bustard & Narver 1975), brown trout (Bremset 2000), Arctic charr (Klemetsen *et al.* 2003), and three-spined stickleback (Sandlund *et al.* 1992) since they are also known to make use of available refuges. Refuge use has also been observed in other animals including amphibians (Bridges 2002; Orizaola & Braña 2003), birds (Hegner 1985; Hogstad 1988; Koivula, Rytkönen & Orell 1995), invertebrates (Richardson & Brown 1992; Turner 1996; Turner & Montgomery 2003), mammals (Cowlishaw 1997), and reptiles (Downes & Shine 1998; Martín & López 1999; Shah *et al.* 2004). Although predator avoidance appears to be the most commonly cited advantage of refuge use in these animals, predator benefits of shelter use may in some cases be secondary to other benefits (Hediger 1977) such as providing thermoregulatory advantages via protection from the rain and radiation (cited in Kappeler 1998) and reductions in metabolic costs (this study).

While there are distinct advantages of using a shelter, refuge sites frequently offer fewer foraging opportunities than in more open habitats

(Krause *et al*. 2000). The cost of refuge use may therefore be a lower food intake and hence reduced growth rates (Allouche & Gaudin 2001; Turner & Montgomery 2003). However, the costs and benefits of seeking shelter from predators are far from straightforward. For instance, in the Damselfly, the growth reduction in response to predation risk could not be fully explained by a decreased food intake, suggesting a role for physiology since they also showed a reduction in the efficiency of converting assimilated food into biomass (McPeek, Grace & Richardson 2001; McPeek 2004). This partly physiologically mediated response may be a by-product of an accelerated metabolism under the ‘fight-or-flight’ response which is associated with an increased heart beat rate and respiration (Slos & Stoks 2008). This may have a direct energetic cost in the sense that less energy is allocated to growth, thereby causing a growth reduction under predation risk (Slos & Stoks 2008). Therefore, the energy requirement of the individual whilst sheltering would be much less than if they were actively feeding. Minimizing the amount of time spent foraging by hiding in sites where there is the greatest opportunity to conserve energy may also enable animals to survive longer periods of food shortage (Valdimarsson & Metcalfe 1998). This energy conservation may also provide longer-term effects such as an increased growth rate compared with individuals without shelter. In salmonids, an increased growth rate during their first year could possibly result in them being able to migrate to sea earlier (Metcalfe & Thorpe 1992).

Comparisons of the energetic costs and benefits of activities such as sheltering or foraging requires a method for measuring MRs of freely moving animals. In the case of fish, while measuring MR by the oxygen consumption rate of a single fish in a static or swimming respirometer is straightforward (Cech 1990), being able to measure the metabolic response of two or more fish in more natural habitats has proved to be more difficult. Although using physiological correlates of MR such as locomotor muscle electromyograms (EMG's) (Briggs & Post 1997; Økland *et al.* 1997; Thorstad *et al.* 2000) and heart rate (Priede & Young 1977; Armstrong 1986; Lucas *et al.* 1991) can work well, their use in small fish is limited, since they rely on the use of potentially invasive monitoring devices which may impede small fish and so affect their energetic costs. In order to look at the MR of fish in different contexts it was therefore necessary to try and develop another way of estimating MR outside the respirometer and which could be used on much smaller fish (Chapter 4). By using opercular rate as a correlate of MR, I was able to measure the MR's of fish when paired and under different levels of competition for shelter, and therefore was able to establish the effect of social environment and its interaction with physical habitat on SMR (Chapter 5). This method could potentially be useful in measuring the MR of other fish species. However, because it relies on visual observation, its use may be restricted to fairly static fish since it would be very difficult to monitor the opercular movements on more active individuals. For some fish, such as those living at deep water depths, underwater videos may be required but this would only be practical when there is excellent visibility.

The use of refuges (especially during periods when predation risk is high) may reduce the physiological costs associated with increased vigilance, so providing indirect benefits as well as the more evident protection from predators. However, refuges may also provide intra-specific protection (Chapter 5). Juvenile Atlantic salmon readily form hierarchies, with the more dominant individuals being aggressive towards subordinates and monopolising the available food supply (Harwood *et al.* 2003). For this reason, the use of shelter would be more beneficial to the smaller fish, which are usually subordinates. Indeed, the extent of this shelter benefit was shown to be highly dependent on the relative size of the adjacent conspecific (Chapter 5). Similar to earlier findings (Chapter 2), the presence of a shelter reduced SMR by approximately 30% in both large and small salmon when they were in isolation. However, the presence of a conspecific, even when a shelter was available, resulted in an increase in SMR in the smaller fish but a reduction in SMR in the larger fish. The effect of being within the same compartment as its conspecific resulted in similar divergent responses, with the elevation of MR being especially marked in the smaller fish. This suggests that smaller fish incur pronounced metabolic costs from being in the presence of larger conspecifics if appropriate shelter is not available. This may reduce the chances of smaller fish surviving periods of reduced food availability since they would have to use up their body reserves at a greater rate to meet the increasing energetic demands.

Size-structured dominance hierarchies have been recognized in many other stream dwelling salmonids including the brown trout (Elliot 1990), steelhead trout (Jenkins 1969), red-spotted masu salmon (Nakano 1995) and coho salmon (Nielsen 1992), and have also been observed in many non-salmonid fish such as the guppy (Gorlick 1976), clown fish (Buston 2003) and the brook charr (Grant, Noakes & Jonas 1989). Subordinate individuals may therefore gain similar benefits of shelter in the presence of an aggressive dominant conspecific in a wide range of fish species. Intraspecific protection may also be relevant for other animals that readily form hierarchical social groups, such as rodents (Noyes, Barrett & Taylor 1982; Aagren, Zhou & Zhong 1989; Van Loo *et al*. 2001) and birds (Hogstad 1989).

It is also possible that smaller fish may have a greater need for protection from predators. With the exception of bird predation, experimental studies generally demonstrate that predation risk decreases as the size of the prey fish increases (Sogard 1997). Indeed, individual variability in body size provides a template for selective mortality processes during early life history stages of teleost fishes (Sogard 1997). This size variability has generated the logically intuitive hypothesis that larger or faster growing members of a cohort gain a survival advantage over smaller conspecifics (Sogard 1997), via enhanced resistance to starvation (Henderson, Holmes & Bamber 1988; Shoji *et al*. 2002), decreased vulnerability to predators (Ellis & Gibson 1995), reduced susceptibility to disease (West & Larkin 1987), and better tolerance of environmental extremes (Wedemeyer, Saunders & Clarke 1980; Johnson & Evans 1996). Fast-start locomotor behaviour is important for escaping from predators and for capturing prey (Hale 1999), and this escape response of prey is strongly related to body size, as reaction distances increase and swimming performance is enhanced (Folkvord & Hunter 1986; Blaxter & Fuiman 1990). This 'bigger is better' hypothesis has also been observed in other organisms including turtles (Janzen 1993), lizards (Huey & Hertz 1984), insects (Stockhoff 1991; Gotthard, Nylin & Wiklund 1994) and crustaceans (Tessier & Consolatti 1989). Therefore, in theory, larger salmon parr would be potentially too large for some predatory fish and will also be faster at escaping from any immediate danger, and so might need shelters less.

However, in some shoaling species of fish, the presence of a conspecific has been shown to lower routine metabolism (Itazawa, Matsumoto & Kanda 1978; Smatresk & Herreid 1980; Ross, Backman & Limburg 1992; Schleuter *et al*. 2007). The most commonly described adaptive advantages of this schooling behaviour are a reduced risk of predation and increased foraging efficiency compared to solitary fish (Pitcher 1986; Pitcher & Parrish 1993). However, if individual metabolism is lowered in groups, it may also represent other potentially selective advantages of this shoaling behaviour such as reduced levels of excitement and/or activity (Smit 1965; Anras, Lagardère & Lafaye 1997; Schleuter *et al*. 2007) and calming effects (Parker 1973; Hettler 1976; Itazawa *et al*. 1978).

In a range of organisms MR correlates positively with an individual's social status (Lahti *et al*. 2002). This has been particularly well studied in birds (Røskaft *et al*. 1986; Högstad 1987; Bryant & Newton 1994) and fish (Clarke 1992; Metcalfe *et al*. 1995; Cutts *et al*. 1998, 1999; Yamamoto, Ueda & Higashi 1998; Cutts *et al*. 2001). In birds, this is often interpreted as 'the cost of being dominant' as more energy would be needed to maintain the higher MR (Lahti *et al*. 2002). However, in salmonids, the higher SMR of a dominant individual has been interpreted as being beneficial, since fish with a high SMR tend to be more aggressive and may have a greater food intake overall, with subsequent higher growth (Cutts, Metcalfe & Taylor 2002). However, in productive habitats, are high-ranking individuals with higher SMR able to simply monopolize the available resources and thereby grow more rapidly than low-ranking individuals, or are they able to digest food at a greater rate? If SMR is directly related to scope for growth, then the speed and size of the SDA response should also correlate directly with SMR, since a rapid and pronounced peak in SDA would reflect faster digestion of food and accretion of tissues. It would also enable more rapid return of appetite after a meal and hence a greater through-put of food. Indeed, there were positive relationships between a fish's SMR and the increase in oxygen consumption following feeding, both in terms of the peak level of oxygen consumption and the overall magnitude of the SDA response (Chapter 3). The high energetic costs during digestion incurred by salmon with high SMR were accommodated by a relatively high peak but short duration of SDA. This phenomenon suggests that not only might fish with a high SMR be extracting more energy from the food, but they might also be processing and assimilating meals faster than in fish with a lower

SMR. Such a link between SMR and food processing exists among species of snake: Secor & Diamond (1998) showed that the Burmese python is able to consume meals relatively more frequently than species of snake with a lower SMR.

Results of the present study therefore suggest that a high SMR in salmon enables a high physiological potential for growth. However, the influence of dominant status on individual growth rates has provided some conflicting results. Some previous studies using Atlantic salmon and other salmonids have demonstrated a positive correlation between SMR and growth under laboratory conditions (e.g. Li & Brocksen 1977; Metcalfe *et al.* 1989; Yamamoto *et al.* 1998) and in the wild (e.g. Nakano 1995; Höjesjö, Johnsson & Bohlin 2002), whilst others conducted in natural or near-natural streams found a lack of correlation (Martin-Smith & Armstrong 2002; Harwood *et al.* 2003; Álvarez & Nicieza 2005). Álvarez & Nicieza (2005) showed that in the wild, the relationship between SMR and growth in brown trout was mainly determined by the variability of the environment.

In Atlantic salmon parr in natural habitats, a lack of positive correlation between MR and growth may therefore be associated, at least in part, by the spatio-temporal variability in resources (Martin-Smith & Armstrong 2002). Therefore, fish with higher SMR's, being able to digest food faster, may do better in areas of the stream that provide relatively predictable resources (Martin-Smith & Armstrong 2002), such as the upstream ends of pools (Nakano 1995). It has been shown that in natural riffles, the relative availability of drifting food can change over a few hours (Martin-Smith & Armstrong 2002). Therefore, dominant fish may have to move among patches to maintain a high food intake. However, Maclean *et al*. (2005) found no evidence that dominant salmon could effectively track changes in food patch quality in a near-natural environment. Furthermore, they observed few effects of dominance rank on patch use compared with smaller scale studies. They suggested that this difference might be attributed largely to visual isolation of patches and costs of sampling the environment, particularly due to predation risk (Lima & Dill 1990).

Predators (Reinhardt 1999) and competitors (Harwood *et al*. 2003; Höjesjö *et al*. in press) may also reduce aggressive behaviour from dominant salmon and thereby increase their access by subordinates to food and preferred habitats. Furthermore, subordinate fish may accrue food by sneaky feeding tactics as they occupy marginal areas of the habitat (Höjesjö, Armstrong & Griffiths 2005).

It is evident that the relationship between dominance, food intake and growth of salmon is complex in natural habitats. The relationship between food intake and growth is defined by the energy budget, which has been a focus of this thesis, and depends on how much energy is lost in metabolic processes. It is clear that dominant fish pay a price in having a relatively high SMR and may also incur high activity costs in competing aggressively for food patches. However, they benefit from grouping effects, whereas subordinate fish lose out in this respect (Chapter 5). Furthermore, dominant salmon can compete most effectively for shelters (Kemp *et al*. 2005) and thereby accrue further metabolic advantages over subordinates (Chapter 2). Independence of growth rate as a function of dominance in some habitats suggests that the energy costs and benefits of different behavioural strategies may balance out. However, it is also evident that physical and social habitat structures play important roles in the behavioural relationships among salmon and can be expected to influence energy budgets and patterns of growth performance.

Size-independent MR's have been shown to correlate positively with river size and summer discharge in brown trout (Álvarez, Cano & Nicieza 2006). This may be due to the fact that productivity tends to be lower and drought effects more severe in small headwaters than in large streams (Allan 1995). Fish populations inhabiting very small streams may therefore have a higher risk of episodes of acute starvation (Álvarez *et al*. 2006), and low-productivity and unpredictable environments might have exerted a disproportionately strong selection on lower maintenance metabolism (Wigley 1985; Hoffmann & Parsons 1997; Harshman, Hoffmann & Clark 1999; Mueller & Diamond 2001). Álvarez & Nicieza (2005) found no correlation between SMR and growth in juvenile brown trout within natural streams of northern Spain. All of the experimental sections in this study were located in very small brooks that were seemingly exposed to great levels of temporal variation in environmental conditions such as acute temperature changes and/or drought. During periods of potential nutritional stress, fish with higher SMR's will face a higher risk of exhausting energy reserves than individuals with lower energetic requirements for subsistence (Álvarez & Nicieza 2005). Thus, the positive relationship between growth and metabolic rate under conditions of high environmental stability may not translate to small rivers (Álvarez & Nicieza 2005) since the cost of maintaining a high SMR would be too high in these environments, and fish with elevated SMR's may therefore do better in larger streams which are more productive and provide an adequate food supply for their energetic needs.

As food level and its variability have been suggested to influence the evolution of SMR (Metcalfe *et al.* 1995; Harshman *et al.* 1999), this may suggest that habitats furthest from the poles, which usually have more stable and abundant food resources, would be a strong factor influencing SMR selection (Lahti *et al.* 2002). Indeed a study investigating the variation in MR between four Finnish brown trout populations (Lahti *et al.* 2002) found that the most southern population had higher SMR's, and this was suggested to be related to the higher phosphorus concentration and subsequent productivity of their natural habitat. However, other studies have found standard metabolism to be higher in montane or high-latitude populations than those from low-latitude areas (e.g. Ayres & Scriber 1994; Álvarez *et al.* 2006; reviewed by Garland & Adolph 1991).

Pronounced temperature gradients with changing altitude or latitude create favourable scenarios for geographic variation in MR (Conover & Schultz 1995). Despite lower temperatures and/or shorter growing seasons of high-latitude environments, northern populations of the Atlantic silverside fish grew more rapidly than southern populations (Conover & Present 1990), suggesting that the capacity for growth increases with latitude (Conover & Schultz 1995). Researchers have found similar latitudinal trends in the striped bass (Conover, Brown & Ehtisham 1997) and largemouth bass (Phillipp & Whitt 1991). This phenomenon, termed 'countergradient variation' (Levins 1969), has been illustrated in various MR processes both within and among a wide variety of poikilotherm species (Bullock 1955). Individuals with a higher capacity for growth, and therefore higher maintenance energy requirements (Chapter 3), may be selected for in environments that do not encounter periods of low resource availability coupled with high environmental temperatures.

Nicieza, Reiriz & Braña (1994) found variation in performance between geographically disjunct populations of Atlantic salmon, with a high-latitude population having a higher digestive performance than a low-latitude population at both high and low temperatures. In the context of a digestive strategy, food turnover rate becomes more important when the availability of food is not limiting (Sibly 1981) or, in poikilotherms, when ambient temperature is low (Nicieza *et al*. 1994). At low temperatures, reduced digestion rates would inhibit further feeding even though food is abundant (Cunjak, Curry & Power 1987; Hart & Gill 1992). High-latitude populations may therefore have selective pressures to maximize digestive performance at both low temperatures (when biotic and abiotic factors limit consumption) and high temperatures (so as to exploit the brief opportunity in midsummer to grow and accumulate reserves) (Nicieza *et al*. 1994), without the advantages of having a high SMR being compromised by any environmental conditions such as extreme high temperatures and/or drought events.

Any benefits of a high SMR, accompanied by a greater digestive performance, would therefore depend on the environment in which it lives. Although habitats at low latitude/altitude may provide an evenly spread supply of resources throughout the year, higher maintenance costs may be risky due to an associated environmental unpredictability. Although high SMR individuals are able to reduce their MR when food is scarce (O'Connor *et al*. 2000), there is a lag in the process and it is not clear whether they can do so to the same extent as those individuals with low SMR at the outset. Also, when low resource availability is also accompanied by extreme high temperatures, individuals with lower starting SMR's would still be at a greater advantage and would be less likely to die of starvation during these periods. Although high latitude/altitude populations may incur periods of low productivity, these may be more predictable and, coinciding with overall lower temperatures, would therefore not compromise any of the associated benefits of high SMR such as a greater digestive performance and scope for growth.

It is clear that the complex interactions between habitat predictability, annual temperature profile and food availability mean that it is difficult with our current state of knowledge to predict what level of MR would be favoured in particular geographical regions or microhabitats. However, further research would be especially profitable in this area, since the wide diversity in standard metabolism within (as well as between) populations of the same species demands an explanation. Although there is supporting evidence that geographical variation in SMR exists (Lahti *et al*. 2002; Álvarez *et al*. 2006), it does not explain variation at a small-scale (within-population) (this thesis). It also opens up further questions on how this variation compares to other populations. Variation in SMR at an individual level may be a consequence of adaptation to specific local conditions. This may result in fluctuating fitness from one year to the next depending on the annual variation in their environment.

This thesis has studied both the within- and between-individual variation in MR in juvenile Atlantic salmon and has underlined the importance of shelter in terms of their resting metabolism and potential implications for growth. However, the complexity of shelter and standard metabolism is significantly influenced by intraspecific interactions, and future work should address these issues on a larger scale, reflecting more natural conditions experienced in the wild. Although the use of ventilation rate as a predictor of MR may be limited outside semi-natural conditions, it may open up the possibilities of obtaining frequent metabolic data to help define and investigate more of the energy budget. This may identify some of the costs/benefits (such as feeding, aggression, movement and growth) associated with varying metabolic strategies, and how this varies with fish density and habitat use.

The potential link between standard metabolism and digestive efficiency also warrants further investigation, since it is not known whether this exists in more natural environments when food availability is patchy and densities of fish are higher. The ability of fish with high SMR’s to re-feed at a greater rate than those with lower SMR’s may only be feasible when there is a constant supply of food, and therefore breaks down when the fish is forced to reduce its resting metabolism when conditions are poor.

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Appendix 1 – Latin names

Common names	Scientific names	Chapter
Arthropoda – Arachnida		
Wolf spider	*Pardosa milvina*	2
Chordata – Actinopterygii		
Arctic Charr	*Salvelinus alpinus*	6
Atlantic salmon	*Salmo salar*	1 - 6
Atlantic silverside	*Menidia menidia*	6
Brown trout	*Salmo trutta*	6
Cichlid fish	*Neolamprologus pulcher*	4
Coho salmon	*Oncorhynchus kisutch*	6
Common blenny	*Blennius pholis*	5
European minnow	*Phoxinus phoxinus*	5, 6
Largemouth bass	*Micropterus salmonids*	6
Northern pike	*Esox lucius*	3
Perch	*Perca fluviatilis*	5
Red-spotted masu salmon	*Oncorhynchus masou ishikawai*	6

Reef fish	*Sparisoma viride*	4
Steelhead trout	*Oncorhynchus mykiss*	6
Striped bass	*Morone saxatilis*	6
Three-spined stickleback	*Gasterosteus aculeatus*	6

Chordata - Amphibia

Cane toad	*Bufo marinus*	2

Chordata - Aves

Adele penguin	*Pygoscelis adeliae*	1
Blue tit	*Cyanistes caeruleus*	2
Great tit	*Parus major*	2
Hoopoe lark	*Alaeman alaudipes*	2
King penguin	*Aptenodytes patagonicus*	4

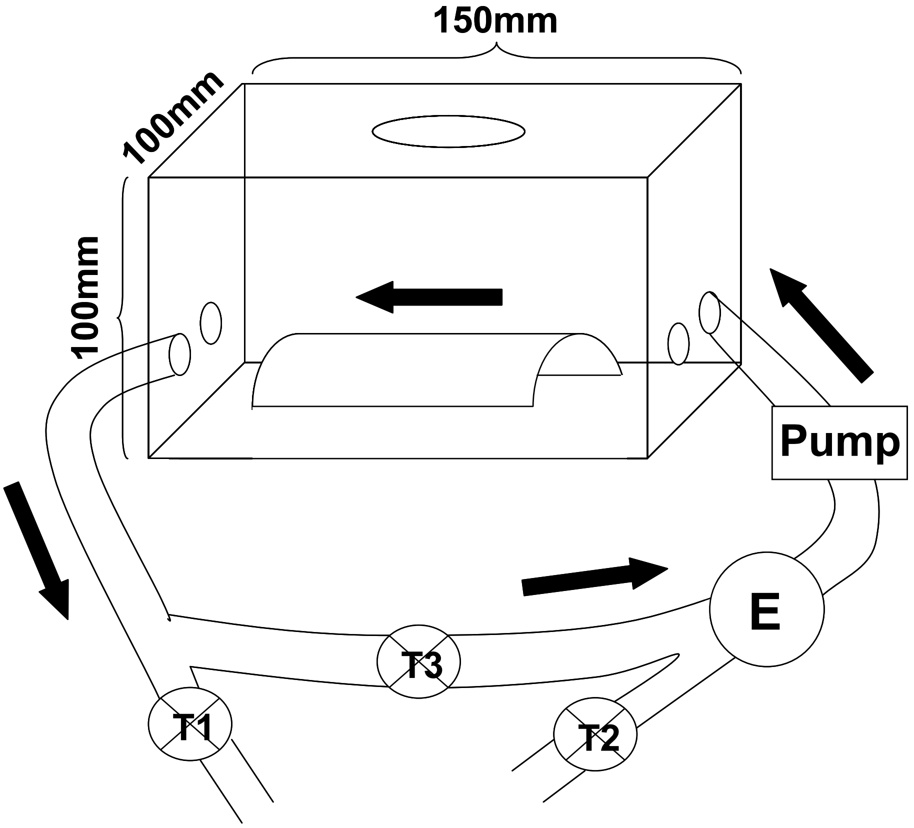
Chordata - Reptilia

Brazilian lizard	*Tropidurus torquatus*	2
Burmese python	*Python molurus*	1, 3

Echinodermata – Asteroidea

Common starfish	*Asterias rubens*	1, 3

Appendix 2 – A diagram of the respirometry set-up



The diagram above shows the experimental set-up of the intermittent flow (or open-closed) respirometry used in chapters 2, 3 and 4 and the arrows indicate the direction on water flow. The respirometry apparatus was housed within its own water bath (not shown in diagram), and this was aerated by three water stones. The level of water within the bath was sufficient to cover the electrode holder (E), as this ensured that the electrode (inserted into the top of the electrode holder) was kept at a constant temperature.

When the system was in the open position (i.e. the chamber was receiving a continual pumped supply of aerated water from the water bath) taps 1 and 2 (T1 & T2) were in the open position whereas tap 3 (T3) was closed. This allowed aerated water to flow continuously through the chamber from the water bath.

When the system was closed in order to take measurements of oxygen consumption, T1 and T2 were turned to a closed position whereas T3 was opened. This prevented aerated water from being pumped into the chamber from the water bath and therefore any changes in the oxygen concentration within the chamber was measured via the electrode.