MATERNAL INFLUENCES ON OFFSPRING SIZE, BEHAVIOUR AND ENERGY METABOLISM

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ABSTRACT

In my thesis I investigate the ecology of maternal influences: the unique ability of mothers to influence, via genetic and non-genetic means, the phenotypic expression of their offspring. My research is presented as a series of standalone chapters that are introduced and then summarised by a general introduction (Chapter 1) and a general discussion (Chapter 6) respectively.

One of the main components of an organism's energy budget is its baseline level of energy metabolism. Individual differences in this cost of self-maintenance (termed in this chapter, resting metabolic rate, RMR) are substantial, but the causes and consequences of this variation are obscure. In Chapter 2, I review the published literature and show that maternal influences (along with other factors) can contribute substantially to variation in offspring RMR. Also, the RMR - fitness relationship appears to be modulated by environmental conditions (e.g. food supply), suggesting that the fitness consequences of a given RMR may be context-dependent. Thus, I propose that broad-scale variation in RMR might persist in natural populations, due to both spatial and temporal variation in environmental conditions and the trans-generational influence of mothers.

To further investigate maternal influences on offspring energy metabolism, I measured the standard metabolic rate (SMR, a measure equivalent to RMR but used in reference to ectothermic animals) of juvenile brown trout (*Salmo trutta*) in response to intra-clutch manipulations of egg cortisol and testosterone (Chapter 3). Although, neither hormone affected offspring SMR (egg testosterone treatment resulted in a likely pharmacological dose), juveniles from cortisol-treated eggs were smaller and subordinate to individuals from control eggs. This indicates that variation in the amount of cortisol deposited in eggs by females, either among clutches or within them, is likely to affect juvenile performance.

In a separate experiment (Chapter 4), I investigated if within-clutch differences in the phenotypes of juvenile brown trout were systematically related to the position where each individual developed during oogenesis. For a given egg size, siblings from dominant mothers were initially larger (but had a lower mass-corrected SMR) if they developed in the rear of the egg mass. However, heterogeneity in the size of siblings from different positions in the egg mass diminished in lower ranking females. Juvenile social status also

varied according to egg mass position, although the direction of this effect depended on their age.

Maternal influences on offspring are not only determined by conditions experienced by females immediately prior to reproduction. In Chapter 5, I investigated whether the juvenile growth rate and adult reproductive traits of female wild Atlantic salmon are related to the performance of their offspring in the wild. Investment in egg size was linked to both the juvenile and adult phenotypes of mothers. Even when controlling for egg size, the influence of these 'past' and 'present' maternal traits extended to offspring performance. Offspring growth was positively related to maternal investment in reproduction and the juvenile growth rate of each mother. The survival and biomass of offspring were also linked to adult reproductive traits but these relationships differed for mothers that had grown at either fast or slow rates as juveniles.

Overall my thesis demonstrates that maternal influences are a substantial source of variation in offspring size, behaviour and physiology, both among and within clutches. My research also underlines the importance of maternal influences for offspring ecology and therefore maternal fitness.

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Candidate’s declaration:

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

Signature of candidate ……..

Date ……..

CHAPTER 1. INTRODUCTION

Phenotypic variation among individuals is envisioned generally as the sum of direct environmental and genetic effects (and their interactions). These sources of phenotypic variation do not, however, account for environmental effects that were experienced in previous generations. Maternal effects – phenotypic variation that may be inherited by an individual independently of the genes provided by its parents and direct environmental effects on developing offspring – are often the cause of such trans-generational influences on phenotypic expression (Mousseau & Fox 1998a). Maternal effects were traditionally perceived as factors that complicated the precise estimate of narrow-sense heritability – the relative contribution of genetic variation to phenotypic variation among related individuals. However, since the release of a landmark publication, *Maternal effects as adaptations* (1998), edited by T.A. Mousseau and C.W. Fox, which summarised the importance of maternal effects in ecology and evolution, interest in their significance now stems from a range of biological sub-disciplines, the diversity of which is reflected in the nomenclature and range of definitions that currently describe this phenomenon. Some examples of this complex terminology are given below:

Maternal effects – "*...part of an offspring's phenotype that does not result from the action of its own genes and the interaction of those genes with its environment*" (Bernardo 1996a) or "*...the causal influence of the maternal genotype or phenotype on the offspring phenotype*" (Wolf & Wade 2009).

Parental effects – "*Together, the transmission of non-genetic developmental factors and developmental plasticity result in an effect of the parental phenotype on offspring phenotype that cannot solely be ascribed to inherited genes*" (Uller 2008).

Inherited environmental effects – "*...those components of the phenotype that are derived from either parent, apart from nuclear genes*" (Rossiter 1996).

Non-genetic inheritance – "*...any effect on offspring phenotype brought about by the transmission of factors other than DNA sequences from parents or more remote ancestors*" (Bonduriansky & Day 2009).

In this thesis, I adopt the definition of Mousseau and Fox (1998a). Where the genetic and non-genetic contributions of a mother to phenotypic variation among offspring cannot be separated, I use the term 'maternal influences' (Green 2008; Venturelli *et al.* 2010).

For many species, early development is a period of high mortality and intense selection. Thus, it is hardly surprising that maternal influences can have a pervasive influence on offspring growth and survival. It is the mother who determines the initial size (and often the subsequent provisioning) of offspring and decides when, where and how they are born or dispersed. Additionally, in mammals and birds, it is generally the mother who cares for the offspring (although in fishes, it is the father who cares for the young in more than 60% of species that have been studied, Gross & Sargent 1985). Environmental effects on the mother can also lead to variation in her growth, condition and physiological state that can be transmitted to offspring via non-genetic resources in the egg, for example as hormones, antibodies, antioxidants and mRNA, that may directly or indirectly influence offspring development (Mousseau & Fox 1998b). Maternal influences have thus been interpreted as a mechanism that allows for an adaptive phenotypic response by offspring to an environmental cue experienced by parents (Bernardo 1996a). Indeed, numerous empirical studies demonstrate that mothers do adjust the phenotypes of their offspring according to changes in the maternal environment (Mousseau & Fox 1998b; Uller 2008). However, not all maternal influences are adaptive. For example, the transfer of pathogens across the placenta may have deleterious consequences for the young, or a mother in poor nutritional condition may be unable to adequately provision her offspring during egg production, both of which would result in reduced prospects for their survival. Furthermore, maternal influences may have a heritable basis themselves, so interpretations of adaptive plasticity may need to be made with a degree of caution (Bernardo 1996a).

OFFSPRING TRAITS THAT ARE SUBJECT TO MATERNAL INFLUENCES:

Egg size

Egg size is crucial because it can profoundly influence the growth and survival of offspring (Mousseau & Fox 1998b). Accordingly, egg size is perhaps the most intensively studied maternal influence (Bernardo 1996a). Large eggs generally result in larger, faster

growing offspring with better survival prospects, particularly in adverse environmental conditions (e.g. Einum & Fleming 1999; Dziminski & Roberts 2006; Donelson, Munday & McCormick 2009; Segers & Taborsky 2011a). In light of such obvious benefits, why do some mothers produce smaller eggs than others? Mothers that lay large eggs must lay fewer eggs due to the trade-off between offspring size and number. Theoretical and empirical investigations of this trade-off reveal an optimal egg size that maximizes maternal rather than offspring fitness: offspring survival rates that maximise maternal fitness may be low compared with those maximising offspring fitness (Smith & Fretwell 1974; Einum & Fleming 2000a). Whilst this may offer a broad explanation for the large variation in egg sizes that has been observed among females in a range of species, environmental conditions experienced by the mother can also influence the size, number and provisioning of eggs that she produces. In some cases, this may be a constraint that reflects the nutritional status of the mother. For example, low food levels experienced by mothers prior to egg production can reduce maternal body condition and reduce the size, energy content and survival of her progeny (Gagliano & McCormick 2006; Donelson, McCormick & Munday 2008; Donelson *et al.* 2009). However, in some cases such egg size plasticity may yield adaptive benefits. In species of *Daphnia*, females can respond to low levels of food by producing larger eggs that are higher in lipid and protein content. The eggs from such females then give rise to larger juveniles that survive better in food-limited environments (Gliwicz & Guisande 1992).

Maternal investment in offspring may be determined by both the 'current' and 'past' environments of a mother (Reznick & Yang 1993). A growing number of studies demonstrate that factors experienced by mothers during their own development as juveniles can also affect the phenotypes of their offspring. Low food availability, poor nutrition or even exposure to predation risk during early ontogeny can cause mothers to produce larger, slower growing offspring, irrespective of the conditions experienced after sexual maturity and adult body size (Huck, Labov & Lisk 1986; Taborsky 2006; Vijendravarma, Narasimha & Kawecki 2010; Segers & Taborsky 2011b).

Juvenile size, energy metabolism and social status

Although egg size can explain much of the initial size variation among juvenile offspring (Chambers & Leggett 1996), other factors can influence the egg size – juvenile

size relationship. Indeed, egg size and composition are not necessarily correlated and variation in egg composition may be as important in an ecological sense as variation in egg size (Bernardo 1996b). The levels of hormones such as cortisol and testosterone that are transferred from the mother to the egg or foetus can vary substantially both within and among clutches and enable the mother to affect offspring sizes by influencing rates of growth (McCormick 1999; Hayward & Wingfield 2004; Groothuis *et al.* 2005; Uller, Astheimer & Olsson 2007).

The causes and consequences of within- and- among clutch variation in egg hormone content have been most widely studied in birds. In many avian species, juveniles that hatch early obtain a competitive advantage because they can be substantially larger than individuals that hatch later (Groothuis *et al.* 2005). By systematically adjusting egg hormone levels within a clutch, particularly androgens, to increase the aggressive or begging behaviours of juveniles (Schwabl 1996), female birds can either compensate for hatching asynchrony to promote survival of late-hatched individuals or even inflate the effects of hatching asynchrony to favour first-laid offspring (reviewed by Groothuis *et al.* 2005). In contrast, between-clutch variation in egg hormone levels can either reflect traits of the mother, such as her territory size and quality (Groothuis & Schwabl 2002), body condition (Love *et al.* 2008) and aggressive behaviour (Whittingham & Schwabl 2002) or temporal and spatial variation in environmental conditions, such as social density and food availability (Groothuis *et al.* 2005).

The precise mechanism by which pre-natal cortisol and testosterone affect growth is unknown, but it may involve indirect effects on other traits. High energy costs of self-maintenance, when measured as minimal rates of energy metabolism (in this Chapter the related terms basal, resting and standard metabolic rate are referred to collectively as 'SMR'), can constrain juvenile growth and even result in a faster rate of mass loss when food is limiting (Steyermark 2002; Killen, Marras & McKenzie 2011). SMR is remarkably variable, often differing by 2 – 3 orders of magnitude among individuals, even after controlling for important factors such as body size and temperature (Metcalfe, Taylor & Thorpe 1995; Steyermark *et al.* 2005; Johnston *et al.* 2007). Little is known of the proximate and broader-scale mechanisms that determine this residual variation in SMR. It has been proposed that SMR may be under parental influence (Pakkasmaa, Penttinen & Piironen 2006; Régnier *et al.* 2010) and recent evidence suggests that some of this

variation may be of maternal origin due the transmission of cortisol or testosterone to eggs (Tobler, Nilsson & Nilsson 2007; Giesing *et al.* 2010; Sloman 2010; Nilsson *et al.* 2011).

In addition to its influence on energy budgets, SMR is known to correlate positively with behavioural traits such as dominance and aggression (reviewed by Biro & Stamps 2010). These in turn may influence developmental or growth rate, since in many species dominant individuals tend to acquire preferential access to resources (Huntingford & Turner 1987). Evidence from birds indicates that mothers can manipulate offspring territoriality, aggression and dominance rank by adjusting levels of egg testosterone (Schwabl 1993; Eising, Muller & Groothuis 2006; Muller, Dijkstra & Groothuis 2009). In contrast, elevated concentrations of glucocorticoids in the embryo seem to have the opposite effect on offspring behaviours by reducing activity, begging and dispersal or invoking risk-averse behaviour (De Fraipont *et al.* 2000; Meylan & Clobert 2004; Rubolini *et al.* 2005; Uller & Olsson 2006). Thus androgens, such as testosterone, and glucocorticoids, such as cortisol, are likely mediators of maternal influences on offspring body size (as separate from egg size), SMR and social status. There are thus many complex interactions between physiology, behaviour and development that may be mediated through the non-genetic influences of the parental generation.

BROAD APPROACH OF THE THESIS AND STUDY ORGANISMS

The main objective of my thesis is to investigate the role of maternal influences in generating individual variation in offspring phenotypes and to address their likely ecological consequences. Admittedly, this is a rather broad topic! Consequently, I address a number of specific questions that are connected to this central theme. For the empirical work presented in this thesis I use stream dwelling members of the family Salmonidae (namely Atlantic salmon *Salmo salar* L. and brown trout *Salmo trutta* L.) as study organisms. These closely related teleost fishes display remarkable variation in their general biology and life history characteristics, both among and within each species (Klemetsen *et al.* 2003). Adults typically spawn in freshwater streams, where females lay hundreds to thousands of eggs in sequentially-spawned gravel nests (‘redds’), where they are fertilised externally by males. After relatively synchronised hatching, the offspring (referred to as alevins) remain in the gravel, sustained entirely by the remnant yolk from the hatched egg (termed the yolk-sac). Once the yolk sac is mostly depleted, the offspring (now termed fry)

emerge from their gravel nests to disperse and begin feeding exogenously. These juvenile salmonids then spend variable amounts of time in their natal stream as 'parr' before migrating, for a period of one or more years, to either marine (anadromous life cycle) or lacustrine (freshwater resident) environments. The majority of growth occurs in these environments, before they return as adults to spawn in their natal stream (although some fish, particularly males, may mature without ever migrating from the natal stream) (Klemetsen *et al.* 2003).

Atlantic salmon and brown trout are ideal study organisms for investigating the causes and consequences of maternal influences, not only on offspring size, but other important offspring traits such as behaviour and physiology. Maternal investment is critical because no further care is provided by either parent after spawning. This means that mothers have limited scope to influence the development of their young other than what they invest in each egg and where they are laid. Moreover, selection during early development is intense: up to 98% of mortality can occur within 2 – 3 months of juveniles emerging from the nest (Elliott 1994). In this context, egg and thus juvenile size are known to be critical for survival (Einum & Fleming 1999; Einum & Fleming 2000b). In Atlantic salmon and brown trout, egg size increases with female body size and large females tend to produce fewer eggs per unit body size than smaller conspecifics. Thus, egg size can vary substantially among females and populations but is usually uniform within individuals (Jonsson & Jonsson 2011). Furthermore, conditions experienced during early ontogeny can also influence investment in eggs by females: fish that grow relatively slowly as juveniles tend to produce larger eggs at maturity, even after controlling for body size at the time of spawning (Thorpe, Miles & Keay 1984; Jonsson, Jonsson & Fleming 1996). Thus, it has been suggested that mothers may 'adjust' the size of their eggs in anticipation of the early growth environment that their offspring may face (Jonsson *et al.* 1996).

After emerging from the nest, fry compete with conspecifics to establish feeding territories, often in high densities (Elliott 1994) especially where suitable habitat is limiting (Armstrong & Nislow 2006). The social structure of salmonids during this period of development is often based around dominance and territoriality (Metcalfe 1998). Large dominant fry are more likely to establish preferable territories near the nest (Metcalfe 1998; Bujold *et al.* 2004), which can promote the dispersal downstream of smaller individuals or subordinates (Bujold *et al.* 2004). Remarkably, individual dominance status of juveniles can be predicted better by energy metabolism than body size: individuals with

higher than average SMR tend to be dominant over those with a lower SMR (Metcalfe *et al.* 1995; Cutts, Metcalfe & Taylor 1999; McCarthy 2001). However, a single 'optimal' strategy in terms of energy metabolism or dominance seems not to apply because individuals that are dominant or have a high SMR do not necessarily experience growth or survival advantages in all conditions (Martin-Smith & Armstrong 2002; Harwood *et al.* 2003; Álvarez & Nicieza 2005).

Given their effects on offspring growth, behaviour and physiology in other taxa, egg hormones may enable salmonid mothers to influence key offspring traits such as body size, social status and SMR. Fish eggs contain appreciable amounts of glucocorticoid and androgen hormones and egg concentrations of these hormones are likely determined by the level in maternal circulation (Hwang *et al.* 1992; McCormick 1999; Tagawa, Suzuki & Specker 2000). Whilst the corticosteroid stress axis of juvenile fish appears fully developed only after hatching, almost immediately after fertilisation, embryonic levels of cortisol decrease and the expression of mineralocorticoid receptors (which like glucocorticoid receptors can also bind to cortisol) increases (Alsop & Vijayan 2008). This indicates that fish embryos are capable of metabolising cortisol and suggests that maternally derived egg hormones may affect offspring before they hatch. Recent manipulative studies in other fish species reveal that between-clutch differences in egg cortisol content reflect environmental conditions experienced by the mother prior to spawning and can have strong effects on juvenile development (McCormick 1998; McCormick 2006; McCormick 2009; Giesing *et al.* 2010). Such data for salmonid fishes are currently lacking, although pharmacological elevations of egg cortisol have been shown to increase the SMR of embryos and reduce the body size of juveniles (Eriksen *et al.* 2006; Sloman 2010).

AIMS AND STRUCTURE OF THE THESIS

A growing number of studies have documented substantial intra-specific variation in SMR. However, the causes of this variation, both proximate and ultimate and its consequences for individual fitness in natural populations remain poorly understood. My first aim is to synthesise the existing knowledge on this topic and indicate gaps in understanding. Thus, in a review of the literature, I ask whether maternal influences are at least partly responsible for within-species variation in SMR, and summarise the known

consequences for fitness of variation in this physiological trait (Chapter 2). Relationships between egg hormone levels and offspring phenotypes have mostly been investigated in birds. Female birds typically produce small clutches and directly influence the post-natal development of their young by providing parental care. Data from taxa with different reproductive tactics, such as fishes, that can produce thousands of offspring and do not provide parental care, are largely absent. Therefore in Chapter 3, I test experimentally whether levels of egg cortisol and testosterone influence offspring body size, SMR and social status in a highly fecund vertebrate (the brown trout). I then investigate if highly fecund mothers can generate systematic variation *within clutches* in these same traits (Chapter 4). Maternal investment in offspring can be determined by environmental conditions experienced by mothers when they are both juveniles and adults. To date, the consequences of these maternal legacies have not been tested in natural populations. Utilising naturally occurring variation in both the juvenile growth rate and adult body condition of mothers, I investigate the relative influence of these factors on the provisioning and subsequent performance of their offspring in the wild (Chapter 5). The findings from these studies are then brought together in a synthesis in the general discussion (Chapter 6).

CHAPTER 2. WHAT CAUSES INTRA-SPECIFIC VARIATION IN RESTING METABOLIC RATE AND WHAT ARE ITS ECOLOGICAL CONSEQUENCES?

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SUMMARY

Individual differences in the energy cost of self-maintenance (resting metabolic rate, RMR) are substantial and the focus of an emerging research area. These differences may influence fitness because self-maintenance is considered as a life history component along with growth and reproduction. In this review, I ask why do some individuals have two to three times the 'maintenance costs' of conspecifics, and what are the fitness consequences? Using evidence from a range of species, I demonstrate that diverse factors such as genotypes, maternal effects, early developmental conditions and personality differences contribute to variation in individual RMR. I review evidence that RMR is linked with fitness, showing correlations with traits such as growth and survival. However, these relationships are modulated by environmental conditions (e.g. food supply), suggesting that the fitness consequences of a given RMR may be context-dependent. Then, using empirical examples, I discuss broad-scale reasons why variation in RMR might persist in natural populations, including the role of both spatial and temporal variation in selection pressures and trans-generational effects. To conclude I discuss experimental approaches that will enable more rigorous examination of the causes and consequences of individual variation in this key physiological trait.

INTRODUCTION

The energy cost of self-maintenance (when measured as minimal rates of energy metabolism) varies remarkably within species. It effectively forms a central component of life history theory which concerns how individuals must allocate a finite energy budget

among the competing interests of growth, reproduction and self-maintenance (Stearns 1992). Compulsory trade-offs among these functions mean that variation in the rate of utilising energy will have likely implications for life history traits and hence fitness. Consequently, there is great contemporary interest in among-individual variation in minimal rates of energy metabolism. In this review, I address two issues: (1) why do some individuals consistently have two or three times the maintenance costs of conspecifics the same size, age and sex; and (2) what are the consequences for fitness? For my purposes, the 'baseline' measures of energy metabolism - basal, standard and resting metabolic rate (BMR, SMR and RMR respectively) are most relevant. When measured on quiescent individuals, at a common temperature and corrected for body mass, these estimate the compulsory energy cost of self-maintenance that is central to life history theory. The definitions of each vary slightly. SMR is the lowest rate of metabolism, measured at a particular temperature, in an inactive and post-absorptive ectotherm (McNab 2002). BMR only differs because it is measured in endotherms and includes the cost of endothermy (McNab 2002). RMR also assumes a post-absorptive state, but is frequently applied to both endotherms and ectotherms and caters for low levels of spontaneous activity (Jobling 1994). Since all three measures represent the minimal metabolism of an individual in a relatively quiescent state, I group them under the term RMR.

Variation in RMR between species is ubiquitous and mostly explained by body mass, temperature, phylogeny and a range of environmental factors (Clarke & Johnston 1999; Glazier 2005 and references therein; see Careau *et al.* 2008; Clarke, Rothery & Isaac 2010). These comparative studies have shown that RMR is a trait of ecological and evolutionary importance but are unable to identify causal mechanisms. Within-species studies are complementary in this respect because they can provide insights into the causal factors underlying variability in RMR. However, attempts at explaining intra-specific variation in RMR have in general been less successful than comparative studies. For example, even after correcting for body mass, temperature and other factors such as age (Moe *et al.* 2009), sex (Rønning, Moe & Bech 2005), season (Broggi *et al.* 2007), dietary history (Cruz-Neto & Bozinovic 2004) and reproductive state (Speakman & McQueenie 1996), three-fold differences in RMR among post-absorptive individuals and even siblings remain unexplained (Metcalfe *et al.* 1995; Steyermark *et al.* 2005; Johnston *et al.* 2007).

Individual variation in RMR appears likely to have consequences for fitness because RMR can constitute up to 50% of an individual's energy expenditure (Steyermark *et al.* 2005). Moreover, RMR correlates with other important measures of metabolic demand (Chappell *et al.* 2007) and a range of fitness-related behavioural traits (Biro & Stamps 2010). Differences in RMR among individuals also appear to be permanent. For example, RMR is repeatable over periods of time ranging from days to years (Nespolo & Franco 2007) even in individuals that have experienced a 20-fold increase in body mass between measurements (McCarthy 2000). Furthermore, individuals seem unable to compensate for periods of intense energy expenditure by lowering their RMR (Wiersma & Tinbergen 2003). Thus, RMR has attracted considerable interest as an important ecological factor that can set rates of resource uptake and allocation to survival, growth, and reproduction (Brown *et al.* 2004). However, hypotheses that attempt to correlate variation in RMR with broad scale ecological variables such as climate and diet are not supported unequivocally at the intra-specific level (Chown & Gaston 1999; Cruz-Neto & Bozinovic 2004) and do not explain the variation in RMR that can occur among siblings.

Using recent evidence from both vertebrate and invertebrate taxa, I first discuss the diverse causes of variation in RMR. Secondly, I review evidence that RMR is linked with fitness. Thirdly, I discuss recent suggestions that the benefits and costs of a relatively high or low RMR may depend on local environmental conditions and that selection on RMR may be constrained by trade-offs, thereby providing an explanation for the persistence of variation in RMR in natural populations and among siblings. I conclude by discussing experimental approaches that can evaluate this hypothesis and enable more rigorous examination of the causes and consequences of intraspecific variation in RMR.

INTRINSIC CAUSES OF INDIVIDUAL VARIATION IN RMR

Local adaptation, heritability and genetic determinants

Broadly distributed species have been used to identify a genetic component to intra-specific variation in RMR that may reflect local adaptation. For example, using the widely distributed isopod *Porcellio laevis*, Lardies and Bozinovic (Lardies & Bozinovic 2008) demonstrated inter-population differences in RMR among F1 generation offspring that had been bred and reared in a common environment. Moreover, the observed differences in

RMR correlated negatively with the latitude of the populations from which the parental generation were sourced (Lardies & Bozinovic 2008). Despite evidence of high within-individual repeatability and (possibly) local adaptation, breeding experiments have generally found the heritability (h^2) of RMR to be low (Nespolo, Bacigalupe & Bozinovic 2003; Nespolo et al. 2005; Rønning et al. 2007; Ketola & Kotiaho 2009; Piiroinen et al. 2010), which is typical for traits related to fitness. However, exceptions do exist (e.g. Sadowska et al. 2005; Nilsson, Akesson & Nilsson 2009), and selective breeding experiments have shown that RMR can respond to selection (Ksiazek, Konarzewski & Lapo 2004), providing evidence for heritability. Such equivocal evidence has led to suggestions that the genetic architecture of RMR may be complex (Arnqvist et al. 2010) or that maternal and environmental effects also influence RMR (Nespolo et al. 2003; Verhulst, Holveck & Riebel 2006). Indeed, different parental configurations of mitochondrial and nuclear DNA can interact with the thermal regime experienced during early development to shape whole-animal RMR (Arnqvist *et al.* 2010).

Maternal effects

Recent evidence suggests that maternal effects can exert a substantial influence on offspring RMR. A possible mechanism underlying such effects is the transfer of hormones from mother to embryo. In oviparous species, concentrations of egg hormones can vary considerably among and within clutches and can have significant effects on offspring phenotypes (Groothuis *et al.* 2005). In relation to RMR, experimental elevation of testosterone levels in zebra finch (*Taeniopygia guttata*) eggs resulted in an increase in offspring RMR that persisted into adulthood (Tobler *et al.* 2007; Nilsson *et al.* 2011). Female three-spined sticklebacks (*Gasterosteus aculeatus*) exposed to the threat of predation produce eggs that have a higher concentration of cortisol and also higher RMR (Giesing *et al.* 2010). Likewise, elevation of cortisol in brown trout eggs increased embryonic RMR (Sloman 2010). Further evidence of a link between hormone levels and RMR in older animals comes from positive correlations between endogenous levels of plasma hormones and RMR (Chastel, Lacroix & Kersten 2003; Ros *et al.* 2004; Steyermark *et al.* 2005) or experiments that manipulate plasma hormone levels or induce stress and find changes in RMR (Buchanan *et al.* 2001; Ros *et al.* 2004). Maternal effects on RMR are not necessarily restricted to hormonal pathways. Eggs laid by female clownfish (*Amphiprion melanopus*) on the periphery of the clutch had a RMR that was on

average 24% lower than that of eggs laid in the centre (Green, Anthony & McCormick 2006). Although variation in maternal provisioning may account for this observation, it is also possible that gradients in dissolved oxygen content influence offspring RMR via their position within the clutch (Green *et al.* 2006).

Biochemical, physiological and behavioural sources of intrinsic variation

The interaction between an individuals' genotype and the environment it experiences during ontogeny is likely to involve effects on a range of biochemical, physiological and behavioural factors that influence intrinsic metabolic demand. Resting individuals consume energy during fundamental processes, such as protein turnover, gluconeogenesis, enzyme activity, nitrogenous waste synthesis and proton transport across the membranes of mitochondria during energy metabolism (e.g. 1997). However, the proportional contribution of these factors to metabolic demand is poorly understood, but may contribute to individual differences in RMR within species. For example, evidence from mice shows that intraspecific variation in the size of the intestines, liver, kidneys and heart accounts for more than 50% of the variation in RMR, despite these organs making up a relatively small proportion (on average approximately 17%) of the total body mass (Konarzewski & Diamond 1995). Likewise, behavioural syndromes or differences in personality (e.g. bold versus shy phenotypes, Sih, Bell & Johnson 2004) may influence individual daily energy expenditure (e.g. due to differences in activity levels), but also RMR: more active individuals may have larger organs than less active individuals, which allow for a higher peak metabolic output, but also need to be maintained at rest (Biro & Stamps 2010). Behavioural differences among individuals may also affect estimates of RMR during respirometry. For example, some individuals are more 'reactive' than others when confined in respirometers, possibly leading to a higher estimate of RMR (Careau *et al.* 2008; Killen *et al.* 2011; Careau *et al.* 2011). This indicates that some individuals may be more susceptible to stress than others, and in terms of RMR, respond more acutely to a range of stimuli. Hence, the variation inherent in intra-specific studies of RMR may partially reflect the wide range of factors that contribute to individual RMR and are overlooked in analyses between species.

EXTRINSIC CAUSES OF INDIVIDUAL VARIATION IN RMR

Physical and biological environment

The expression of RMR can also be affected by environmental conditions experienced during and after development. For example, developmental temperature is known to be a strong determinant of later-life RMR (Steyermark & Spotila 2000; Le Lann *et al.* 2010). Furthermore, challenges to the immune system and levels of conspecific density experienced during early development can also influence later-life RMR (Steyermark & Spotila 2000; Le Lann *et al.* 2010). For example, in birds, juvenile RMR can be reduced by increases in brood density during early development. Whereas another study showed that adult RMR can be higher in response to being raised in an enlarged brood (Burness *et al.* 2000; Verhulst *et al.* 2006). In eastern chipmunks (*Tamias striatus*), juvenile parasite load can significantly increase adult (non-parasitised) RMR when measured a year later (Careau, Thomas & Humphries 2010). This effect on RMR likely results from up-regulated immune function, since challenges to the immune system elicit a temporary increase in RMR (Ots *et al.* 2001; e.g. Freitak *et al.* 2003) which is similar to that observed in parasite-infected individuals (Nilsson 2003; Careau *et al.* 2010).

Resting metabolic rate in adulthood may also be affected by early growth conditions. Growth compensation in juvenile zebra finches (following a temporary reduction in dietary protein content) resulted in an elevated RMR once those birds became adults (Criscuolo *et al.* 2008). This suggests that the long-term energy costs of a higher RMR may be outweighed by the immediate benefits of catching up in body size (reduced predation risk, for example). Supportive evidence from biomedical and epidemiological studies shows that poor quality nutrition during early development can have irreversible effects on traits likely to affect RMR such as organ size, nutrient metabolism and enzyme physiology (Desai & Hales 1997). Conversely, a reduction in diet quantity (calorie restriction) during development can reduce RMR (O'Connor, Taylor & Metcalfe 2000; Brzek & Konarzewski 2001; Moe *et al.* 2004; Moe, Stolevik & Bech 2005; Rønning *et al.* 2009; Roark & Bjorndal 2009). However, this reduction is reversible once conditions improve (Schew 1995; O'Connor *et al.* 2000), suggesting that it may be a mechanism that conserves energy when food is limiting (O'Connor *et al.* 2000; Brzek & Konarzewski 2001; Moe *et al.* 2005; Rønning *et al.* 2009).

RMR is also known to fluctuate over short periods of time in response to both physical and social stimuli. Juvenile Atlantic salmon (*Salmo salar*) without access to overhead shelter can incur 30% higher resting metabolic costs than those with a shelter, even if the shelter is not used (Finstad *et al.* 2004; Millidine, Armstrong & Metcalfe 2006). The presence of conspecifics can also affect individual RMR. For example, in juvenile Atlantic salmon, the close proximity of a smaller conspecific was found to cause a 40% reduction in RMR, whereas the presence of a slightly larger fish caused RMR to nearly double. This divergence in RMR occurred in the absence of activity and the presence of a transparent barrier that prevented physical interactions between the fish (Millidine, Metcalfe & Armstrong 2009). A similar deviation in RMR between dominant and subordinate individuals has been reported in other species. Sloman et al. (2000) measured the RMR of individual brown trout before and after size-matched pairs were allowed to establish a social hierarchy. After pairing, the RMR of subordinate fish increased by nearly 30%, whereas that of the dominant decreased by 10%.

DOES RMR AFFECT FITNESS? EVIDENCE FOR CONTEXT-DEPENDENT EFFECTS AND TRADE-OFFS

Studies that have investigated links between RMR and fitness have used a range of proxies including growth, reproductive output (number and size of propagules), reproductive fitness (number of surviving offspring), senescence and survival/lifespan. However, predicting the direction of the relationship between RMR and fitness is difficult because logical arguments can be made for both negative and positive trends (Boratynski & Koteja 2010). The ‘compensation’ hypothesis proposes that individuals with a low RMR will have higher fitness because they have lower self-maintenance costs and can devote more energy to growth and reproduction. Conversely, the ‘increased intake’ hypothesis (for explanations of each see Boratynski & Koteja 2010 and references therein) predicts that individuals with a high RMR will have higher fitness than low RMR individuals because they generally have larger internal organs (Chappell *et al.* 2007) and higher maximum metabolic rates (Chappell *et al.* 2007; Biro & Stamps 2010). This greater ‘metabolic machinery’ (Biro & Stamps 2010) might allow for higher sustained energy throughput, thus enabling greater assimilation of energy for growth and reproduction (McNab 1980).

However, high rates of resting metabolism may also carry a cost in terms of increased mitochondrial production of reactive oxygen species (ROS) that cause damage to important biological molecules (e.g. proteins, lipids, nucleic acids), accelerating cellular senescence and ultimately death. On this basis, a higher RMR has been assumed to decrease lifespan through an increased production of reactive oxygen species – the 'free radical' hypothesis of aging (Harman 1956). However, comparative studies show that this hypothesis is too simplistic, since a high RMR does not necessarily result in either greater ROS production or reduced lifespan (Brand 2000). The only study to my knowledge that has studied the relationship between RMR and lifespan at an intra-specific level found that individual mice with a higher RMR tended to survive longer (Speakman *et al.* 2004). This was attributed to higher levels of uncoupling proteins in the mitochondria, which increase the conductance of protons across the mitochondrial inner membrane. Such 'uncoupled' mitochondria require more oxygen per unit of ATP produced but produce fewer ROS. Hence, greater mitochondrial uncoupling is thought to increase overall energy consumption (and so mass-specific RMR) but generate less oxidative stress, resulting in an inverse relationship between RMR and lifespan (Speakman *et al.* 2004).

Relationships between RMR and growth, reproductive output, reproductive fitness and reproductive senescence have been subject to greater scrutiny and are summarised in Table 2.1. Laboratory studies that use *ad libitum* levels of food have failed to find any relationship between RMR and reproductive output, leading to speculation that there is no direct physiological link between the two traits (Hayes, Garland & Dohm 1992). However, this is consistent with life-history theory because unlimited access to energy is unlikely to cause trade-offs in allocation among self-maintenance, growth and reproductive processes. In this respect, a positive relationship between RMR and reproductive output has been demonstrated in natural conditions (Table 2.1), where food levels and other important factors may be more variable.

When considering growth as a measure of fitness, there is evidence for and against both the 'compensation' and 'increased-intake' hypotheses. The majority of laboratory studies use *ad libitum* levels of food and reveal that high RMR individuals show faster rates of growth, supporting the latter hypothesis (Table 2.1). However, where food is restricted, high RMR individuals do not grow any faster than those with lower RMR's, and can lose mass faster than low RMR individuals when completely food-deprived (Killen *et al.* 2011).

Similarly, brown trout with high RMR's had higher growth rates when fed *ad libitum* in captivity, but not when they were released in four natural streams. No correlation was found between RMR and growth in two of the streams, whereas in the other two, growth and RMR were negatively correlated (Álvarez & Nicieza 2005), lending support to the 'compensation' hypothesis.

In regard to the association between RMR and survival, positive, negative and variable relationships have been reported, with the latter differing among sexes and seasons (Table 2.1). Information on the relationships between RMR and reproductive performance is scarce. Directional selection on RMR varied between sexes and among seasons in a study of free-living bank voles (*Myodes glareolus*), but overall reproductive fitness was positively correlated with RMR (Boratynski & Koteja 2010). Conversely, an analysis of cross-sectional data on a population of wild great tits showed no relationship between RMR and rates of reproductive senescence (Bouwhuis, Sheldon & Verhulst 2011). Currently, few studies have considered RMR in the context of sexual selection. However, positive relationships have been demonstrated between RMR and secondary sexual characters, such as the duration and rate of acoustic calls and the production of olfactory attractants (Reinhold *et al.* 1998; Radwan *et al.* 2006; Ketola & Kotiaho 2010).

Table 2.1. Representative summary of relationships between RMR and fitness-related traits obtained in laboratory (L), semi-natural (S) and field conditions (F). Positive (+ve), negative (-ve) and non-significant (ns) relationships between RMR and each trait are shown. Also indicated (in the case of laboratory experiments), are whether ad libitum (AL) or restricted (R) rations were employed. Ration level is denoted as being not applicable (NA) in field experiments.

trait	Species	setting	food ration	relationship	reference
growth	Atlantic salmon (*Salmo salar*)	L	AL	+ve	(McCarthy 2000)
	Masu salmon (*Oncorhynchus masou*)	L	AL	+ve	(Yamamoto, Ueda & Higashi 1998)
	Brown trout (*Salmo trutta*)	L	AL	+ve	(Álvarez & Nicieza 2005)
	Snapping turtle (*Chelydra serpentina*)	L	AL	–ve	(Steyermark 2002)
	Zebra finch (*Taeniopygia guttata*)	L	AL & R	ns under R ration +ve under AL ration	(Mathot *et al.* 2009)
	Brown trout (*Salmo trutta*)	F	NA	ns in 2 streams -ve in 2 streams	(Álvarez & Nicieza 2005)
reproductive output	Lab mice (*Mus domesticus*)	L	AL	ns	(Hayes *et al.* 1992; Johnson, Thomson & Speakman 2001; Johnston *et al.* 2007)
	Cotton rat (*Sigmodon hispidus*)	L	AL	ns	(Derting & McClure 1989)
	House sparrow (*Passer domesticus*)	F	NA	+ve	(Chastel *et al.* 2003)
reproductive fitness	Bank vole (*Myodes glareolus*)	F	NA	+ve	(Boratynski & Koteja 2010)

senescence	Great tit (*Parus major*)	F	NA	ns	(Bouwhuis *et al.* 2011)
survival	radiated shanny (*Ulvaria subbifurcata*)	L	AL & R	–ve under R ration ns under AL ration	(Bochdansky *et al.* 2005)
	Bank vole (*Myodes glareolus*)	S	NA	dependent on sex & season	(Boratynski & Koteja 2009; Boratynski *et al.* 2010)
	Garden snail (*Helix aspersa*)	S	NA	–ve	(Artacho & Nespolo 2009)
	Brown trout (*Salmo trutta*)	F	NA	–ve	(Álvarez & Nicieza 2005)
	Red squirrel (*Tamiasciurus hudsonicus*)	F	NA	–ve	(Larivée *et al.* 2010)
	Short-tailed field vole (*Microtus agrestis*)	F	NA	+ve	(Jackson, Trayhurn & Speakman 2001)

The influence of food availability on the relationship between RMR and growth in laboratory experiments, and the absence of a general trend between RMR and survival in natural settings (where food levels and other important environmental factors may vary) indicate that a single optimal RMR may not exist. It is unlikely that either a high or low RMR will be favoured in all conditions and at all times when natural environments can be so variable. Indeed, the strength and direction of selection on RMR is known to operate differently according to sex and season (Boratynski & Koteja 2010; Boratynski *et al.* 2010). Some authors have also speculated that selection on RMR may be modulated by environmental factors such as the availability of resources (Álvarez & Nicieza 2005; Steyermark *et al.* 2005), which can fluctuate substantially in space and time. Thus, the relationship between RMR and fitness may depend, at least partly, upon the quality of environmental conditions – what I propose to call the 'context dependence' hypothesis that links RMR and fitness. High RMR individuals are likely to have relatively high fitness when environmental conditions are favourable and *vice versa* when they are poor (food supply being the most obvious factor, but gradients in other environmental variables may be applicable). In comparison, low RMR individuals may be somewhat buffered against the environment due to their lower costs of maintenance. I predict that low RMR individuals will have relatively high fitness in such conditions but lower fitness than high RMR individuals in favourable environments. The 'context dependence' hypothesis is perhaps best understood when considering how resource availability (i.e. food supply) can interact with individual RMR to influence growth rate.

Growth is dependent on both access to food and the ability to convert ingested food into new tissue. Relatively high RMR individuals tend to be more aggressive and dominant over those with low RMR's (Biro & Stamps 2010), giving them preferential access to food (Metcalfe *et al.* 1995). Where resources are abundant or predictable, individuals with relatively high RMR's can therefore exhibit faster growth rates than low RMR individuals (Table 2.1). They may also have a greater physiological capacity for growth, as they can digest and process meals faster (Millidine, Armstrong & Metcalfe 2009) and have higher digestive efficiency (Ksiazek *et al.* 2004; Ksiazek, Czerniecki & Konarzewski 2009). This may be advantageous in highly seasonal environments (e.g. high latitudes) where conditions can be favourable for growth only for a limited period of time. Evidence from the Atlantic silverside *Menidia menidia*, a broadly distributed species of marine fish, shows that individuals from high latitude populations tend to have higher RMR's and a larger

specific dynamic action (SDA, i.e. investment of energy in food digestion). They also consume more food and have higher food conversion efficiencies than those from low latitude populations (see Arnott, Chiba & Conover 2006 and references therein). Likewise, selective breeding of mice for high RMR results in a higher rate of food consumption and assimilation of new tissue (Ksiazek *et al.* 2004). Furthermore, when exposed to a sudden and unpredictable decrease in ambient temperature, mice selectively bred for high RMR are less likely to enter a negative energy balance because they can consume and digest more food, if it is freely available (Ksiazek *et al.* 2009). The advantages of a high RMR, such as rapid growth potential, may however, be realised only in environmental conditions that can offset the higher costs of routine maintenance, for example where food is abundant, accessible, predictable or defendable by aggression. If these conditions are not satisfied, individuals with high RMR's may not benefit from any growth advantage or may even experience lower rates of growth and/or survival (Table 2.1). Thus, low RMR individuals may be more resilient in adverse conditions due to their lower maintenance requirements. Such effects need not only relate to food supply: juvenile Atlantic salmon lost energy reserves over the winter faster when no in-stream cover was available. However, this energy loss was least in fish with a relatively low RMR (Finstad *et al.* 2007). Also, only individuals with relatively low RMR may be able to use habitats where foraging costs are relatively high, as in the case of salmonid fishes feeding on invertebrates carried in stream currents (Armstrong, Millidine & Metcalfe 2011). Furthermore, individuals with high RMR are also known to engage in riskier behaviour (Huntingford *et al.* 2010; Killen *et al.* 2011). Thus, the benefits of a high RMR (e.g. high social status and growth capacity) might be traded off against costs such as an increased predation risk. Thus, I propose that, variation in RMR might be maintained for the following reasons. First, selection on RMR is unlikely to remain static in space and time (alternatively, organisms may only encounter brief episodes of selection on RMR). Second, trade-offs may constrain the directional evolution of RMR. And third, individual RMR may be shaped by maternal effects (which could be influenced by the environment experienced by the mother), early developmental conditions or an interaction between the genotype and either the current or the parental environment.

FUTURE DIRECTIONS – TESTING HYPOTHESES REGARDING THE CAUSES AND CONSEQUENCES OF INDIVIDUAL VARIATION IN RMR

The causes of intraspecific variation in RMR, on both proximate and ultimate levels, are poorly understood and require further investigation. Maternal effects and environmental factors operating during early ontogeny offer a proximate mechanism needing greater scrutiny. Moreover, the interaction between environment and genotype during this period may also be critical (Arnqvist *et al.* 2010). On a broader level, the mechanisms maintaining intraspecific variation in RMR remain speculative. Environmental heterogeneity has attracted attention as a candidate factor (Álvarez & Nicieza 2005; Steyermark *et al.* 2005; Armstrong *et al.* 2011, this review), and both observational and experimental studies may contribute to the evaluation of this hypothesis. In the case of the former, the scale of individual variability in RMR among natural populations (as opposed to mean differences) that are exposed to different environmental conditions has not been measured. When measured in a common environment, one might predict that variability in RMR would be higher among individuals originating from populations that inhabit stochastic rather than stable environments. Alternatively, experimental tests of this hypothesis might involve longitudinal studies that monitor the growth and survival of individuals with known RMR's in semi-natural conditions where environmental conditions such as food availability and habitat complexity can be manipulated.

While RMR can sometimes be associated with components of fitness in free living animals, the causal mechanism underlying these associations is usually unclear. This occurs because most studies rely on natural variation in RMR and so the relationships could be driven by a third, unidentified, factor. Ideally, RMR should be manipulated independently of other trait(s) that may influence performance. Selective breeding for high and low RMR's is a useful approach (Ksiazek *et al.* 2004). However, selection experiments are time-consuming and can be performed in controlled conditions only where other selective forces are largely absent. Additionally, the genetic architecture of metabolic traits may be complex (Arnqvist *et al.* 2010). Thus, it could be difficult to select for RMR alone and not for correlated traits that also influence fitness. A promising approach would be to manipulate RMR during early ontogeny in the laboratory and then monitor the performance of the animals in semi-natural or natural conditions. Recent studies suggest that this can be achieved by hormonal manipulation of the developing embryo or by

altering competitor density or protein intake during ontogeny (Burness *et al.* 2000; Verhulst *et al.* 2006; Tobler *et al.* 2007; Criscuolo *et al.* 2008). However, it is currently unclear whether these experimental manipulations affect other traits that may also influence fitness.

A major obstacle confronting researchers interested in individual variation RMR is separating cause from effect. For example, RMR is often correlated with levels of plasma hormones (Ros *et al.* 2004; Steyermark *et al.* 2005; Rønning *et al.* 2009) and manipulation of plasma hormone levels can affect RMR (e.g. Ros *et al.* 2004), suggesting causality. However, both RMR and plasma hormone levels can also correlate with organ size (Steyermark *et al.* 2005). Thus the causal factor in these relationships is obscure - do large organs and/or high hormone levels cause a high RMR, or do large organs or high hormone levels result from high RMR?

I also emphasise the value of longitudinal studies where RMR and related traits are measured repeatedly within the same individual, since these may reveal information that is not observed in short-term or cross-sectional studies. Biro and Stamps (2010) suggested that longitudinal studies are necessary to reveal if correlations between RMR and behaviour are temporally consistent. This suggestion is applicable to other phenotypic traits and also studies investigating the causes of individual variation in RMR. Maternal effects and environmental factors experienced during early development can affect the expression of RMR (Steyermark & Spotila 2000; Burness *et al.* 2000; Green *et al.* 2006; Verhulst *et al.* 2006; Tobler *et al.* 2007; Criscuolo *et al.* 2008; Careau *et al.* 2010; Giesing *et al.* 2010; Le Lann *et al.* 2010; Sloman 2010; Nilsson *et al.* 2011), but most of these studies made a single measurement of RMR (usually in early life), neglecting measurements during later life stages and thus the repeatability of any effect. Longitudinal studies that have examined individual variation in RMR in relation to performance in free-living animals have also revealed important information regarding the strength and direction of selection on RMR (Boratynski & Koteja 2009; Boratynski & Koteja 2010; Boratynski *et al.* 2010). Estimates of lifetime reproductive success in relation to RMR are however absent and, with the exception of studies on a single species of fish (Álvarez & Nicieza 2005) and snail (Artacho & Nespolo 2009), current knowledge of the fitness consequences of variation in RMR in free-living animals is restricted to investigations conducted on short-lived mammals overwintering at high latitudes. Data from other study

systems, for example, species with longer life expectancies and different thermoregulatory strategies that inhabit lower latitudes are required to evaluate the generality of conclusions drawn from the currently narrow range of study systems.

CHAPTER 3. EGG HORMONES IN A HIGHLY FECUND VERTEBRATE: DO THEY INFLUENCE OFFSPRING SOCIAL STRUCTURE IN COMPETITIVE CONDITIONS?

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SUMMARY

Social status can vary considerably among individuals and has significant implications for performance. In addition to a genetic component, social status may be influenced by environmental factors including maternal effects such as pre-natal hormone exposure. Maternal effects on traits determining social status have previously been examined in species where mothers provide parental care for relatively few offspring and therefore directly influence post-natal development. However, the generality of conclusions arising from these investigations is unclear because species that employ different reproductive strategies have not been studied. I investigated the hypothesis that egg steroid hormone levels influence the social status of juvenile brown trout (*Salmo trutta*). I manipulated intra-clutch levels of cortisol and testosterone in eggs from 15 mothers using dilute hormone baths at the time of fertilisation, and examined their effects on traits that correlate with social status in juveniles (including standard body size, aggression, competitive ability and standard metabolic rate, SMR). Hormone treatment did not affect whole animal or mass-corrected SMR at the critical developmental stage when juveniles switch from reliance on a maternally-provisioned yolk-sac to independent feeding. However, juveniles from cortisol-treated eggs were smaller at this developmental stage. They were also less aggressive than, and subordinate to, fish from untreated eggs in socially competitive conditions, even after correcting for the observed effect of cortisol on body size. Egg testosterone treatment resulted in a likely pharmacological or toxicological dose with subsequent effects on both body size and behaviour in independently feeding juveniles. Results from this study demonstrate that variation in the amount of cortisol deposited in eggs by spawning females influences juvenile social status and performance. The effects of elevated egg cortisol in fish are similar to the actions of embryonic glucocorticoids

reported in other vertebrate taxa with very different reproductive strategies, suggesting a widespread mechanism for the effects of maternal stress on offspring. Possible adaptive aspects of this relationship are discussed.

INTRODUCTION

Individual variation is becoming increasingly recognised as an important characteristic of wild populations in a diverse range of ecological disciplines such as population biology (Bolnick *et al.* 2003), behavioural ecology (Sih *et al.* 2004) and physiology (Williams 2008). Within hierarchical societies, social status can vary substantially among individuals and has profound effects on their relative fitness (Clutton-Brock 1988). In a range of taxa, high social status can confer enhanced access to resources, earlier sexual maturation, higher survivorship (Huntingford & Turner 1987) and is positively correlated with basal energy expenditure (Biro & Stamps 2010). Intra-specific variation in this 'idling' cost of metabolism (hereafter I collectively refer to the related terms, basal, resting and standard metabolic rate, as SMR) is widespread and can be considerable, varying more than two-fold amongst conspecifics after correcting for allometry (Steyermark *et al.* 2005).

In vertebrates, hormones transferred from the mother to the egg or foetus vary substantially within and/or between clutches or litters and have a considerable impact on offspring phenotypic traits, such as growth, behaviour and immune function (McCormick 1999; Groothuis *et al.* 2005; Uller *et al.* 2007) . Recent evidence from birds suggests that intra-specific variation in SMR may be caused by the action of maternally derived hormones, since elevated levels of egg testosterone increase post-natal SMR (Tobler *et al.* 2007), (but see Eising *et al.* 2003). Androgens, such as testosterone, and glucocorticoids, such as cortisol, are likely mediators of maternal effects on SMR and social status because maternal levels of these hormones fluctuate in response to endogenous processes and exogenous environmental variables. They are also readily transferable to developing offspring (Zarrow, Philpott & Denenber 1970; Groothuis & Schwabl 2008), permitting conditions experienced by the mother to affect offspring phenotype. Moreover, a potential effect of these hormones on SMR is suggested by positive correlations between endogenous levels and SMR (Buchanan *et al.* 2001; Ros *et al.* 2004) and social dominance in several vertebrates (Dloniak, French & Holekamp 2006; Clutton-Brock *et al.* 2006).

Hitherto, attention has almost exclusively focussed on identifying the effects of maternal hormones within vertebrates that produce relatively few offspring (Groothuis *et al.* 2005; Kapoor & Matthews 2005; Uller *et al.* 2007). The effects of hormones in vertebrates such as fishes that spawn many eggs almost simultaneously is not clear, although some key studies have identified effects on behaviour, physiology and survival (Gagliano & McCormick 2009; Sloman 2010). Stream-dwelling salmonid fishes are ideal species in which to investigate questions regarding hormone mediated effects on development and the causes and consequences of variation in social status and related traits, such as SMR. First, their eggs contain substantial concentrations of steroids, which can vary both within and among clutches (Stratholt, Donaldson & Liley 1997; Suter 2002; Sloman 2010). Second, the social structure of salmonids is often based around dominance and territoriality (Metcalfe 1998), which can affect individual performance, by influencing rates of growth and age at maturation (Metcalfe 1998). Furthermore, SMR varies substantially among siblings and is a better predictor of social status than body size in this family (Metcalfe *et al.* 1995). Individuals with higher than average SMR tend to be more aggressive and dominant over those with a lower SMR (Metcalfe *et al.* 1995). However, dominant or high SMR individuals may experience growth or survival advantages only where resources are predictable or defendable by aggression (Martin-Smith & Armstrong 2002; Harwood *et al.* 2003; Álvarez & Nicieza 2005). It has also been predicted that specific combinations of water velocity and food availability will result in relatively high growth of dominant, high SMR individuals, whereas others will favour subordinate, low SMR fish (Armstrong *et al.* 2011). Therefore, variation in SMR and associated behavioural strategies may enable mothers to maximise offspring survival via heterogeneous advantage (Griffiths & Armstrong 2001) by producing a range of offspring phenotypes for a range of environmental conditions.

In the present study I test the hypothesis that increased egg testosterone and/or cortisol will increase offspring SMR and/or social status using brown trout (*Salmo trutta*, Linnaeus, 1758) as a study system. Females of the genus *Salmo* deposit hundreds to thousands of eggs in sequentially spawned gravel nests, providing no further care. Early-life social status and SMR are likely to be particularly important because newly-emergent young must compete with conspecifics to establish feeding territories in crowded conditions (Elliott 1994) and within a narrow range of suitable habitats (Armstrong & Nislow 2006). Moreover, there is recent evidence that levels of cortisol in ovarian fluid can increase egg

metabolic rate and offspring aggressiveness in this species (Sloman 2010). I manipulated egg concentrations of testosterone and cortisol and investigated the subsequent effects on several fundamental correlates of social status in salmonid fishes; body size, SMR and behaviour.

MATERIALS AND METHODS

Egg hormone manipulation

Egg hormone manipulations were performed at the Marine Scotland freshwater hatchery at Almondbank, Perthshire, Scotland. Fifteen female brown trout (hatchery F1 generation of wild Loch Broom fish) were anaesthetised, measured for fork length (L_F, range 290 – 344 mm) and somatic mass (total mass minus ovarian mass, range 224.6 – 376.8 g). The eggs from each female were removed by stripping and fertilised *in vitro* with milt from a single male, yielding 15 full-sibling families. In the minutes following fertilisation, salmonid eggs undergo 'water hardening' where water is absorbed into the egg and the egg membrane hardens to protect the developing embryo. Hormone manipulations were made during this hardening phase as follows. Within 1 min of fertilisation, the eggs from each family were divided evenly into three batches, corresponding to cortisol-treated (cort), testosterone-treated (T) and a control with each batch placed in its own 250 ml plastic beaker. This procedure produced 45 groups (15 families × 3 treatments) of eggs. The cort and T treatments involved temporarily immersing the egg batches in ca. 200 ml fresh water solution containing each respective hormone at a concentration of 200 $\mu g\ l^{-1}$. In a previous study of brown trout, this treatment method and dose was assumed to cause a physiologically relevant increase in egg steroid content because two days after treatment, steroid concentrations in treated eggs had fallen to (or below) those observed in un-treated controls (Suter 2002). Each hormone (cort - Sigma, hydrocortisone 98%, T - Fluka, testosterone 99%) was dissolved initially in 100 % ethanol, prior to dilution in fresh water. Control eggs were placed in fresh water containing the same concentration of ethanol (0.002%) as the hormone treatments. The eggs were immersed in each treatment solution for 2 h with periodic mixing of the beakers to ensure that all eggs were equally exposed to each treatment solution. Similar techniques for administration of hormones to eggs have been employed successfully in salmonids (Stratholt *et al.* 1997; Suter 2002) and other teleosts (McCormick 1999; McCormick &

Nechaev 2002). The eggs were then rinsed thoroughly in fresh water and transferred to stainless steel hatchery baskets. To confirm elevation of egg hormone levels, immediately after rinsing, 10 eggs from each treatment per family were frozen at -80° C for subsequent analysis. Despite efforts to ensure that egg hormone levels were elevated within physiologically relevant limits, the T treatment led to egg levels being significantly outside this range (see *Results*). Thus, I focus my discussion on the effects of egg cort only. However, given that the behavioural effects of each hormone were assessed in triads composed of one fry from each of the three treatments (see below), I still present results regarding the effects of egg T-treatment on offspring body size, SMR and behaviour, despite the likely pharmacological dose.

Determination of egg hormone levels

Egg hormone levels (cort and T) were quantified by radioimmunoassay (RIA) in the 10 families for which behavioural and SMR data were obtained (see below). First, hormones were extracted from egg samples using a modification of a protocol used previously on salmonid eggs (Eriksen *et al.* 2006). Briefly, six to eight eggs per sample were divided between two extraction tubes, weighed (0.0001 g), and crushed using a glass rod. To each tube, 1 ml of physiological saline was added together with 50 µl (ca. 5000 cpm) of radioactive-labelled cortisol to calculate hormone recovery during the extraction process. This solution was vortexed, incubated for 45 min at 37ºC and extracted in 5 ml of diethyl ether (DEE) while vortexed for 60 s, followed by centrifuging (2000 rpm, 5 min, 4ºC). The extract was snap-frozen and the ether/hormone phase decanted into a fresh tube. This process was repeated twice (addition of 5 ml DEE, 30 s and 15 s vortex respectively) before all extracts from each sample were combined, rinsed with 2 ml of 70% methanol, dried under nitrogen, and stored at -20ºC prior to RIA.

Subsequently, extracts were thawed and dissolved in 200 µl of phosphate-buffered-saline with gelatine (PBSG). From this solution, a subsample of 20 µl was taken, mixed with scintillation cocktail (Ultima Gold, Perkin Elmer) and radioactivity (^{3}H) counted on a liquid scintillation counter. Subsequently, 20 µl of sample was used for cort determination using kits purchased from Orion Diagnostica (‘Spectria’, Espoo, Finland) and 50 µl was used for T determination using kits purchased from Beckman Coulter GmbH (‘DSL-4000’, Standort, Sinsheim). Standards for each assay were prepared using dilution series from pre-

prepared stock and ranged from 4 to 500 ng ml^{-1} for cort and 0.04 – 20 ng ml^{-1} for T. Egg dilution curves run parallel to the standards in both cases. Recoveries were calculated by comparison to non-extracted labelled hormone and averaged 76% (range 69 – 84%). Intra-assay CV was 1.6% and 2.6% for C and T respectively, while inter-assay CV (3 assays) was 6.7% and 7.4% respectively for pools of controls with low (44 ng ml^{-1}) and high (125 ng ml^{-1}) cort concentrations, and 16.4% and 7.2% respectively for controls with low (0.2 ng ml^{-1}) and high (15 ng ml^{-1}) T concentrations. For both the cort and T assays, the controls consisted of a pooled sample of plasma that had been taken from a mature female trout.

Offspring development

To test whether egg hormone treatment influenced offspring body size and condition, 10 juveniles from each treatment per family were preserved in a 30 % ethanol solution (Gagliano, Kowalewsky & McCormick 2006) at hatching and at the onset of independent feeding after consumption of the maternal yolk sac approximately 8 weeks later (referred to hereafter as alevins and fry respectively). The alevins and fry were subsequently measured for standard length (SL, snout tip to insertion of caudal fin rays, with callipers, to 0.01 mm) and mass (to 0.0001 g). Morphological data were obtained for all 15 families at the alevin stage and for a sub-sample of 10 families at the fry stage.

Offspring rearing conditions

Once they reached the first-feeding stage (51-60 days after hatching), fry were transferred from the Almondbank hatchery to the University of Glasgow for screening of behaviour and SMR. Groups of approximately 15 sibling fish from a single treatment per family were held prior to observations in sections of a re-circulating aquarium system composed of four linked glass tanks (180 × 25 cm) through which water flowed at (mean ± s.e.) 1.28 ± 0.03 cm s^{-1} at a depth of 15 cm. The tanks were fitted with white plastic longitudinal and mesh transverse dividers to produce compartments of size 20 × 12.5 cm. Approximately 8-15% of the water in the system was changed daily for routine cleaning and maintenance. Each day the fry were fed *ad libitum* amounts of frozen bloodworm. A minimum of 3 days before screening of SMR and behaviour, the fry were anaesthetised and batch-marked according to hormone treatment (but in different combinations so that

later behavioural observations were conducted blind) with different colour Visible Implant Elastomer tags (VIE, Northwest Marine Technology, Washington, USA) with a single mark between the dorsal fin and lateral line.

Offspring SMR

Open flow respirometry was employed to investigate whether egg hormone treatment affected offspring SMR. Randomly chosen fry (25-58 days after reaching first feeding) were placed in glass respirometry chambers (internal diameter 9·7 mm, length 75 mm, vol. 1·14 ml) through which water flowed at a constant rate under gravity from a header tank. The out-flowing water drained to a sump tank, from which it was pumped back to the header tank through an ultra-violet steriliser to reduce bacterial respiration. Oxygen levels in both tanks were kept saturated by air stones. A rack of chambers arranged in parallel allowed the oxygen consumption rates of 12 fry (chosen such that there was one fish from each treatment from each of four families) to be measured on the same day. An extra chamber was always left without a fish and acted as a control. The fry were left to acclimate in the chambers overnight and measurements commenced 17-20 h later, by which time they had settled and evacuated their guts. Previous studies of juvenile salmonids have demonstrated a stable oxygen consumption rate after this period of acclimation (Metcalfe *et al.* 1995). To minimise activity, fry were kept in semi-darkness by placing a black cloth over the respirometry chambers, while low flow rates (0.29 ± 0.01 l h^{-1} mean ± s.e.) removed the need for active swimming. Flow rates were calculated by collecting the water outflow from each tube in a beaker over a measured time period (minimum 2 min) and then weighing it (to 0.01 g).

The reduction in water oxygen concentration due to fry respiration was measured with a Fibox 3 temperature compensated oxygen meter (Loligo Systems, Denmark). A flow-through fiber-optic cell with integrated planar oxygen sensor (PSt3 oxygen sensitive coating, Presens, Denmark) was connected temporarily to the outflow of each respirometry chamber. The flow through cell was calibrated approximately 17-20 h before measurements began each day with a two-point calibration of oxygen free water and oxygen saturated water. Oxygen free water was prepared by dissolving ca. 1 g sodium sulphite (Na_2SO_3) in ca. 100 ml of water. Oxygen saturated water was prepared by

simultaneously stirring and aerating ca. 100 ml of header tank water. Metabolic rates ($\dot{V}O_2$, ml O_2 h^{-1}) of individual fish were calculated according to the equation:

$$\dot{V}O_2 = V_W \cdot \Delta C_W \cdot \beta O_2$$

where V_W is the flow rate (l h^{-1}) of water through the respirometry chamber, ΔC_W is the percentage difference in oxygen concentration between water in-flow and out-flow and βO_2 is the capacitance of oxygen in the water. Measurements of outflow water oxygen concentration and temperature were logged (software Oxyview PST3v602) every 10 s for each fish over a 5 min period (minimum) or until the oxygen concentration had stabilised (to account for fluctuations caused by sensor movement between chambers). The oxygen concentration of water exiting a fish-less control chamber was measured at the beginning and conclusion of each measurement day. Two replicate sets of measurements of metabolic rate were made for each fry between 9:00 am and 16:00 pm (with a minimum interval of 45 min between measurements). A third reading was taken if the second value was not within ± 20 % of the first. SMR was calculated as the average of these measurements and includes any slight diurnal variation in metabolism equally for all fish. During each measurement of metabolic rate, the activity rate (time in seconds not spent in an inactive state) of each fry was monitored over a 3 min period using an angled mirror positioned below the respirometry chambers because they could not be observed from above. Fry were isolated from each other visually. The temperature of water in the respirometry chambers averaged (± s.e.) 12.91 ± 0.03° C. The fry were anaesthetised and weighed (to 0.001 g) after measurements of metabolic rate.

Offspring behaviour

After being screened for metabolic rate the fry were allocated randomly into triads containing one sibling from each treatment group. Each triad was then placed in a compartment of the stream tank. The area of these compartments (0.025 m^2) approximate the predicted territory size (0.026 m^2) of a first feeding brown trout fry (30 mm L_F) (Grant & Kramer 1990), thereby increasing the likelihood that the three fish would compete. The fry were then allowed to acclimate for 2 days prior to the 2 day period of behavioural observations. During this period, fry were fed *ad libitum* amounts of bloodworm that were

pippetted just beneath the water surface at the upstream end of each compartment. Behavioural trials were conducted in a temperature-controlled room (mean water temperature 9.4° C ± s.e. 0.1).

The effects of hormone treatment on fry social status were examined using three general protocols adapted from previous studies: relative spatial position of each fish within the simulated feeding territory (Metcalfe, Valdimarsson & Morgan 2003), competition for food items (Cutts *et al.* 1999) and aggressive interactions (Cutts, Adams & Campbell 2001). These protocols are referred to hereafter as territory quality, competitive ability and aggression, respectively. Observations were made through the glass side wall of each compartment.

Territory quality was measured by recording the position of each fry within a compartment six times daily, with at least 45 min between observations. Spatial positions were quantified in two dimensions by dividing both the horizontal and vertical axes of each compartment into three equal lengths, creating nine equally-sized zones. The position of the eye was used when recording the zone occupied by each fish. Dominant juvenile salmonid fry prefer to occupy central-downstream positions within simulated feeding territories, often maintaining position just off the substrate with subordinates confined to the periphery (Metcalfe *et al.* 2003). Therefore, fry positioned in the lower two thirds of the water column at the centre and downstream areas of each compartment were given a score of +1, those in the remaining corners of the compartment were given a score of -1 as were those resting on the substrate (except if they were against the downstream transverse mesh divider, in which case they scored -2). All other positions were given a score of 0. The 2 days of observations yielded 12 spatial position records for each fry. Total scores for territory quality therefore ranged from -24 (an individual always assuming the most subordinate positions) to +12 (an individual always assuming a preferred position).

After each recording of territory quality, competitive ability was measured by introducing a single piece of bloodworm (ca. 1 - 2 mm long to avoid satiation) to each triad using the same technique employed during the acclimatisation period. If a fry made no attempt to acquire the food item it received a score of -1, an unsuccessful attempt in the absence of competition from the other two fry received a score of 0, a successful uncontested attempt or an unsuccessful contested attempt both received a score of +1 and a

successful contested attempt received a score of +2. Thus, my scoring system may be conservative with respect to the 'success' of dominants because successful uncontested attempts might result from other triad members being too afraid to compete with a dominant individual. The 2 days of observation yielded 12 contest records for each fry. Total scores for competitive ability could therefore range from -12 (an individual not making any effort to acquire food) to +24 (an individual successfully acquiring all items of food under competition).

At a haphazardly selected time during each observation day, aggressive interactions between triad members were recorded over a three minute period. To quantify aggression I recorded the initiators (dominant behaviour) and recipients of overt aggressive interactions, involving chasing and biting. The initiator of either a chase or a nip that resulted in a visible subordinate response was given a score of +2 and the fish responding a score of -1. If there was no response then a score of +1 was awarded to the initiator and a score of 0 to the individual not responding. This scoring system therefore awarded high positive scores to aggressive, dominant individuals. Behavioural and SMR data were obtained for 49 triads of fry from the same 10 families (range: 4-5 replicates per family) that were measured for body size at the first-feeding stage.

Data analysis

Analyses of the effects of hormone treatment on offspring phenotypes were performed using linear mixed effect (LME) models, with family as a random and treatment as a fixed categorical factor. First-feeding fry body condition was calculated according to the formula, condition = body mass/$SL^{3.9}$, where the exponent is the slope from a linear regression of log body mass against log SL of control fry ($r^2 = 0.64$, n = 130). The repeatability of whole-fry SMR was calculated from the first and second measurements of SMR (Lessells & Boag 1987). Treatment effects on mass–corrected SMR were examined with fry body mass, fry activity level during respirometry, and respirometry chamber water temperature as continuous variables. Treatment effects on whole animal SMR employed the same model but omitted fry body mass as an explanatory variable.

To validate the scoring criteria assigned to my classifications of territory quality I examined how the competitive ability (i.e. food items acquired) of an individual varied in

relation to the spatial position it occupied most frequently during observations (hereafter termed 'modal position'). I ranked these modal positions on a four point scale, based on their corresponding territory quality scores (see '*Offspring behaviour*' above) that ranged from +1 to -2 and then compared the competitive abilities of fry occupying these four ranks of modal position. Since patterns of behaviour differed between triads, individual competitive ability scores were normalised prior to analysis (by subtracting the mean score of all 147 fry from each individual's score and dividing this value by the standard deviation for all fry). These normalised scores were then used as the response variable in an LME model with family as a random variable, fry modal position as a fixed categorical variable and fry body mass and proportion of time spent in the modal position as continuous variables. The latter variable was included because the spatial position of some individuals varied more than others. The competitive ability score obtained by a fry was influenced by its modal position in the stream tank ($F_{3, 141}$=34.37, p <0.001) and was positively related to the amount of time it occupied that position ($F_{1, 141}$=6.26, p <0.05) and its body mass ($F_{1, 141}$=5.47, p <0.05). After controlling for the effects of body mass and proportion of time spent in modal position, fry with modal positions awarded scores of +1 obtained higher competitive ability scores (adjusted mean ± s.e: 0.56 ± 0.08) than those with modal positions awarded scores of zero (-0.12 ± 0.15), -1 (-0.54 ± 0.12) and -2 (-1.33 ± 0.20). These results confirm that my scoring criteria of territory quality reflected performance at those positions.

The territory quality and competitive ability protocols yielded data with a different range of possible values compared to the aggression protocol. To account for this, the raw values for each of these protocols were normalised within triads by subtracting the triad mean from each individuals score and dividing this by the triad standard deviation. Treatment effects on the normalised scores were examined with fry age (days since first feeding), body mass (g) and mass-corrected SMR as continuous explanatory variables. Associations between the normalised scores of the three behavioural protocols were described using Pearson correlations and then summarised with principal component analysis (PCA), omitting data for the few trials where no aggression was observed (n = 13). This resulted in a single principal component (PC1) summarising all three behaviours as a general index of fry social status. Individual PC scores were then analysed in an LME model comparing treatments (fixed categorical variable) with family as a random variable and fry body mass, age (days since first feeding) and mass-independent SMR as

continuous variables. To identify potentially collinear explanatory variables, variance inflation factors (VIF) were calculated for the explanatory variables in each of the general linear mixed models examining variation in fry behaviour. VIF's were less than 2 in all cases indicating that collinearity was not an issue (O'Brien 2007).

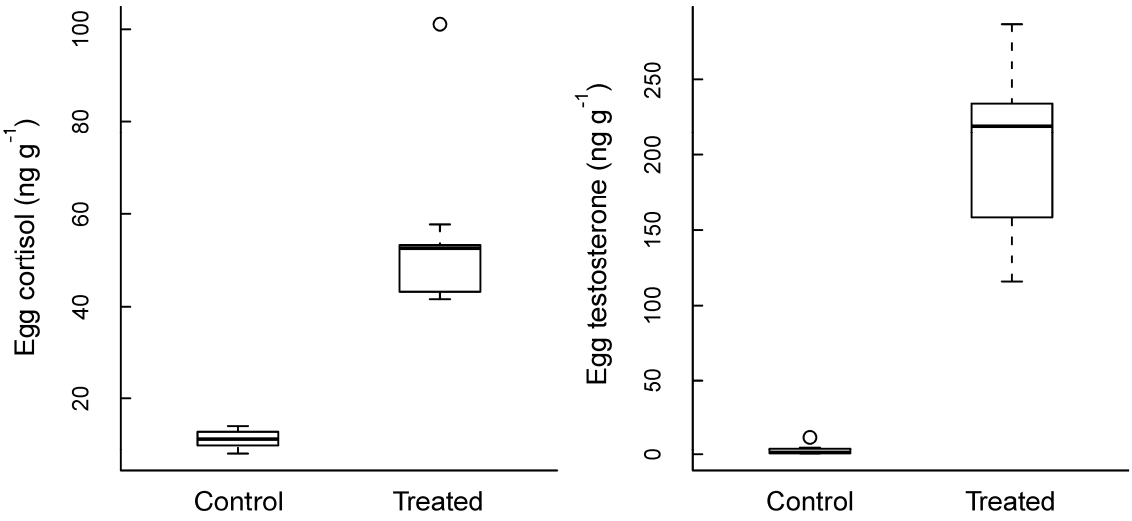
In all LME models, interaction terms were included initially and all non-significant terms were dropped sequentially (beginning with least significant interactions) to produce minimal adequate models. Significant differences among treatment groups were examined with pair wise least significant difference (LSD) comparisons. All statistical models were validated to check that the underlying assumptions of general linear modelling were satisfied; normality of residuals was examined with frequency histograms and homogeneity of variance was assessed by plotting standardised residuals against the fitted values and explanatory variables from each model. Analyses were performed with SPSS *v.* 15·0 (www.spss.com); two-tailed *p* values less than 0.05 are considered significant; means are quoted ± s.e.

RESULTS

Egg hormone concentrations

Both the cort and T baths successfully elevated egg hormone levels (Fig. 3.1a, b). The cort bath elevated mean egg concentrations above controls (control, 11.14 ± 0.65; treatment, 55.04 ± 5.40 ng g^{-1}; paired t-test, $t_9 = 8.04$, $p < 0.0001$). This increase is within the physiologically relevant range, lying within two standard deviations of the mean observed in eggs from 15 female brown trout kept in sections of a large semi-natural stream prior to spawning, assayed using the same technique described above (mean 30.26, s.d. 35.87, range 3.22 – 122.47 ng g^{-1}). The T treatment also elevated mean egg concentrations above controls (mean ± s.e.; control, 2.95 ± 1.12; treatment, 203.85 ± 17.47 ng g^{-1}; paired t-test, $t_9 = 11.70$, $p < 0.0001$). This elevation was greater than two standard deviations of the mean observed in eggs from the same 15 females from the semi-natural stream (mean; 36.60, s.d.; 31.07, range 1.64 – 107.34 ng g^{-1}).

Figure 3.1. Boxplots of hormone concentration in (a) cortisol treated eggs and (b) testosterone treated eggs in comparison to controls. A single value for each hormone was determined from a pooled sample of 6 – 8 eggs from the same 10 families for which behavioural and SMR data were obtained. Median egg hormone concentration, upper and lower quartiles, range and outliers indicated by line, box, error bars and dots respectively.



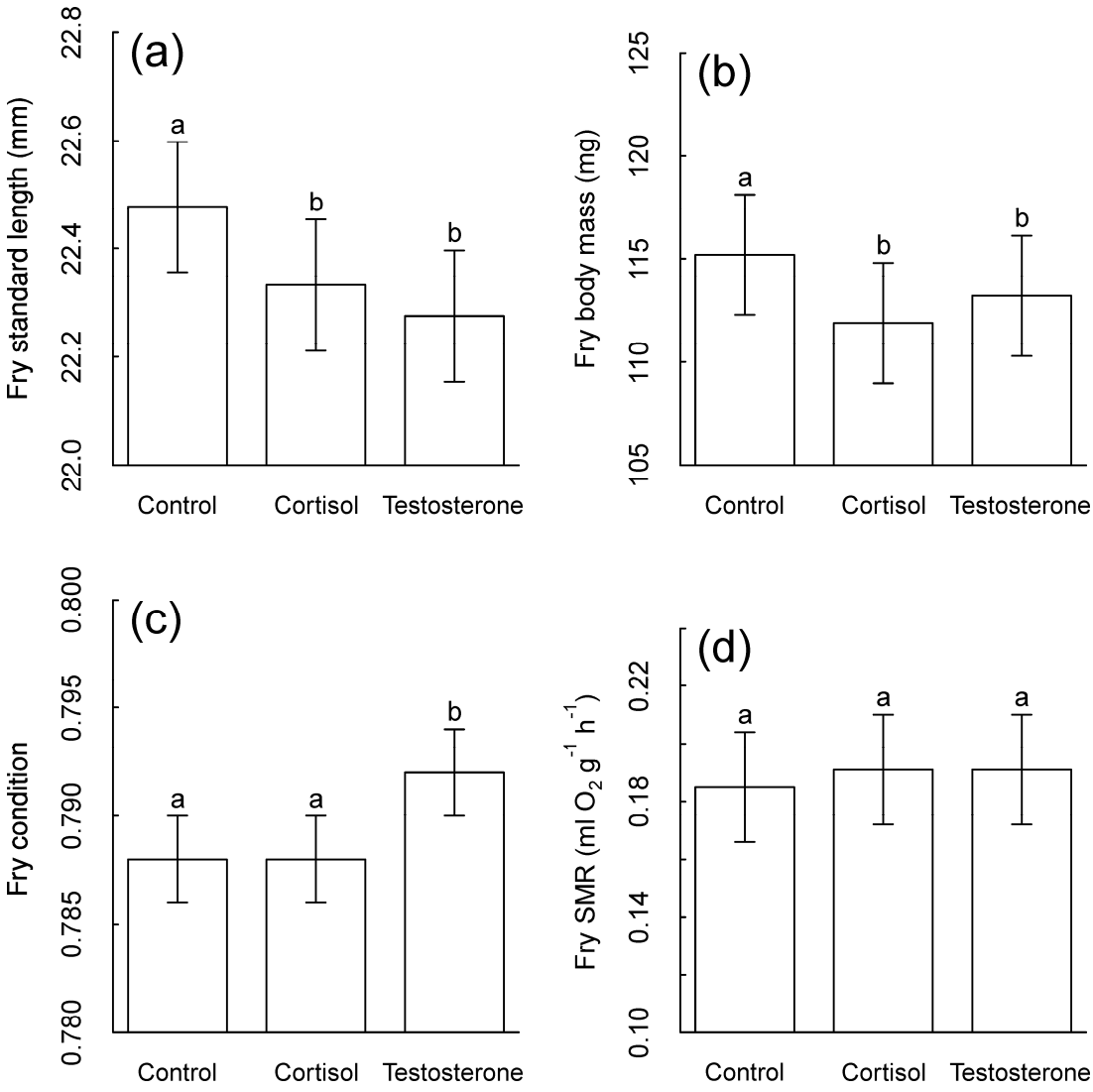
Body mass, length and SMR

At hatching, neither alevin SL nor body mass differed significantly among the treatment groups (Table 3.1; overall mean ± s.e. SL, 18.40 ± 0.02 mm; body mass, 88.86 ± 0.38 mg) However, by the time the fish had utilised their maternal yolk supplement and were ready to feed independently (51-60 days after hatching), standard length, body mass and body condition differed significantly among treatments (Table 3.1). Fry from cort and T-treated eggs were smaller and lighter than those originating from controls (LSD post-hoc tests, standard length: p <0.01 and p <0.0001 respectively, Fig. 3.2a; body mass: p <0.01 and p <0.05 respectively, Fig. 3.2b). The T treatment had a greater effect in reducing skeletal length than body mass, resulting in these fry being in better body condition than those from cort-treated and control eggs (LSD post-hoc tests, p <0.01 for both, Fig. 3.2c). Fry SMR (expressed as total oxygen consumption per fish) was significantly repeatable within individuals (r = 0.77, $F_{152, 153}$=7.92, p <0.0001), but was not influenced by hormone treatment when expressed on a mass-corrected or whole animal basis ($F_{2, 133.18}$=0.07, p=0.931, Fig. 3.2d and $F_{2, 134.26}$=0.1, p =0.909, respectively).

Table 3.1. Results from linear mixed effect models of egg hormone treatment on body length (SL), mass and condition of brown trout at the hatching (alevin) and first feeding (fry) stages of development. Body condition was not calculated for the alevin stage. All analyses included family as a random factor.

	treatment effect		
response variable	d.f.	*F*-statistic	*p*-value
alevin SL	2,433	0.28	0.759
alevin body mass	2,433	0.43	0.652
fry SL	2,375	6.97	<0.001
fry body mass	2,375	6.16	<0.01
fry body condition	2,375	5.29	<0.01

Figure 3.2. Mean (± s.e.) standard length (a), body mass (b), body condition (c) and SMR (mass-corrected) of fry originating from eggs with elevated concentrations of cortisol and testosterone. Fry morphological (n = 10 sibling fry per family per treatment) and SMR (n = 4 -5 sibling fry per family per treatment) data were obtained from the same 10 families. Different letters represent significant differences between treatment groups, after controlling for among-family differences (see text for statistical analyses).



Behaviour

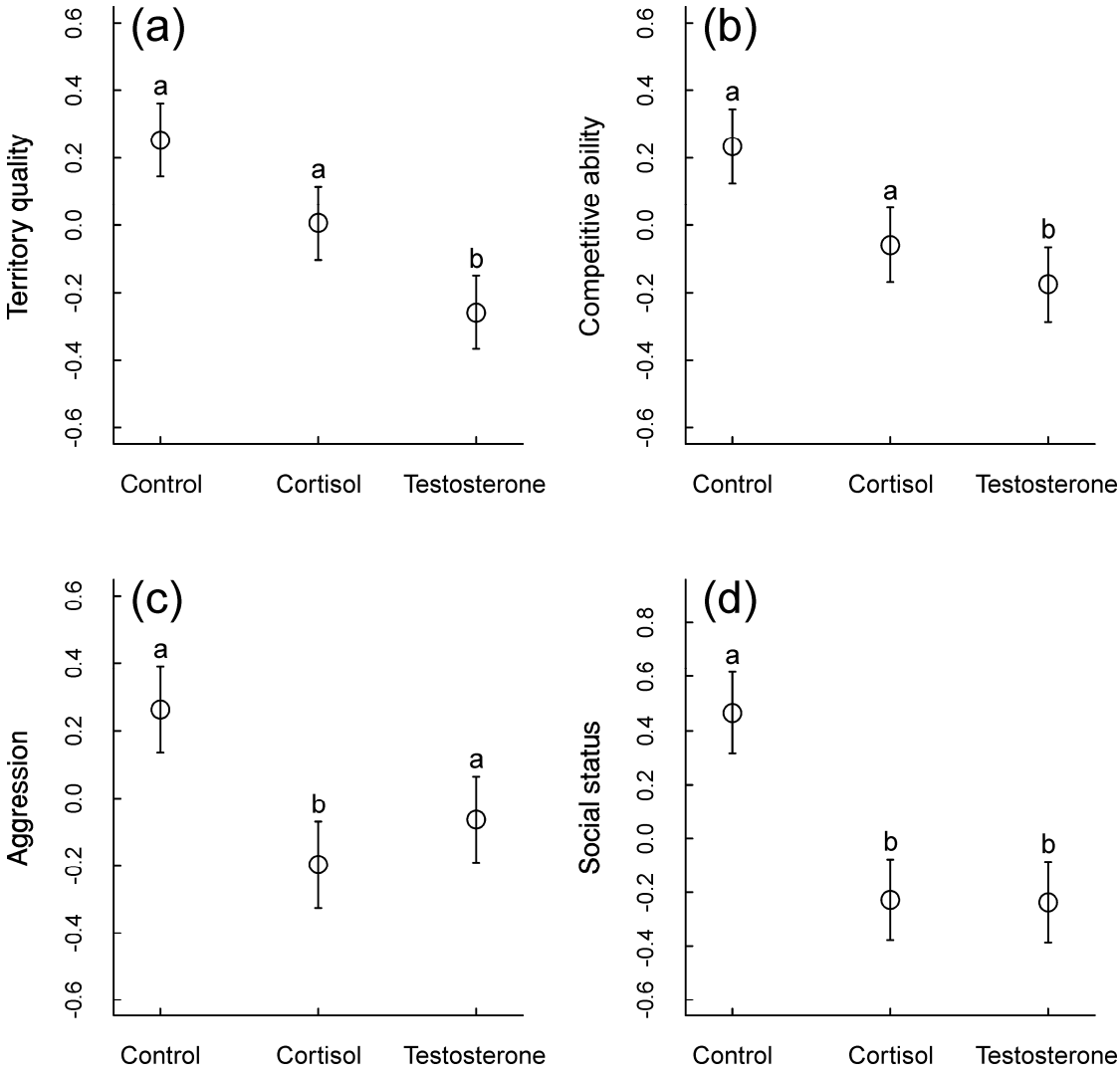
The differences in body size had implications for fry performance in simulated feeding territories, with body mass being the strongest determinant of, and affecting positively the quality of territory acquired, competitive ability and aggression (Table 3.2). However, egg hormone treatment had a significant independent effect on each behavioural attribute after controlling for body mass (Table 3.2). Thus, fry from T-treated eggs, despite being in better body condition, obtained poorer quality territories and were less competitive than

control fry of the same size (LSD post-hoc tests, territory quality p <0.01, Fig. 3.3a, competitive ability p <0.05, Fig. 3.3b). They were also less aggressive, however this effect was only significant at the p <0.1 level (LSD post-hoc test, p = 0.08, Fig. 3.3c). Hormone treatment also affected fry aggression (Table 2), with fry from cort treated eggs being less aggressive than control fry of the same size (LSD post-hoc test, p <0.05, Fig. 3.3c). Mass-corrected fry SMR was not a significant predictor of these behavioural attributes.

Table 3.2. Parameter estimates from linear mixed effect models of egg hormone treatment on behavioural performance of brown trout fry. Analyses controlled for fry age, body mass and mass-corrected SMR, and included family as a random factor. Non-significant terms (other than egg hormone treatment) were excluded from the final analyses. The effect of testosterone is represented by the intercept, estimates for the control and cortisol treatments are given in comparison to this value. See text for details of pair wise comparisons between treatments.

model	estimate	SE	d.f.	*t*-statistic	*p*-value
territory quality					
intercept (testosterone)	-1.338	0.280	143	-4.77	<0.0001
control	0.511	0.152	143	3.35	<0.01
cortisol	0.264	0.152	143	1.73	0.085
body mass	7.610	1.857	143	4.10	<0.0001
competitive ability					
intercept (testosterone)	-1.145	0.287	143	-3.98	<0.0001
control	0.409	0.156	143	2.62	<0.05
cortisol	0.116	0.156	143	0.75	0.457
body mass	6.836	1.904	143	3.59	<0.0001
aggression					
intercept (testosterone)	-1.121	0.342	104	-3.27	<0.01
control	0.325	0.181	104	1.80	0.075
cortisol	-0.135	0.181	104	-0.75	0.458
body mass	7.394	2.208	104		<0.001
social status					
intercept (testosterone)	-1.597	0.402	104	-3.98	<0.0001
control	0.702	0.212	104	3.31	<0.01
cortisol	0.009	0.212	104	0.04	0.968
body mass	9.515	2.590	104	3.67	<0.0001

Figure 3.3. Mean (± s.e.) territory quality (a), competitive ability (b), aggression (c) and social status (d) of fry originating from eggs with elevated concentrations of cortisol and testosterone in comparison to controls. Data are plotted as z-scores except for (d) which are principal component scores, based on a summation of the other three behavioural variables. Behavioural data were obtained for the same fry that were measured for SMR. Different letters represent significant differences between treatment groups (see text for statistical analyses).



Pearson correlations indicated that the three behaviours were significantly associated (competitive ability vs. territory quality, r = 0.746; aggression vs. territory quality, r = 0.686; aggression vs. competitive ability, r = 0.611; n = 108 and p <0.0001 for each correlation). PCA indicated that a single PC summarised 78% of the variation in the three behaviours, with high PC1 scores indicating individuals of high social status. Fry social status (i.e. PC1 score) was significantly affected by hormone treatment and fry body size (Table 3.2), with fry from cort and T-treated eggs being subordinate to control fry of the same size (LSD post-hoc tests, p <0.01 for both, Fig 3.3d).

DISCUSSION

The results from this study provide, to my knowledge, the first experimental evidence of a link between egg cort levels and offspring social status in fish. However, my prediction that increased egg levels of cort would increase offspring SMR and/or social status was rejected. Instead, offspring from cort-treated eggs were smaller at first feeding and this size difference partly explained their reduced status when exposed to socially competitive conditions. But even when controlling for body size, offspring from cort-treated eggs were less aggressive and of lower social status. Elevation of egg T levels resulted in offspring that were less competitive, occupied poorer quality territories and were also of lower social status than controls of the same size. However, this was in response to a likely pharmacological dosage of eggs. Thus, the biological significance of the T data in a functional context is unknown. Accordingly, I focus the remainder of the discussion on the effects of egg cort only, although T data are presented for the benefit of future studies, and for those that are interested in pharmacological effects of androgens. Offspring SMR was not associated with social status nor was whole-animal or mass-corrected SMR affected by cort (or T) treatment. These findings suggest that the effects of egg cort on social status are not mediated through an altered programming of SMR at this development stage. Although I did not detect statistically significant differences in fry SMR among treatment groups (when measured after the emergence stage), cort-treated fry were smaller. This suggests that cort-treated individuals may have had a higher SMR earlier in development (as found by Sloman 2010). In a range of vertebrates, prenatal exposure to increased androgens or glucocorticoids can have a substantial influence on offspring growth and behaviour (McCormick 1999; Groothuis *et al.* 2005; Kapoor & Matthews 2005; Uller *et al.* 2007; Love & Williams 2008; Chin *et al.* 2009). Despite a growing research focus on this topic, the effects of these hormones have largely been examined in birds and mammals. To my knowledge, the few studies investigating the consequences of embryonic hormone exposure in alternative study systems, such as fish, have not addressed their impact on both growth and behaviour, nor done so under relevant social conditions.

Body size effects of increased egg cortisol

Offspring that hatched from eggs with increased cort were smaller at the critical development stage of first feeding (hereafter, fry). This result is congruent with evidence

from other vertebrates that elevated egg or foetal glucocorticoids reduce offspring growth and body size (McCormick 1999; Hayward & Wingfield 2004; Kapoor & Matthews 2005; Meylan & Clobert 2005; Love & Williams 2008; Chin *et al.* 2009). However, my findings contrast with a study of trout which showed that elevated egg cort caused a transient increase in fry body mass (Sloman 2010). Unlike the present study, the fish investigated by Sloman (2010) had been feeding independently for approximately one week prior to the development of variation in body mass. Together, the results of this study and that of Sloman (2010) confirm that egg hormones have a strong effect on trout development, but the direction of this effect may depend upon factors such as egg hormone dose, food conditions or the use of hatchery versus wild stock.

In the present study the effects of increased egg cort on offspring body size were not evident until the point of complete yolk sac consumption This is a critical stage when the juveniles switch from being largely quiescent in the gravel, provisioned with maternal yolk, to active foraging in open water (Armstrong & Nislow 2006). What caused the reduction in fry body size? There is some debate about whether maternal cort might affect offspring body size among mammals via direct transfer of cort to the embryo or indirectly by influencing provisioning of the embryo. Consistent with previous studies (e.g. McCormick 1999; Love & Williams 2008), my results indicate the former, given that egg cort was manipulated after maternal provisioning had ceased (although I cannot exclude the possibility that cort of maternal origin might also have influenced egg provisioning). The proximate effects of egg glucocorticoids on fish development remain virtually unknown. Other studies have shown that increased egg cort can increase the somite pulsations, lateral flexions, heart rates and SMR of developing fish embryos (McCormick & Nechaev 2002; Gagliano & McCormick 2009; Sloman 2010), resulting in less endogenous energy being available for growth and a smaller larval size after hatching (McCormick & Nechaev 2002). My elevation of egg cort might have caused a transient increase in egg or alevin SMR, but with noticeable effects on growth not evident until the fry stage. This explanation would account for the absence of an effect on fry SMR but presence of an effect on fry body size. Regardless of the mechanism underlying the effects of cort, body size in newly-emerged salmonids can have important fitness consequences because competition for feeding sites is intense amongst fry and larger individuals may outperform smaller conspecifics in certain conditions (Einum & Fleming 2000b, this study).

Behavioural effects of increased egg cortisol

Studies based on natural bird and reptile populations demonstrate that elevated embryonic glucocorticoids affect fitness-related behavioural traits in offspring such as activity, begging and dispersal and invoke anti-predator behaviour and risk-averse behaviour (De Fraipont *et al.* 2000; Rubolini *et al.* 2005; Uller & Olsson 2006; Love & Williams 2008; Chin *et al.* 2009). I also observed significant size-independent treatment effects on behaviours related to social status. This demonstrates that the performance implications of increased egg cort levels for juvenile salmonids extend beyond reductions in body size. I encouraged the expression of social rank by providing spatially-limited simulated feeding territories (the area available for each triad approximated that required by a single fish). In these conditions, cort-treated individuals were less aggressive and were generally subordinate in comparison to control fry. In contrast, Sloman (2010) found that cort-treated trout fry were more aggressive against their mirror image than controls. However, in contrast to this study, she used fish that were reared and tested in isolation and so had never encountered aggression. Experience of social interactions may modulate the tendency to initiate aggression, and my results suggest that, under the more natural conditions used in my study, the net effect of the cort treatment was to reduce the likelihood of fish attaining high status when competing for territories against conspecifics.

Ecological consequences of variation in egg cortisol

The results of the current study suggest that elevated levels of egg cort may be detrimental in juvenile brown trout. Indeed, the phenotypic responses of vertebrate offspring to egg hormone content are often viewed solely from an offspring perspective and labelled simply as positive or negative. However, recent evidence in birds suggests that increased levels of glucocorticoids in eggs laid by low quality mothers may match maternal quality to offspring demand, ultimately increasing maternal fitness (Love & Williams 2008). Current interest in the evolution of developmental plasticity has generated a number of hypotheses that identify hormones as a likely mediator of adaptive environmental effects on early development. These hypotheses propose that environmental influences during development may prepare the offspring phenotype for conditions it will likely encounter later in life (Monaghan 2008). Substantial within and among clutch variation in the androgen content of bird eggs has lent support to the hypothesis that avian

mothers may 'program' offspring in anticipation of post-hatching conditions (reviewed by Groothuis *et al.*, 2005). Whether female teleost fish can actively manipulate egg hormone deposition is currently unknown. If egg hormone levels reflect those of the mother, however, my manipulation of egg cort levels might simulate an effect that would occur in mothers that are stressed prior to spawning. The apparently detrimental effects of cort observed in the current study may then be a consequence of the experimental conditions under which it was assessed: rearing the fry in relatively benign conditions may have constituted a mismatch between the actual environment of the offspring and the environment 'anticipated' by the elevation of egg cort levels.

Alternatively, it is possible that variable hormone deposition in eggs may represent a maternal bet-hedging strategy for producing within or among clutch heterogeneity in offspring phenotypes. Several studies in avian taxa have demonstrated intra-clutch variation in yolk cort, leading to the hypothesis that such variation may be related to the benefits of enhancing or reducing the competitiveness of offspring (Love *et al.* 2008; Love *et al.* 2009). This has been little studied in fish but is the subject of ongoing research. In comparison with birds, ectothermic species such as fish have very different reproductive systems. Female salmonids produce hundreds or thousands of eggs and provide no further care after oviposition. Additionally, a development period of several months separates egg-laying and offspring emergence from the nest, meaning that spawning females may have difficulty anticipating the environment likely to confront their emerging offspring. Thus, the likelihood that salmonid mothers can adaptively 'program' offspring to match a particular future environment appears low. Instead, it seems more plausible that fitness may be enhanced in mothers that generate heterogeneous offspring via variability in egg cort levels (either within or among egg batches). Emergence is a period of critical importance for juvenile salmonids because an individual's chances of survival depend largely on its ability to compete and defend an area within its natal stream (Elliott 1994). From the perspective of maternal fitness, variation in offspring social status and body size at this time may be beneficial. Large dominant fry are more likely to establish territories near the nest site (Metcalfe 1998; Bujold *et al.* 2004), which would promote the dispersal of smaller subordinates to potentially productive territories in areas of lower population density downstream (Bujold *et al.* 2004). Heterogeneity in social status and body size among settled individuals near clumped nests may also be beneficial because it may reduce aggression among siblings and conspecifics of the same age class. The microhabitat (e.g.

water depth and velocity, stream topography) within spawning streams is highly variable over small spatial scales, which may suit a range of different phenotypes. This may explain why field experiments using juvenile salmonids have demonstrated that subordinate individuals can perform as well dominant conspecifics within the same stream (Martin-Smith & Armstrong 2002; Harwood *et al.* 2003). Moreover, genetically heterogeneous groups of juvenile salmonids are known to achieve considerably higher biomass than homogenous groups, which probably results from a broader utilisation of microhabitats and niches (Griffiths & Armstrong 2001). Similar principles may apply within different contexts. Mothers may therefore generate heterogeneity in offspring via non-genetic mechanisms, such as hormones, that increase the prospects of offspring survival in unpredictable environments and therefore the maternal contribution to populations.

I have presented novel data demonstrating that egg cort content affects fish offspring body size and social status in competitive conditions. These results concur with the general effects of elevated embryonic glucocorticoids that have been observed across vertebrate taxa with different life history strategies, underlining a general mechanism for the adjustment of offspring phenotypes. In salmonids, I suggest that this mechanism may have evolved to increase maternal fitness by producing variability in offspring. Further investigations in semi-natural settings with variation in social density and resource availability, will help elucidate the causes and consequences of hormone-mediated maternal effects.

CHAPTER 4. WITHIN-CLUTCH DIFFERENCES IN THE PHENOTYPES OF JUVENILE FISH DEPEND ON THEIR LOCATION WITHIN THE EGG MASS AND MATERNAL DOMINANCE RANK

SUMMARY

Theory suggests that when the post-natal environment of offspring is unpredictable, mothers may gain fitness advantages by diversifying the traits of offspring within a clutch. I investigated if within-clutch differences in the phenotypes of juvenile fish were systematically related to the position in the egg mass where each individual developed during oogenesis. I sampled eggs from the front, middle and rear thirds of the egg mass in female brown trout of known dominance rank. In the resulting juveniles, I then measured traits that are related to individual fitness: body size, social status and standard metabolic rate (SMR). When controlling for differences among females in mean egg size, siblings from dominant mothers were initially larger (but had a lower mass-corrected SMR) if they developed from eggs at the rear of the egg mass. However, heterogeneity in the size of siblings from different positions in the egg mass diminished in lower ranking females. Location of the egg within the egg mass also affected the social dominance of the resulting fry, although the direction of this effect varied with developmental age. This study provides the first evidence of a systematic basis for within-clutch differences in the phenotypes of offspring in a highly fecund organism.

INTRODUCTION

Maternal investment in offspring is a critical life-history decision that is under strong selective pressure (for recent reviews see Green 2008; Marshall, Allen & Crean 2008; Uller 2008). Environmental effects on the mother can lead to variation in her growth, condition and physiological state that can be transmitted to offspring via non-genetic resources provided to eggs (Mousseau & Fox 1998). Mothers adjust the phenotype of their offspring (e.g. size, energy content or biochemical composition) in response to a range of environmental factors including food availability, photoperiod and the intensity of inter- and intraspecific competition (Mousseau & Fox 1998b). The pattern or extent of maternal

investment can depend on the mother's physiological state or dominance status. For example, in oviparous vertebrates the rank of a mother within a dominance hierarchy can influence the amount of androgen hormones transferred to her eggs (Whittingham & Schwabl 2002; Tanvez et al. 2008). Such variation can have implications for her fitness because egg androgens influence offspring growth, survival and behaviour (Groothuis et al. 2005). Indeed, there are numerous examples in the literature demonstrating that phenotypic variation among juveniles is influenced by environmental conditions which affect the state of the mother (Verboven et al. 2003; Gagliano & McCormick 2006; Warner, Lovern & Shine 2007).

Mothers can also adjust the phenotypes of their offspring within a clutch. For example, in many species of birds there can be substantial differences between the size of the first and last-laid eggs within a clutch (Slagsvold et al. 1984). This size disparity has been interpreted as a maternal tool for manipulating the size of the brood in response to prevailing environmental conditions (Slagsvold et al. 1984). What about mothers that are unable to predict the conditions that their offspring are likely to experience (e.g. because of a prolonged egg development time)? Numerous theoretical arguments predict that mothers may gain fitness advantages by producing variable offspring phenotypes (typically size) in unpredictable environments (see Crean & Marshall 2009 and references therein). Recent evidence from teleost fish and marine invertebrates demonstrates that within-clutch variation in offspring size does indeed increase when environments are unpredictable (Crean & Marshall 2009). Such bet-hedging interpretations of within-clutch variation in maternal investment seem particularly applicable to taxonomic groups, such as fishes, that spawn large clutches of small eggs almost simultaneously. After spawning, embryos, larvae and juveniles of many fishes receive little or no parental care, meaning that mothers can influence the development of their young only through their investment in each egg. Furthermore, egg development may be prolonged (up to 6 months in salmonids) and juvenile fish inhabit environments with high spatial and temporal heterogeneity, conditions under which variation in sibling phenotypes can be expected to increase maternal fitness (Crean & Marshall 2009). Finally, in many species, intense competition among juveniles for feeding territories results in high density-dependent mortality of juveniles (Elliott 1994). The combination of pronounced environmental heterogeneity, prolonged egg development and high density-dependent mortality means that clutches of sibling fish that

display high phenotypic variation potentially have higher average survival probabilities and, consequently, enhance maternal fitness.

The survival and growth of juvenile salmonid fish in freshwater streams is typically influenced by a dominance/territoriality based social structure (Metcalfe 1998). Two phenotypic traits primarily determine juvenile success in these systems. First, larger eggs give rise to larger juveniles that have a survival advantage under poor growth conditions (Hutchings 1991; Einum & Fleming 1999). Second, competitive ability is positively related to juvenile standard metabolic rate (SMR) (McCarthy 2001). SMR is the lowest rate of oxygen consumption, when measured in an inactive, post-absorptive ectotherm and corrected for temperature (McNab 2002). SMR thus represents the minimal energy cost of maintenance. Juvenile salmonids with higher SMR's can process food faster and so potentially gain a further growth advantage through their ability to feed more frequently (Millidine *et al.* 2009). Like the relationship between egg size and juvenile fitness, environmental conditions are also likely to affect the particular SMR phenotypes that most benefit juvenile salmonid fishes during their early development. In productive environments juveniles with a relatively high SMR are more likely to be competitively dominant, allowing them to gain productive territories and grow faster (Metcalfe *et al.* 1995; McCarthy 2000). However, when food is limiting or patchy, a high SMR may be of no advantage because gains in food intake are unable to offset the higher 'maintenance costs' of this phenotype, leading to no relationship between SMR/dominance and growth (Reid, Armstrong & Metcalfe 2011; Burton et al. 2011b). Metabolic rate is a fundamental physiological trait that is correlated with many aspects of individual physiology and behaviour (Careau et al. 2008; Biro & Stamps 2010) and, correspondingly, is linked with fitness (Burton et al. 2011b). In the absence of environmental heterogeneity, mothers might be predicted to produce a single optimum metabolic rate that varies minimally among siblings within a given clutch. However, natural environments tend to be highly variable and correspondingly, 2 – 3 fold variation in SMR has been reported within-clutches by a number of authors (reviewed by Burton et al. 2011b). While the precise mechanism that controls this variability remains unknown, maternal provisioning of eggs with steroid hormones is a likely candidate because hormones, such as cortisol, can influence energy metabolism (Sloman 2010), and also affect juvenile growth rates and behaviour (Hayward & Wingfield 2004; McCormick 2006; Uller & Olsson 2006; Burton et al. 2011a).

The role of maternal hormones in controlling within- and among-clutch variation in offspring phenotype has been widely studied in taxa producing small clutches, such as birds (Groothuis et al. 2005). In many avian species, egg levels of testosterone or cortisol systematically increase or decrease over the laying sequence of the clutch and can have substantial effects on offspring phenotypes (Groothuis et al. 2005; Love et al. 2008). For fishes, preliminary evidence suggests that cortisol levels in the eggs of female trout can vary systematically according to the position of eggs within the ovary during oogenesis (Suter 2002). Here I test whether the phenotype of juvenile trout differs systematically according to the position in the egg mass where individual eggs were located during oogenesis. In addition, because of the known role of maternal dominance status on offspring investment, I test whether this relationship may be modulated by the social status of the mother.

MATERIALS AND METHODS

Maternal dominance ranking and crosses

The experiment was based on clutches taken from 12 female brown trout (*Salmo trutta*, hatchery F1 generation of wild fish from Loch Broom in Perthshire, Scotland). However, to ensure that these females covered a broad spectrum of dominance ranks, the sampled clutches were selected from a larger pool of spawning female trout. Therefore at the start of the experiment, approximately 6 weeks prior to spawning, 34 female brown trout were anaesthetised, weighed (range 427 – 1363 g), measured for fork length (L_F, range 32 – 47 cm) and implanted with a passive integrated transponder (PIT) tag in the intra-peritoneal cavity. The trout were sorted randomly into 4 tanks (2 m diameter) each containing 8 - 9 individuals and were allowed to recover and acclimatise for one week during which an excess of opaque grey PVC pipe shelters (40 cm long, 15 cm diameter) were provided in an otherwise bare tank. The tanks were illuminated by natural light supplemented by overhead fluorescent lights during daylight hours. The size of the pipes was originally chosen so that only a single fish could shelter within each one at any one time, although I note that on a few occasions two fish were observed sharing the same tube. Shelters were weighted to hold them stationary on the floor of the tank.

Following acclimatisation, the simple shelters were removed and replaced with a single shelter so that there was competition for this scarce resource, allowing us to assess dominance. This method of dominance ranking was used in place of more commonly used methods such as competition for food (e.g. Metcalfe et al. 1995) because female trout are largely non-feeding in the weeks prior to spawning. However, during this time they prefer habitats with overhead cover (Armstrong et al. 2003). This habitat preference allowed us to quantify relative dominance by recording priority of access to the single shelter. The shelter was a PVC pipe of approximately the same dimensions as used earlier, but this time it was fitted with a PIT tag detector that monitored the presence and identity of any PIT-tagged trout residing within it (Wyre Micro Design Ltd, Poulton-le-Fylde, Lancashire, UK). The sensitivity of the detectors was adjusted to ensure that tags were only recorded when inside the shelter. Shelters were connected to a computer that automatically recorded the PIT tag detections every minute.

PIT tag records were analysed daily, and whenever an individual fish accounted for more than 50% of the occupancy records on at least 3 of 5 consecutive days it was judged to be the dominant fish in the tank, and was removed to a separate holding tank. The process was then repeated with the remaining fish, thereby assigning a dominance rank to each fish based on a serial removal process (following the protocol of Metcalfe et al. 1989). The procedure was terminated when there were 2-3 fish remaining per tank, and these fish were given a joint low rank in cases where their dominance could not be determined unambiguously. Daily shelter occupancy varied between fish during the ranking period, ranging from 0 records per day (shelter not used at all by that fish) to 1166 (shelter used for 32% of the day). Fish tended to use the shelter more intensively during daylight hours, typically taking occupancy between 6 - 8 am and emerging at ~9 pm. Nevertheless, on several occasions fish occupied shelters overnight and, hence, the shelter-use data were expressed as percentage occupancy per day (24 h).

To gain an indication of whether my measure of dominance was repeatable, I commenced a second serial removal (with the same individuals present in the same tanks) as soon as possible after the first set was completed. Unfortunately, the female trout ovulated and became ready to spawn earlier than expected and I could only complete the first phase of the second serial removal. Therefore, dominance behaviour during the second test was ranked based on overall occupancy of the shelter during the first two days of the

second trial. Individual dominance scores, although based on slightly different methods, were consistent across the two trials (Spearman's rank correlation, rho = 0.84, n = 25, $p < 0.0001$) and were thus averaged to quantify the relative dominance (hereafter 'dominance rank') of each fish. Maternal dominance values are presented on a decreasing scale (e.g. the most dominant fish was given rank 1 and the most subordinate fish given rank 7, see Table S1, *Supplementary Material* for further details). Ovulation status was determined by netting and lightly squeezing the sides of each female, at the time of transfer between experimental and holding tanks during the serial removal procedure, to detect the presence of loose eggs within the body cavity. A total of 8 trout died during the ranking period, so that my sample size of ranked fish was 26 (7 fish in each of 3 tanks, 5 fish in 1 tank). The 12 clutches used in the experiment were selected from 18 females that were ready to spawn on the 4th November of 2010, and were chosen to represent as wide a spectrum of dominance ranks as possible. Thus, 4 females were selected from each of the 3 tanks (L_F range 360 – 463 mm; mass range 533.1 – 1042.5 g, measurements made after removal of eggs; see Table S1, *Supplementary Material* for further details).

A possible criticism is that my dominance ranking method of adult females might have measured a preference for shelter or 'shyness', especially because I did not quantify the frequency and intensity of aggressive interactions among mothers. However, overt displays of aggression are not necessarily a requirement for the establishment and maintenance of a social hierarchy (Sapolsky 2005). Moreover, the female fish actively sought out the shelters in each tank, and were frequently observed trying to enter shelters that were already occupied. In several cases, 3 – 4 fish were clustered around the outside of an occupied shelter and shelters were always re-occupied by a new fish after the 'resident' fish had been removed as part of the serial removal process. Hence, it would seem counterintuitive that the most 'shy' individual could out-compete the others and gain access to the shelter.

For each selected fish, separate batches of eggs were obtained from the front, middle and rear third of the egg mass (by dissection). To do this, an incision was made along the mid-ventral line from the anus to the pectoral girdle and the egg mass in the abdominal cavity divided by eye along its length into roughly equal thirds; each third was removed, weighed to obtain total egg mass, and then fertilised using milt from a single male across all batches. The spatial configuration of eggs within the ovaries is likely maintained from

vitellogenesis (the main period of egg growth, where the developing eggs are bound within a cellular sheath known as the follicle layer) through to ovulation (after the follicle layer of each ovary has ruptured and eggs are released into the abdominal cavity). With the exception of one fish, subsamples of eggs were taken from each female and preserved in buffered formalin for later counting to determine individual egg mass. Unfortunately I did not collect egg mass measurements from each region of the egg mass.

Offspring rearing conditions

The fertilised eggs were transferred to the University of Glasgow, where they were reared at 5°C in egg baskets that retained the separate ovary position and family group identities. After hatching, the water temperature was slowly increased so that it reached ~10°C when the juveniles were ready to begin independent feeding (after consumption of the maternal yolk sac). Once they reached the first-feeding stage (55-59 days after hatching), groups of 50 sibling juveniles from each ovary position per family were transferred to round 5 litre plastic containers (sides and floor replaced with stainless steel mesh) that were suspended in one of five re-circulating 1 m^2 tanks (average temperature, 9.45 °C ± s.e. 0.02). The top of each container was half-covered with black plastic to provide overhead shelter. Containers holding siblings from the 3 different egg mass positions were grouped together in triplicates and rotated haphazardly within each tank once per week, so that all juveniles experienced similar developmental conditions. Juvenile mortality among the different regions of the egg mass during the rearing period was low (mean percentage mortality ± s.e.; front of egg mass, 6 ± 1%, middle of egg mass; 5 ± 1%, rear of egg mass, 6 ± 2%, n = 12 families).

Approximately 20% of the water in each tank was changed every 2 - 3 days during routine cleaning, and juveniles were fed ad libitum amounts of chopped bloodworm and powdered food daily (Micro Harmony, EWOS, West Lothian, Scotland). A minimum of 4 hours before being placed in respirometry chambers for measurement of SMR (see below), the juveniles were anaesthetised and marked according to egg mass position with different colour Visible Implant Elastomer tags (VIE, Northwest Marine Technology, Washington, USA); each fish was given a single mark between the dorsal fin and lateral line and the colour code was alternated so that subsequent behavioural observations were conducted blind with respect to egg mass position. I believe that use of the term SMR is justified

(over the closely related term RMR) because juveniles were left to acclimate in the chambers overnight and measurements of oxygen consumption did not commence until17 - 20 h later.

Offspring phenotype

Representative egg mass values (referred to hereafter as 'egg sizes') were estimated by counting the number of eggs in a weighed subsample (range 3.9 – 11.8 g) from each female. To test whether position within the egg mass influenced subsequent offspring body size, 10 juveniles from each egg mass position per family were sacrificed and preserved in 5 % buffered formalin (Fleming & Ng 1987) at the onset of independent feeding, but before they were given exogenous food. The preserved juveniles were subsequently weighed (to 0.0001 g).

Open flow respirometry was employed to investigate whether position within the egg mass influenced offspring SMR. The protocol followed that of Burton et al. (2011a) with minor modifications. Randomly chosen juveniles (between the ages of 18-49 days after the first feeding stage) were placed in cylindrical polypropylene respirometry chambers (vol. 20 ml) through which O_2-saturated and UV-sterilised water was pumped at a constant rate by a peristaltic pump from a header tank. A rack of chambers arranged in parallel allowed the oxygen consumption rates of 18 juveniles (chosen such that there was one fish from each egg mass position from each of six families) to be measured on the same day. The juveniles were left to acclimate in the chambers overnight and measurements commenced 17 - 20 h later, by which time they had settled and evacuated their guts. Previous studies of juvenile salmonid fry have demonstrated a stable oxygen consumption rate after this period of acclimation (Metcalfe et al. 1995). To minimise activity, juveniles were kept in semi-darkness by placing a black cloth over the respirometry chambers, while low flow rates (average 0.18 l h^{-1} ± s.e. 0.001) removed the need for active swimming. Flow rates were calculated by collecting the water outflow from each tube in a beaker over a measured time period (minimum 2 min) and then weighing it (to 0.001 g) and water temperature averaged 10.9 ± s.e. 0.02° C.

The reduction in water oxygen concentration due to juvenile respiration was measured with a Fibox 3 temperature compensated oxygen meter (Loligo Systems, Tjele, Denmark).

A flow-through fiber-optic cell with integrated planar oxygen sensor (PSt3 oxygen sensitive coating, Presens, Regensburg, Germany) was connected temporarily to the outflow of each respirometry chamber. The flow through cell was calibrated with a two-point calibration of oxygen free water and oxygen saturated water. Oxygen free water was prepared by dissolving ca. 1 g sodium sulphite (Na_2SO_3) in ca. 100 ml of water. Oxygen saturated water was prepared by simultaneously stirring and aerating ca. 100 ml of header tank water. Metabolic rates ($\dot{V}O_2$, ml O_2 h^{-1}) of individual fish were calculated according to the equation:

$$\dot{V}O_2 = V_W \cdot \Delta C_W \cdot \beta O_2$$

where V_W is the flow rate (l h-1) of water through the respirometry chamber, ΔC_W is the percentage difference in oxygen concentration between water in-flow and out-flow and βO_2 is the capacitance of oxygen in the water. The oxygen concentration of water flowing into each chamber was determined in reference to the water exiting an empty (fish-less) control chamber. Measurements of the control chamber were made at the beginning and conclusion of each day. Two measurements of the control chamber were made to confirm that the O_2 saturation of the inflow water, and the performance of the O_2 sensor, were stable throughout the day. Measurements of outflow water oxygen concentration and temperature were logged (software Oxyview PST3v602, Presens, Regensburg, Germany) every 10 s for each fish over a 5 min period (minimum) or until the oxygen concentration had stabilised (to account for fluctuations caused by sensor movement between chambers). Three replicate measurements of metabolic rate were made for each juvenile between 08:00 and 15:00 h (with a minimum interval of 90 min between measurements). SMR was calculated as the average of these measurements and so accounts for any diurnal variation in metabolism equally for all fish. During each measurement of metabolic rate, the activity rate (time in seconds not spent in an inactive state) of each juvenile was monitored over a 3 min period using an angled mirror positioned below the respirometry chambers because they could not be observed from above. These data were collected to enable any potential among-individual variation in activity levels to be accounted for in subsequent statistical analyses. The juvenile were anaesthetised and weighed (to 0.001 g) after measurements of metabolic rate. SMR data were obtained for 8 – 9 juveniles from each egg mass position for each of 12 families (n = 301 juveniles in total).

After being screened for metabolic rate the juveniles were allocated into family triads containing one sibling from each egg mass position. Each triad was then placed in a compartment of a stream tank through which water was re-circulated with flow rate of 1.3 cm s^{-1} as described in Burton et al. (2011a). The area of these compartments (0.025 m^2) approximated the predicted territory size (0.026 m^2) of a first feeding juvenile brown trout (30 mm L_F) (Grant & Kramer 1990), thereby increasing the likelihood that the three fish would compete. The juveniles were then allowed to acclimate for 2 days prior to a 2-day period of behavioural observations. During the acclimation period, the juveniles were fed ad libitum amounts of bloodworm that were pippetted just beneath the water surface at the upstream end of each compartment. Behavioural trials were conducted in a temperature-controlled room (average water temperature 10.6 °C ± s.e. 0.02). The relationship between position within the egg mass and subsequent offspring behaviour was assessed according to the protocols described in (Burton et al. 2011a). Briefly, I assessed the relative social status of the three individuals in each tank by monitoring their ability to compete for food and territory space, together with the outcome of any overt aggressive interactions. These three indicators of social status are referred to as competitive ability, territory quality and aggression hereafter. Each triad was only tested once. Behavioural data were obtained for 8 juveniles from each egg mass position for each of 12 families (n = 288 total). Due to practical constraints it was only possible to measure a relatively small number of fish at a time. Thus, it was unavoidable that fish of different ages were used throughout the course of the experiment.

Data analysis

To analyse relationships between egg mass position and subsequent offspring phenotypes, I fitted linear mixed effect (LME) models, with family as a random factor and egg mass position (front, middle or rear) as a fixed categorical variable. Details of specific models are outlined below. The relationship between juvenile body mass at the first feeding stage of development and egg mass position was analysed with egg size and maternal dominance rank as additional explanatory variables (including all two way interactions). This analysis omitted data from one of the families because the preserved egg samples were missing.

Correlations among the first, second and third measures of SMR for each fish were calculated using Kendall's coefficient of concordance. The relationship between SMR of the juveniles and position within the egg mass was modelled with water temperature, individual activity level, age (days since the first feeding stage of development) and maternal dominance rank as additional continuous variables (including the two way interactions between age, dominance rank and egg mass position). Activity level was included here to account for potential inflation of SMR with increased activity, and age was included to account for potential changes in SMR during early development. Respirometry batch (i.e. date of SMR measurement) was included as an additional random variable to account for potential among-batch differences in measured metabolic rates. Prior to analysis, rates of standard metabolism were corrected for differences in body mass by calculating the residuals from a regression of SMR on body mass (both values log transformed).

The territory quality and competitive ability protocols yielded data with a different range of possible values compared to the aggression protocol. To account for this, the raw values for each of these protocols were normalised within each triplicate by subtracting the triplicate mean from each individual score and dividing this by the standard deviation of the triplicate. Associations between the normalised scores of the three behavioural protocols were described using Kendall's coefficient of concordance and then summarised with principal component analysis (PCA), omitting data for the few trials where no aggression was observed (n = 18 from 96 trials). This resulted in a single principal component (PC1) summarising all three behaviours as a general index of juvenile social status. Individual PC scores were then analysed in an LME model comparing egg mass positions (fixed categorical variable) with family as a random variable and juvenile body mass, age (days since first feeding), residual SMR and maternal dominance rank as continuous variables (including all two way interactions). Fry age was included into this model to account for potential changes in behaviour during ontogeny. Body mass and mass-corrected SMR were included as covariates because they are known correlates of behaviour in salmonid fishes (see Introduction). This analysis omitted data for the eighteen trials where aggression was not observed.

Model selection and validation was performed according to protocols outlined in Zuur et al. (2009). Likelihood ratio tests were used to sequentially compare the log-likelihoods

(an indicator of model goodness-of-fit) of nested models that were parameterised using maximum likelihood (ML) criteria. More parsimonious models were retained if an increase in the log-likelihood ratio statistic was statistically significant ($p < 0.05$). Final LME models were then re-fitted with restricted maximum likelihood (REML). Prior to model selection, I calculated variance inflation factors (VIF) for all candidate explanatory variables. A threshold value of 3 – 5 can be used to remove collinear variables (Zuur et al. 2009). VIF's were less than 2 in all cases, indicating that my results are not sensitive to collinearity. All statistical analyses were conducted in R version 2.13.1 (R Development Core Team 2011).

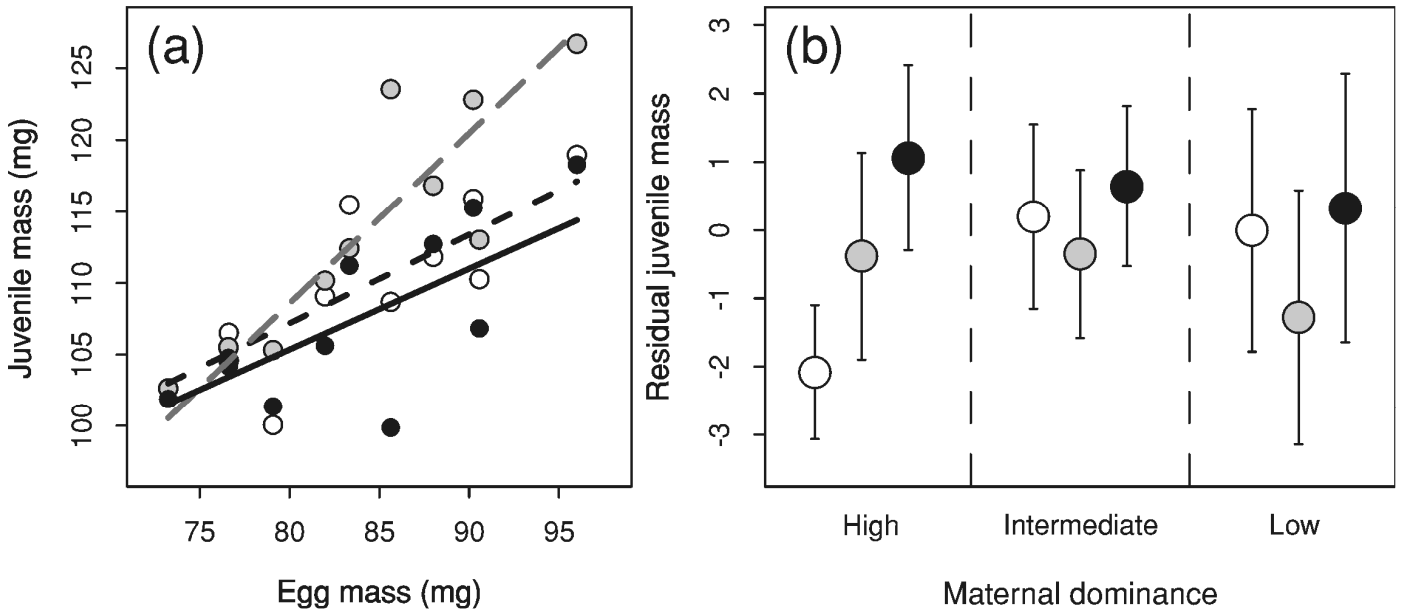
RESULTS

Egg size, maternal dominance rank and position within the egg mass all influenced the size of juveniles at the first feeding stage (i.e. after they had absorbed their yolk reserves and begun feeding on exogenous food). Overall, egg size had a positive effect on juvenile body size (Table 4.1, Fig. 4.1a). However, the strength of this effect was contingent upon the location within the mother's egg mass from which the egg had originated (hereafter referred to as 'egg mass position'). In females that produced small eggs, egg mass position had little effect on juvenile body size. However, as egg size increased, juveniles from the middle part of the egg mass were larger than those from the front and the rear of the egg mass (egg size × egg mass position interaction, Table 4.1, Fig. 4.1a).

Table 4.1. Summary of the final linear mixed effect model explaining variation in the mass of juvenile trout at the first feeding stage of development. The analysis controlled for the effects of egg size (mean value per family), maternal dominance rank and the position within the egg mass from which the fish originated (front, middle and rear). Parameter estimates are given as treatment contrasts with juveniles originating from the front of the egg mass set as the intercept. Family was included as a random variable. Treatment contrasts between juveniles from the middle and rear part of the egg mass (when the rear part is set as the intercept) are given in bold.

	estimate ± SE	*t*-statistic	*p*-value
intercept (front of egg mass)	55.88 ± 14.03	3.981	<0.001
egg size	0.62 ± 0.18	3.500	<0.001
maternal dominance	0.54 ± 0.81	0.660	0.510
middle of egg mass	-38.69 ± 13.47	-2.871	<0.01
rear of egg mass	1.99 ± 13.47	0.148	0.883
maternal dominance × middle of egg mass	-1.82 ± 0.78	-2.325	<0.05
maternal dominance × rear of egg mass	0.24 ± 0.78	0.307	0.759
egg size × middle of egg mass	0.57 ± 0.17	3.332	<0.01
egg size × rear of egg mass	-0.06 ± 0.17	-0.332	0.740
middle vs. rear of egg mass	-40.68 ± 13.47	-3.019	<0.01
maternal dominance × middle vs. rear of egg mass	-2.06 ± 0.78	-2.632	<0.01
egg size × middle vs. rear of egg mass	0.62 ± 0.17	3.664	<0.001

Figure 4.1. (a) Relationship between juvenile body mass at the first feeding stage of development and egg mass (mean value per family). (b) Relationship between residual juvenile body mass and maternal dominance. In both cases juvenile mass depends on the position within the egg mass (front, middle or rear) from which the juveniles originated. In (a) the lines are the predicted values for each egg mass position from the final LME model (see Table 4.1 for statistical analysis). Clear circles/black dashed line = front of egg mass, grey circles/grey line = middle of egg mass, black circles/solid black line = rear of egg mass. The predicted values are based on a female of average dominance (2.95). Juvenile mass data are mean family values (n = 10 per egg mass position). In (b) data are plotted as mean residual values (± s.e.) averaged by egg mass position across females of different dominance status: clear circles - front of egg mass, grey circles - middle of egg mass, black circles - rear of egg mass. High dominance; mothers ranked between 1.0 and 2.0 (n = 4), Intermediate dominance; mothers ranked between 2.5 and 3.0 (n = 4), Low dominance; mothers ranked between 4.0 and 5.5 (n = 3). The residuals were derived from a LME model of juvenile body mass with family as a random factor. Mean family egg mass, egg mass position and their interaction were included as explanatory variables because the juvenile mass - egg mass relationship differed among sections of the egg mass (see Table 4.1). Although maternal dominance is treated as a continuous variable in the analysis (Table 4.1), effects have been plotted categorically to aid visual interpretation.



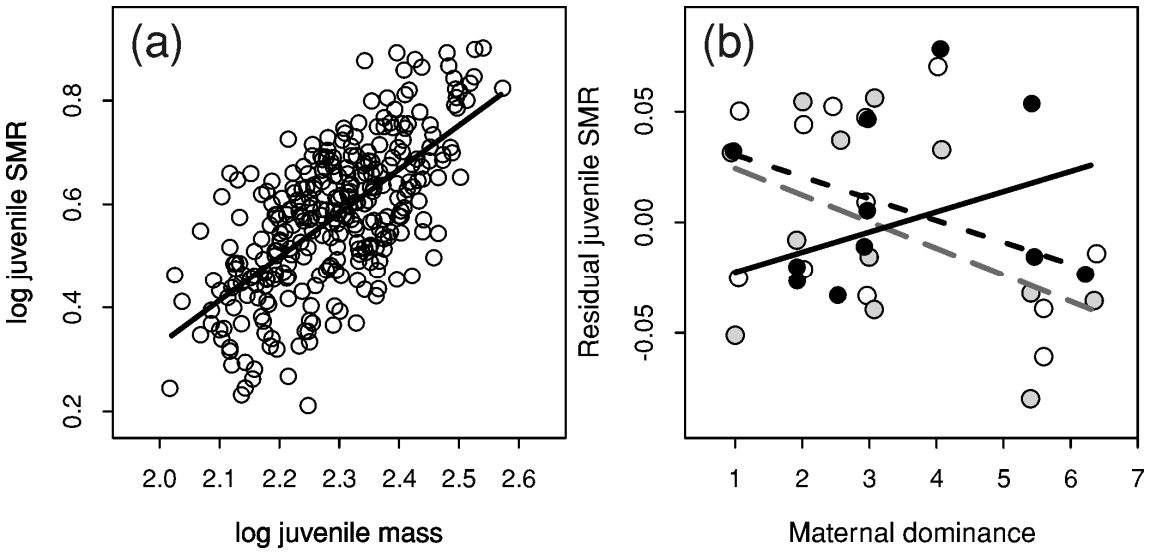
Maternal dominance rank also influenced juvenile body size, independently of egg size, although the strength and direction of this effect was dependent on egg mass position. Thus, as maternal dominance decreased, the mass of juveniles from the middle part of the egg mass decreased relative to that of juveniles from the front and rear of the egg mass (Table 4.1, Fig. 4.1b). In the full sample of female spawners, maternal dominance ranks were not associated with body size (general linear model: $F_{1,23} = 1.62$, $p = 0.22$), nor were the size of eggs produced by individual females related to their body size or dominance rank (sequential removal of terms in general linear model: body size, $F_{1,20} = 0.51$, $p = 0.48$: maternal dominance rank, $F_{1,21} = 1.09$, $p = 0.31$). Together these results indicate that both dominant females and females that laid large eggs (irrespective of their own body size) produced offspring that differed in body size depending upon the position within the egg mass where those individuals developed. Conversely, the size of juveniles from subordinate females or females laying small eggs differed less with respect to their developmental position within the egg mass.

The three replicate measurements of SMR (when expressed as total oxygen consumption per individual) were highly correlated with each other (Kendall's coefficient of concordance, W = 0.826, 300 d.f., $p < 0.0001$). The mean of these three SMR values was log-linearly related (Fig. 4.2a) to the individual's body mass according to the equation log SMR = 0.847 (log body mass mg) – 1.366 ($r^2 = 0.44$, n = 301, $p < 0.0001$). After correction for the effect of body mass, both maternal dominance rank and egg mass position influenced the standard metabolism of juveniles. Juvenile offspring of dominant mothers had relatively higher metabolic rates if they originated from the front and middle parts of the egg mass (Table 4.2, Fig. 4.2b). However, as maternal social status decreased, this trend reversed: juveniles from the rear section of the egg mass of subordinate mothers had higher SMR's than those from the front and middle of the egg mass (maternal dominance × egg mass position interaction, Table 4.2, Fig. 4.2b).

Table 4.2. Summary of the final linear mixed effect model explaining variation in the residual standard metabolic rate (SMR) of juvenile trout. The analysis controlled for the effects of maternal dominance rank and the position within the egg mass from which the fish originated (front, middle and rear). Parameter estimates are given as treatment contrasts with juveniles originating from the front of the egg mass set as the intercept. Family and respirometry batch were included as crossed random variables. Treatment contrasts between juveniles from the middle and rear of the egg mass (when the rear part is set as the intercept) are given in bold.

	estimate ± SE	*t*-statistic	*p*-value
intercept (front of egg mass)	0.04 ± 0.03	1.302	0.194
maternal dominance	-0.01 ± 0.01	-1.221	0.223
middle of egg mass	-0.004 ± 0.03	-0.140	0.889
rear of egg mass	-0.07 ± 0.03	-2.550	<0.05
maternal dominance × middle of egg mass	-0.002 ± 0.01	-0.279	0.780
maternal dominance × rear of egg mass	0.02 ± 0.01	2.465	<0.05
middle of egg mass vs. rear of egg mass	0.07 ± 0.03	2.406	<0.05
maternal dom. × middle of egg mass vs. rear of egg mass	-0.02 ± 0.01	-2.724	<0.01

Figure 4.2. (a) Relationship between log standard metabolic rate (SMR) and log body mass of juvenile trout. Line represents the predicted values from the LME model describing the relationship between SMR and body mass. (b) Relationship between SMR (residual values corrected for the effect of body mass) of juvenile trout and maternal dominance depends on the position within the egg mass from which the juveniles originated. Lines are the predicted values for each egg mass position from the final LME model (see Table 4.2 for statistical analysis). Clear circles/black dashed line = front of egg mass, grey circles/grey line = middle of egg mass, black circles/solid black line = rear of egg mass.



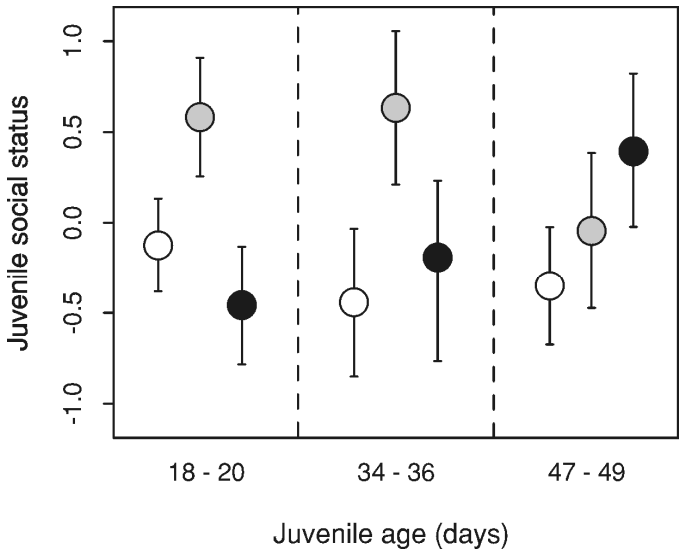
Observations of juvenile social status based on competitive ability, territory quality and aggression behaviour revealed that a clear dominance hierarchy quickly formed between siblings from different positions in the egg mass. For example, overt displays of aggression were observed in more than 81 % of triads. Correlation analyses indicated that the three behaviours assessed here were significantly associated (Kendall's coefficient of concordance, W = 0.650, 233 d.f., $p < 0.0001$). Thus juveniles that were good competitors tended to have better quality territories and also tended to instigate and win a greater number of aggressive encounters. PCA demonstrated that the first PC summarised 68% of the variation in behaviour, with high PC1 scores indicating offspring of high overall social status (good competitors that behaved aggressively and occupied high-quality territories).

To assess whether the observed variation in social status of juveniles was related to the position within the egg mass from which they originated and/or to their SMR, I analysed the variation in PC1 scores using LME (as above). Contrary to my findings for juvenile body size and SMR, maternal dominance rank did not directly influence juvenile social status (Table 4.3, this term was removed from the minimal model). Similarly, neither mass-corrected SMR nor juvenile size affected juvenile social status (Table 4.3). However, social status was significantly influenced by the position in the egg mass from which juveniles originated, but the direction of this effect changed as juveniles grew older (Table 4.3). The first and last measurements of SMR on replicate batches of fish covered a time span of 32 days, so that the age (since the start of exogenous feeding) of the fish in each triad ranged from 18 to 49 days. During the earliest period of fry feeding (age = 18 – 20 days) juveniles from the middle of the egg mass had higher social status than those from the front and rear sections. This relationship was still evident among fry of intermediate ages (age = 34 – 36 days), but by 7 weeks of age dominance in offspring increased from front to middle to rear positions in the egg mass (age × egg mass position interaction, Table 4.3, Fig. 4.3).

Table 4.3. Summary of the final linear mixed effect model explaining variation in the social status of juvenile trout. The analysis controlled for the effects of age and the position within the egg mass from which the fish originated (front, middle and rear). Parameter estimates are given as treatment contrasts with juveniles originating from the front of the ovary set as the intercept. Family was included as random variable. Treatment contrasts between juveniles from the middle and rear ovary (when the rear ovary is set as the intercept) are given in bold.

	estimate ± SE	*t*-statistic	*p*-value
intercept (front of egg mass)	-0.26 ± 0.46	-0.567	0.571
Age	0.01 ± 0.01	0.616	0.539
middle of egg mass	1.54 ± 0.66	2.343	<0.05
rear of egg mass	-0.75 ± 0.66	-1.141	0.255
age × middle of egg mass	-0.04 ± 0.02	-2.223	<0.05
age × rear of egg mass	0.02 ± 0.02	0.916	0.361
middle of egg mass vs. rear of egg mass	2.29 ± 0.66	3.484	<0.001
age × middle of egg mass vs. rear of egg mass	-0.06 ± 0.02	-3.139	<0.01

Figure 4.3. Relationship between social status of juvenile trout and position within the egg mass from which the juveniles originated is dependent on their age. Early; juveniles aged between 18 – 20 days (n = 12 juveniles per egg mass position), Intermediate; juveniles aged between 34 – 36 days (n = 12 juveniles per egg mass position), Late; juveniles aged between 47 – 49 days (n = 10 juveniles per egg mass position). Clear circles - front of egg mass, grey circles – middle of egg mass, black circles – rear of egg mass. Data are mean (± s.e.) principal component scores (PC1), derived from a PCA of the aggression, competitive ability and territory quality measures of behaviour averaged across all 12 families for each egg mass position. Although age is treated as a continuous covariate in the analyses (Table 4.3), effects have been plotted at discrete time periods to aid visual interpretation. Behavioural data were obtained for the same juveniles that were measured for SMR (see Table 3 for statistical analyses).



DISCUSSION

This study provides the first evidence for systematic differences in the phenotypes of offspring within a clutch in a highly fecund species. My study also shows that within clutches, maternal influences may be expressed as differences in offspring size, behaviour and physiology. Within-clutch heterogeneity in the body size, energy metabolism and social status of siblings was partly attributable to the location within the egg mass in which they developed as eggs. However, when considering juvenile size and SMR, the strength and direction of the effect of egg mass position was related to the dominance rank of the mother, suggesting that ecological factors, such as environmental conditions or competitor densities, can also influence how position within the developing egg mass affects offspring phenotypes. My results also show that the relationship between egg size and subsequent juvenile size is modulated by egg mass position, and that the effects of position within the egg mass on subsequent offspring social status can change with juvenile age.

Steroid hormones are likely mediators of offspring plasticity within clutches because maternal hormone levels are under environmental influence, are transferred to eggs and have affect many offspring traits including growth (McCormick 1999; Eising et al. 2001), physiology (Tobler *et al.* 2007; Sloman 2010) and behaviour (Uller & Olsson 2006; Muller *et al.* 2009). Preliminary evidence indicates that concentrations of maternally-derived cortisol are higher among eggs from the anterior part of the ovary in trout (Suter 2002). Thus, female fish could theoretically produce the range of phenotypes reported here within clutches via the differential transfer of hormones to eggs, dependent on position within the egg mass. However, it is unlikely that the current results can be attributed solely to hormonal effects. For example, the largest differences in juvenile size with respect to position within the egg mass were observed in females that produced the largest eggs. Although egg hormones can have strong effects on juvenile growth, systematic differences in egg size among regions of the egg mass is a more plausible explanation in this study because egg size explains more than 70% of the variation in the size of juvenile fishes (Chambers & Leggett 1996). Within-clutch variation in egg size is generally low in salmonids: less than 3 % of the variation in egg size is due to differences within clutches (Jonsson & Jonsson 2011). However, within-clutch variation in egg size may be increased in some situations (e.g. captive rearing, Einum & Fleming 1999). This suggests that certain

environments may result in a systematic component to the provisioning of individual eggs (in terms of composition or size) among females that produce large eggs or differ in dominance status.

My results also indicate that ontogenetic changes in juvenile behaviour are related to their developmental position within the ovary, since the relative social status of juveniles from the front, middle and rear parts of the ovary changed with age. If siblings are provisioned differentially according to their position within the ovary, it is not inconceivable that variation in their social rank may be expressed at different stages of ontogeny. It is worth noting that in this study juvenile age also represents the period of time spent by juveniles in their rearing containers prior to measurement of SMR and social status. Thus, aggressive interactions among juveniles in the rearing environment may have decreased with age (e.g. due to reduced density caused by the sequential removal of individuals for measurements) and in the experimental arena, affecting the development of social hierarchies among siblings among the older age groups. Nevertheless, I did not observe any age-related change in aggression levels within triads, and the mechanism underlying the age-related changes in the relative social status of juveniles from the front, middle and rear parts of the egg mass requires further research.

The results of this study suggest that optimal strategies for investment across the clutch vary among mothers of different social rank. Thus dominant mothers may accrue fitness benefits by producing heterogeneity in offspring that is more pronounced among regions of the egg mass. Conversely, maternal fitness in subordinate mothers may be maximised by spreading differences in offspring phenotypes more uniformly across the egg mass. Alternatively, it has been suggested that within-clutch variation may be a constraint resulting from an inability of mothers to allocate resources evenly among siblings (e.g. Einum & Fleming 2004). If dominant mothers are required to expend more energy in maintaining their social rank they might have less energy available for reproduction and be constrained to invest more variably across the egg mass. Evidence from birds shows that mothers can be constrained in their investment in individual offspring because the last-laid egg is often smaller, more poorly provisioned and the resulting chicks have a lower probability of survival (Groothuis et al. 2006). Nevertheless, I do not believe that the dominant mothers in my experiment were in poorer physiological condition than subordinates and thus constrained in their investment in offspring because of the likely

metabolic benefit conferred through their access to shelter (Millidine *et al.* 2006). Indeed, it would seem more plausible that subordinate mothers might be in poorer physiological condition because they were often observed to be actively swimming in their attempts to dislodge a fish resting in the shelter.

With the current data set I cannot determine whether or not the observed relationships between maternal dominance, egg mass position and offspring phenotype serve an adaptive function. Nevertheless, it is possible that the intra-clutch variation in the distribution of offspring phenotypes in relation to egg mass position may be ecologically important. Brown trout are known to be very cautious and overhead cover (e.g. submerged logs, undercut banks) is a critical habitat requirement for their spawning. For example, it has been reported that over 80% of nests are located within 1.5 m of cover (Witzel & MacCrimmon 1983). It is likely, therefore, that dominant females acquire preferential access to spawning areas with close proximity to suitable cover. Given that female trout can spawn multiple nests (Jonsson & Jonsson 2011), greater heterogeneity in egg traits among regions of the egg mass may equate to higher variation in offspring phenotypes among nests. Hence, it is possible that dominant mothers are better able to determine where (and when) they spawn their nests and perhaps benefit from greater differences in egg/offspring traits between nests. Indeed, recent empirical evidence shows that enhanced diversity in phenotypes (and genotypes) within populations may increase the number of juveniles that survive and the amount of biomass produced (Griffiths & Armstrong 2001; Ellers et al. 2011). This has led to suggestions that a similar principle may apply within-clutches as a bet-hedging mechanism against environmental uncertainty and to match variation in offspring to spatial variation in their environment (Armstrong *et al.* 2011).

In summary, I show the first evidence of a systematic component to the distribution of within-clutch heterogeneity in offspring size, behaviour and physiology in a highly fecund species. Furthermore I show that these differences can reflect maternal dominance rank and egg size. Variation in the composition of eggs (e.g. egg hormone concentrations, relative lipid content, presence of antioxidants) from different regions of the ovary warrants further investigation as a mechanistic explanation for the results present here. Overall, my study suggests that optimal strategies for investment in offspring may vary among mothers of different social rank, and that the results of different investment strategies might change with ontogeny during early juvenile development. More broadly,

my results are consistent with the hypothesis that mothers can enhance their fitness by programming the phenotypes of their offspring during egg development.

SUPPLEMENTARY MATERIAL

Table S.1. Body size, dominance rank and egg size data for the 12 female Brown trout selected for the measurement of sibling size, metabolic rate and social status in relation to their position within the egg mass.

tank	female	dominance rank	final length (cm)	final weight (g)	egg weight (mg)
1	792C	5.5	43.7	1042.5	90.2
1	B4F8	1.0	43.0	1004.5	85.6
1	51C8	4.0	42.7	853.3	83.3
2	9758	3.0	46.3	534.8	82.0
2	B06B	2.0	39.5	832.2	88.0
2	3ECA	1.0	36.0	533.1	76.6
3	A0B8	3.0	41.5	768.2	73.2
3	45F8	2.5	43.0	936.6	76.6
3	4193	5.5	44.5	995.4	90.6
4	0E4E	6.3	41.5	706.5	sample missing
4	8DOA	2.0	41.0	826.3	79.1
4	4E7A	3.0	40.5	937.9	96.0

CHAPTER 5. MATERNAL INFLUENCES VIA MULTIPLE PATHWAYS: LINKS BETWEEN PARENTAL LIFE HISTORY TRAITS AND OFFSPRING PERFORMANCE IN WILD ATLANTIC SALMON

SUMMARY

Both the juvenile and adult environments experienced by a mother can affect her investment in offspring. However, the implications of these maternal legacies, both juvenile and adult, for offspring fitness in natural populations are unclear. I investigated whether the juvenile growth rate and adult reproductive traits (length, body condition and reproductive investment at spawning) of female wild Atlantic salmon were related to the growth and survival of their offspring. Adult salmon captured on their upstream migration were used to create experimental full-sib clutches of eggs, which were mixed and then placed in artificial nests in a natural stream that lacked salmon due to a migration barrier. Four months later I re-sampled the stream to obtain family-level estimates of offspring size and survival. Mothers that had grown slowly as juveniles but had invested heavily in reproduction and were in relatively poor body condition at spawning produced the largest eggs. Larger eggs resulted in larger juveniles and higher juvenile survival. However, after controlling for egg size, offspring growth was positively related to maternal juvenile growth rate and reproductive investment. The predictors of offspring survival and biomass (i.e. reproductive success) varied with the juvenile growth rate of the mother: if females grew slowly as juveniles, their reproductive success was positively related to their body size. In contrast, the reproductive success of females that grew quickly as juveniles was unrelated to their body size and was instead related positively to the size of egg they produced and to a lesser extent their own body condition. My results show that maternal influences on offspring in the wild can be complex, with reproductive success related to the early life performance of the mother as well as her state at the time of breeding.

INTRODUCTION

In addition to their genetic contribution, mothers can adjust the phenotypic development of their offspring in response to prevailing environmental circumstances. Such maternal

influences (the combined effect of maternal phenotype and maternal genotype, Venturelli *et al.* 2010) can have pronounced effects on both maternal fitness and offspring performance (for recent reviews see Green 2008; Marshall *et al.* 2008; Uller 2008) and influence population ecology (Benton, St Clair & Plaistow 2008; Venturelli *et al.* 2010). Remarkably, however, factors experienced by females during early development can also affect the phenotypes and fitness of their offspring, as shown in laboratory experiments on hamsters *Mesocricetus auratus*; (Huck *et al.* 1986), cichlid fish (*Simochromis pleurospilus*; Taborsky 2006) and *Drosophila* (Vijendravarma *et al.* 2010). However, there is little evidence that similar long-term effects are found in wild populations.

Here I report results from a field experiment investigating maternal influences on offspring arising from variation in both the early growth rate and the adult condition of mothers. Atlantic salmon (*Salmo salar*) are an ideal species in which to investigate such effects, due to the contrast in early and late-life environments experienced in their lifecycle. They spawn in rivers and streams, where juveniles live until smolting (the physiological and morphological preparation for marine life). On becoming smolts, the fish then migrate to sea, where most of their growth occurs. After one or more winters at sea, they return to spawn in fresh water (Klemetsen *et al.* 2003). The body condition (hereafter, 'somatic condition') of adult salmon returning from the sea is a strong indicator of lipid reserves and can vary substantially among individuals, most probably due to conditions experienced at sea (Todd *et al.* 2008). Variation in the somatic condition of females as they enter fresh water is likely to influence their production of eggs (Todd *et al.* 2008) and also their somatic condition as they prepare to spawn (from a few weeks to many months later), because the relative lipid content of somatic tissue declines rapidly during the fresh water migration to the spawning grounds (Jonsson, Jonsson & Hansen 1997).

Maternal somatic condition at spawning has been shown in other fish species to influence the number, size, energy content and survival of offspring (Reznick & Yang 1993; Gagliano & McCormick 2006; Donelson *et al.* 2009). However, the relevance of somatic condition at spawning time is not necessarily clear, because it might reflect both the state of the fish returning to fresh water and the extent to which soma is converted to gonad (Roff 1992). Reproductive investment is likely influenced by present environmental conditions, but also those experienced by the adults during early development. The transformation into smolts and seaward migration only occurs during spring; fish that fail

to smolt remain in fresh water for at least another year (Metcalfe 1998). Thus, the fastest growing fish will smolt a year or more ahead of those that grow at a slower rate. This period of juvenile growth results in a phenotypically plastic response in reproductive investment by adults: fish that grow relatively slowly as juveniles (i.e. smolt at older ages) produce larger eggs at maturity, even after controlling for body size at the time of spawning (Thorpe *et al.* 1984; Jonsson *et al.* 1996). However, these earlier studies did not examine the consequences for offspring of these maternal traits. Using wild Atlantic salmon in their natural environment, I investigate here the consequences of variation in the somatic condition, reproductive investment and early growth of mothers for the growth and survival of their offspring.

MATERIALS AND METHODS

Selection of maternal fish and crosses

Atlantic salmon undertaking their spawning migration were captured at a fish trap on the River Blackwater, Ross Shire, northern Scotland. I randomly selected 83 ripe females that had spent a single winter at sea before returning to fresh water to spawn (one sea winter or 1SW fish), using body size distributions to distinguish 1SW from multi-sea winter (MSW) fish. A sample of scales was collected from each female (for subsequent determination of age at smolting), and their fork length (L_F, to 0.5 cm) and body mass (to 0.1 g) recorded prior to the stripping of their clutch of eggs, which was then drained of ovarian fluid and then weighed (to 0.1 g; referred to hereafter as ‘clutch mass’).

Clutch mass was subtracted from body mass to give the somatic mass of each female, which was then used as the measure of female mass in the subsequent calculation of somatic condition because it is not confounded by reproductive mass. I defined somatic condition as somatic mass relative to body length, determined by calculating the residuals of a linear regression (both variables log-transformed) of somatic mass against fork length for all 83 female fish. Similarly reproductive investment was defined as the residuals of clutch mass regressed on fork length (both variables log-transformed). Since I was interested in the relative importance of somatic condition and reproductive investment on offspring performance, the subset of 36 clutches used in the field experiment were selected to maximise variation in these traits. I thus excluded ‘average’ condition individuals (i.e.

those located closest to the regression lines of somatic mass and clutch mass against fork length) to create four distinct groups of maternal fish (each represented by 9 females, size range, L_F 53.5 – 62.5 cm, somatic mass 1079.2 – 1835.9 g) that differed in their reproductive investment and somatic condition: (1) fish in relatively good somatic condition with high reproductive investment, (2) fish in relatively poor somatic condition with low reproductive investment, (3) fish in good somatic condition with low reproductive investment and (4) fish in poor somatic condition with high reproductive investment.

A sub-sample of approximately 10 g of eggs from each of the 36 selected clutches was weighed (to 0.01 g) and preserved with 5% buffered formalin (Fleming & Ng 1987). Fecundity was determined by calculating the number of eggs in each weighed sub-sample and extrapolating this value to the total clutch mass of each female. Individual eggs from these sub-samples were later weighed (to 0.0001 g, n = 10 per clutch) to calculate the mean mass of individual eggs (hereafter egg mass) per female. The remaining eggs from each female were fertilised *in vitro* with sperm from one of 36 wild anadromous males to create 36 full sibling families. Adipose fin clips were removed from the parental fish to enable offspring parentage assignment. The fertilised eggs were transferred to the Scottish and Southern Electricity hatchery at Contin, where they were reared as separate family groups under ambient water temperatures until the eyed stage. Egg mortality was recorded until egg stocking (see *Field Experiment*). When the eggs reached the eyed stage of development, sub-samples of approximately 100 eggs from each family were transferred to the Marine Scotland freshwater hatchery at Almondbank, Perthshire, Scotland. These sub-samples were reared as separate family groups under ambient water temperatures until the onset of independent feeding (when juveniles switch from being largely quiescent in the gravel, provisioned with maternal yolk, to active foraging in open water), when 10 per family were preserved in 5% buffered formalin and later weighed (to 0.0001 g) to provide data on juvenile size at emergence.

Scale readings subsequently confirmed that all the selected female spawners had spent one year at sea (1SW), but varied in their rate of early growth (the number of years they had spent as juveniles in fresh water before turning into smolts and migrating to sea). Those that had grown faster (= Fast Early Growth, or FEG, females) had reached the size threshold necessary for seaward migration (Metcalfe & Thorpe 1990) earlier, and had

become smolts as 2-year-olds, whereas slower growing females had taken 3 years to reach the smolt stage (= Slow Early Growth or SEG females). Hence, early growth affects total age at spawning and may correlate with other variables such as size at smolting. Scale reading is commonly used in fisheries management to determine the age and growth patterns of individual fish. The process is comparable to counting the rings on a tree as specific marks appear on scales that indicate an individual's age and growth (e.g. McCarthy, Friedland & Hansen 2008).

Field experiment

The growth and survival of offspring from these females was assessed in a small tributary (Gleann Mèinich) of the River Conon. This mid-altitude stream (240 m.a.s.l.) provides suitable habitat for salmon juveniles but natural spawning is prevented by dams further downstream which act as barriers to upstream migration of adult fish. Both older juvenile salmon that had been stocked as eggs in previous years and a natural population of resident brown trout (all age classes) were present in this stream, which is also open to mammalian and avian predators of juvenile salmon. These conditions provide an ideal setting for investigating maternal effects on offspring that are subject to natural selection pressures. On 22 March 2010, the stream was seeded with eyed-stage eggs from each of the 36 selected females (n = 1250 per family, n = 45,000 total). All eggs were first pooled and thoroughly mixed, and then dispersed throughout an 860 m length of stream in 43 artificial gravel nests that were spaced at intervals of approximately 20 m (suitable spawning habitat permitting). This corresponded to a stocking density of approximately 1050 eggs per nest, and created natural spawning densities for this species (Fleming 1996). No other salmon eggs were stocked in this stream in this year.

During the period 12–14 July 2010 (approximately 2 months after juveniles were estimated to have emerged from the gravel nests and begun independent feeding, hereafter 'emergence'), four hundred metres of the stream was electrofished to obtain data on the density and sizes of surviving experimental juveniles. Beginning at the lowest nest site, the stream was divided into 2 m-long sections that were sampled with a single electrofishing pass. On each day of electrofishing for juveniles I conducted multiple electrofishing passes in a 10 m-long test section of stream (riffle habitat) to estimate my capture efficiency. Electrofishing efficiency estimated by the Zippin method (Bohlin *et al.* 1989) indicated

that the first pass caught on average 53 % of the experimental juveniles present and that temporal variation in electrofishing efficiency was relatively low (mean = 0.53, standard deviation = 0.09, n = 3).

All fish (i.e. experimental and older juvenile salmon and brown trout) caught within a section were anaesthetised with MS 222. A distinctly bi-modal distribution of lengths was used to separate experimental salmon fry (n = 1288; fork length (L_F) range 28 – 46 mm) from non-experimental conspecifics belonging to older age classes (n = 419; L_F range 63 – 124 mm) and brown trout (n = 103; L_F range, 31 – 212 mm). Conspecifics from older age classes and brown trout were allowed to recover from anaesthesia in enclosures placed within the stream before being released. Given the large number of experimental fish captured, it was not practical to measure them accurately in the field and so they were given a lethal dose of anaesthetic before being preserved in 100% ethanol for subsequent measurement of body size and tissue sampling. This design enabled the capture location of all fish to be recorded at a 2 m scale, so that the effect of local density on growth could be estimated (see *Data Analysis*).

All preserved experimental fish were subsequently weighed (0.001 g) and fin-clipped for microsatellite analysis of parentage. To account for shrinkage in body mass caused by preservation in ethanol, I weighed a sub-sample of juveniles (n = 167) immediately after terminal anaesthesia and again after 3 days of storage in 100 % ethanol. Shrinkage in body mass was analysed using a regression ($F_{1,165} = 12786$, $p<0.0001$, $r^2 = 0.98$) between fresh (M_{B1} range, 0.305 – 1.241 g) and preserved body mass values (M_{B2} range, 0.181 – 0.778 g). The relationship between M_{B1} and M_{B2} is described by the equation, $M_{B1} = 1.51M_{B2} + 70.69$, which was used to convert measurements of juveniles preserved in ethanol to estimates of fresh mass prior to statistical analysis.

Genotyping and parentage analysis

Genotyping was provided by commercial suppliers (Landcatch Natural Selection Ltd, Alloa, Scotland) using a panel of markers that they had customized for their internal use. DNA was extracted from the fin clips of all parental fish and recaptured offspring using a Biosprint DNA Tissue kit (Qiagen, Crawley, UK) following the manufacturer's protocol. Genotyping was performed using a single multiplex panel of 10 informative microsatellites

scattered across the genome. A Mastercycler gradient thermal cycler (Eppendorf, Hamburg, Germany) was used for optimization and genotyping of the markers, and ABI 377 sequencer (Applied Biosystems, USA) used for the fluorescent detection of the allelic profiles. Analysis for parentage assignment by exclusion was carried with the programme Vitassign 8.3 (Vandeputte, Mauger & Dupont-Nivet 2006). Allowing a maximum of two allele mismatches, 1,283 juveniles (>99%) were uniquely assigned to a single set of parents, and only four not assigned, mostly due to a failure of PCR amplification. Twenty three juveniles could not be assigned to any combination of parents because they had unusual microsatellite fingerprints. These individuals were most probably juvenile brown trout that had been misidentified as salmon during field work.

Data analysis

I measured the following characteristics of juveniles and their performance: egg size, size at emergence, size at recapture (2 months after fry emergence from the gravel), and family-level survival rate of juveniles (i.e. total number recaptured per family). Juvenile fitness in Atlantic salmon can be reliably assessed by monitoring growth and survival soon after emergence from the nest because dispersal is limited and almost invariably there is intense competition and high mortality at this time (Einum *et al.* in press). Not all juveniles were recaptured hence my measure of survival is a relative estimate of this trait (hereafter termed ‘relative juvenile survival’).

To analyse maternal effects on egg size, juvenile size at emergence and juvenile size when re-captured I fitted linear mixed effect (LME) models that incorporated the following maternal traits as a general fixed structure: maternal fork length (hereafter ‘maternal body size’), reproductive investment, somatic condition, rate of early growth (i.e. FEG or SEG) and (where appropriate) the family’s mean value for egg size (hereafter ‘family egg size’), plus all two way interactions between these variables. Details of specific models that contained (or omitted) additional explanatory variables are specified below. Previous research in this catchment has demonstrated that juvenile body size is strongly influenced by the density of conspecifics from the same age class within an upstream distance of 11 m (Einum *et al.* 2011). I was unable to estimate upstream densities on a 1 m scale, so I calculated the numbers of experimental juvenile salmon within 10 m upstream of a focal individual. Using the same spatial scale, I also calculated upstream densities of older

conspecifics and of brown trout because they could affect the growth of juvenile salmon. These densities were included as additional explanatory variables in the analysis of juvenile size at recapture.

In each LME model, maternal identity was included as a random variable to control for non-independence of siblings. In the analysis of juvenile size at re-capture, the capture location of each individual was included as an additional random factor to control for spatial and temporal correlations among fish that inhabited the same sections of stream and were re-captured on the same day. In the analysis of juvenile size at re-capture, measurements of juvenile size and family egg size values were ln transformed to meet assumptions of normality.

I used generalised linear models (GLM's) with a negative binomial error distribution to analyse variation in maternal fecundity and relative juvenile survival (i.e. as count data). Both GLM's incorporated the explanatory variables in the fixed structure described previously. The link functions for the negative binomial generalised linear models (GLM's) analysing variation in maternal fecundity and offspring survival rate were selected by comparing the AIC values that resulted from each full model when fitted with a Gaussian-, log- or square root- link function.

I also calculated the theoretical contribution of each mother to the biomass of the experimental salmon population, had the number of eggs placed in the stream reflected the fecundity of each female (rather than been made equal for all females regardless of their fecundity). For each family, the juvenile biomass per egg stocked (sum of the recaptured juvenile body mass values for each family, divided by number of eggs stocked per family) was thus multiplied by the mother's fecundity. This value was then used as a response variable in a generalised least squares (GLS) model with all the explanatory variables in the general fixed structure (including all two way interactions). In this model, I included a fixed variance structure (VarFixed) that allowed for residual variance to increase with family egg size.

Model selection and validation was performed according to protocols outlined in Zuur *et al.* (2009). Prior to statistical analysis, I calculated variance inflation factors (VIF) for all candidate explanatory variables used in each statistical model. In all cases, VIF's were less

than 4, indicating that collinearity among explanatory variables was unlikely to have affected my analyses (Zuur *et al.* 2009). For model selection, likelihood ratio tests were used to sequentially compare the log-likelihoods (model fit) of nested models (using maximum likelihood, ML). More parsimonious models were retained if an increase in the log-likelihood ratio statistic was statistically significant ($p < 0.05$). Final GLS and LME models were then re-fitted with REML. All statistical analyses were conducted in R version 2.13.1 (R Development Core Team 2011).

RESULTS

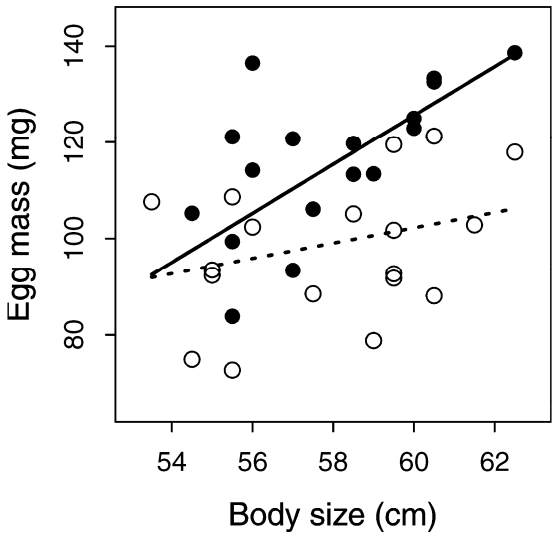
Offspring size and number

Individual egg mass varied more than two-fold among families and was positively related to maternal body size and reproductive investment, whereas it was negatively related to somatic condition (Table 5.1). The largest eggs were thus produced by large females that (for their body length) had the poorest body condition but had heavy ovaries. Egg size was also influenced by a female's age at smolting (and hence presumed rate of early growth; Table 5.1), with SEG females producing larger eggs than FEG females despite the two types of female not differing in body size by the time of spawning (L_F mean ± s.e. FEG = 57.94 ± 0.63cm; SEG = 57.86 ± 0.52cm; t-test, $t_{32} = 0.10$, $p = 0.92$). However, the strength of this effect of a female's early growth on egg size was dependent on her body size at the time of spawning, as indicated by a significant early growth rate × maternal body size interaction (Table 5.1). Thus while early growth rate was unrelated to egg size if the females were small by the time of spawning, large SEG spawners produced significantly larger eggs than large FEG spawners (Fig. 5.1).

Table 5.1. Summary of the optimal linear mixed effect model explaining variation in mean egg size among female salmon. The analysis initially controlled for the following maternal traits: body size, reproductive investment, somatic condition and rate of early growth (SEG versus FEG; see text for definitions and details of the analysis). Parameter estimates are given as treatment contrasts with fast early growth (FEG) females represented by the intercept. Family was included as a random variable.

	estimate ± SE	*t*-statistic	*p*-value
intercept (FEG)	7.16 ± 53.23	0.14	0.893
maternal body size	1.59 ± 0.92	1.73	0.084
reproductive investment	83.33 ± 17.58	4.74	<0.0001
somatic condition	-237.84 ± 82.04	-2.90	<0.01
SEG	-186.81 ± 85.22	-2.19	<0.05
maternal body size × SEG	3.50 ± 1.50	2.38	<0.05

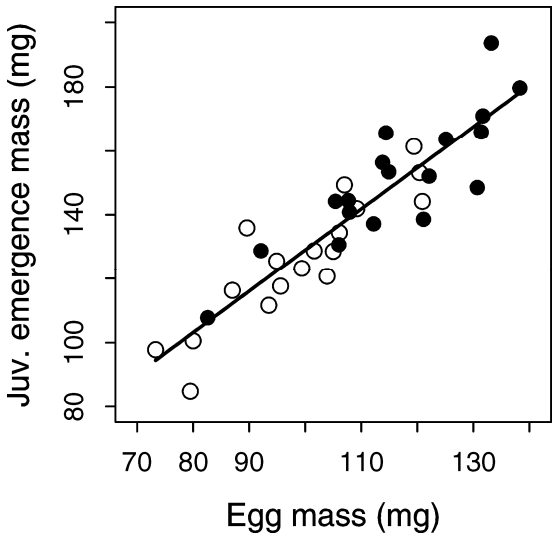
Figure 5.1. The relationship between a female's body size and the mean size (mass) of her eggs for females with fast and slow rates of early growth (FEG – open circles, SEG – filled circles, respectively. The predicted values from the optimal LME model for FEG and SEG females are represented by the dashed and solid lines respectively. See Table 5.1 for analysis and *Supplementary Material* for details of the data used to plot the predicted values.



Fecundity was lower in SEG than FEG females (mean fecundity ± s.e.; FEG 2406.87 ± 118.45; SEG 1993.63 ± 115.31, parameter estimate for SEG females in comparison to FEG females ± s.e.; -0.22 ± 0.06, *z*-value = 3.66, $p < 0.001$) and was positively related to reproductive investment (parameter estimate ± s.e.; 1.06 ± 0.28, *z*-value = 3.81, $p < 0.001$)

but unrelated to maternal body size. Family egg size had a strong positive effect on the mass of newly-emerged juveniles and was the only explanatory variable retained in the model describing variation in the size of offspring at this stage of development (parameter estimate ± s.e.; 1.28 ± 0.10, *t*-value = 12.54, $p < 0.0001$, Fig. 5.2); thus the early growth rate of females did not affect the initial size of their offspring apart from indirectly through its link with egg size.

Figure 5.2. The relationship between mean egg mass and mean mass of juveniles at the time of emergence. The predicted values from the optimal LME model are represented by the solid line. Data are mean family values, and are shown separately for females with fast and slow rates of early growth (FEG – open circles, SEG – filled circles, respectively), although maternal early growth did not influence this relationship. See text for analysis.



Offspring performance in natural conditions

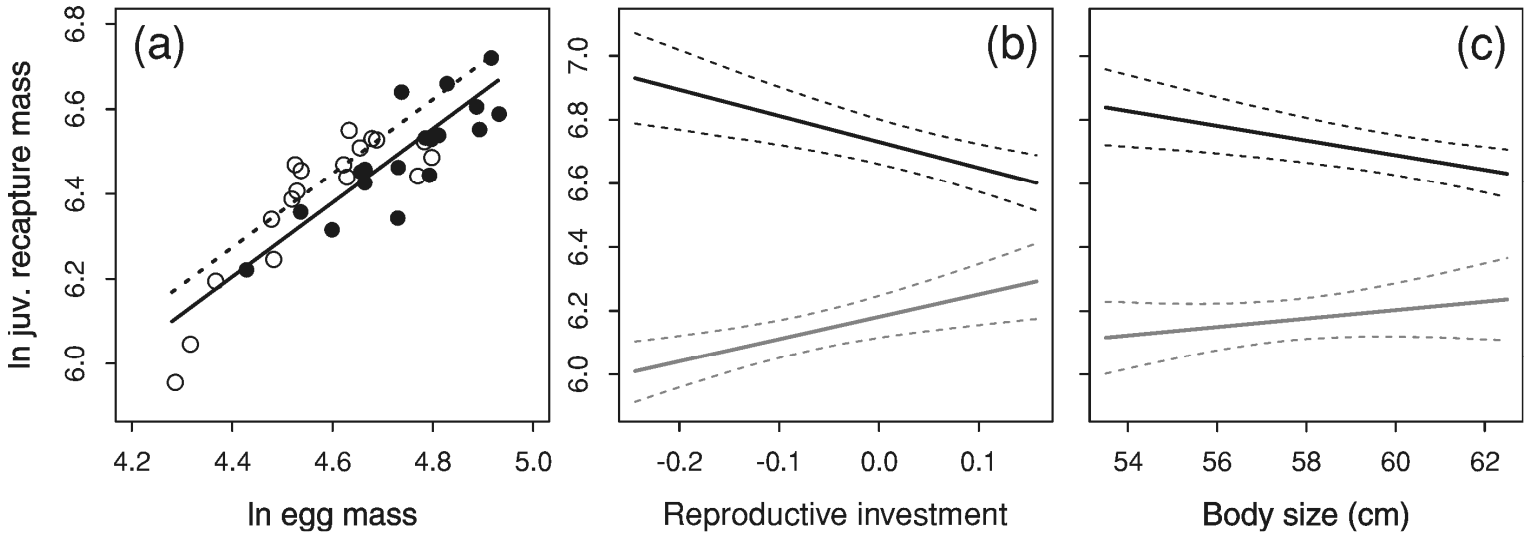
The mean body size of 2 month old juveniles varied more than 200 % among families, ranging from 389.5 ± 14.8 (s.e.) to 838.3 ± 41.1 mg (n = 36 families, 10 – 63 recaptured individuals per family). Size at 2 months of age was positively related to family egg size, maternal body size and maternal reproductive investment (Table 5.2). Local densities of salmon from the same year class and older year classes had positive and negative effects on juvenile size, respectively, whereas trout density was not significant (Table 5.2). Even after controlling for other maternal traits and local population densities, there was a link with early maternal growth rate: for a given egg size, SEG mothers produced slower-

growing offspring than did FEG mothers (Table 5.2, Fig. 5.3a). Furthermore, early growth rate was influenced by significant interactions between family egg size and both maternal body size and reproductive investment (Table 5.2). Thus, as maternal reproductive investment increased, the difference in subsequent size between juveniles that hatched from large and small eggs diminished (Fig. 5.3b). Likewise, the discrepancy in size among juveniles from large and small eggs lessened in offspring from larger females (Fig. 5.3c).

Table 5.2. Summary of the optimal linear mixed effect model explaining variation in mass of juvenile salmon (ln transformed) recaptured 2 months after emergence. The analysis initially controlled for the same variables listed in Table 5.1 but included the effects of egg size (mean value per family, ln transformed) and upstream densities of salmon of the same age, older year class salmon, and trout (see text for definitions and details of the analysis). Parameter estimates are given as in Table 5.1. Family and stream capture location of each individual (recorded at a 2 m scale) were included as crossed random variables.

	estimate ± SE	*t*-statistic	*p*-value
intercept (FEG)	-12.36 ± 7.28	-1.698	0.090
maternal body size	0.26 ± 0.12	2.058	<0.05
reproductive investment	10.81 ± 2.83	3.819	<0.001
ln egg size	4.13 ± 1.56	2.657	<0.01
SEG	-0.07 ± 0.02	-2.884	<0.01
same-age salmon density	1.82E-03 ± 3.55E-04	5.114	<0.0001
older salmon density	-2.54E-03 ± 9.42E-04	-2.692	<0.01
reproductive investment × ln egg size	-2.36 ± 0.61	-3.842	<0.001
maternal body size × ln egg size	-0.06 ± 0.03	-2.133	<0.05

Figure 5.3. (a) The relationship between the mean mass of eggs from each family and the mean mass of the resulting juvenile salmon (recaptured 2 months after emergence, FEG – open circles, SEG – filled circles). The predicted values from the optimal LME model for FEG and SEG females are represented by the dashed and solid lines respectively. Both axes are on a logarithmic scale. Panels (b) and (c) show interactions between maternal reproductive investment and maternal body size with egg size respectively. The solid and dashed lines are the predicted values and 95 % confidence intervals from the optimal LME model (see text for analysis). Black and grey lines refer to predictions for large (mass = 138 mg) and small (72 mg) eggs respectively. Data points are omitted in (b) and (c) to aid visual interpretation. See Table 5.2 for analysis and *Supplementary Material* for details of the data used to plot the predicted values.

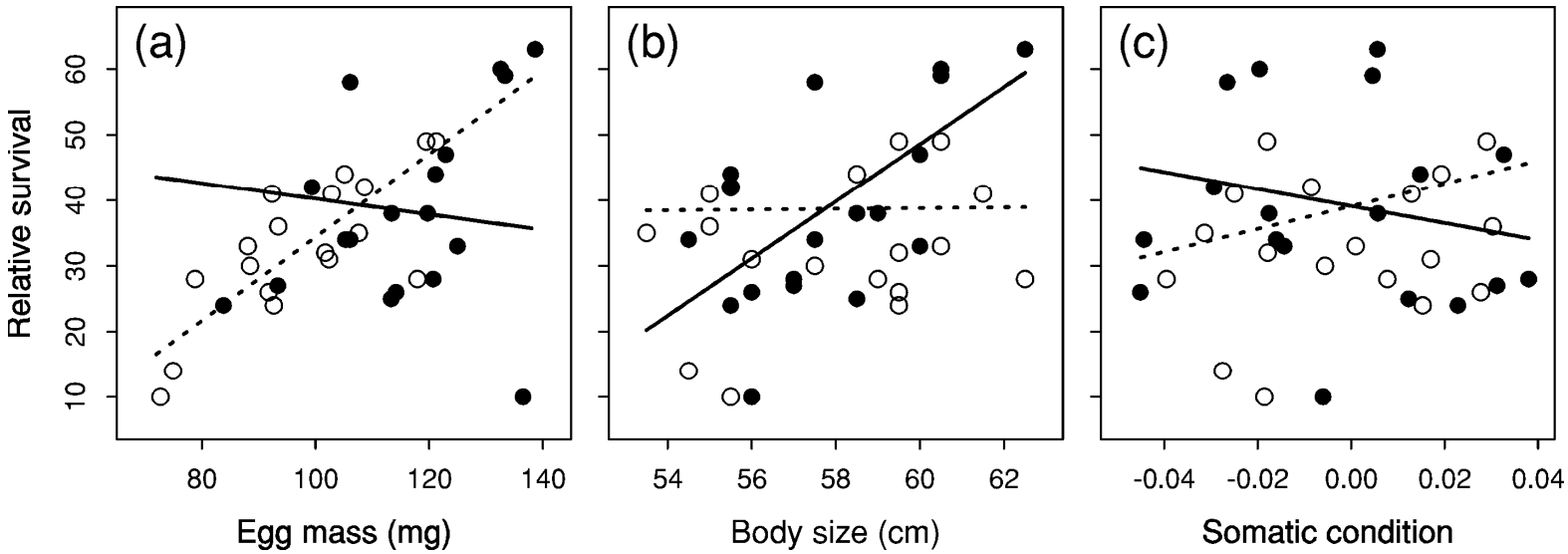


Relative juvenile survival (recapture rate) was highly variable, differing more than six-fold among families despite each female contributing an equal number of eggs to the stream (Fig. 5.4). This result was unlikely to be caused by egg and juvenile mortality prior to emergence, as combined egg and hatchling mortality in the subsamples of experimental eggs that were retained in the hatchery after stocking was uniformly low, averaging (mean ± s.e.) 1.14 ± 0.25 % across the 36 families. In absolute terms, relative juvenile survival was higher for offspring of SEG versus FEG females (mean ± SE; FEG 32.94 ± 2.52; SEG 38.33 ± 3.45). However, the factors contributing to offspring survival differed between FEG and SEG females. For offspring of FEG females, survival was positively related to family egg size and maternal somatic condition (Table 5.3, Fig, 5.4a,c). In contrast, early juvenile survival of offspring from SEG females was positively related to maternal body size, with larger SEG females giving rise to more surviving juveniles (Table 5.3, Fig. 5.4b).

Table 5.3. Summary of the optimal generalised linear model (negative binomial distribution with gaussian-link function) explaining variation in relative survival rates of juvenile salmon from different mothers. The analysis initially controlled for the same variables listed in Table 5.1 but included the effects of egg size (mean value per family). See text for details of the analysis. Parameter estimates are given as in Table 5.1.

	estimate ± SE	*z*-statistic	*p*-value
intercept (FEG)	-31.78 ± 41.69	-0.762	0.446
maternal body size	0.05 ± 0.80	0.067	0.947
somatic condition	173.55 ± 90.50	1.918	0.055
egg size	0.63 ± 0.14	4.564	<0.0001
SEG	-168.38 ± 77.70	-2.167	<0.05
egg size × SEG	-0.75 ± 0.21	-3.508	<0.001
maternal body size × SEG	4.30 ± 1.50	2.861	<0.01
somatic condition × SEG	-303.20 ± 123.39	-2.457	<0.05

Figure 5.4. Relationships between the relative survival rate of juveniles (i.e. number recaptured) and (a) the mean size (mass) of eggs from which they hatched, and (b) the body size and (c) somatic condition of their mothers. FEG females are represented by open circles and SEG females by filled circles. The predicted values from the optimal generalised linear model for FEG and SEG females are represented by the dashed and solid lines respectively. See Table 5.3 for analysis and *Supplementary Material* for details of the data used to plot the predicted values.

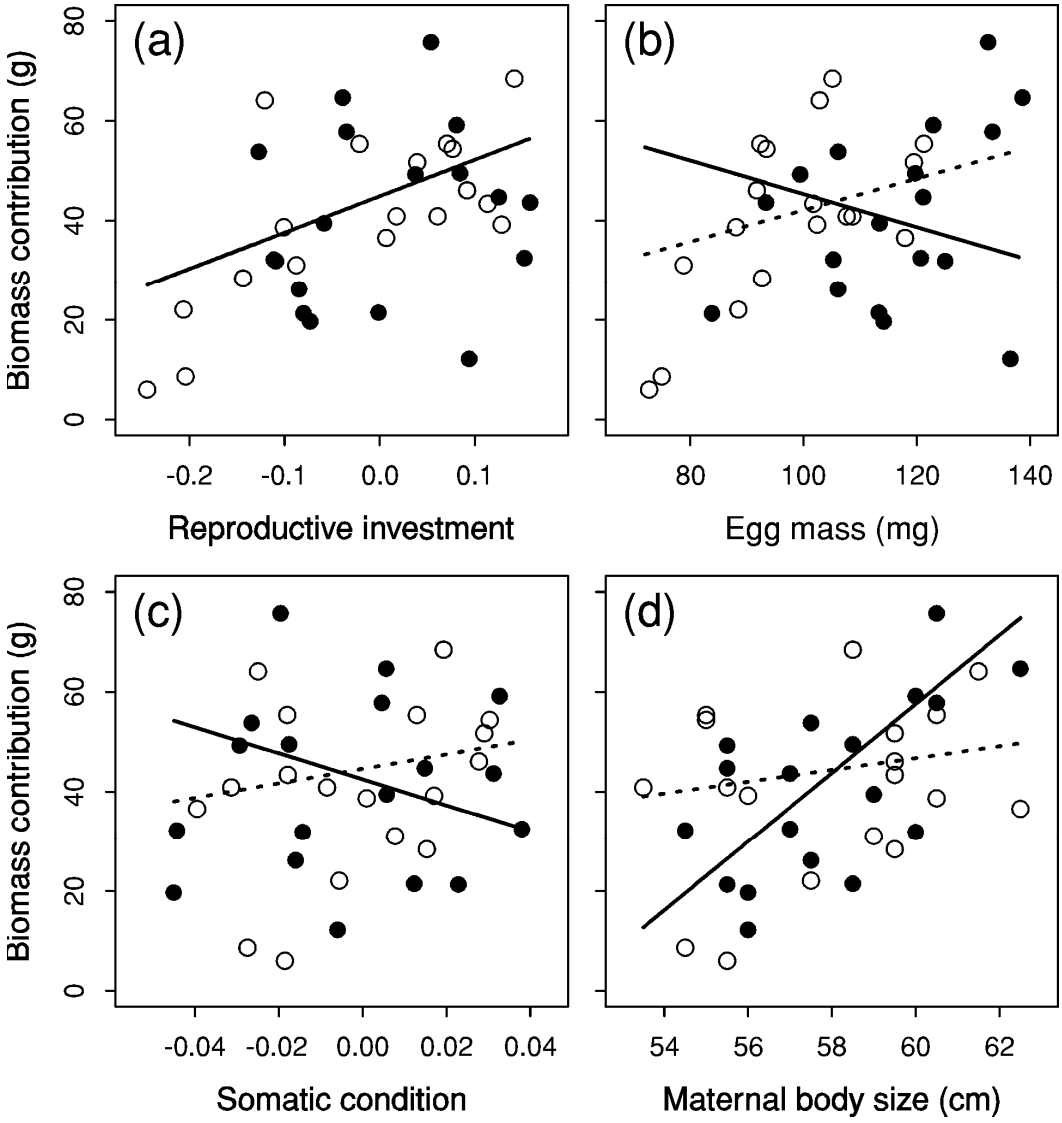


The contribution, in terms of predicted offspring biomass, of each mother to the experimental population would have varied substantially among families if the numbers of eggs per family stocked in the stream had reflected maternal fecundity, rather than being made equal for all families and assuming similar competitive conditions to those experienced in my experiment (Fig 5.5). Overall, predicted offspring biomass two months after emergence (g) was similar among FEG and SEG mothers (mean ± SE; FEG 40.61 ± 4.02; SEG 40.82 ± 4.13) and was related positively to reproductive investment (Table 5.4, Fig. 5.5a). However, when investment in reproduction is accounted for there are further effects of maternal body size, egg size and remaining somatic condition, each of which depend on early growth rate. Overall the relationship between maternal body size and offspring biomass production was stronger among SEG females (Table 5.4, Fig. 5.5d). Increases in egg size and somatic condition were related positively to offspring biomass among FEG mothers, but these relationships were negative in SEG mothers (Table 5.4, Fig. 5.5b,c).

Table 5.4. Summary of the optimal generalised least squares model explaining variation in the theoretical contribution of each mother to the biomass of the experimental salmon population. The contribution of each mother was determined by calculating the amount of offspring biomass produced per egg stocked and multiplying this value by her fecundity. The analysis initially controlled for the same variables listed in Table 5.1 and included the effects of egg size (mean value per family). See text for details of the analysis. Parameter estimates are given as in Table 5.1.

	estimate ± SE	*z*-statistic	*p*-value
intercept (FEG)	-57.05 ± 63.36	-0.900	0.376
maternal body size	1.18 ± 1.20	0.989	0.331
somatic condition	145.27 ± 147.41	0.985	0.333
reproductive investment	72.99 ± 29.33	2.489	<0.05
SEG	-263.93 ± 120.43	-2.191	<0.05
egg size	0.32 ± 0.29	1.091	0.285
maternal body size × SEG	5.72 ± 2.34	2.442	<0.05
somatic condition × SEG	-406.24 ± 181.76	-2.235	<0.05
egg size × SEG	-0.65 ± 0.36	-1.818	0.080

Figure 5.5. The theoretical contribution of each mother to the biomass of the experimental salmon population depends on (a) their investment in reproduction, (b) the mean size (mass) of eggs they produce, (c) their somatic condition and (d) their body size. The contribution of each mother was calculated by multiplying the biomass of sibling juveniles produced per egg stocked in the stream with maternal fecundity. FEG females are represented by open circles and SEG females by filled circles. In (a) the solid line is the predicted values for all females (i.e. FEG and SEG) from the optimal generalised least squares model. In (b) to (d), the dashed and solid lines are the predicted values plotted separately for FEG (dashed line) and SEG (solid line) females respectively. See Table 5.4 for analysis and *Supplementary Material* for details of the data used to plot the predicted values.



DISCUSSION

Differences in somatic condition, reproductive investment and rate of early growth among mothers exerted a large influence on the size of eggs and therefore, newly-emerged

juveniles. When the surviving juveniles were recaptured after c. 2 months in a natural stream environment, substantial variation was present among families in both juvenile body size and relative rates of survival. This occurred despite stocking equal numbers of eggs from each family in the same sections of stream. Moreover, much of the variation in these traits could be explained by maternal characteristics such as somatic condition, reproductive investment or rate of early growth, independently of egg size (and in the case of juvenile body size, important ecological factors such as local densities of conspecifics). My results also show that maternal influences on offspring size and survival in a natural setting can be complex, as indicated by the influence of interaction terms between various maternal characteristics on each offspring trait that was measured. Overall, this experiment provides direct evidence from a natural system that a mother's reproductive success is linked to both her early growth conditions and the factors that constitute her 'current reproductive state' (i.e. reproductive investment and somatic condition).

Size is of critical importance for early life performance (Kestrel & Munch 2010), with large juveniles being especially favoured under adverse conditions (e.g. Einum & Fleming 1999; Dziminski & Roberts 2006; Segers & Taborsky 2011a). Accordingly, differences among families in egg size had significant implications because larger eggs resulted in larger juveniles, both at emergence and after a period of c. 2 months in natural conditions. Juveniles from larger eggs also had a higher rate of survival than juveniles from small eggs. However, juvenile performance was not determined solely by egg size. Increases in maternal reproductive investment tended to result in faster juvenile growth for a given egg size. Furthermore, juveniles hatching from small eggs were more able to match the body size of fry from much larger eggs if their mother was either large at the time of spawning or had invested disproportionately in reproductive tissue (see Fig. 5.3b,c). Indeed, recent research shows that hatching from a relatively small egg does not necessarily prevent an individual fish from reaching a body size equal to juveniles from larger eggs: expression levels of the growth hormone receptor (*GHR*) gene can be higher in juveniles that hatch from small eggs, resulting in a faster rate of growth and similar final body size, when compared to juveniles from large eggs (Segers, Berishvili & Taborsky 2011).

In terms of the maternal phenotype at spawning, females that invested relatively little in reproduction produced smaller eggs, had a reduced fecundity, and for a given egg size had slow growing offspring and a low total juvenile biomass. Larger eggs were associated with

poor maternal somatic condition, apparently in contrast to some previous studies (e.g. Donelson *et al.* 2009), but this result may be because the poor somatic condition was a consequence of the investment in eggs. However, the early-life maternal phenotype was also important. Mothers who grew slowly when young (SEG females) tended to produce larger eggs at maturity, as found previously (Thorpe *et al.* 1984; Jonsson *et al.* 1996; Taborsky 2006). These offspring had a high survivorship, but the average relative contribution of SEG and FEG mothers to the population in terms of predicted offspring biomass was equal, presumably because FEG mothers produce more, albeit smaller, eggs. Interestingly though, the relationships between egg size and the survival and biomass of offspring differed among FEG and SEG females. For FEG females these measures of were positively related to egg size, whereas negative relationships were evident among SEG mothers. Although the mechanisms underlying this result are unclear, it is possible that FEG females, who by definition grew fast as juveniles, may be of higher than average quality and so are able to produce higher quality eggs irrespective of their body size at the time of spawning. Thus, a stronger correlation between egg size and egg 'quality' may be present among such females, as indicated by the relatively fast growth and better rates of survival of their offspring compared to those hatching from the same size of egg produced by SEG females. Offspring survival and biomass production were more closely related to maternal body size in SEG than in FEG mothers. This suggests that small SEG females may be producing lower quality eggs, even if their eggs tend to be relatively large. Thus, maternal body size at the time of spawning may be more important for females that had experienced slow rates of juvenile growth. It is also worth noting that I recorded a positive relationship between the upstream density of conspecifics from the same age class and the body size of surviving juveniles, when the inverse of this relationship is generally observed (Einum *et al.* 2011). Presumably, the pattern in my study reflected spatial variation in habitat structure and quality such that areas that supported high densities also allowed fast growth.

My results collectively suggest that different kinds of offspring (as well as offspring of different size) may be produced by mothers that vary in their current reproductive state and their rate of growth as a juvenile. If this is the case, do such maternal influences constitute an adaptation or a constraint (Marshall & Uller 2007)? Currently, little empirical or theoretical evidence exists with which to evaluate these possibilities in relation to maternal somatic condition and reproductive investment. Links between parental ontogeny and

offspring phenotype might enable parents to 'program' offspring phenotypes to match the conditions that offspring are likely to encounter in early life (e.g. Bateson *et al.* 2004). If juvenile and adult ecologies differ, as is the case with Atlantic salmon, cues from conditions experienced by adults in the run up to spawning may be poor predictors of the environment offspring are likely to encounter, and so maternal investment in offspring may be better determined by a mother's own experience as a juvenile (Taborsky 2006).

Fast Early Growth and Slow Early Growth female salmon appear to produce different types of offspring, but these might be suited to different ecological conditions. For example, growth rate in juvenile salmon declines with the altitude of the stream they inhabit. Even within a relatively small altitudinal range; the mean size of 1 year old fish can decline by over 33 % with an increase in altitude of only 270 m (Egglishaw & Shackley 1985; Baum *et al.* 2004). High-altitude tributaries are known to produce a higher proportion of SEG fish (Shearer 1992), presumably because a minimum size threshold must be reached before juveniles migrate to the sea (Metcalfe & Thorpe 1990). Thus, it is possible that FEG females are more likely to originate from lower altitude, more food-rich tributaries, while SEG females are more likely to come from higher altitude streams with poorer growth opportunities. Given such differences in early growth performance, it is possible that FEG and SEG females are producing eggs/offspring that are suited to these different niches. Alternatively, mothers that developed slowly as juveniles may be low quality individuals, that then produce lower quality offspring (Monaghan 2008). It is not possible to separate these hypotheses with the current experiment as it was conducted in a relatively homogeneous environment – all eggs were stocked at a uniform density into a single section of a mid-altitude stream. Further research is needed where the effects of maternal reproductive investment, somatic condition and juvenile growth rate are disentangled experimentally and the growth and survival of offspring then evaluated under a range of environmental conditions.

SUPPLEMENTARY MATERIAL

Figure 5.1. The predicted values from the optimal LME model for FEG and SEG females (dashed and solid lines respectively) are based on a female of average somatic condition (-0.002) and reproductive investment (-0.009).

Figure 5.3. In (a) to (b) the predicted values are based on average upstream densities of 46.3 same-age salmon and 12.4 older juvenile salmon respectively. Additionally in (a) they are based on a female of average body size (fork length = 57.9 cm) and reproductive investment (-0.009), in (b) they are based on a female of average body size (fork length = 57.9 cm) and (c) they are based on a female of average reproductive investment (-0.009).

Figure 5.4. In (a) the predicted values are based on a female of average body size (fork length = 57.9 cm) and somatic condition (-0.002), in (b) a female of average somatic condition who produced eggs of average mass (105.9 mg) and in (c) a female of average body size who produced eggs of average mass.

Figure 5.5. In (a) the predicted values are based on a female of average body size (fork length = 57.9 cm) and somatic condition (-0.002) who produced eggs of average mass (105.9 mg). In (b) to (d) the predicted values are based on a female of average reproductive investment (-0.009). Additionally in (b) the predicted values are based on a female of average body size and somatic condition, in (c) a female of average body size who produced eggs of average mass and in (d) a female of average somatic condition who produced eggs of average mass.

CHAPTER 6. GENERAL DISCUSSION

In nature, individual animals of the same species, age and sex can differ remarkably in their size, physiology and behaviour. Understanding the causes and consequences of this variation is an emerging field in ecology and evolution (Bolnick *et al.* 2003; Sih *et al.* 2004; Williams 2008). The research presented in my thesis explores a key component of this phenomenon: maternal influences (Green 2008; Venturelli *et al.* 2010). Maternal influences describe how phenotypic or genotypic variation among mothers in one generation can affect the phenotypic development and performance of their progeny in the subsequent generation

In my review of the published literature (Chapter 2), I concluded that RMR is an important phenotypic trait with consequences for individual fitness. Implicit in much of the literature is an assumption that either a high or low rate of RMR should be favoured by phenotypic selection. Instead, I offered an alternative proposal, the 'context-dependence' hypothesis, summarising evidence that high rates of energy metabolism are linked with high rates of growth and survival when conditions are 'good', (e.g. abundant and predictable food) and vice versa in 'poor' conditions. Consequently, I proposed that large variation in energy metabolism might persist within populations because environmental conditions frequently change from 'good' to 'poor' over time. This agrees with current theory on patterns of phenotypic selection in nature (Siepielski, DiBattista & Carlson 2009). Furthermore, using evidence from a range of species, I demonstrated that diverse factors, such as genotypes, early developmental conditions and maternal influences (e.g. through the transfer of maternal hormones to the egg) can contribute to within-species variation in RMR.

In light of the relationships between egg hormones (e.g. testosterone and cortisol) and offspring RMR that have been reported in a range of vertebrates (see Chapter 2), I employed brown trout, *Salmo trutta* (L.), as a study species in Chapter 3 to address the hypothesis that offspring energy metabolism will be influenced by levels of egg cortisol and/or testosterone. Stream-dwelling salmonid fishes such as trout are ideal species in which to investigate questions regarding hormone mediated effects on the development of variation in phenotypic traits such as energy metabolism. Firstly, their eggs contain substantial concentrations of steroid hormones, which can vary both within and among

clutches (Stratholt *et al.* 1997; Suter 2002; Sloman 2010) and secondly, SMR can vary 2 – 3 fold, even among siblings (Metcalfe *et al.* 1995; McCarthy 2001). The results of the testosterone manipulation were difficult to interpret due to it resulting in unnaturally high levels of hormone. Contrary to predictions, manipulation of egg cortisol levels had no influence on offspring RMR.

However, the relationships between levels of egg cortisol and the growth and social status of juvenile brown trout (Chapter 3) were similar to the general trends observed in avian species. This occurred despite the absence of parental care and hatching asynchrony that have been identified as causes of egg hormone variation in birds (Groothuis *et al.* 2005; Love *et al.* 2008). In birds, egg hormone levels (testosterone and cortisol, for example) commonly increase or decrease with laying order across the clutch. This has been interpreted as a maternal mechanism for the favouritism or neglect of certain offspring (Groothuis *et al.* 2005; Love *et al.* 2008) because females are able to influence the post-hatching development of their offspring by providing parental care. In contrast, female salmonids, like most fishes, produce relatively large clutches of small eggs that are spawned almost simultaneously. Egg development in salmonids typically occurs over a protracted period (e.g. > 5 months, Elliott 1994) and parental care ceases after egg-laying (apart from a brief period of nest guarding in some species). This reduces the ability of females to influence and/or predict the conditions their offspring will experience after they emerge from their gravel nests.

My findings thus suggest a general link between maternally derived cortisol and offspring phenotype among vertebrates, but indicate that the mechanisms underpinning the evolution of these systems may vary according to reproductive mode. In highly fecund mothers such as salmonid fishes, I propose that egg hormones may favour the production of variation in offspring phenotypes, either among-or within-clutches. Why? Juvenile salmonids inhabit environments with high spatial and temporal heterogeneity, conditions under which variation in sibling phenotypes may promote maternal fitness (Crean & Marshall 2009). Additionally, juveniles typically emerge from the nest into high densities and accordingly competition is intense. This has led to suggestions that heterogeneity in offspring, either within- or among- clutches, may broaden their utilisation of different ecological niches, increasing the prospects of offspring survival and therefore the maternal contribution to the population (Griffiths & Armstrong 2001; Armstrong *et al.* 2011).

Empirical evidence, presented in Chapter 4, is supportive of this possibility. I demonstrated that within a single clutch, female brown trout can produce substantial differences in offspring size, social status and energy metabolism. Moreover, in mothers that were dominant or produced large eggs, the distribution of variation in sibling phenotypes (size and energy metabolism) was linked systematically to their position within the clutch prior to spawning. This suggests that environmental conditions prior to spawning or intrinsic differences among individuals (e.g. dominant vs. subordinate) can affect how highly fecund mothers, such as fishes, allocate resources within a clutch.

In chapter 5, I demonstrated, using wild female Atlantic salmon, *Salmo salar* (L.), that variation in offspring investment strategies among mothers that adopt different life history strategies can affect the growth and survival of their offspring in natural conditions. Female salmon that had grown slowly as juveniles, but had invested heavily in reproduction and were in relatively poor body condition at spawning, produced larger eggs on average. In accordance with previous findings, larger eggs resulted in larger juveniles and higher juvenile survival (Chambers & Leggett 1996; Einum & Fleming 2000b). However, for eggs of a given size, offspring growth was positively related to the level of maternal investment in reproduction and remarkably, the juvenile growth rate of the mother. Furthermore, reproductive success (offspring survival and biomass per female) in the stream was also related to the juvenile growth rate of the mother. Among females that grew slowly as juveniles, reproductive success was positively related to maternal body size at spawning. In contrast, the reproductive success of females that grew quickly as juveniles was instead related positively to the size of egg they produced and to a lesser extent maternal body condition. These findings add to recent evidence of an important relationship between early developmental conditions and patterns of offspring investment (Taborsky 2006; Vijendravarma *et al.* 2010; Segers & Taborsky 2011b).

FUTURE DIRECTIONS

Maternal ‘control’ over variation in offspring investment, either among-females or within-clutches is not necessarily a prerequisite for effects on maternal fitness (Marshall & Uller 2007). Differences in offspring provisioning, either among- clutches or within them, may passively reflect the maternal state prior to spawning. For example, in female brown trout, levels of plasma cortisol increase markedly as spawning nears (from 10.3 to 44.1 ng

ml^{-1}, between November and late December) and there is among-female variation in this pattern (Pickering & Christie 1981). A similar pattern in cortisol levels have been reported in chum salmon (*Oncoryhnchus keta*) as they migrate from the coast to their freshwater spawning grounds (approximate values - coast; 200 ng ml^{-1}, midway; 200 ng ml^{-1}, spawning grounds; 600 ng ml^{-1}, Onuma *et al*. 2003). There are no published descriptions of the pattern of pre-spawning levels of plasma testosterone in female brown trout or Atlantic salmon. However, in chum salmon plasma testosterone tends to increase after the fish have entered freshwater but then decrease as the fish approach the spawning grounds (approximate values - coast; 200 ng ml^{-1}, midway; 400 ng ml^{-1}, spawning grounds; 150 ng ml^{-1}, Onuma *et al*. 2003). Thus, it is possible that egg hormone levels may simply correlate with those of the maternal plasma. Furthermore, in experiments that manipulate the egg hormone content of fish, the definition of a 'physiologically relevant' increase is vague. Some authors have compared whether the hormone content of manipulated eggs falls to the level observed in control eggs a few days after treatment (Suter 2002; Sloman 2010). Whereas other authors compare the hormone content of manipulated eggs immediately after treatment to the distribution of values observed within the source population of that species (McCormick 1999). Ultimately, more baseline data from wild fish populations that documents variability in egg hormone content and the factors that influence it may help elucidate both the degree of maternal control over egg hormone content and also define more clearly the physiologically relevant limit of egg hormone manipulations.

Analysing the hormone content of eggs from different regions of the egg mass is a logical starting point to investigate the mechanisms underlying the distribution of offspring phenotypes within a clutch that were presented in Chapter 4. In fishes, relationships between egg hormone levels and the growth and survival of resulting juveniles have only been examined in laboratory conditions. Given that the outcome of maternal influences often depends on the environment (Rossiter 1998), we really need data from natural or semi-natural conditions that relate egg hormone levels to the fitness of larval fishes and hence their mothers. Ideally, this evaluation of the fitness consequences should be extended to older life stages. For example, the data presented in Chapter 3 suggest that hatching from an egg containing a relatively high level of cortisol is likely to result in a smaller body size and inferior social status at the fry stage. However, whether this is maintained over subsequent life history stages of an individual is not known. Salmonids

are ideal organisms in which to investigate these questions. Naturally occurring populations are often excluded from habitable streams by migration barriers to spawning adults (e.g. as used in Chapter 5). This means that such streams could be stocked with groups of hormone manipulated eggs/juveniles of differing parentage. Over time, the relative performance of individuals from treatment and control groups could be compared by determining the parentage of re-captured survivors using molecular markers. If females generate adaptive variation in offspring phenotypes, e.g. via cortisol transfer to the egg (as hypothesised in Chapter 3), then one would predict higher juvenile survivorship or biomass production in sections of stream stocked with egg batches of heterogeneous (but within the natural physiological range), rather than homogenous cortisol content.

In chapter 5, I showed that, when controlling for egg size, offspring growth in field conditions was positively related to the juvenile growth rate of the mother. I proposed that females who experienced either relatively fast or slow growth as juveniles may produce offspring that are suited to their own developmental conditions: SEG (slow early growth) females are more likely to come from high altitude streams with poorer opportunities for growth, whereas FEG (fast early growth) females are more likely to originate from low altitude, more food-rich tributaries. If this is the case, what offspring traits might FEG and SEG mothers benefit by influencing? Juvenile salmon in high altitude streams grow more slowly, even within a relatively small altitudinal range, because higher altitude streams are colder and less productive, with a shorter growing season (Egglishaw & Shackley 1985; Baum *et al.* 2004). Given their likely poorer opportunities for growth, the offspring of SEG females would benefit from having a large nutrient reserve during the critical phase after emergence from the nest. Indeed, SEG mothers tend to produce larger eggs (Thorpe *et al.* 1984; Jonsson *et al.* 1996) and the size of salmon eggs is a strong predictor of their nutritional composition (Berg *et al.* 2001).

However, growth in juvenile salmon is also dependent on both access to food and the ability to convert food into new tissue. Access to food depends not only on the availability of food in the environment, but also the competitive phenotype of the individual. When juvenile salmonids emerge from their nests, there is intense competition for feeding territories, resulting in density-dependent survival and growth (Einum *et al.* 2008). The particular phenotypes that are favoured in this 'critical early period' for survival (Elliott 1994) depend on the environment. Larger eggs give rise to larger juveniles, which have a

survival advantage under poor growth conditions (Hutchings 1991; Einum & Fleming 1999). Moreover, competitive ability can be more related to SMR than body size (McCarthy 2000) and juveniles with higher SMR's can also process food faster (Millidine *et al.* 2009). In productive environments this means that juveniles with a relatively high SMR are more likely to be dominant, gain productive territories and grow faster (Metcalfe *et al.* 1995; McCarthy 2000). However, this more competitive phenotype is not necessarily suited to poor environments. When food is either limiting or unpredictable, a high SMR may be of no advantage because gains in food intake are unable to offset the higher 'running costs' of this phenotype, leading to no relationship between dominance and growth (Reid *et al.* 2011; Reid, Armstrong & Metcalfe 2012). Similarly, a high SMR is of little advantage if ambient temperatures are low, since fish (irrespective of their social status or access to food) cannot grow fast at cold temperatures (Forseth *et al.* 2001).

It would thus seem plausible that females who grew slowly as juveniles and took longer to reach the smolting stage might produce offspring that in comparison with faster early growing females: (a) are provisioned better, (b) have a lower SMR, (c) are less aggressive, and (d) are favoured, in terms of growth and survival, in low food/high altitude environments but are likely outcompeted in high food/low altitude environments. This may result from maternal effects (as found for offspring SMR, Chapter 2) or local adaptation, arising from the fact that salmon tend to spawn in their natal environment (Garcia de Leaniz *et al.* 2007). If maternal effects are the underlying cause, mothers who experience different growth trajectories as juveniles may influence the growth and survival of their offspring by altering levels of important substances such as carotenoids, hormones and hormone receptors (Tyndale *et al.* 2008; Bazyar Lakeh *et al.* 2010; Segers *et al.* 2011).

PRACTICAL APPLICATIONS

Maternal influences are often incorporated into fisheries management programmes which aim to protect older, larger females on account of their higher fecundity and the larger, higher quality young they produce. Whether the offspring of particular parents are 'programmed' for particular environmental conditions currently attracts great interest from disciplines ranging from medical epidemiology to evolutionary biology (Bateson *et al.* 2004). However, this concept is largely absent from conservation and population supplementation programmes which would presumably benefit from prior knowledge that

offspring from specific parents would perform better in some environments than in others. Virtually all wild Atlantic salmon stocks are subject to management interventions, often in the form of stocking hatchery-reared eggs or juveniles to supplement wild stocks (Waples, Ford & Schmitt 2007; Naish *et al.* 2008). Management programmes for salmonid fishes currently do not take into account variation in the life history of the parental brood stock when deciding where to release eggs or juveniles. Many salmonid populations are maintained or enhanced by stocking eggs or fry, obtained from wild females, into areas of apparently suitable juvenile habitat which cannot be accessed by wild fish, e.g. due to natural or man-made barriers such as hydroelectric power dams that prevent the upstream migration of spawning adults (Waples *et al.* 2007; Naish *et al.* 2008). Normally, eggs from different females are fertilised artificially, mixed and then planted out (either as eggs or fry) without considering whether offspring from particular females are likely to be more suited to certain habitats. Invariably there is little evaluation of the success rate of the stocking policy adopted (Waples *et al.* 2007; Naish *et al.* 2008). This may not be the most effective way of managing such supplemented populations. Therefore, if the differences in offspring phenotype among FEG and SEG mothers, reported in Chapter 5, are adaptive, then the success rate of stocking programmes could be significantly increased if the distribution of eggs among stocking sites is not random but accounts for both the size of eggs and the phenotype of the female who produces them, so as to match types of egg to particular environments within a catchment.

Maternal influences may have other inadvertent consequences for fish conservation and aquaculture. In fisheries supplementation programmes and aquaculture, brood stock females (of either wild or hatchery origin) tend to be held in stressful conditions prior to spawning. The fish are generally maintained at very high densities and exposed to routine hatchery practices such as transportation, confinement, handling and cleaning. Stressors in the hatchery environment may thus affect offspring performance by influencing female body condition, which correlates with juvenile survival (Chapter 5) or by affecting egg hormone content. For example, a confinement stressor applied to maturing female brown trout, elevated their plasma cortisol levels above those observed in control fish (stressed individuals; 6.9 ± 1.2 ng ml^{-1}, control individuals; 2.6 ± 0.8 ng ml^{-1}) (Campbell, Pottinger & Sumpter 1994). In the same study, plasma testosterone levels of the stressed females were significantly reduced in comparison to controls (stressed individuals; 38.7 ± 5.6 ng ml^{-1}, control individuals; 57.3 ± 6.1 ng ml^{-1}) (Campbell *et al.* 1994). If egg hormone levels

reflect those of the mother, hatchery stressors that alter maternal hormone levels outside of the natural changes that occur before spawning (e.g. Pickering & Christie 1981) might be transmitted to eggs with effects on offspring growth and social status (Chapter 3). Indeed, it seems likely that the hormone content of salmonid eggs reflects maternal plasma levels during late oogenesis. A physical stressor applied to adult female coho salmon (*Oncorhynchus kisutch*), during late oogenesis elevated plasma cortisol levels (227.5 ± 61.5 ng ml^{-1}) above those observed in control fish (141.0 ± 42.7 ng ml^{-1}). After the fish had ovulated, egg cortisol content (25.3 ± 0.77 ng g^{-1}) was significantly higher in the disturbed than in the undisturbed females (9.90 ± 0.94 ng g^{-1}) (Stratholt *et al.* 1997). Other studies on rainbow trout and brown trout suggest that exposure of adults to stress may cause a reduction in egg size, juvenile size and survival rates of progeny (Campbell, Pottinger & Sumpter 1992; Campbell *et al.* 1994; Contreras-Sanchez *et al.* 1998). Thus, if egg hormone levels are changed from naturally occurring levels by stressors imposed upon mature females in the hatchery environment, juvenile performance during early development in supplemented populations or aquaculture may be affected.

CONCLUSION

In my thesis I have demonstrated that maternal influences are a substantial source of individual variation in offspring phenotypes. I have shown that the relationship between a mother and her progeny can encompass variation in offspring size, behaviour and physiology, both among clutches and within them. My research also adds to evidence from a range of taxonomic groups that egg hormones such as cortisol are important agents in mediating maternal influences on offspring. Furthermore, my research underscores the remarkable complexity of maternal influences which can stem from conditions experienced by mothers, both as adults and during their own development as juveniles and have pervasive consequences for offspring ecology in natural populations.

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