Decoupling aggression and risk-taking: patterns of variation in two species of freshwater fish

María de Lourdes Ruiz-Gómez

Thesis submitted in fulfilment of the requirements for the degree of Doctor in Philosophy

Department of Ecology and Evolutionary Biology

Faculty of Biomedical and Life Sciences

University of Glasgow

Declaration

I declare that the work described in this thesis is my own, unless otherwise is stated. No part of the work shown here has been submitted as part of any previous examination. Supervision by F. Huntingford has helped to develop ideas throughout the thesis.

María de Lourdes Ruiz-Gómez

ABSTRACT

An extensive literature has documented the existence of suites of correlated behavioural traits (called behavioural syndromes) in a range of vertebrate species, as well as in some invertebrates. The existence and persistence of such behavioural syndromes is of both fundamental and applied interest and the main aim of the work described in this thesis was to examine sources of individual variation in risk-taking and aggression, as well as the circumstances under which those behaviours could be uncoupled. The study used two species of freshwater fish that have become something of a model to study behaviour: the three spine stickleback (*Gasterosteus aculeatus*) and the rainbow trout (*Oncorhynchus mykiss*).

In **chapter 1** I give a background on the current research on individual variability in behaviour, behavioural syndromes and coping strategies, with special reference to fish; as well as considering the implications of behavioural syndromes for evolutionary biology and aquaculture. C**hapter 2** describes a long-term experiment on boldness, aggression and the relationship between them in sticklebacks that grew at different rates under two different competitive regimes. In one treatment (the low interaction condition) food was dispersed, while in the other (the high interaction condition), food was clumped. Fish were fed to excess in both treatments. Analysis of the relationship between risk-taking and some morphological variables showed that, in general, shy fish were heavier and longer than both bold and behaviourally intermediate fish, independently of their body condition. Fish from the low interaction condition were more aggressive than those from the high interaction feeding regime. Boldness and aggression were positively associated only in the fast growing fish from the high interaction competitive regime.

Limited evidence suggests that individual personalities may influence reproductive success and other fitness-related traits in complex and context-specific ways. In the study described in **Chapter 3**, I used an indirect approach to relate fitness to personalities in sticklebacks. Specifically, I related hatching date of fry (used as an indirect measure of parental fitness) to their personalities (boldness and aggression). Individuals that hatched early were

bolder than late hatched fish, whereas most of the shy individuals were found among the late bred fish. There were no detectable differences in aggression between early and late hatched fish, but there was a relationship between boldness and aggression independent of hatching date.

In **chapters 4 and 5**, I describe studies of rainbow trout from two lines selected for breeding for low (LR) or high (HR) post-stress plasma cortisol response that have become something of a model system for studies of coping strategies in fish. In addition to striking differences in cortisol responsiveness, LR and HR fish show patterns of brain biochemistry, risk-taking and aggression that are typical of so-called proactive and reactive animals respectively.

The results reported in **chapter 5** strengthen this interpretation, by comparing behavioural flexibility and response to novelty in 3rd generation LR and HR rainbow trout. After being trained individually to find food in one arm of a T-maze, HR fish were able to found food strikingly faster than LR trout when the resource was moved to a different position. In contrast, LR fish were much less distracted by the presence of an unfamiliar object. Previous studies have shown that proactive animals develop and follow routines more strictly than do reactive animals, while the latter are more aware of changes in their environment. My results therefore give further support to the characterisation of LR and HR rainbow trout as showing proactive and reactive coping strategies.

In **chapter 4** however, I complicate this interpretation by showing that the relationship between boldness and aggression is flexible. Following transport from the UK to Norway, HR and LR fish switched behavioural profiles. In contrast to the results of previous studies, HR fish fed sooner in a novel environment and became dominant over LR fish in pairwise aggressive interactions. One year after transport, HR fish still fed sooner than LR fish, but no difference in social dominance was found. Among offspring of transported fish, no differences in feeding rates were observed, but as in pre-transported 3rd generation fish, HR fish lost fights for social dominance against size matched LR opponents. Transported fish and their offspring retained their distinctive physiological profile throughout the study, with HR fish showing consistently higher post-stress cortisol levels at all sampling points. Therefore the striking difference in cortisol responsiveness in these two strains of trout is on its own not sufficient to maintain distinct behavioural phenotypes.

The work described in this thesis therefore extends current understanding of individual variability in behaviour and of behavioural syndromes by identifying circumstances under which risk-taking and aggression are uncoupled in two species of freshwater fish. It also suggests some potential consequences for fitness. In **chapter 6** these results are discussed on the light of current research on animal personalities, behavioural syndromes, coping strategies and their implications for evolutionary biology. Particular reference is made to the existing literature on fish and the implications of those findings for aquaculture are also discussed.

Acknowledgments

This work would not have been possible without the help and support of many people. First of all I would like to thank my supervisor Felicity Huntingford for being such a great supervisor (the best to be fair) during these 3 years. Thanks Felicity for all your time and patience, for sharing your knowledge, for being so understanding and supportive in all respects and for giving me the fastest feedback I have ever seen, thanks to this I finished this soon. Thank you also for having me in your house several times which made me feel less far from home, (this thank you extends to Jim as well).

I am also indebted to Dr. Øyvind Øverli who had me in his lab for more time than he should, thank you Øyvind for all you help, patience, knowledge, company, fishing trips (to have a rest from the fish) ah! and thanks for the scooter as well, I'm still going to claim it back! A big thank you also goes to Dr. Erik Höglund for hosting me in his lab and for being such good company. Thank you Erik for all what you did (and the things you let me do!), for your advice and help so I could finish my experiment, I hope we can keep collaborating. Tom Pottinger and Alison Bell gave me a very helpful feedback on some of my chapters. I am also thankful to my examiners Prof. Carin Magnhagen from the Swedish University of Agriculture Sciences and Prof. Neil Metcalfe from the University of Glasgow for their excellent comments and for making the examination a very smooth enjoyable process.

I would like to thank to the Norwegian University of Life Sciences, the Danish Institute of Fisheries Research, the Scottish Centre for Ecology and the Natural Environment and the Division of Ecology and Evolutionary Biology for the use of their facilities, special thanks to Øle Madvig Larsen, Graham Law and John Laurie for helping me with fish husbandry and quite a few other technical matters. I am also grateful to all people at DEEB especially to Lorna Kennedy for all her help from before the start of my PhD and for all the sweeties that kept me conscious on the less sunny days.

Many people around Glasgow, Norway and Denmark made these years an unforgettable experience. Silje, Sunny, Celine, Miran, Nick, Tania, Jonathan, Leandro, Caro, Jen, Flavia, Fabio, Pierre thank you for being a great company and for your friendship. Gume, Sue, Jas and Liliana have been of great support, providing me many times with a warm bed where to lay my head on, and listening to all my moaning and swearing when things were just not going very well. Thank you guys for being great friends, for all the good fun and for making me feel that home was not too far. Thank you to my office mates for all your support and for never complaining about my mess and sometimes my bad mood.

My friends in Mexico, too many to name, have always been here despite the distance. Thank you for always listening, making me laugh and reminding me every single day that this worth all the while. All of you have helped me to complete this stage in very many aspects, thank you for your constant (long-distance) encouragement and for just being my friends.

A mi familia, padres y hermano muchas gracias por su amor, paciencia y comprensión. Sin su apoyo este hubiera sido un proceso mucho más difícil.

Finally, I'm undoubtedly grateful to the Autonomous University of the State of Mexico and CONACyT, Mexico for funding this PhD without which, surely it would not have been even started. I would like also to thank all the little fishes that helped me (involuntarily) to obtain such interesting findings, and also to the ones that did not help at all; this thesis is on you!

María de Lourdes Ruiz-Gómez

Table of contents

CHAPTER 1 GENERAL INTRODUCTION

CHAPTER 2 VARIATION IN RISK-TAKING, AGGRESSION, AND BEHAVIOURAL SYNDROMES IN STICKLEBACKS THAT GROW AT DIFFERENT RATES IN TWO COMPETITIVE ENVIRONMENTS

CHAPTER 3 DIFFERENCES IN RISK-TAKING, AGGRESSION AND THEIR RELATIONSHIP IN STICKLEBACKS THAT HATCH AT DIFFERENT TIMES DURING THE BREEDING SEASON

CHAPTER 4 BEHAVIOURAL PLASTICITY IN RAINBOW TROUT (*Oncorhynchus mykiss*) WITH DIVERGENT COPING STRATEGIES

CHAPTER 5 COPING STRATEGIES IN RAINBOW TROUT: FLEXIBILITY AND RESPONSE TO NOVELTY

CHAPTER 6 GENERAL DISCUSSION

ACCOMPANYING MATERIAL

List of tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

CHAPTER 1 GENERAL INTRODUCTION

General Introduction

Individual variation in behaviour

It has long been recognized that individual animals show variability in their behavioural responses to particular stimuli; for example, one animal may feed vigorously whereas another of the same species may ignore identical food. In some cases, such variation in behaviour is the result of differences in the general environment. For example, the presence of a predator may suppress feeding in certain cases. In other cases, individual differences in response to a particular stimulus might result from changes within the individual concerned. For example, a recent meal or a predatory attack would suppress feeding. In yet other cases, individual variation in behaviour might result from inherited differences between the animals concerned. For example, inherited differences in growth hormone production are known to influence appetite in several vertebrates. In addition, there may be interactions between these sources of variation; for example, animals may show inherited differences in the extent to which their behaviour (feeding, for example) is altered by experience (prior predatory attack, for example). All these different kinds of effect, as well as interactions between them, have been reported in the case of individual variation in risk-taking and aggression, which are the topics of this thesis.

Individual differences in risk-taking

Animals as diverse as mice (Benus *et al.*, 1991), birds (Dingemanse & Rèale, 2005), fish (Budaev, 1997) and invertebrates (Sinn & Moltschaniwskyj, 2005) show individual variation in behaviours such as risk-taking, aggression and exploration. Some of this variation is the result of prior experience; in other cases, differences in risk-taking may be a result of social interactions. For example, Frost and collaborators (2007) found that in rainbow trout (*Oncorhynchus mykiss*); individuals can modify their behavioural responses after observing a conspecific's behaviour. In perch (*Perca fluviatilis*) social learning affected foraging behaviour and, indirectly, boldness (Magnhagen & Staffan, 2003).

Variability in risk-taking can also be mediated by underlying genetic mechanisms. For example, selection over generations has produced strains of mice with different latency to attack a conspecific (short-SAL and long-LAL) (Benus *et al.*, 1991; Vanoortmerssen & Bakker 1981). Wild and lab reared great tits (*Parus major*) also show genetically mediated divergence in the way they explore a new environment. Birds have been bred for that trait in two lines (fast and slow explorers) and a response to selection has been consistently found for over four generations, showing a high degree of heritability (54±5% Dingemanse *et al.*, 2002; Drent *et al.*, 2003).

Consistency in risk-taking and "personality" in animals

It is still debatable whether behavioural phenotypes are consistent across contexts and situations, or whether behavioural responses can be flexible. Few studies have looked at within-individual consistency in behaviour (also termed repeatability) and the results seem to be ambiguous. For example, Beauchamp (2000) found that individual zebra finches (*Taenopygia guttata*) that explored a particular feeding area, consistently visited the same feeding spot across trials. In dumpling squids (*Eupryma tasmanica*), differences in boldness were consistent and repeatable across time and two contexts. These were a feeding test where squids were presented with live food in the presence of the experimenter and a threat test where individuals were touched (Sinn *et al.*, 2008). However, in great tits selected for fast or slow exploration of a new environment, consistency over time and across situations was found only between lines. Individual behaviour varied, fast explorers were more consistent than slow explorers and slow explorers were flexible in their responses (Carere *et al.*, 2005).

Several different terms have been used to describe consistent individual differences in behaviour. Where these exist, the term "personality" has been applied primarily to humans (Goslin, 2001). Recently, Rèale and collaborators (2007) proposed use of the terms "temperament", "personality" and "individuality" as synonyms for describing behavioural differences in non-human animals that are consistent over time and across situations.

Behavioural phenotypes are usually not bimodal variables, unless artificial selection for behavioural or physiological characteristics has been carried out, for example, in the case of mice (Benus *et al.*, 1991) or rainbow trout (Pottinger & Carrick, 2001). Instead it seems that individuals often vary along a continuum. Generally, the extremes of the behavioural range are the subjects of study. For example, much interest is focused on the bold-shy continuum, which reflects the willingness of an animal to take risks in potentially dangerous situations (Wilson *et al.*, 1994) such as in the presence of predators. Aggressiveness, defined as the delivery of a potentially harmful stimulus to another animal of the same species (Huntingford & Turner, 1987) is also a well-studied personality trait. Other commonly measured behavioural axes include exploration-avoidance, activity and sociability (Gosling & John, 1999).

Correlations between behavioural traits

Studies show that single aspects of behaviour may vary consistently across situations and over time; however, correlations between behaviours in different contexts have also been found in several species. Sih and collaborators (2004) coined the term "*behavioural syndromes*" to describe "suites of correlated behaviours which are expressed within a given behavioural context or across different contexts". For example, in the three spined stickleback (*Gasterosteus aculeatus*) Huntingford (1976) found that the levels of aggression shown by individual males to conspecifics during the breeding season correlated with boldness towards a predator outside the breeding season, whereas Bell & Stamps (2004) showed that also activity in a novel environment and boldness under predation risk were correlated in the same species. In the cricket (*Gryllus integer*), aggressiveness towards size matched opponents was correlated with activity in a new, potentially dangerous environment (Kortet & Hendrick, 2007).

Coping strategies

Another dimension of consistent variability that has been extensively studied in birds, mammals and to some extent in fish is the proactive-reactive axis. This involves consistent correlations between risk-taking behaviour, aggression, dominance, response to new environments and aspects of metabolic and stress physiology. The term coping strategies has been used to describe "a coherent set of behavioural and physiological stress responses, which is consistent over time and characteristic to a certain group of individuals" (Koolhaas *et al.*, 1999). Genetic bases of such variability have also been identified in some cases, with animals responding to bidirectional selection of either behavioural traits such as aggressiveness, exploration, risk-taking (Benus *et al.*, 1991; Dingemanse & Goede, 2004) or physiological traits (post-stress cortisol levels) over generations (Pottinger & Carrick, 2001).

When challenged, proactive and reactive animals respond in one of two different ways. On one hand, the behavioural profile of proactive animals, compared to reactive ones, is to control the situation (by means of the fight/flight reaction), showing higher levels of aggressiveness, dominance, risk-taking or boldness, activity and active avoidance. The physiological response to changes in proactive animals tends to be a low hypothalamic-pituitary-adrenal axis activity (indicated by low levels of plasma cortisol) and predominantly a high sympathetic activation (shown by high levels of catecholamines). On the other hand, reactive animals are characterized by an acceptance of the change, responding with relative immobility and lack of initiative (showing a conservation/withdrawal response). Reactive copers tend to be shy and easily defeated by proactive individuals. Physiologically, reactive animals show parasympathetic-hypothalamic-activation (high levels of cortisol and low levels of catecholamines) (Kolhaas *et al.*, 1999; Korte *et al.*, 2005). Studies in rats, mice and pigs show that one of the main differences between proactive and reactive animals is the degree of behavioural flexibility and distractability (in terms of how changes are perceived) between phenotypes. Reactive animals tend to show flexible behaviour, perhaps because they are more aware of and reactive to any change in the environment. They are also easily distracted by change, whereas proactive animals are characterized by a rigid behaviour and the ready formation of routines (Benus *et al.*, 1991; Bolhuis *et al.*, 2004).

Decoupling behavioural syndromes

It is still not clear whether behavioural correlations of the kind discussed above can be decoupled. If behavioural traits in a syndrome are tightly correlated because they are driven by underlying mechanisms, such as common reliance on a single hormone, syndromes should be difficult to break. One source of information suggests that experiences could shape an individual's behavioural tendencies across a range of contexts. Some components of the neuroendocrine machinery, such as hormonal expression, are also modifiable by experience (Burmeister *et al.*, 2005; Burmeister *et al.*, 2007; Watt *et al.*, 2007); therefore, they are likely to reflect some degree of plasticity in associated behavioural traits.

The correlation between behavioural traits may also vary substantially if different correlations are favoured in different environments. This view has been supported by a recent study in which 12 populations of sticklebacks were explored for the existence of behavioural correlations. Activity, aggressiveness and exploration of new environments all correlated positively with each other, but this correlation was only present in environments with high predation pressure (Dingemanse *et al.*, 2007). This suggests that some environmental conditions such as the presence of predators can generate correlations between behaviours. Bell & Sih (2007) have shown the way predation pressure can modulate the expression of behavioural syndromes.

Figure 1.1 shows that boldness and aggression were not correlated in sticklebacks from a population characterised by low levels of predation pressure (see Bell, 2005 for details).

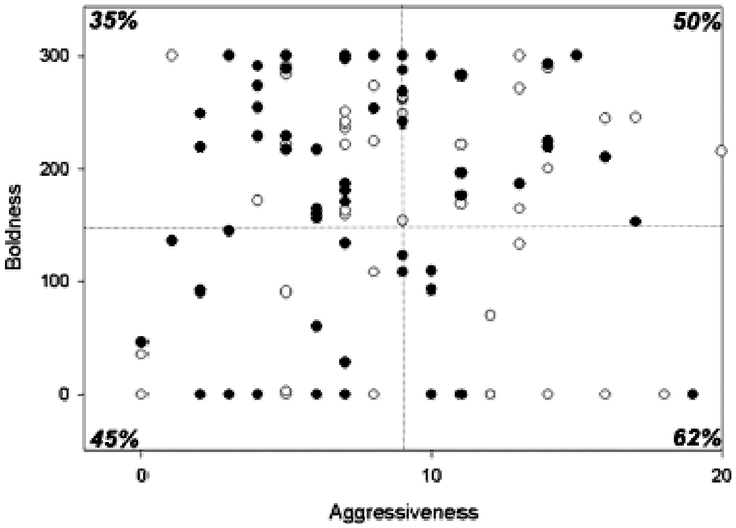


Figure 1.1 Boldness and aggressiveness were not correlated in individuals from a population of sticklebacks characterised by a low predation regime. The graph shows the survivors represented by open circles and individuals consumed by the predator represented by closed circles after fish were exposed to real predation. The percentages of stickleback's survival after predation are shown in each quartile (From Bell & Sih, 2007).

Bell & Sih (2007) subsequently exposed fish from that population to predation; interestingly, this generated a correlation between boldness and aggression (figure 1.2). This was caused by a combination of the predator feeding selectively on fish with particular behavioural phenotypes (bold fish were likely to be eaten) and behavioural flexibility of the surviving individuals. Therefore, there are circumstances under which behavioural syndromes are likely to be decoupled.

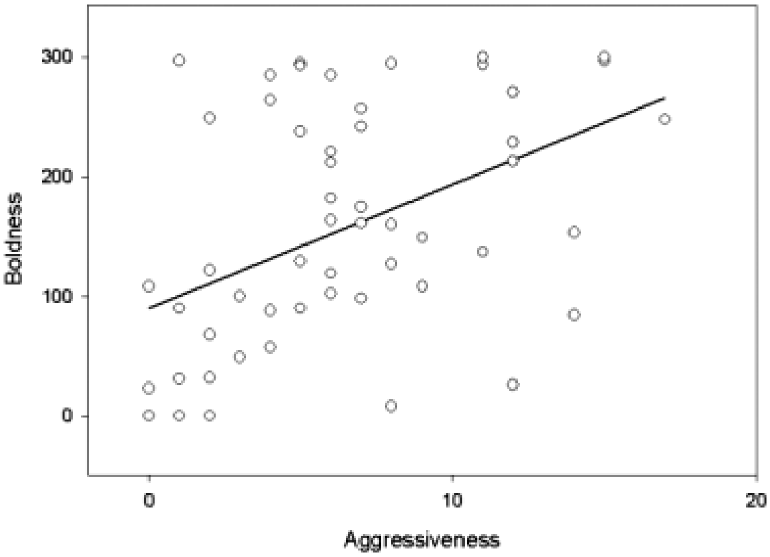


Figure 1.2 Boldness and aggressiveness covaried each other after sticklebacks from a population that previously did not show the correlation were exposed to real predation (From Bell & Sih, 2007).

Behavioural syndromes and coping strategies in salmonid fishes

A number of studies have documented the existence of consistently correlated behavioural traits in other fish species, for example, brown trout (*Salmo trutta*) (Sundström *et al.*, 2004) and grayling (*Thymallus thymallus*) (Salonen & Peuhkuri, 2006). There is relatively little information on the proximate mechanisms governing such behavioural variability in fish, although Bell and collaborators (2007) reported a correlation between individual risk-taking behaviour and brain biochemistry in sticklebacks. However, there is a good deal of information on possibly coping strategies in rainbow trout (Øverli *et al.*, 2005; Pottinger & Carrick, 1999; Schjolden & Winberg, 2007).

In the rainbow trout, a number of behavioural differences have been reported in strains selected for high (high responsive, or HR trout) and low (low responsive, or LR trout) cortisol responsiveness to a standardized stressor; details of the selection programme can be found in Øverli *et al.* (2005), Pottinger & Carrick (1999) Schjolden & Winberg (2007). Cortisol responsiveness is an individual, heritable characteristic in rainbow trout. The estimated heritability for stress-induced cortisol response in the HR and LR strains is of h2 = 0.41 (Fevolden *et al.*, 1999; Pottinger & Carrick, 1999). Behaviourally, fish from the LR strain tend to become socially dominant over HR fish (Pottinger & Carrick, 2001). HR fish show higher levels of locomotor activity in the presence of an intruder, whereas LR strain resume feeding earlier in isolation than do fish from the HR strain (Øverli *et al.*, 2002). The strains also differ in their cognitive ability; LR fish retain a conditioned response longer than HR fish do (Moreira *et al.*, 2004). The behaviours shown by the HR line (including reduced appetite, reduced ability to win aggressive encounters, enhanced locomotion during acute stressful challenges) are consistent with previously reported effects of cortisol in non-mammalian vertebrates (DiBattista *et al.*, 2005; Gregory & Wood, 1999; Øverli *et al.*, 2002). In non-selected rainbow trout, a negative correlation between the plasma cortisol response to stress and the levels of aggression shown towards subordinate fish has been found (Øverli *et al.*, 2004), as well as between cortisol responsiveness and risk-taking measured as the willingness to feed in novel environments (Øverli *et al.*, 2006b). Other hormones and neuropeptides are also involved in the regulation of these behaviours, so it seems unlikely that only cortisol is responsible for controlling all behavioural aspects of stress coping style (Koolhaas *et al.*, 2007; Øverli *et al.*, 2007).

Fitness consequences of individual variation in behaviour

The fitness consequences of individual variation in personality and their correlations have not been extensively studied. However, knowledge of the relationship between consistent individual behaviour and fitness may help to understand some of the ecological and evolutionary aspects of their expression (Gosling & John, 1999; Sih *et al.*, 2004). For example, Wilson and collaborators (1994) suggested that personality traits may be adaptive if individuals at the extreme ends of the behavioural distribution have the higher fitness. Various mechanisms, including frequency-dependent selection, have been suggested to maintain variation in the traits (Dall *et al.*, 2004). Examples of personality traits related to fitness have been shown in bighorn sheep ewes (*Ovis canadensis*); in which individual variation in boldness correlates positively with survival during seasons of high predation pressure (Rèale & Festa-Bianchet, 2003). Bluebirds (*Sialia mexicana*) aggressively defending their nesting territories do not invest in parental care, which results in low reproductive success for those birds compared to less aggressive individuals (Duckworth, 2006).

In great tits (*Parus major*), speed of exploration is related positively to aggressiveness and competitive ability (Verbeek *et al.*, 1999). Dingemanse and collaborators (2004) found that the fitness consequences of personality for fast and slow exploring birds were reflected in annual adult survival. However, the results were sex-dependent and varied between years. In this case, temporal variation in the competitive regime (food abundance) seemed to play a role in the maintenance of both phenotypes in this population. In poor winters, where food was scarce, slow exploring males and fast exploring females survived better, whereas the opposite was true for winters with abundant food, when possibly competition was more relaxed. Overwinter offspring survival was related to the mother's personality and also fluctuated with food abundance. Later, it was found that assortative mating between animals of different personalities of fast and slow explorers was likely to produce more surviving offspring (Both *et al.*, 2005).

In fish, there are few studies looking at the fitness consequences of personality traits and behavioural syndromes. Most of the work has been orientated to the study of individual and population differences of single behaviours or to the expression of covarying behaviours. However, from the few examples I can mention, Dugatkin (1992) found that guppies that show higher levels of predator inspection have a higher mortality rate than the more cautious individuals. Godin & Davis (1995) found that the same trait also had positive fitness consequences, because predators were significantly less attentive to, and less likely to attack, guppies that inspected them. Therefore, more work is needed in order to unveil the relationship between variation in behavioural phenotypes and fitness in fish.

Ecological and evolutionary implications of the boldness-aggression syndrome

If individuals vary consistently in behaviour, this may consequently affect how they will behave in different contexts. For example, the work by Huntingford (1976) showed that sticklebacks that were aggressive towards conspecifics during the breeding season, when such behaviour is possibly advantageous in for example, defending nesting territories, were also consistently bold against predators outside the breeding season, which might set them at high risk of predation (Bell & Sih, 2007).

The evolutionary implications of correlated behavioural phenotypes have been seen from two perspectives (Sih *et al.*, 2004). Firstly, the “constraint hypothesis” suggests that when two behavioural traits are controlled by the same underlying mechanism, for example a hormone, the behaviours might not be able to evolve independently. When selection favours one behavioural trait, it consequently will have an effect on a correlated behaviour, because it may be difficult or may take a long time to modify the shared proximate mechanism. Therefore, whereas behavioural correlations could promote fitness under one condition they could also be maladaptive under other circumstances. Hence, behavioural correlations could act as a constraint on the evolution of such behaviours and the correlation between behaviours would become a general characteristic of a species (Bell, 2005; Sih *et al.*, 2004). The “adaptive hypothesis” on the other hand, suggests that selection will favour correlated behaviours only in the contexts where such correlation is adaptive, whereas the correlation would be decoupled when it is maladaptive (Bell, 2005; Sih *et al.*, 2004).

In 2005, in a study describing behavioural variation between two populations of sticklebacks, Bell clearly showed how both hypotheses could work in two different populations (figure 1.3). Figure 1.3a shows that boldness and aggression are tightly correlated in one population (such as in the example by Huntingford, 1976; see above). The constraint hypothesis (figure 1.3b) will predict that common underlying mechanisms would cause boldness and aggression to be correlated within and between populations. On the other hand, the adaptive hypothesis (figure 1.3c) would suggest that individual levels of boldness and aggression are not correlated within populations though the average behaviours correlate positively between populations, 1.3c (i). Other assumptions can be that boldness and aggression are correlated, but the direction of the correlation is reversed in the two hypothetical populations, 1.3c (ii). Another scenario could be that boldness and aggression are correlated in one, but not in other population, 1.3c (iii).

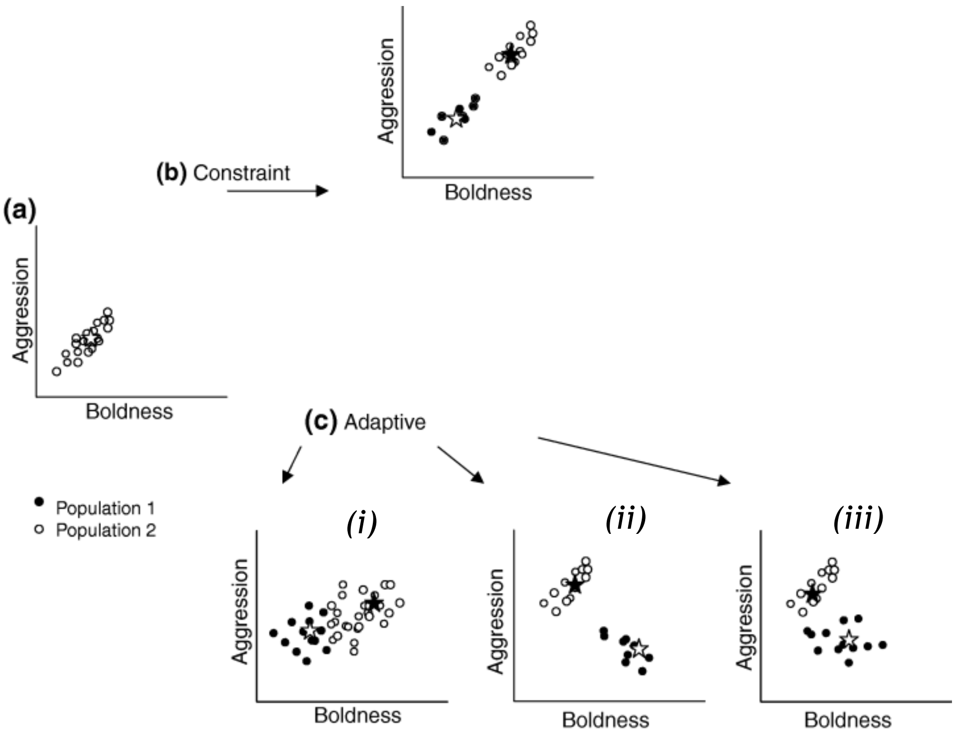


Figure 1.3 (a) shows that aggression and boldness are positively correlated in one population. The predictions for the constraint and adaptive hypotheses on the expression correlated behaviours are shown in (b) and (c) respectively. Closed and open circles represent different populations and stars show the mean for each population (From Bell, 2005).

Empirical evidence supports the adaptive view as a plausible explanation of the expression of behavioural correlations. For example, now it is becoming more clear that environmental conditions such as predation pressure can determine whether individual behaviours are coupled or not (see Bell, 2005; Bell & Sih, 2007; Dingemanse *et al.*, 2007). Therefore, consistent variation in behaviour does not necessarily constrain the evolution of behavioural phenotypes, because only under some conditions will behavioural correlations be favoured. However, more evidence is needed in order to elucidate under which particular circumstances behavioural correlations can be uncoupled and also to understand the role of proximate mechanisms in the expression of behavioural correlations.

Implications for aquaculture of the link between boldness and aggression

Aquaculture is an industry in expansion; the most recent estimated global aquaculture production was of approximately 66.7 million tonnes and the projections show that, due to the demands on the fish market, this increase will be sustained (FAO, 2007). Recently, the welfare of farm animals has become a concern and there is increasing pressure on the authorities to legislate on this matter; fish farming is not an exception. Research on fish behaviour, physiology and neurobiology is influencing the way fish are viewed. Now it is known that fish are complex animals capable of learning and more recently there is evidence that fish may well possess the neurological anatomy and the physiological and cognitive abilities necessary to feel pain (Portavella *et al.*, 2003; Sneddon, 2003b), although this issue is still controversial (Rose, 2002). Therefore, there has been an increase in research related to fish welfare and the introduction of legislation and guidelines to safeguard fish wellbeing.

However, apart from obvious signs of distress, the concept of exactly what constitutes good welfare for a farmed fish is unclear and still under debate. Although there is no agreement on how to assess fish welfare, there is a general consensus that a number of different indicators must be selected (Huntingford *et al.*, 2006). Possible indicators include health and condition (Goede & Barton, 1990), variation on feed intake and growth rates, physical damage, environmental monitoring. Measures of the behavioural and physiological stress response also provide a possible framework for assessing farmed fish welfare.

Studies on wild animals have shown how individual variability in behaviour, and their possible correlations, influence characteristics that are important for aquaculture, such as growth rates (Biro & Stamps 2008; Ward et al., 2004), resumption of feeding after disturbance (Øverli *et al.*, 2002) and reaction to novelty (Brown *et al.*, 2007). Studies have also revealed that domestication has the potential to modulate behaviour. Comparisons of the behaviour of farmed and wild fish reared in “common garden” experiments show that farmed fish tend to take more risks in potentially dangerous situations and in some circumstances to be more aggressive than wild fish (Sundström *et al.*, 2004).

This may indicate that under aquaculture conditions, if individual behaviours are tightly correlated, domestication and selection for desirable characteristics for aquaculture, such as fast growth rates, would also inadvertently select for behaviours that can compromise welfare, for example aggression. If this were a general trend, this would possess a problem for fish farming with evident implications for welfare, because the best fish for production systems should be bold individuals that are eager to feed, ideally showing fast growth rates. But, given a link between aggression and boldness, fish that show an appropriate combination of behavioural traits (risk takers and non-aggressive) are likely to be scarce in production systems.

The consequences of aggression and boldness in aquaculture are various. For example, during feeding, agonistic interactions between fish often increase. This may result in heterogeneous growth, since bold-aggressive fish could potentially get most of the food. It also could lead to the development of infectious diseases as a result of injury, the product of biting (Ashley, 2007). Therefore, how fish are fed may help to reduce aggression in production systems. Food is commonly delivered clumped either in time (food delivered at specific times) or in space (through various feeders). This could potentially promote fierce competition, because fish have to interact closely when the resource is presented (Robb & Grant, 1998). A study showing that variation in the feeding environment could modulate behavioural phenotypes and hence potentially decouple boldness and aggression was carried out by Ruzzante & Doyle (1991). Medaka fish (*Oryzias latipes*) bred under two feeding environments, one in which food was clumped (the high interaction environment) and other in which food was given dispersed (the low interaction environment), with food being given in excess in both cases. Fish were investigated for aggression on each condition. Differences in aggression between lines selected for slow and fast growth were evident. Fast growing fish held in the high interaction environment were less aggressive than any other group of the low interaction, both in the presence and in the absence of food. Therefore, it seems that variation in the competitive regime could potentially decouple boldness from aggression.

Different behavioural responses can also have influence on how individuals respond to stress or so-called coping styles (Koolhaas *et al.*, 1999, Korte *et al.*, 2005). In fish, the best documented example is rainbow trout selected for high and low response to a stressor, for which the post-stress cortisol response is correlated with distinctive behavioural profiles. The importance of stress in fish welfare is well known. Stress is an adaptive response to changes; however, the detrimental effects of stress are evident when the sources of stress are unavoidable, prolonged and repeated (Koolhaas *et al.*, 2007). Many of the aquaculture practices such as transport, handling, feeding techniques, human presence, stocking densities can compromise the welfare of the fish because all those practices potentially elicit chronic stress responses. Chronic stress has implications in several individual conditions. For example, the resistance to disease can be affected by stress because stress can suppress immune function (Koolhaas, 2008). Stress also leads to reduction in growth rates and consequently to poor production (Strand *et al.*, 2007). Since individuals are not equally affected by stressful stimuli, knowledge of the extent to which individual fish respond to farming practices would be a helpful to fish welfare research.

Choice of model species

Due to their characteristics and a wealth of biological data available, the three-spined stickleback (*Gasterosteus aculeatus*) and the rainbow trout (*Oncorhynchus mykiss*) have both become something of a model to study behaviour, ecology and evolution in fish. I used both species to address my research on individual variation on behavioural phenotypes in fish.

The three-spine stickleback (*Gasterosteus aculeatus*) is a small fish inhabitant of freshwater lakes and streams, with a wide natural distribution. Since sticklebacks are relatively easy to catch and hold under laboratory conditions, a good deal of research has been done on this species. The biology of the species as well as its life history traits are very well documented. Work using sticklebacks as a research species is increasing and research ranges from behaviour, evolution, ecology, physiology, toxicology, parasitology to molecular genetics (Östlund-Nilsson *et al.*, 2007). Because its whole genome has just been recently released, this species is becoming a major research organism.

Sticklebacks are widely used in behavioural research. One of the most studied characteristics of sticklebacks is its peculiar reproduction. Male build the nests from vegetation, sand, and other detritus, binding the material together with a glue-like substance secreted from the kidneys. In spring, males defend nesting territories; the sequence of territorial, courtship and mating behaviours have been described in detail. Parental behaviour is mainly carried out by males, involving nest maintenance and fanning of the eggs to ensure good oxygenation. Outside the breeding season, the stickleback tends to form shoals, especially when young (Östlund-Nilsson *et al.*, 2007; Wootton, 1984). Other studies on stickleback behaviour also include research on cognitive processes, such as spatial learning, social learning and personality traits. The relationship between behaviour and physiology has also been recently reported (Bell *et al.*, 2007). Due to all those characteristics and all the information available, I used this species to examine risk-taking, aggression and their relationship under different conditions.

The rainbow trout (*Oncorhynchus mykiss*) is a species native to tributaries of the Pacific Ocean in Asia and North America. Its one of the most intensively studied salmonid fishes, because of its economic importance as a farm species and as a sport fish. In addition, it is similar to other salmonids of high economic importance. Many aspects of the life history of the rainbow trout have been studied. In spite of having a long lifespan compared to other species such as the stickleback, in rearing conditions the rainbow trout is easy to spawn. It is also tolerant to a wide range of environments and handling. Therefore, several studies have used the rainbow trout as a research species in areas including physiology, behaviour, ecology and genetics (Øverli et al., 2005; Sneddon, 2003a).

In the present study, I used two strains of rainbow trout that have been selected for their plasma cortisol responsiveness to a standardized stressor for over 4 generations (the HR and LR rainbow trout strains described above, details in Øverli et al., 2005; Pottinger & Carrick, 1999; Schjolden & Winberg, 2007). The high and low responding strains were initially bred for studies aiming to improve performance in aquaculture. Later it became clear that they are also a valuable model for studying the links between behaviour and physiology, so they have become a model to study proactive and reactive coping strategies in fish.

Aims of the present study

With this background, the broad objective of the present study was to examine various aspects of individual variation in behaviour and their correlations in fish. This was achieved through the following specific aims:

The study described in **chapter 2** was aimed at examining risk-taking, aggression and the relationship between them in sticklebacks that grow at different rates in two competitive environments. This main aim was addressed via the questions detailed below.

- What is the distribution of risk-taking phenotypes in the study site and how does it relate to morphological traits such as length, weight, and condition factor?
- Is the relationship between risk-taking and the morphological traits the same after 10 weeks exposure to different feeding regimes?
- How is the behaviour shown by individuals in a novel environment and how does it relate to fish size?
- Do absolute levels of aggression or risk-taking differ in relation to feeding regime and between fast and slow growing fish?
- What is the relationship between aggression and risk-taking in fish subjected to different regimes?

In the study detailed in **chapter 3**, I related fry hatching date of sticklebacks (used as a possible indirect measure of parental fitness) to their personalities (risk-taking and aggression). The study was developed through behavioural observations that allowed me to give an answer to the following questions:

- Do early and late hatching sticklebacks vary in risk-taking phenotype?
- Do early fish differ from late fish in how they respond to a standard conspecific?
- Do early fish tend to dominate late fish (or the converse) if behavioural style is allowed for?
- Is the relationship between boldness and aggression different in fish with different hatching dates?

For the work in **chapter 4**, I used fish from the high (HR) and low (LR) stress responsive strains of rainbow trout to quantify risk-taking, aggression and their relationship in individual fish. To address this, the following questions were addressed:

- Does transport have a long-term effect on the behaviour of HR and LR strains?
- Is any change in behaviour passed on to the next generation of offspring?
- Do changes in water chemistry (salinity) enhance the effects of transport on behaviour, cortisol, sodium, chloride and glucose in HR and LR adult fish?

For the work in **chapter 5**, I measured individual risk-taking behaviour in high and low responsive rainbow trout, evaluating individual behavioural consistency of this trait and its relation to post-stress cortisol levels. Further characterisation of the LR and HR rainbow trout as proactive and reactive copers was addressed by the following questions:

- Do HR and LR rainbow trout differ in their behavioural flexibility to changes?
- Do HR and LR rainbow trout vary in the extent to which they distracted by environmental variation?

Finally, the results of the four studies and their impact on the knowledge of personalities and behavioural syndromes in fish, together with an insight on their implications for behavioural ecology, evolution and aquaculture, are discussed in **chapter 6**.

Chapter 2 Variation in risk-taking, aggression, and behavioural syndromes in sticklebacks that grow at different rates in two competitive environments

Introduction

Behavioural syndromes

Individual variation in behaviour has been long recognized. Animals differ in their responses to a myriad of conditions. One category of measured behaviour involves risk-taking in a variety of situations, such as feeding and mating , and also aggression. Although such single aspects of behaviour may vary across situations and over time, correlations between them have been found in several species. Where such differences are consistent over time and across situations, they can be characterised as behavioural syndromes (Bell, 2007; Sih *et al.*, 2004). In fish, correlations between behaviours have been described in some species such as: *Gasterosteus aculeatus* (stickleback: Bell, 2005; Bell & Sih, 2007; Dingemanse *et al.*, 2007); *Danio rerio* (zebrafish: Moretz *et al.*, 2007) and *Salmo trutta* (brown trout: Sundström *et al.*, 2004). A syndrome involving boldness (a term that hereafter will be used to describe the readiness of a fish to take risks) and aggression has been frequently observed. A landmark study that illustrates such a correlation was the carried out by Huntingford (1976), who described that the level of aggression shown by individual breeding males of the three-spined stickleback (*Gasterosteus aculeatus*) was a consistent trait that co-varied with boldness shown towards a predator outside the breeding season.

Such findings have ecological and evolutionary implications, suggesting that when behavioural traits in a syndrome are tightly correlated, they might not be free to evolve independently (Sih *et al.*, 2004). Environments or selection regimes favouring one type of behaviour (i.e. boldness) could lead changes in a completely different behaviour (i.e. aggression), so that in particular situations or contexts one of them might be at maladaptive levels. For example, aggressive sticklebacks could gain an advantage over non-aggressive fish in the form of faster food acquisition (which potentially leads to fast growth), establishing territories or gaining mates. However if aggression is tightly coupled with boldness towards a predator, it would put such individuals into potential risk in high predation environments, which in turn could be maladaptive. However, in environments where predation risk is low, they would flourish. Growth-mortality tradeoffs have been suggested to explain how individual behavioural variability is maintained. Stamps (2007) has suggested that some individuals show fast growth by adopting a risky strategy foraging in the presence of predators, as well as defending aggressively food patches. Those behaviours imply a trade-off between growth and mortality and therefore, they potentially will be correlated across individuals. This is supported by the fact that boldness, activity and/or aggressiveness are often positively related to food intake rates, growth and other life-history traits (Biro & Stamps, 2008).

There is still uncertainty as to whether behavioural syndromes are fixed or can be decoupled. In sticklebacks, studies looking at behavioural syndromes are extensive and correlations between aggression and boldness (Huntingford, 1976; Bell & Stamps, 2004; Bell, 2005) have been found. It has also been suggested that behavioural syndromes are consistent between feeding and shoaling contexts (Ward *et al.*, 2004). However, recent studies in wild populations of sticklebacks have shown that behavioural syndromes are present in some populations but not in others, depending on environmental factors such as food availability and predation pressure. For example, Bell (2005) found that boldness and aggression were phenotypically and genetically correlated in a population with apparently high levels of predations risk, but those two behavioural traits did not correlate in a second population where predation risk was apparently low. This suggests that predation pressure (among other things) might drive the expression of behavioural syndromes.

This has been supported by a recent study carried out by Dingemanse and collaborators (2007), in which 12 populations of sticklebacks were examined for behavioural correlations. Indeed, behavioural syndromes were correlated with the presence of predators. The authors conclude that natural selection may favour correlations between behaviours only in certain types of environment. This may involve a degree of flexibility in the behavioural responses; however, it is not well known to what extent and under which particular circumstances correlated behaviours are likely to be uncoupled. An outstanding study has shown how predation pressure can modulate the expression of behavioural syndromes (Bell & Sih, 2007). Sticklebacks from a population with low levels of predation pressure that hence did not show the boldness and aggression syndrome (Bell, 2005) were exposed to a predation regime. Exposure to predation generated the boldness and aggression behavioural syndrome, and this correlation was caused by a combination of the predator picking fish with particular behavioural phenotypes and behavioural flexibility of the other surviving individuals.

Implications for aquaculture

Behavioural syndromes may have implications for production and welfare of farmed fish (Huntingford, 2004; Huntingford & Adams, 2005). This is because long-term selection for important traits in aquaculture, such as high feeding rates or fast growth (a possible reflection of boldness), may inadvertently select for undesirable traits such as high levels of aggression and competition. For example, if the correlation between aggression and boldness cannot be decoupled, fish showing the suitable traits for aquaculture (non-aggressive and bold) would be in short supply. However if there are conditions in which boldness and aggression can be decoupled, these behavioural traits could perhaps be selected in the desirable direction (non aggressive and bold fish) for aquacultural purposes.

It is known that domestication can increase aggression in production systems (Ruzzante, 1994; Einum & Fleming, 1997; Metcalfe *et al.*, 2003), although other results suggest a decrease (Hedenskog *et al.*, 2002) or no effect (Yamamoto & Reinhardt, 2003). However, the direction and intensity of the relationship between growth, aggression and boldness may depend on the environmental conditions under which the competition for food takes place.

In a study on medaka fish (*Oryzias latipes*), Ruzzante and Doyle (1991) measured levels of aggression in lines selected for slow and fast growth. Both kind of line were subjected to two different feeding regimes, differing in the extent the fish had to interact with each other in order to get food. The feeding environment was established as high interaction, when food was clumped; and low interaction, when it was dispersed over the tank. Selection for growth was effective in both environments, but differences in behaviour between fast and slow growers were only seen in the high interaction environment. In this environment, the fast growing fish were less aggressive than slow growing fish, both in the presence and in the absence of food. Ruzzante and Doyle (1991) did not look at whether the populations they were using show behavioural syndromes prior or after their experiment, which sets the question whether behavioural syndromes can be uncoupled by modifying the environmental conditions. Borrowing Ruzzante and Doyle's set up, but using fish of known risk-taking phenotypes, in the work described in this chapter, I looked at whether variation in the feeding environment can modulate agonistic and bold responses and the relationship between them, and what the implications are for growth.

Aims

The overall objective of this part of my research was to examine the effects on boldness, aggression and the relationship between them in sticklebacks that grow at different rates in two competitive environments. This was addressed via a series of questions detailed below.

- What is the distribution of risk-taking phenotypes in the study site and how does it relate to morphological traits such as length, weight, and condition factor?
- Is the relationship between risk-taking and the morphological traits the same after 10 weeks exposure to different feeding regimes?
- How do individual fish behave in a novel environment and how does this relate to fish size?
- Do absolute levels of aggression or risk-taking differ in relation to feeding regime and between fast and slow growing fish?
- What is the relationship between aggression and risk-taking in fish subjected to different regimes?

Originally, the fish from the very top and bottom 5 individuals of the growth distribution were going to be used to set up a breeding programme to follow the same experiment in the subsequent generation. However, due to an outbreak of disease, this part of the project was abandoned.

Materials and Methods

Fish

The sticklebacks used for the experiments came from a wild population, the River Endrick in the Loch Lomond catchment, Scotland. Juvenile fish were caught and kept at the Scottish Centre for Ecology and the Natural Environment

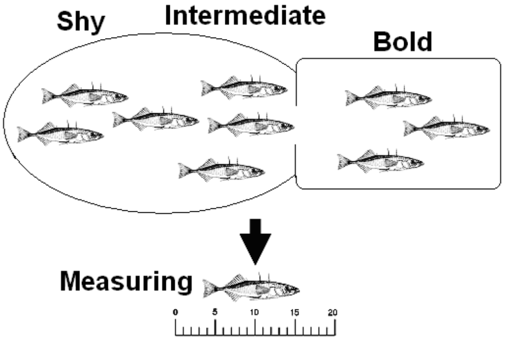
(SCENE) until used in the experimental set up. Holding tanks were supplied with flow-through fresh water from Loch Lomond, held at ambient temperature and with photoperiods that reflected the natural conditions.

Experimental design

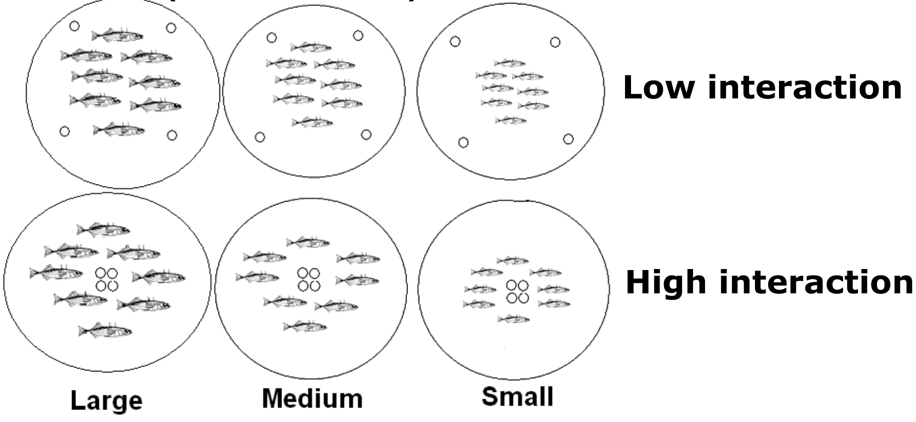
In this section I will give a broad explanation of the experimental design, details being given later. For logistic reasons, including fish availability and size and to avoid unnecessary stress, fish were not individually marked. This means that I could not trace individual behaviour during the first part of the experiment. The decision of not marking the fish was made in order to minimize stress, diseases such as fungal infections and to avoid the possibility of marking interfering with growth. The very low mortality rate (6 out from 271 fish) found during the experimental period justified this decision.

All the fish captured were initially screened for risk-taking behaviour in groups of 9 fish and assigned to one of 3 groups (bold, intermediate or shy, Figure 2.1a).

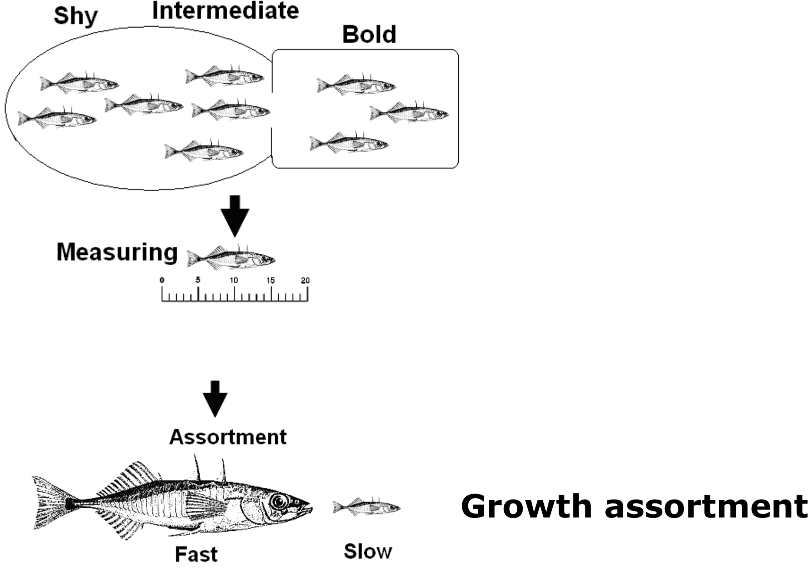
a) Initial group screening



b) Establishment of the competitive regimes and groups
(n+45 for each tank)



c) Final group screening



d) Individual screening for exploratory and agonistic behaviour

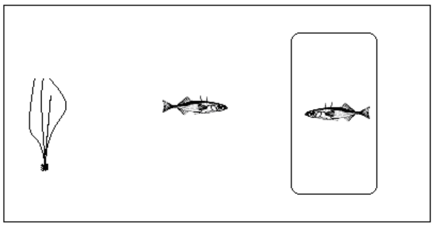


Figure 2.1. Diagram showing the experimental design. a) risk-taking in groups at the start of the experiment, followed by morphological measures; b) establishment of the experimental groups, 3 low and 3 high interaction groups of 45 fish each of known risk-taking profiles; c) risk-taking in groups at the end of the experimental period followed by morphological measures, selection of the top and bottom 10 fish of the weight distribution for individual behavioural assays; d) individual screening for risk-taking and agonistic behaviour.

Following behavioural screening, fish were measured for weight and length. Three pairs of weight-matched groups with the same distribution of behavioural phenotypes were then formed. The competitive feeding environment was manipulated to give one of two different conditions following Ruzzante and Doyle (1991). Food was given either always clumped (high interaction environment) or always dispersed (low interaction environment). Feeding and growth experiments started on December 2006 and were followed for 10 weeks, stopped as soon as the first signs of breeding condition were detected (end of March 2007).

Originally, I intended to have 3 replicate groups of equal size for both feeding conditions; however, due to the size distribution of the initial stock and the number of fish available, I had to use three size categories (small, medium, and large fish) in each feeding condition (see figure 2.1b). After the feeding period finished, all fish in each group were rescreened for risk-taking in groups and measured for weight and length (see figure 2.1c). The 10 heaviest and the 10 lightest fish in each size and feeding category were separated and used to test individual exploratory and agonistic behaviour (figure 2.1d).

Screening for risk-taking in groups

Risk-taking was measured as the time taken by individual fish to leave a group in a safe, darkened area. It hence represents the willingness of a fish to explore a new, potentially dangerous environment. This method has already been used to assess risk-taking or boldness both in the wild (Brown *et, al.*, 2004) and in the laboratory (Bell, 2005; Brydges *et al.*, 2008). The test arena consisted in a 200 litre glass aquarium (dimensions (LxWxH): 1 X 0.40 X 0.50 meters) lined on three sides with white self-adhesive plastic. The front wall had a cardboard screen with a small slit that allowed the observer to record the behaviour without disturbing the fish. The tank had gravel as a substratum and was filled with water from Loch Lomond. A dark settling chamber was placed in a corner of the tank; the chamber had a door that, once opened, permitted the fish to see the lit, novel environment. The novel environment consisted of the remaining open space, supplied with a petri dish containing food (frozen *Chironomid* larvae) and two artificial plants placed in the middle. Food was not supplied the day before to ensure that during the test all the fish were hungry and they had to make the decision to leave a safe area in order to eat. A group of nine fish was allowed to settle in the chamber for an hour, after which the door was gently opened, giving the fish the option to enter the novel environment or not. Fish were familiar to each other in the sense that they were held in the same holding tanks for over 6 weeks. From a pilot experiment, criteria were developed to distinguish between risk-taking phenotypes; bold, intermediate and shy fish were categorized primarily according to their emergence sequence, but with time limits according to table 2.1. When all fish of each risk-taking phenotype were out, the door was gently closed and the fish that had emerged were netted out and separated into bold, intermediate and shy groups.

Table 2.1. Criteria used to distinguish between risk-taking phenotypes in groups of nine sticklebacks. Risk-taken was measured as the time it took a fish in a group to emerge from a safe, darkened area into a well lit, potentially dangerous environment. Fast emergence was regarded as a bold, risk-taking response and failure to emerge as a shy, risk-avoiding response. Since fish were screened in groups, occasionally more than 3 fish came out at the same time. The times chosen to discern between phenotypes were generated from a pilot experiment based on the distribution of times taken by a large sample of fish to leave the refuge.

Risk-taking phenotype	Criteria
Bold	The first c. three fish to enter the novel environment before six minutes
Intermediate	The second group of c. three fish that emerge before 21 minutes
Shy	Fish that remained in the settling chamber after 21 minutes

Establishment of the different competitive regimes

After screening for risk-taking, fish in each behavioural category were measured for length and weight. Fish in each of the bold, intermediate and shy categories were ranked by size. From the size distribution and according to the overall behavioural distribution, groups of 17 bold, 16 intermediate, and 12 shy fish were used to form each of the 6 experimental tanks (n=45 per tank). This was done to ensure that all the experimental groups contained the same distribution of risk-taking phenotypes, with pairs of high and low interaction groups being weight-matched (table 2.2). Fish were held in 200 litre plastic tanks (1m diameter x 60cm height) with flow through water supply from Loch Lomond. A clump of artificial plants was placed at one side of the tank to provide the fish with a refuge. Tanks were kept outdoors covered by a mesh screen, so fish were held in natural temperature and photoperiod, but safe from predators.

Table 2.2. Mean weight (± SE) of sticklebacks in each of the experimental groups. Groups were of 45 fish.

Experimental group size	Feeding condition	Mean weight ± SE	
Small	High interaction	0.228 ± 0.003	t=2.398 p=0.15
	Low interaction	0.265 ± 0.004	
Medium	High interaction	0.329 ± 0.008	t=2.369 p=0.16
	Low interaction	0.365 ± 0.008	
Large	High interaction	0.535 ± 0.014	t=2.567 p=0.10
	Low interaction	0.574 ± 0.017	

The feeding regimes were established as follows: the high interaction or clumped feeding condition consisted of 4 opaque plastic containers (4X4 cm) placed together in the middle of the tank. For the low interaction or dispersed condition, the same number of containers were distributed separately around the tank. Rations were divided equally between the plastic containers. Fish were fed 5% of body weight per day. Assuming that there was no variation in size among the fish, a model of stickleback growth developed by Allen and Wootton (1982) was used to adjust food ration to fish growth every week, to avoid stressing the fish by repeated anaesthesia and measurements. Since weight (mg) can be measured more accurately than length in fish, changes in this characteristic were used to predict the specific growth rate (mg day-1) in sticklebacks. At a given temperature, the growth model that shows a reasonable linear relationship between Specific Growth Rate (SGR) and the logarithm of the ration when ration is expressed as a percentage of the fishes' initial body weight, takes the form:

$$SGR = A + B1 * \ln X1 + B2 * \ln X2$$

Where:

X1 is the ration, X2 is the initial body weight, and A, B1 and B2 are the regression coefficients at different temperatures (Allen & Wootton, 1982). Table 2.3 shows the values used for each tank per week.

Table 2.3.Values used to predict weekly growth rate of sticklebacks based on the formula SGR = A + B1 * ln 5 + B2 * ln X2. Table shows the calculated values of body weights per tank. The body weight at week 10 was the actual value measured at the end of the experimental period. The regression coefficients from the specific growth rate (SGR) and the logarithm of the ration at different temperatures were taken from Allen & Wootton, (1982).

WEEK	AVERAGE TEMPERATURE	AVERAGE INITIAL BODY WEIGHT (X2)						REGRESSION COEFFICIENTS		
		HIGH INTERACTION			LOW INTERACTION					
		SMALL	MEDIUM	LARGE	SMALL	MEDIUM	LARGE	A	B1	B2
1	7.28	0.22	0.33	0.49	0.28	0.392	0.644	-0.0075	0.0075	0.00001
2	6.85	0.23	0.37	0.49	0.285	0.397	0.648	-0.006	0.00761	0.00001
3	6.85	0.23	0.37	0.49	0.285	0.397	0.648	-0.006	0.00761	0.00001
4	3.57	0.23	0.37	0.49	0.286	0.397	0.648	-0.0045	0.00676	0.00001
5	5.20	0.23	0.37	0.49	0.286	0.398	0.648	-0.0252	0.00801	0.00362
6	6.78	0.23	0.37	0.49	0.287	0.397	0.649	-0.006	0.00761	0.00001
7	5.00	0.23	0.37	0.49	0.286	0.397	0.648	-0.0252	0.00801	0.00362
8	5.71	0.23	0.37	0.49	0.287	0.398	0.649	-0.0252	0.00801	0.00362
9	6.71	0.23	0.37	0.49	0.287	0.398	0.649	-0.0252	0.00801	0.00362
10	5.28	0.28	0.40	0.56	0.338	0.458	0.700	FINAL WEIGHT		

Fish assortment by growth

After the fish had been re-tested for risk-taking behaviour and weighed at the end of the 10 week feeding regime, the 10 fish from the top and bottom of the weight distribution of each tank (figure 2.2) were classified as fast and slow growers respectively. This gave a total of 12 groups (120 fish): slow and fast growing fish from three replicates (small, medium, large) from the clumped and dispersed feeding conditions. These fish, together with the fish that remained from the assortment (to be used as stimulus fish in subsequent tests, see below), were transported to the Division of Ecology and Evolutionary Biology, University of Glasgow aquaria facilities to be screened for individual risk-taking and aggression.

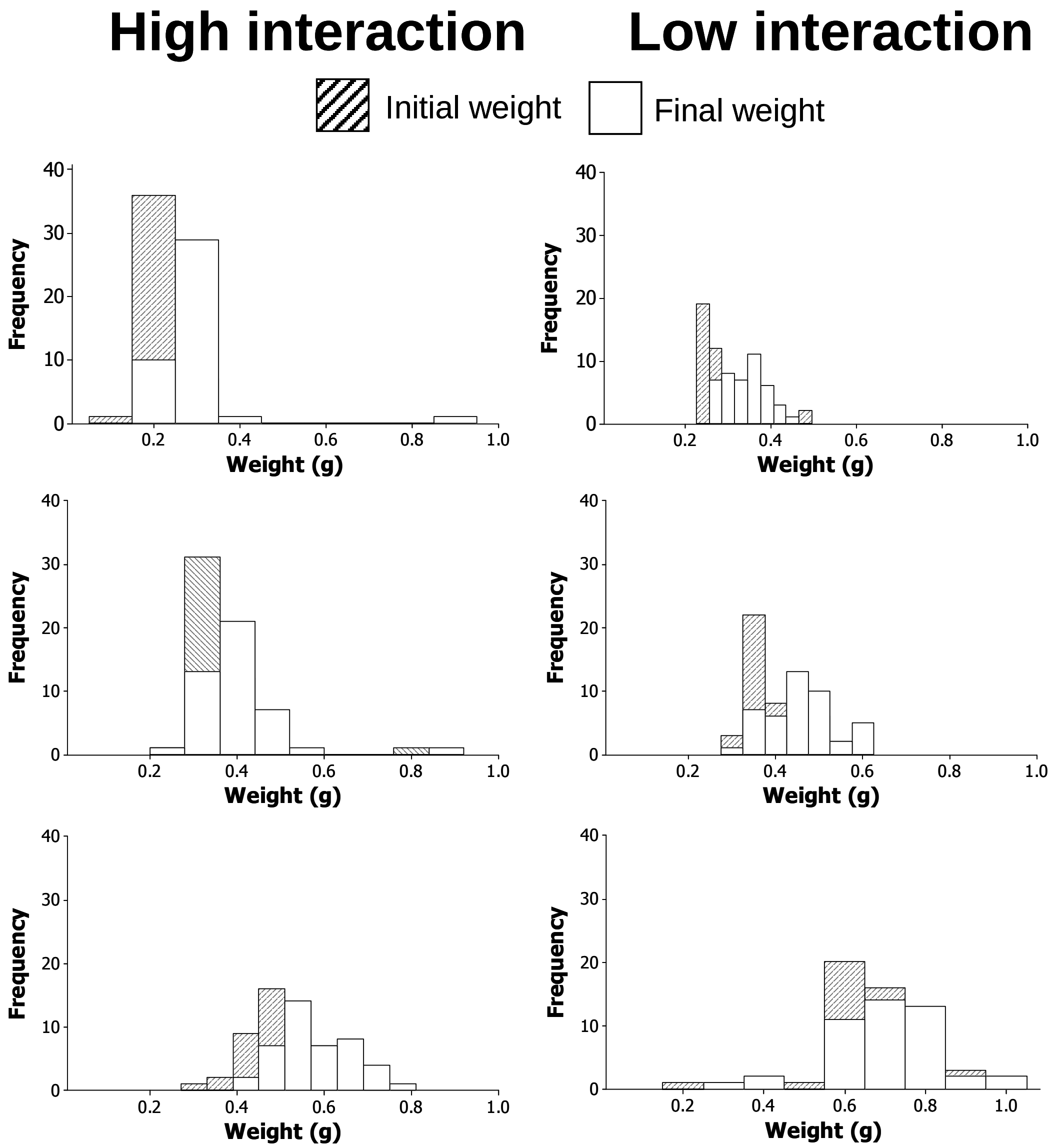


Figure 2.2. Weight distribution of sticklebacks in each of the experimental tanks at the beginning and end of the 10 weeks feeding period.

Individual screening for exploratory behaviour (boldness)

The fish selected for fast and slow growth from all tanks were tested individually for exploratory behaviour in a novel environment (boldness) and aggression towards a conspecific, following the experimental set up used by Bell (2005). The test arena (shown in figure 2.3) consisted of a 37 litre glass aquarium (dimensions (LxWxH): 20 X 10 X 12.5 inches) supplied with copper free fresh water and a corner filter, the bottom being evenly covered (1cm) with gravel substratum. Three walls were lined from the outside with white paper to avoid exterior disturbance and to keep the fish isolated from each other. A settling chamber, a piece of opaque plastic pipe 20cm diameter X 30cm tall containing a piece of plastic weed, was placed in one end of the tank. The tank also had a clump of plastic weed half way along the back wall. A 1 litre clear plastic bottle full of water placed at the opposite end to the settling chamber was used for the aggression test (see below). A 30 watt strip lamp above each aquarium gave even light to the whole tank. The front of the tank was covered with a screen made with black fabric, behind which the observer was able to record the behaviour of the focal fish through a slit. The walls of the tank were marked to divide it into 5 vertical lines (main lines) and one horizontal line in the middle, dividing the tank in 12 sections.

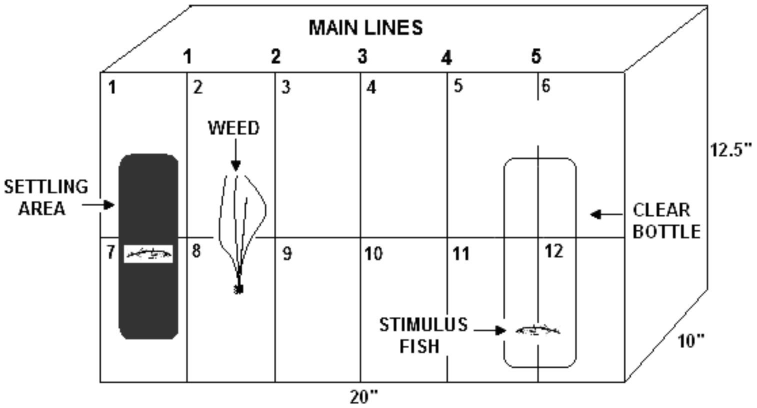


Figure 2.3. Diagram of the observations tank used to assess individual risk-taking and aggression. Focal fish were placed into the settling, dark area overnight. Next day risk-taking behaviour was assessed after which a stimulus fish was placed into a clear plastic bottle located in the middle of the tank in order to test agonistic behaviour towards a conspecific.

Twelve observation tanks were available for screening, allowing one fish from each of the 12 categories (detailed above) to be screened on a given day. The test was carried over 10 days (not consecutive). Three observers were delegated the task of recording the behaviour on a given day. Observations were carried out blind with respect to the identity of the fish. Focal as well as stimulus fish were kept in the observation room during the experimental period in opaque plastic containers. The containers were supplied with corner filters and fish were fed daily *ad libitum* on *Chironomid* larvae. Photoperiod reflected the natural conditions at the time of the experiment. The temperature was fixed at 12.0 C.

Each focal fish was allowed to settle in the chamber overnight. The hanging lamp was turned on manually and the corner filter removed from each tank at least one hour before the test started. Fish were observed for 15 minutes, starting immediately after the settling tube was gently lifted and removed from the aquarium. This allowed the fish to see the rest of the tank from the settling area, but provided it with a safe place (a piece of weed) to stay. The latency to emerge from the settling area was recorded, as well as the latency to cross each of the 5 vertical lines for the first time. The total number of sections entered at least once and the time spent swimming were also recorded. Time swimming was defined as the time the fish spent showing steady swimming around the tank, hence, exploring the new environment. This was used instead of the total time spent swimming, because some fish swam rapidly up and down the tank, or followed their reflection on the tank's walls, which was not considered exploratory behaviour.

Individual screening for aggression

When the test for exploratory behaviour had finished, fish were allowed to settle in the test tank for at least 45 minutes. A broadly size-matched conspecific (stimulus fish) was placed gently inside the plastic bottle at the far end of the tank. Non-breeding sticklebacks are known to fight (Bakker, 1986) and this same method had been successfully used to test levels of aggression in juvenile sticklebacks by Bell (2005). Stimulus fish were not familiar to the focal fish, which were the fish that remained from the selection for growth, mixed from all the experimental groups, since they were kept in communal holding tanks separate from the test fish prior to experimentation. For this test, the first time at which the focal fish faced the stimulus fish directly from one body length or less was recorded (first facing). The total time spent facing and the number of bites (attacks) given were also recorded. The behaviour of the stimulus fish was scored from 0 (a fish that did not show any movement) to 5 (a fish that showed active agonistic behaviour towards the focal fish). At the end of the experiment, each fish was weight and length measured and any sign of breeding coloration was recorded. During observations, it was clear that some focal fish attacked the stimulus fish (biting the bottle); however, other individuals only approached the stimulus fish, without showing any clear signs of aggressive displays (such as raised spines or bites), in a possible attempt to shoal with the stimulus fish. Therefore, I quantified this shoaling tendency as time spent near the stimulus fish once latency to approach and attack rate had been taken into account (see below).

Statistical analysis

Differences in the morphological variables (weight, length, and condition factor) between risk-taking phenotypes before and after exposure to the feeding regimes were tested using General Linear Model ANOVA, followed by a Tukey *post-hoc* analysis. A *Chi-square* analysis was used to examine the change in the initial distribution of the risk-taking phenotypes at the end of the feeding period. Individual exploratory behaviour was examined for effects of confounding factors. All the variables (time swimming, latency to explore, time to cross each 1-5 lines and total areas used) were regressed on date and observation sequence during each day. General linear model ANOVA was used to test for any effect of the tanks and observer. A t-test was run to see if signs of breeding coloration in the focal fish interfered with the recorded exploratory and aggressive behaviours. Principal Components Analysis (PCA) was used to examine the relationship between the exploratory behaviour variables. Differences in individual aggression, boldness, activity and shoaling behaviour by size, feeding regime and fast and slow growth were assessed with a General Linear Model ANOVA followed by a Tukey *post-hoc* analysis. Pearson correlation was used to investigate the presence of behavioural syndromes. All the tests were carried out using MINITAB v15 software.

Results

Risk-taking behaviour in groups and its relation to morphological variables at the start of the experimental period

A total of 387 fish were screened for risk-taking in 43 groups of 9 fish. 40.4% of the screened fish were categorized as bold, 36.2% as intermediate, and 23.4% as shy. The number of fish in each risk-taking category was expected to be a third of the total. However, sometimes more than three fish came out from the settling chamber within a given criterion interval, so the number of bold, intermediate and shy fish was not always 3 in each trial.

Table 2.4a shows the mean and standard deviation of weight, length, and condition factor for each risk-taking category. ANOVA General Linear Model (see table 2.4b) revealed that shy fish tended to be heavier and longer than both bold and intermediate fish. Bold and intermediate fish did not differ in these variables. However, bold fish were in better condition than intermediate fish, with no difference in condition factor between bold and shy fish.

Table 2.4 a) Mean (± SD) of weight (g), length (cm), and condition factor in sticklebacks for each risk-taking category at the start of the experimental period.

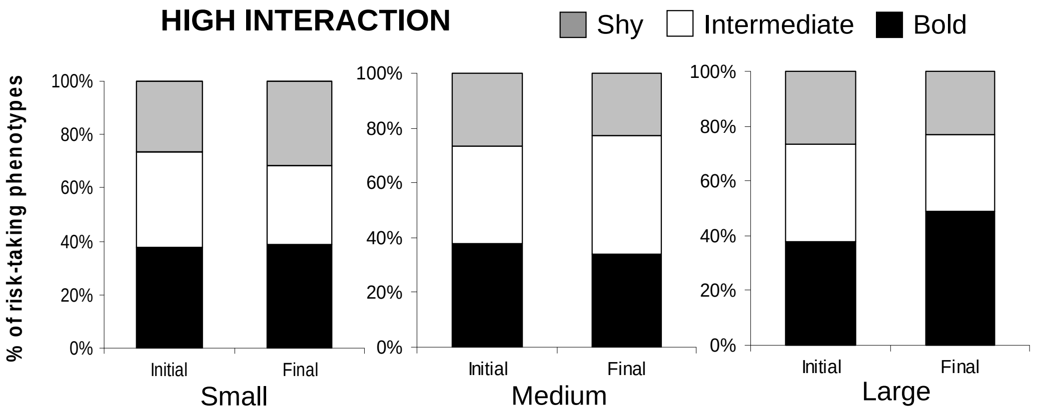
	Bold	Intermediate	Shy
Weight	0.377 ± 0.016	0.362 ± 0.014	0.459 ± 0.020
Length	0.306 ± 0.004	0.311 ± 0.004	0.335 ± 0.005
Condition factor	12.560 ± 0.209	11.719 ± 0.223	11.91 ± 0.289

b) Results of GLM ANOVA for the comparisons of weight, length, and condition factor in sticklebacks for each risk-taking category at the start of the experimental period.

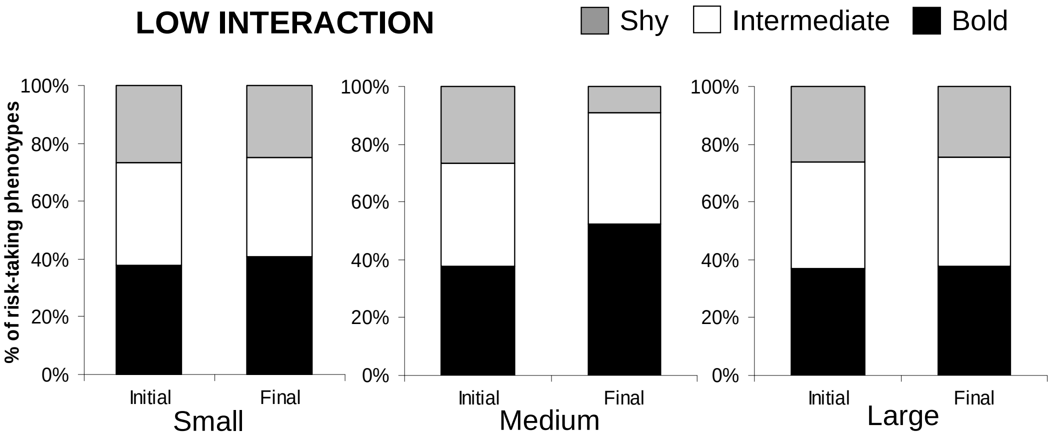
GLM ANOVA	Tukey *post-hoc*	Bold	Intermediate
Weight $F_{270.2}$=8.58 p=0.0001	Bold	-	p=0.7777
	Shy	p=0.002	p=0.0003
Length $F_{270.2}$=9.11 p=0.0001	Bold	-	p=0.7032
	Shy	p=0.0001	p=0.0025
Condition factor $F_{270.2}$=3.81 p=0.023	Bold	-	p=0.0216
	Shy	p=0.1483	p=0.8345

Distribution of the risk-taking phenotypes and their relationship to morphological variables after the feeding period

Figure 2.4 shows the initial and final distribution of the bold, intermediate and shy phenotypes for each tank. The method followed to assess risk-taking was based on emergence sequence of 9 fish in a group, so differences between initial and final distributions for any of the groups measured were not expected.



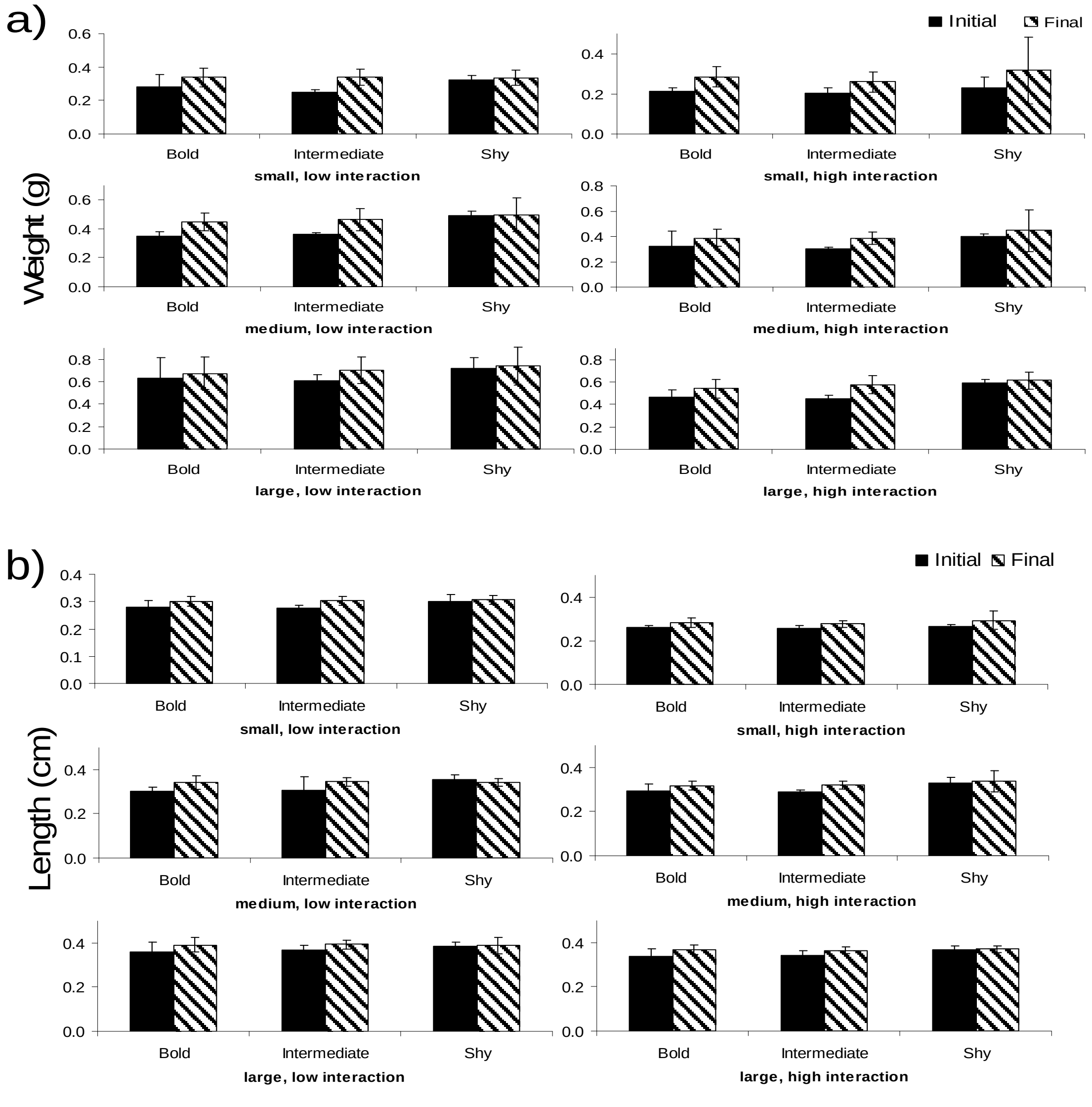
(X^2= 0.457, DF=2, p=0.796) (X^2= 0.553, DF=2, p=0.759) (X^2= 1.129, DF=2, p=0.569)



(X^2= 0.160, DF=2, p=0.923) (X^2= 4.920, DF=2, p=0.085) (X^2= 0.032, DF=2, p=0.984)

Figure 2.4. Initial and final distribution of the sticklebacks' behavioural phenotypes in each size group and feeding regime used in this experiment.

Figure 2.5 shows the initial and final measurements for the morphological variables in each tank. As expected, all the fish grew in weight and length. For weight (Figure 2.5a), there were no differences among bold, intermediate and shy phenotypes in any of the tanks by size: small, high interaction ($F_{40,2}$=0.99, p=0.379); small; low interaction ($F_{42,2}$=0.01, p=0.989); medium, high interaction ($F_{43,2}$=1.68, p=0.200); medium, low interaction ($F_{43,2}$=0.90, p=0.413); large, high interaction ($F_{42,2}$=2.71, p=0.079) and large, low interaction ($F_{44,2}$=0.76, p=0.476). Likewise, there were no differences in length among the risk-taking phenotypes in any of the groups (Figure 2.5b). Small, high interaction ($F_{40,2}$=1.21, p=0.310); small, low interaction ($F_{42,2}$=0.28, p=0.754); medium, high interaction ($F_{43,2}$=1.63, p=0.208); medium, low interaction ($F_{43,2}$=0.07, p=0.937); large, high interaction ($F_{42,2}$=0.29, p=0.750) and large, low interaction ($F_{44,2}$=0.11, p=0.898). Figure 2.5c, shows that there were differences in condition factor among bold, intermediate and shy fish, but only for the large groups. In the large groups from the high interaction condition ($F_{42,2}$=6.83, p=0.003), shy (p=0.008) and intermediate fish (p=0.016) were in better condition than bold fish; shy and intermediate fish did not differ in condition factor (p=0.920). In the large low interaction tank ($F_{44,2}$=3.35, p=0.045), shy fish were in better condition than bold fish (p=0.037), while bold and intermediate (p=0.721) and shy and intermediate (p=0.159) fish did no differ in their condition factor. For the other groups, there were no differences in condition factor: small high interaction ($F_{40,2}$=0.82, p=0.447), small low interaction ($F_{42,2}$=1.09, p=0.347), medium high interaction ($F_{43,2}$=0.09, p=0.918), medium low interaction ($F_{43,2}$=0.55, p=0.582).



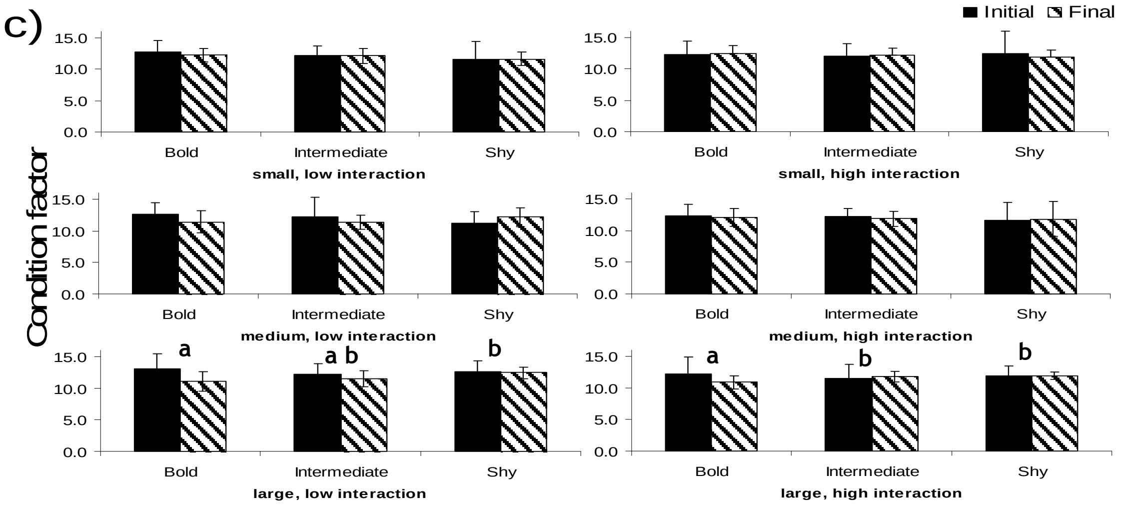


Figure 2.5. Mean ± SE of a): weight, b): length and c): condition factor for each size group and feeding condition of sticklebacks at the start (initial) and at the end (final) of the 10 week experiment. Significant differences were observed only in c where different letters stand for p<0.05.

Individual exploratory behaviour

Confounding effects: During the behavioural observations it was not possible to control for some variables that could affect exploratory behaviour. Those include date of test, observation tank number, focal fish breeding coloration, observer and sequence in which each observer screened the fish a given day (observation sequence). Statistical analyses were carried out to find out whether these variables had an effect on the behaviour. The results in table 2.5 show that date of observation had an effect on the results (positive for time swimming, latency to explore, and total areas, and negative for the latency to cross lines 1 to 5). In order to control for these effects, the residuals from the regression analysis on the raw data for each variable on date were used. Although the experiment was stopped as soon as the first signs of breeding coloration were shown by some males. Breeding coloration was coded as showing any evidence of red colouration on the chin = 1 and no red colouration = 0. I would expect to find more fish showing breeding coloration in the large size groups than in the small size groups. However, the difference is marginal (see table 2.6; X^2=6.017, DF= 1, p=0.056). There were no differences in breeding coloration among the fast and slow growers (X^2=1.239, DF= 1, p=0.266). In addition, this variable did not affect the behavioural performance of the fish.

Table 2.5. Regression analysis of possible confounding variables on all measurements of individual behaviour in sticklebacks.

	Date	Observation sequence	Breeding coloration	Tank	Observer
	Linear regression			ANOVA	ANOVA
Time swimming	R^2=15.2% p=0.001	R^2=0.5% p=0.697	R^2=0.01% p=0.876	$F_{113,11}$=0.47 p=0.918	$F_{113,2}$=0.27 p=0.761
Latency to explore	R^2=11.7% p=0.001	R^2=0.1% p=0.971	R^2=0.01% p=0.825	$F_{113,11}$=1.02 p=0.438	$F_{113,2}$=0.70 p=0.499
Latency to cross line 1	R^2=12.1% p=0.001	R^2=0.1% p=0.691	R^2=0.3% p=0.266	$F_{113,11}$=0.72 p=0.717	$F_{113,2}$=1.33 p=0.270
Latency to cross line 2	R^2=8.7% p=0.001	R^2=0.5% p=0.464	R^2=0.3% p=0.259	$F_{113,11}$=1.50 p=0.144	$F_{113,2}$=2.30 p=0.105
Latency to cross line 3	R^2=7.5% p=0.003	R^2=1.3% p=0.222	R^2=0.3% p=0.245	$F_{113,11}$=1.83 p=0.059	$F_{113,2}$=1.38 p=0.255
Latency to cross line 4	R^2=6.2% p=0.008	R^2=2.6% p=0.086	R^2=0.01% p=0.784	$F_{113,11}$=1.44 p=0.165	$F_{113,2}$=0.84 p=0.435
Latency to cross line 5	R^2=4.5% p=0.023	R^2=1.6% p=0..183	R^2=0.01% p=0.735	$F_{113,11}$=1.73 p=0.078	$F_{113,2}$=0.47 p=0.624
Total areas	R^2=3.7% p=0.041	R^2=0.6% p=0.397	R^2=0.1% p=0.842	$F_{113,11}$=1.09 p=0.374	$F_{113,2}$=1.45 p=0.240

Table 2.6 Proportion of male sticklebacks in breeding condition (showing red chin) in each experimental group at the end of the experimental period.

Holding condition	Growth	Size	Proportion of males in breeding condition
clumped	fast	small	0.3
		medium	0.3
		large	0.3
	slow	small	0.0
		medium	0.1
		large	0.2
dispersed	fast	small	0.2
		medium	0.1
		large	0.2
	slow	small	0.0
		medium	0.1
		large	0.5

Condensing exploratory behaviour variables: In order to examine the relationship between the behaviours measured in the exploration test, and, if possible, to condense them for further analysis, a Principal Components Analysis (PCA, loads shown in table 2.7) was run with all the variables. Two components accounted for 80% of the variance. For the first component (which explained 69% of the variance), all measurements that involved latency (to explore and to cross each of the main lines) had negative loadings. Hence a fish with a high score in this component would be a fish that emerged fast from its shelter and visited the whole tank rapidly. Therefore this component was named “boldness”. The second component (which explained 11% of the variance) had positive loadings for time spent swimming and latency to cross lines 2 to 5. High scores on this component would be given to fish that spent more time swimming and crossing the lines. I termed this as an “activity” component. For each fish the score of both components was stored and used in further analysis as the boldness and activity variables.

Table 2.7. Loading of the “boldness” and “activity” components from the Principal Component Analysis that condensed the variables recorded on the exploratory behaviour test. % refers to percentage of variance explained by component.

Variables	PC1 “Boldness” 69%	PC2 “Activity” 11%
Latency to explore	-0.332	-0.330
Time spent swimming	0.242	0.737
Latency to cross line 1	-0.381	-0.008
Latency to cross line 2	-0.392	0.173
Latency to cross line 3	-0.397	0.242
Latency to cross line 4	-0.385	0.261
Latency to cross line 5	-0.368	0.319
Total areas used	0.302	-0.300

Boldness and activity in the experimental groups

Means and standard errors for boldness and aggression for all the experimental groups are shown in tables 2.8 and 2.9. The results indicate that there were no differences in boldness and activity for any of the tested groups and conditions.

Table 2.8. Between and within group comparisons of boldness and activity scores of groups of sticklebacks exposed to two different feeding environments.

		Boldness		Activity	
Overall 12 groups		$F_{112,11}=1.10$ p=0.366		$F_{112,11}=1.39$ p=0.191	
		Mean ± SE		Mean ± SE	
Size	Small	-0.061 ± 0.398	$F_{112,2}=1.31$ p=0.275	0.118 ± 0.128	$F_{112,2}=1.04$ p=0.358
	Medium	-0.404 ± 0.404		-0.195 ± 0.200	
	Large	0.477 ± 0.356		0.079 ± 0.170	
Feeding regime	Dispersed	0.19 ± 0.31	t=0.85 p=0.398 DF=110	-0.049 ± 0.137	t=0.51 p=0.612 DF=110
	Clumped	-0.19 ± 0.32		0.050 ± 0.139	
Growth	Fast	0.32 ± 0.28	t=1.41 p=0.161 DF=107	0.155 ± 0.126	t=1.57 p=0.119 DF=109
	Slow	0.30 ± 0.35		-0.147 ± 0.146	

Table 2.9 Mean and SE of boldness and activity scores in each of the 12 assorted groups of sticklebacks.

Size	Feeding regime	Growth assortment	Boldness		Activity	
			Mean	S.E.	Mean	S.E.
Large	Clumped	Slow growth	-0.432	0.775	-0.098	0.263
		Fast growth	0.505	0.531	0.546	0.254
	Dispersed	Slow growth	1.174	0.842	-0.455	0.443
		Fast growth	0.687	0.545	0.454	0.256
Medium	Clumped	Slow growth	-0.499	0.861	-0.604	0.530
		Fast growth	0.131	0.869	-0.095	0.224
	Dispersed	Slow growth	-1.392	0.756	0.092	0.261
		Fast growth	0.283	0.687	-0.169	0.558
Small	Clumped	Slow growth	-1.484	0.850	0.048	0.321
		Fast growth	0.507	0.728	0.601	0.225
	Dispersed	Slow growth	0.571	0.843	0.171	0.212
		Fast growth	-0.122	0.723	-0.362	0.213

Aggression and shoaling

Some focal fish showed clear aggression towards the stimulus fish. This was quantified by attack rate (the number of attacks per minute spent facing the stimulus fish). This variable was negatively influenced by date of observation (R^2=3.6% p=0.049); therefore, the residuals from the regression analysis of the attack rate data on date were stored as the aggression variable. Other focal fish approached the stimulus fish, but instead of attacking, appeared to be trying to shoal with it. To quantify this tendency independently of time spent near the stimulus fish to attack, a multiple regression was carried out of the time the focal fish spent facing the stimulus fish on attack rate and the latency to face. The model showed that time facing was related significantly ($F_{2,110}$t=6.05, p=0.003) to both attack rate (β=124.41±52.24, p<0.05) and latency to face (β=-3.159±1.634, p<0.05). The residuals for this model were stored to be used as an indicator of shoaling that was independent of time to react to the stimulus fish and to levels of aggression.

Only a few fish showed red chins, a sign of breeding condition for male sticklebacks (see table 2.6 above). Breeding coloration did not have an effect on overall aggression (t=1.61, p=0.118, DF=33). There were no differences in aggression ($F_{112,2}$=0.69, p=0.506) between fish of the small (mean ± SE: -0.0172 ± 0.0276), medium (mean ± SE: -0.0082 ± 0.0244) and large groups (mean ± SE: 0.0261 ± 0.0314). Fish previously held in the dispersed feeding regime (mean ± SE: 0.0336 ± 0.0228) showed more aggression toward the stimulus fish than did fish held in the clumped condition (mean ± SE: -0.0342 ± 0.0219; $F_{107,1}$=5.14, p=0.025). However, the *post-hoc* test revealed that these results are mainly driven by the interaction of feeding regime and size ($F_{107,2}$=6.37 p=0.002). Large fish in the dispersed regime showed the higher levels of aggression (p=0.04, see table 2.10 for mean values). Independently of feeding regime, aggression between fast (mean ± SE: 0.0272 ± 0.0233) and slow growers (mean ± SE: -0.025 ± 0.0218) did not differ (t=1.66 p=0.100 DF=110). There were overall differences in aggression when all the 12 groups were analyzed together ($F_{112,11}$=2.58 p=0.006), but the *post-hoc* analysis did not reveal any specific difference (all p>0.05, see table 2.10for mean and SE in each group).

There were no differences in shoaling for any of the tested groups and conditions. Overall result for size ($F_{108,2}$=1.29, p=0.279). Mean ± S.E. for small fish: -9.7 ± 13.8, medium fish: 19.0 ± 12.8 and large fish: -10.6 ± 18.3. Overall feeding regime (t=0.47, p=0.640, DF=106), dispersed (mean ± SE: 4.1 ± 12.2), clumped (mean ± SE: -4.1 ± 12.5). Independently of feeding regime, there were no differences in shoaling (t=0.37 p=0.712 DF=106) between fast (mean ± SE: 3.3 ± 12.0) and slow growers (mean ± SE: -3.2 ± 12.6). There were no overall differences in shoaling either when all the 12 groups were analyzed together ($F_{108,11}$=0.86 p=0.586 see table 2.10 for mean ± SE in each group).

Table 2.10 Mean (± SE) of aggression (attack rate) in each of the six experimental groups and 12 assorted for growth groups of sticklebacks. Values in parenthesis stand for (mean ± SE).

Size	**Feeding regime**	**Growth assortment**	**Aggression**		**Shoaling**	
			Mean	**S.E.**	**Mean**	**S.E.**
Large	**Clumped n=10** (-0.0547±0.041)	**Slow growth**	-0.043	0.056	-27.2	42.5
		Fast growth	-0.068	0.063	28.1	30.4
	Dispersed n=10 (0.1027±0.040)	**Slow growth**	0.061	0.061	-14.8	37.1
		Fast growth	0.148	0.050	-24.3	37.1
Medium	**Clumped n=10** (0.0311±0.034)	**Slow growth**	0.011	0.059	4.9	28.0
		Fast growth	0.051	0.036	12.3	28.7
	Dispersed n=10 (-0.0520±0.032)	**Slow growth**	-0.053	0.047	48.3	23.6
		Fast growth	-0.050	0.048	8.6	21.3
Small	**Clumped n=10** (-0.0863±0.034)	**Slow growth**	-0.098	0.053	-4.3	20.2
		Fast growth	-0.076	0.047	-33.9	26.7
	Dispersed n=10 (0.0450±0.037)	**Slow growth**	-0.046	0.035	-32.9	28.5
		Fast growth	0.136	0.053	35.4	29.9

Behavioural syndromes

Figure 2.6 shows the only significant correlation found, which was for boldness and aggression. Table 2.11a shows the values for the overall correlations (independently of size and feeding regime) among the main variables measured: activity, boldness, aggression and shoaling.

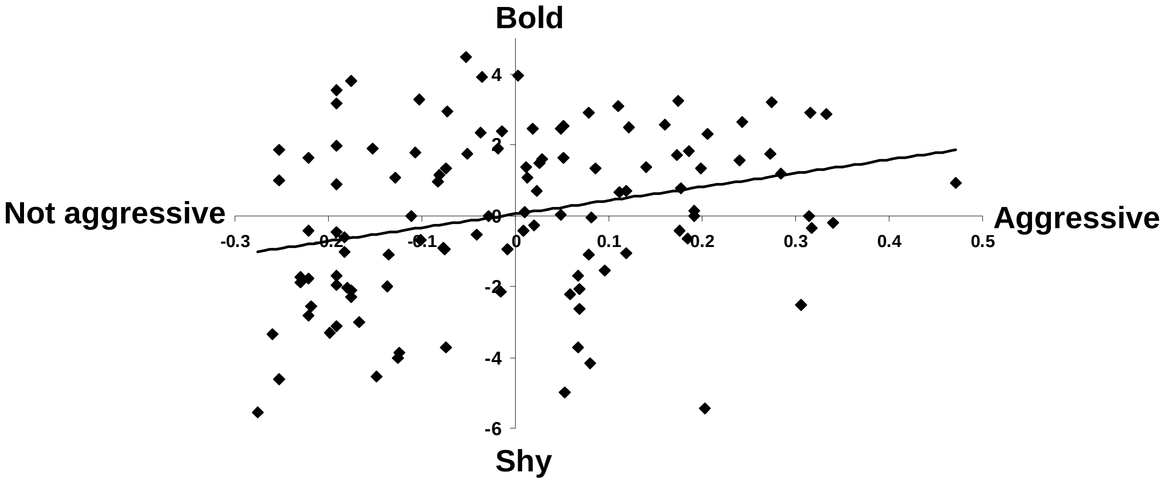


Figure 2.6. Relationship between boldness and aggression in sticklebacks held during 10 weeks under controlled conditions. r=0.27, p=0.003.

Since there were no differences in boldness and aggression between small, medium and large size fish, I only investigated whether the correlation between boldness and aggression was present for each feeding regime for the fast and slow growing fish. Table 2.11b and figure 2.7 show that only for the fast growing fish in the high interaction regime (given food clumped) the correlation was significant (r=0.464, p=0.015).

Table 2.11. a) Product-moment correlation coefficient between the 4 variables of the individual behaviour of sticklebacks.

	Aggression	Shoaling	Boldness
Shoaling	r=-0.0014 p=0.971		
Boldness	*r=0.278 p=0.003*	r=-0.082 p=0.400	
Activity	r=-0.016 p=0.867	r=0.049 p=0.617	r=-0.004 p=0.963

b) Product-moment correlation coefficient between boldness and aggression in sticklebacks assorted for slow and fast growth in the clumped (high interaction) or dispersed (low interaction) feeding regimes

Feeding regime	**Correlation values**
Fast growth, high interaction (n=30)	*r=0.464 p=0.015*
Fast growth, low interaction (n=30)	r=0.117 p=0.562
Slow growth, high interaction (n=30)	r=0.214 p=0.275
Slow growth, low interaction (n=30)	r=0.331 p=0.074

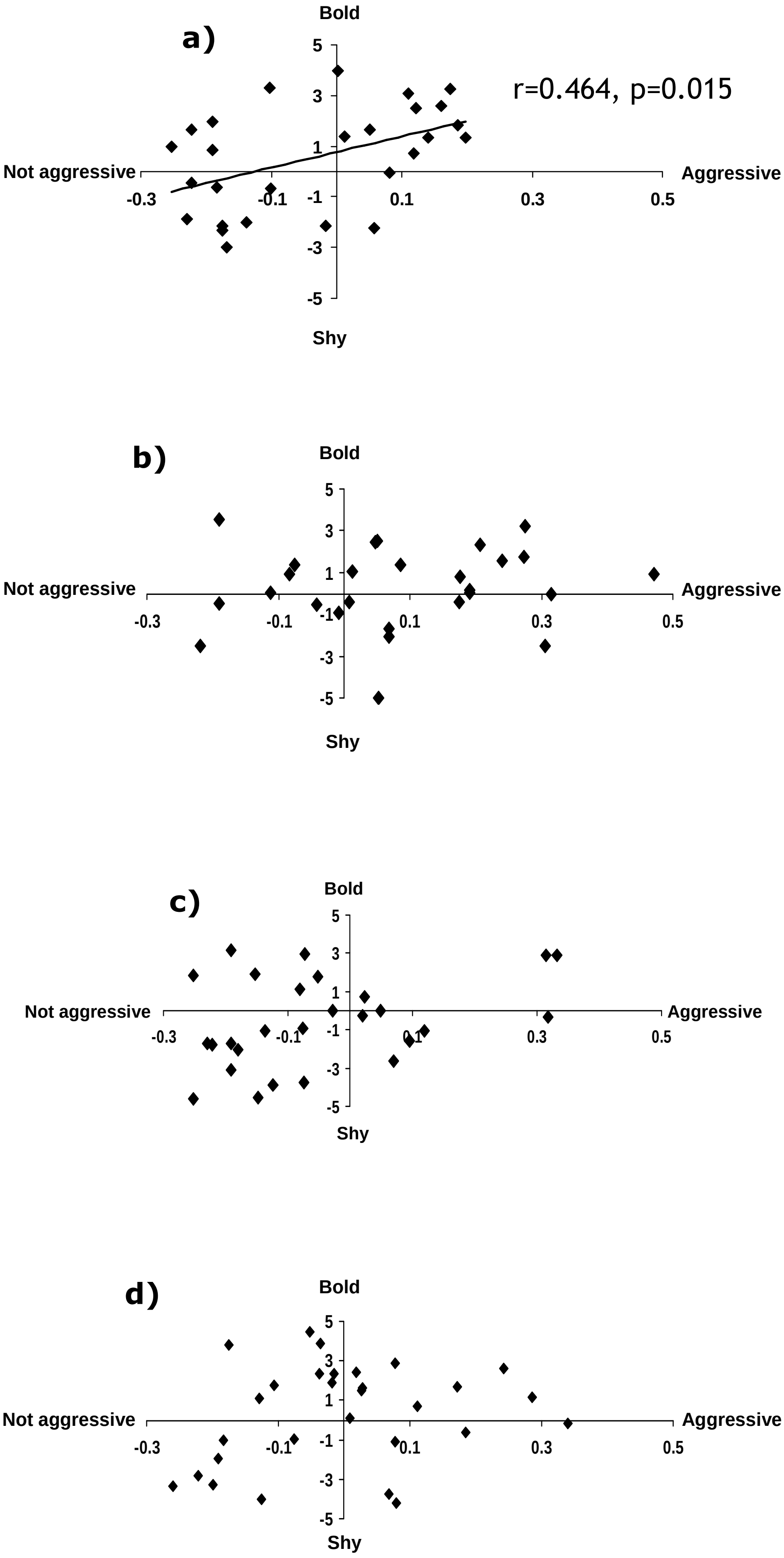


Figure 2.7. Correlation between boldness and aggression in sticklebacks in the experimental groups: a) fast growth, high interaction; b) fast growth, low interaction; c) slow growth, high interaction and d) slow growth, low interaction, at the end of the experimental period.

Discussion

In this study, groups of wild-caught sticklebacks with a natural distribution of risk-taking phenotypes were exposed to 2 different competitive regimes (low and high interaction) and the effects on boldness, aggression and the relationship between them was examined. In this discussion I target the specific aims listed previously.

Distribution of risk-taking phenotypes and relationship to morphology (Aims 1 and 2)

At the initial screening for risk-taking, shy fish were heavier and longer than bold and intermediate fish. This agrees with Brown & Braithwaite (2004), who found that in 8 populations of *Brachyraphis episcopi*, small fish tended to emerge sooner from a shelter, or were bolder, than large fish. In male Iberian rock lizards (*Lacerta monticola*) the tendency of smaller animals being bolder has been found (Lopez *et al.*, 2005). Smaller fish have smaller nutrient reserves than bigger fish and may have to deal with higher levels of hunger; therefore, their motivation to leave a safe area will be high, even if it compromises their safety. However, in the present study bold and shy fish were in equivalent condition, both better than intermediate fish at the start of the experiment. This would suggest that bold fish are not simply bold because of hunger, but that some other mechanisms may be involved.

The results found in my study are somewhat different from previous studies in which bold animals were found to grow faster than shy, as reported, for example, by Ward and collaborators (2004). They found that bold sticklebacks grew faster and were more competitive, in the sense of capturing more prey items and doing so more rapidly, than shy individuals. However, it is important to mention that comparisons between both studies are not straightforward, since fish were subjected to different environmental and experimental conditions. For example, the fish used by Ward et al. (2004) were caught at larger sizes (larger than 40mm), the feeding environment was less competitive, because fish were kept in groups of 10 fish, the experimental period lasted about 5 weeks and fish were screened individually for risk-taking behaviour.

The method used in the present study to screen for boldness in unmarked fish was based on emergence sequence, so I was not able to detect any changes in the distribution of the risk-taking phenotypes after 10 weeks of exposure to the different experimental regimes. At the end of this period the only morphological correlate of risk taking phenotype was found in the large size groups, where shy fish were in better condition than bold fish in both feeding regimes.

Body size has complex implications for fitness. On the one hand, although larger size may indicate bigger body reserves, it may also make an individual more visible to predators, and this could be the reason why bigger sticklebacks tended to stay under cover. On the other hand, it is known that sticklebacks have a whole suit of adaptations that protect them against predation, such as bony armour and spines. Those adaptations are developed with age, making older fish less palatable to predators; therefore, the larger more protected fish might tend to take more risks compared to smaller fish. Since the fish used in this study were juveniles, they had probably not developed their armour and spines completely. Since, in addition, they were not subjected to immediate predation risk, the indication is that lower body reserves might be the main motivation driving smaller fish to leave the safe area.

In the present study, bold fish were in poor body condition compared to shy fish at the end of the experimental period, even though food was plentiful. In the context used here, individuals that were prone to take risks paid a price in the form of reduced body condition. Conversely, timid fish benefited from their low level of risk-taking. One reason for shy fish having better condition may be due to social learning. It is known that naïve fish are able to use the information generated by experienced conspecifics to gather resources without having to incur the costs of exploration or the risk of predation (Templeton & Giraldeau, 1996; Brown & Laland 2003). Thus in this experiment, social learning could be the strategy used by shy fish to gather food resources. Evidence suggests that bold animals are also more aggressive and tend to explore more the environment than shy animals; since these behaviours are costly, they tend to have a higher metabolic rate. This higher energy expenditure may be another reason that in this study bold fish are in a poorer condition.

Individual behaviour and its relationship to feeding regime (Aims 3 to 5)

Individuals show differences in growth even if they are maintained under conditions that permit maximal growth rates. Since fish within groups were tightly size-matched, I used differences in weight at the end of the study to classify fish into fast and slow growers. Due to the weight distribution of the initial fish stock, I used groups of small, medium and large size-matched fish in two feeding conditions; thence, fish fell under one out of 12 categories. Fish in each category were screened for individual exploratory and agonistic behaviour. Through PCA I identified two components of individual exploratory behaviour: boldness and activity. These components have been identified previously in sticklebacks by Bell (2005). Two components of response to a conspecific were also distinguished: aggression and shoaling. It is known that by running multiple comparisons on a given data set, there is an increasing likelihood of getting a significant result by chance alone. However, relatively few comparisons were made in the present study and the significance threshold found was low enough to indicate real biological effects and not statistical artefacts due to multiple comparisons.

Overall, there were no differences in boldness and activity in fish classified by size, by feeding regime or by growth rate. In a previous study, Bell (2005) found no differences in activity in a novel environment between 2 populations of sticklebacks, whereas the levels of boldness were different. A possible explanation for the lack of differences in risk-taking between groups in the present study is that the experimental tanks contained the same distribution of risk-taking phenotypes at the start of the feeding experiment, and groups were formed with size-matched fish. The distribution of behavioural phenotypes was maintained until the end of the experiment. This could influence the individual behaviour of the fish since individual behaviour in fish is to some extent influenced by the social environment. For example, Magnhagen (2007) found that YOY perch (*Perca fluviailis*) adjust their behaviour (boldness and exploratory behaviour) as a response to the behaviour of conspecifics in the same group, shy fish becoming somewhat bolder in the presence of bold fish, and the converse.

Another possible explanation is that, in studies where individual differences in risk-taking behaviour have been found, fish were screened soon after being brought from the wild (Ward *et al.*, 2004; Bell & Sih, 2007, Dingemanse *et al.*, 2007; Brydges *et al.*, 2008). In contrast, in the present study, fish were held under laboratory conditions for about two months before the experimental assays were conducted. In addition, some studies used fish with larger sizes (Ward *et al.*, 2004; Dingemanse *et al.*, 2007). Working with only this kind of fish may bias the results, since there is evidence that shy fish tend to be bigger than bold fish, as was found in this study and by Brown & Braithwaite (2004). This assumption needs further evidence, since recently Brown *et al.* (2007) found a positive, strong relationship between boldness scores and body mass per unit of length in the poeciliid *Brachiopsis episcopi*. However, they also found that small fish tended to emerge from shelter sooner than large fish and showed a greater tendency to approach a novel object. They suggested that fish can alter their behaviour as they grow, perhaps through experience.

Regarding the response to a conspecific, shoaling behaviour did not differ in any of the tested groups. This result is somehow hard to interpret since previous studies of aggression in juvenile sticklebacks using a fish confined behind glass have not distinguished between fish nudging at glass to attack and fish doing the same in an attempt to shoal. Therefore, aggression has been measured as the PCA scores resulting from the sum of agonistic interactions and the time the focal fish spent facing the stimulus fish (Bell, 2005). However, during my observations, there were fish that showed clear agonistic behaviour towards the stimulus fish, whereas others were only attracted to it. Hence, that supports my decision to describe them as different behaviours.

The individual levels of aggression were not different among size categories, or between fast and slow growers. However overall, fish held in the low interaction environment were more aggressive than those held in the high interaction environment. This result was mainly due to an interaction of the feeding regime with size, since the biggest differences were found in fish from the large size groups. At first glance, this result is surprising, since it has been argued that clumped food increases aggressiveness through an increase of immediate competition over food in a number of species (reviewed in Huntingford *et al.*, 2004). It is interesting to note that this effect of experimental feeding regime was evident after the fish had been assorted by growth, transported to a new facility to be screened for individual behaviour in a different set up (in glass tanks confronted visually to a standard conspecific). It would seem that some sort of medium-term effect of experience of different competitive regimes produced this difference in behaviour.

In my study the levels of aggression in the dispersed feeding condition, especially for the large fish, are partially in line with Ruzzante and Doyle's (1991) study. These authors found that levels of aggression in medaka fish (*Oryzias latipes*) were lower in fish held in a clumped food, high interaction environment than in a dispersed food, low interaction environment. A possible explanation for my result could be that, when food is given in excess but clumped, the feeding environment does indeed provide a place of high interaction. In such a case, due to the large number of individuals eating at the same time, it was not possible for the fish to establish and defend a territory. The associated costs of aggression made fighting over food uneconomic, since there was plenty of food for all the fish. In contrast, when food is dispersed among several patches, the number of fish foraging in each patch at the same time is reduced. As a consequence, it may be worthwhile for a fish to engage in aggressive behaviour, defending a feeding territory even if there is plenty of food. Such a scenario is supported by Grant and collaborators (2002), who found that juvenile convict cichlids (*Archocentrus nigrofasciatum*) showed decreased aggression in response to clumped food only when food was given in excess. They suggested it was due to a degree of behavioural flexibility of the fish in response to food abundance. To explain my finding, it is also necessary to suggest that the behavioural changes induced in this way are sufficiently persistent to influence how fish behave 2-3 weeks later and in a different context. This is not implausible, since it is known that previous experience during agonistic interactions can have marked effects on subsequent behaviour in fish (Frost *et al.*, 2007). The fact here my result was due mainly to the large size groups may be due to the size of the food containers which were of the same size for all groups so that tanks holding larger fish could make clumped condition even more clumped compared to the groups containing smaller fish.

Behavioural syndromes

In this study, in order to identify possible behavioural syndromes, I examined correlations among the variables that were recorded during the individual behavioural observations. According to the way the variables were defined by the PCA analysis, the only possible correlations were those among aggression and activity, boldness and shoaling, activity and shoaling and boldness and aggression. Overall the only correlation was a weak (r=0.278) but significant positive association between boldness and aggression. There was no correlation between any other of the tested behaviours, such as activity and aggression and shoaling and activity or boldness. At group level, when looking an at the effect of competitive feeding environment and growth on the expression of behavioural syndromes, the only significant correlation was found in the fast growth fish from the high interaction or clumped feeding condition. Only 6 comparisons were made, so with a level of significance of <0.01 it is reasonable to assume that this relationship is real and not an artefact of multiple statistical comparisons.

A positive relationship between boldness and aggression has been found several times in sticklebacks (Huntingford, 1976; Bell, 2005; Bell & Stamps, 2004; Dingemanse *et al.*, 2007), zebra fish (Moretz *et al.*, 2007) and brown trout (Sundström *et al.*, 2004). Recent evidence suggests that boldness and aggression are correlated only under certain circumstances. For example, in wild caught sticklebacks, boldness and aggression covary only in fish from sites with predators (Bell, 2005; Dingemanse *et al.*, 2007; Brydges *et al.*, 2008).

The fact that an association between boldness and aggression has been found only in sites with high predation regimes (Bell, 2005; Dingemanse *et al.*, 2007) could be explained through 3 different mechanisms. 1) It could be due to natural selection acting over evolutionary time favouring a causal link only in sites where sticklebacks coexist with predatory fish. This is the explanation suggested by Dingemase *et al.*, (2007) 2). The association could be generated through selective mortality of fish from the shy-aggressive and/or bold-unaggressive quadrants of the behavioural distribution. This is supported by a recent study conducted by Bell & Sih (2007); see figures 2.8a and b), in which a predator, in this case rainbow trout, ate shy individuals which were at the same time aggressive, generating a statistical correlation between boldness and aggression after predation that was absent before. 3) The relationship could arise as a result of flexible, experience-induced changes in the behaviour of some categories of fish. Thus, by studying the behaviour of individually identified fish that were not eaten by the predator, Bell & Sih (2007) demonstrated such a shift in behaviour, with sticklebacks that were aggressive and timid before exposure to a predator becoming less aggressive after it (moving from the bottom right hand quadrant of the boldness/aggression space towards the bottom left half quadrant). From their study it seems that in the wild, behavioural syndromes involving aggression and boldness arise as a result of both selective predation on and phenotypic plasticity in shy and aggressive fish.

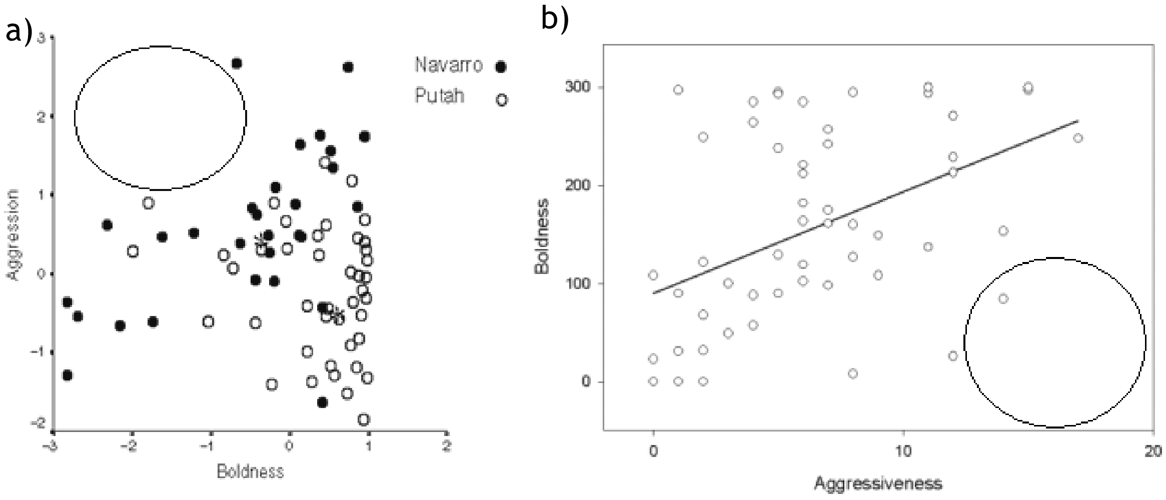


Figure 2.8. Relationship between boldness and aggression in a): two populations of sticklebacks that differ in their predation risk (reprinted from Bell, 2005) and b): fish from the Putah River exposed to predation (from Bell & Sih, 2007). The circles show the area of the distribution where there were not fish distributed.

In the present study, in the group for which the correlation between boldness and aggression is significant (fast growing fish from the high interaction condition), there is a striking lack of fish that are aggressive and shy, whereas for the rest of the groups fish seem to be distributed across all the quadrants (see figure 2.7a in the results section). Since the fish in my experiments were not subjected to predation and very little mortality occurred, the lack of aggressive-shy fast growing fish from the high interaction condition must be due to phenotypic plasticity of the fish. It would seem that timid fish with a tendency to fight over food learned to be less aggressive towards conspecifics during 10 weeks of exposure to a high interaction feeding regime. This would have to be the case for fast growing fish only, perhaps because these were actively feeding individuals that would have experienced frequent social interactions at the feeding dish.

The study of correlations between behaviours has attracted interest because when two behaviours are tightly correlated across time and contexts, this may potentially impose evolutionary constraints on the ability of the individual behavioural patterns to respond to selection. If behavioural correlations can be uncoupled (as shown in Bell, 2005; Dingemanse *et al.*, 2007; Brydges *et al.*, 2008), this would indicate the presence of a degree of behavioural plasticity as has been proposed by the adaptive hypothesis, hence not imposing constraints (Sih *et al.*, 2004; Bell, 2005). Hence, it is important to know under what conditions such correlations can exist, or to put it the other way round, under what conditions such relationship can be decoupled.

The results presented in this chapter show that individual variation in risk-taking and aggressive behaviour is not fixed; levels of aggression in particular showed individual differences across experimental groups. It was found that certain circumstances, in this case manipulation of the feeding environment, may alter expression of boldness and/or aggression in a subset of fish, generating a behavioural syndrome. Recent work by Bell & Sih (2007) show that both, selective predation on specific behavioural phenotypes and behavioural shifts in fish with certain behavioural combination contribute to the appearance of a boldness and aggression correlation in fish from a low predation site following exposure to predation. My results extend current understanding by showing that social interactions (and in particular competition for localized food) can have a similar effect.

Chapter 3 Differences in risk-taking, aggression and their relationship in sticklebacks that hatch at different times during the breeding season

Introduction

When observing a group of animals, it is possible to distinguish behavioural differences among individuals, both between and within species. Individual variation in behaviour is sometimes characterized into “personalities” (Gosling, 2001) or “temperaments” (Rèale *et al.*, 2007). Examples are provided by the bold-shy continuum and by variable levels of individual aggression (Wilson *et al.*, 1994). Particular interest has been devoted to the study of the extremes of the behavioural distribution (i.e. shy-bold, aggressive-non aggressive animals), since they constitute two different strategies of reaction to the range of environmental conditions that individuals encounter (Korte *et al.*, 2005). Such behaviours can sometimes characterize a population or a species, with individual levels varying across the behavioural range. It is not just that single behaviours vary; correlations between sets of behaviours have also been found, leading to the use of the term “behavioural syndromes” (Sih *et al.*, 2004). The study of how single behaviours and their correlations vary among individuals and between species have implications in areas such as ecology (Rèale *et al.*, 2007), aquaculture (Huntingford & Adams, 2005) and conservation biology (Bremmer-Harrison *et al.*, 2004).

One of the most commonly studied personality dimensions in animals is the shy-bold continuum (Wilson *et al.*, 1994). This refers to the fact that some individuals are prone to take risks and explore new environments, while others tend to avoid any kind of risks and freeze or hide when exposed to novelty. In many species, populations consist of individuals falling across the whole of bold to shy continuum; however, it is interesting to know how natural selection acts upon the two extremes of the behavioural distribution (bold and shy) when they are opposite strategies to cope with the environment. Another individually-variable behaviour that has received a great deal of attention is aggression. Aggressive behaviour is advantageous, but it also imposes costs to the aggressive individual. There is evidence that aggressive individuals for example, have advantage in the monopolization of food, mates or territories, but they may also pay a cost due to the energetic demands and increased risk of injury or death (Briffa & Sneddon, 2007).

Identifying correlations between individual behaviours could help to understand how selection shapes behavioural phenotypes. Correlations between behaviours can indicate either constraints to the independent evolution of behaviours or suites of behaviours favoured by selection (Sih *et al.*, 2004; Bell, 2005; Duckworth, 2006). Evidence is pointing to the last as the most plausible reason. In sticklebacks for example, only under particular environmental circumstances such as high predation pressure, the correlation between boldness and aggression has been found (Bell, 2005; Dingemanse *et al.*, 2007).

The fitness consequences of boldness and aggression and any correlation between them have not been extensively studied. It can be suggested for example that individuals that take greater risks and/or behave aggressively and that are therefore willing to accept the associated costs, must gain additional fitness benefits from doing so that timid non-aggressive individuals do not enjoy. For example, in birds, a number of reports show that consistent individual variation in boldness correlates negatively with survival (Rèale & Festa-Bianchet, 2003), but positively with reproductive success (Both *et al.*, 2005). In fish, boldness has been found to covary with growth (Ward *et al.*, 2004) and body mass (Brown & Braithwaite, 2004), whereas aggressive individuals are successful gaining mates (Morell *et al.*, 2005) and food (Magnhagen & Borcherding, 2008). All those characteristics have fitness consequences; for example, in a study linking aggression and reproductive success in bluebirds it was found that birds that defend their nesting territories aggressively do not invest enough in parental care, resulting in low reproductive success. Therefore, it is unclear why aggression is a characteristic of this population even though less aggressive individuals are favoured (Duckworth, 2006). However, evidence suggests that aggressive individuals are able to colonize new environments (Duckworth & Badyaev, 2007).

In great tits (*Parus major*), speed of exploration has been found to be a heritable personality trait (Dingemanse *et al.*, 2002) and this relates to aggressiveness and competitive ability (Verbeek *et al.*, 1999). Fitness consequences of fast and slow explorers have also been found. Adult personalities are related not only to adults' success, but also to their offspring survival (Dingemanse *et al.*, 2004). Assortative mating between animals of different personalities has been found to be one of the underlying causes of the variation in fitness among those birds (Both *et al.*, 2005). In fish, there are few studies looking at the fitness consequences of personality traits and behavioural syndromes. Most of the work has been orientated to the study of individual and population differences of single behaviours or to the expression of behavioural syndromes. But for example, Dugatkin (1992) found that guppies that show higher levels of predator inspection have a higher mortality rate than the more cautious individuals. However, Godin & Davis (1995) found that the same trait also had positive fitness consequences because predators were significantly less attentive to, and less likely to attack, guppies that inspected them. Biro and collaborators (2003) have shown that age-0 rainbow trout modulated their risk-taking behaviour in the presence of a predator and this varied with food availability. Fish in an environment with abundant food were relatively risk-aversive compared to rainbow trout held in an environment with less food available.

The examples mentioned above have studied the fitness consequences of animal personalities by looking at the profile of individuals of known personality and by measuring some aspects of fitness. Based on the knowledge that animal personalities and fitness are related, another approach (which has not been applied yet) could be to study traits that are known to be important for fitness (such as date of first breeding) and relate these to personality. The characteristics of the stickleback's reproductive biology make this species a fine model to test this approach. In sticklebacks breeding typically takes place at some time between March and early August and the season can be divided in early (March-May) and late (June-August) seasons. During the breeding season, a well fed female may produce up to 20 clutches (Wootton, 1984), but generally less than five (Baker, 1994). Females tend to die at the end of the reproductive period (Wootton, 1984), although a few females can survive to a second reproductive period. The usual breeding times for a single female stickleback have been found to be restricted only to one reproductive period, either reproducing early or late in the season (Saito & Nakano, 1999).

Evidence suggests that personalities influence reproductive success and other fitness-related traits. Assuming a degree of heritability of animal personalities and also assuming that early and late caught fry are the offspring of sticklebacks that bred early and late during the season, I used this indirect approach to relate fitness to personalities in sticklebacks. Specifically, I related the fry's hatching date (used as an indirect measure of parental fitness) to their personalities (boldness and aggression). The study was developed through behavioural observations that allowed an answer to the following questions:

- Do early and late hatching sticklebacks vary in risk-taking phenotype?
- Do early fish differ from late fish in how they respond to a standard conspecific?
- Do early fish tend to dominate matched-size late fish (or the converse) if behavioural style is allowed for?
- Is the relationship between boldness and aggression different in fish with different hatching dates?

Materials and methods

Fish

Fry of the three-spined sticklebacks (*Gasterosteus aculeatus*) were obtained from a wild population (the River Endrick) in the Loch Lomond catchment, Scotland. Three sampling sessions were carried out; each sampling was separated in time from each other for a month (end of May, June and July 2007). During each sampling, the very smallest, developmentally immature fish fry were caught, ensuring that we sampled recently hatched fish and not that these were slow growing fish. Fish were transported to the aquaria facilities of the Graham Kerr Building, University of Glasgow for further tests and held at ambient temperature and with photoperiods that reflected the natural conditions at the moment. I assumed that collecting the smallest fish during each sample, I captured fish that hatched at 3 different times (early, intermediate and late) throughout the breeding season. Fish (early n=43, intermediate n=28, and late hatching fish n=54) were kept in glass tanks until further behavioural screening. The time fish spent under controlled conditions was 25 weeks for early bred fish, 21 weeks for intermediate hatched fish and 17 weeks for late bred fish. Food rations were adjusted to the size of the fish, smaller fish had more food available, so that at the moment the behavioural observations took place January 2008 all the fish had approximately the same size.

Distribution of morphological variables and risk-taking phenotypes by hatching date

Since fry were sampled at different times, food rations were adjusted to try to equate size between early and late caught fish. Figure 3.2 shows the mean ± S.E. of weight, length, and condition factor of early, intermediate and late hatching fish.

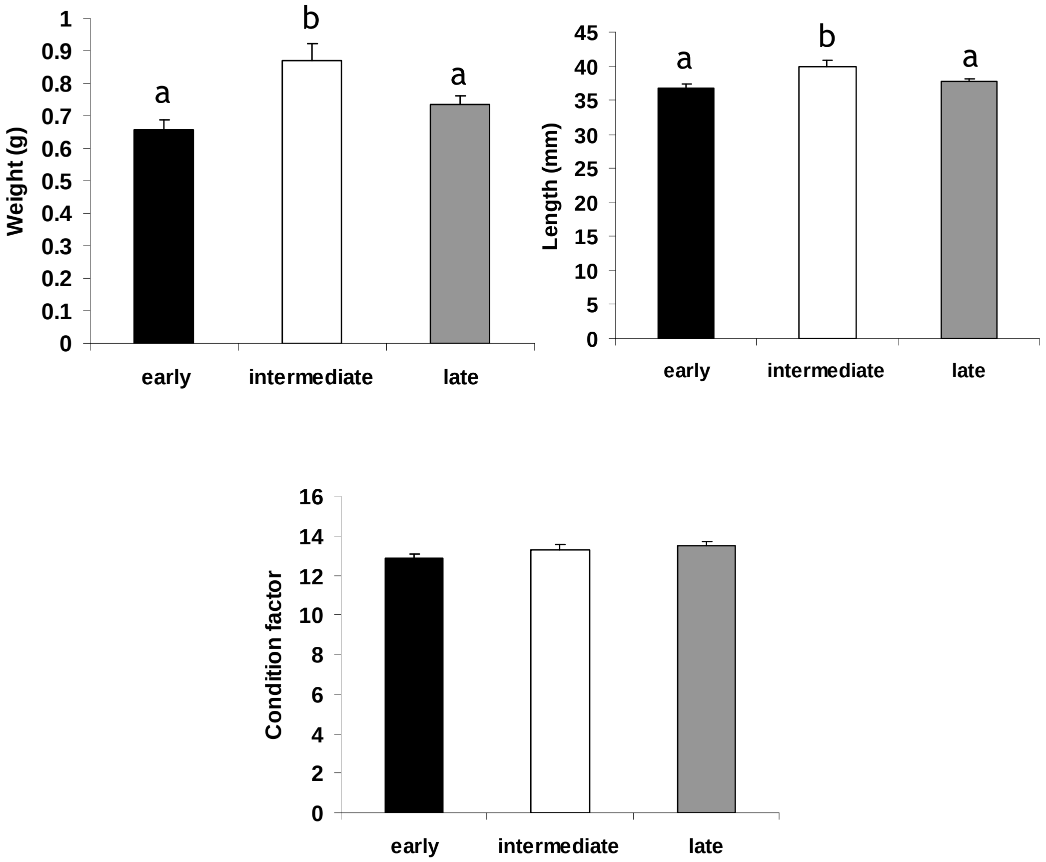


Figure 3.1. Mean ± S.E. of: a) weight, b) length, and c) condition factor of early, intermediate and late hatching fish after being held under controlled conditions. Different letters stand for p < 0.05.

After being held in captivity, there were overall differences in weight among early, intermediate and late hatching fish ($F_{122,2}$=8.41 p=0.001). Tukey *post-hoc* analysis revealed that differences were driven by intermediate hatching fish, which were heavier than early (p=0.002) and late fish (p=0.022). However, early and late fish did not differ in their weights (p=0.175). There were also overall differences in length ($F_{122,2}$=5.40 p=0.006). Intermediate hatching fish were longer than early (p=0.0043) and late fish (p=0.0047), whereas there were no differences between the lengths of early and late fish (p=0.5296). In general, fish from the three hatching dates did not differ in their body condition ($F_{122,2}$=2.45 p=0.091).

Screening for risk-taking in groups

After being held in the lab, all fish from each hatching category were weighed and length measured. Fish were marked by a dye of alcian blue in three different positions in order to distinguish between fish caught in the early, intermediate and late hatching dates. Fish were screened for risk-taking in groups and categorized as bold, intermediate and shy phenotypes. This method has been described previously in chapter 2, so will only be mentioned here briefly. The cut-off emergence times for measuring risk-taking were set from a previous pilot experiment. Bold fish were those that emerged first from a shelter into a lit space, whereas shy fish were those that did not come out of the safe area.

The test arena consisted in a 200 litre glass aquarium (dimensions (LxWxH): 1 X 0.40 X 0.50 meters) lined on three sides with white self-adhesive plastic. The front wall had a cardboard screen with a small slit that allowed the observer to record the behaviour without disturbing the fish. The bottom of the tank was covered 1cm gravel as a substratum, and was filled with cooper free water. A dark settling chamber was placed in a corner of the tank; the chamber had a door that, once opened, permitted the fish to see the lit, novel environment. The novel environment consisted in the remaining open space supplied with a petri dish containing food (frozen *Chironomid* larvae) and two clumps of artificial plants.

Groups of 9 marked fish (3 early, 3 intermediate and 3 late hatching fish) were screened at a time. The fish were allowed to settle in the chamber for an hour, after which, the door was gently opened giving the fish the option to enter the novel environment or not. The criteria chosen to distinguish between risk-taking phenotypes are described in table 2.1 (chapter 2). When all fish of each risk-taking phenotype were out the door was gently closed and the fish that had emerged were netted out. The corresponding hatching date mark was registered and fish were separated into bold, intermediate and shy groups from the early, intermediate and late hatching date.

Screening for aggression

Set up

After the risk-taking test, fish were screened for aggression towards a conspecific. The test arena consisted in a clear plastic aquarium (32 X 16 X 23 cm, front X side X height) half divided by a removable opaque white plastic wall. Two air-stones were placed one on each side of the tank to provide the fish with enough oxygen through the test. The bottom of the tank was covered by a 1cm gravel substratum and a piece of artificial weed in the middle provided the fish with a refuge. 12 tanks were available at a given day. Three sides of the tank were lined from the outside with white paper to diminish exterior disturbance. A cardboard screen with a slit in the middle was placed in front of the tank, from which the observer recorded the behaviour without disturbing the fish. A row of 30 watt strip lamps above of the aquaria gave even light to all the tanks. Fish were not fed the day before being tested. A pair of fish, one on each side, was assigned to each tank. 30 minutes before the tests, the room lights were turned off; after the test finished the lights were turned on again to keep the remaining fish under controlled light conditions. Fish were allowed to settle overnight and the next morning the test took place. At the end of the test the water in each tank was changed so that the new tested fish did not have odour cues that interfered with their performance the following day.

One aim of the test was to find out if early and late hatched fish from the bold and shy behavioural phenotypes differ in how they respond to a standard conspecific. Bold and shy fish from the early and late hatching categories: bold early (n=14), bold late (n=14), shy early (n=11) and shy late (n=11) were paired against a random selected intermediate hatching fish. Stimulus fish were used twice however; a single fish was not used two days in a row. In order to test whether early fish (n=10) tend to dominate a separate sample of late hatching fish (n=10) or the converse when behavioural style (bold and shy) is allowed for, early and late hatching sticklebacks were paired against each other.

Behavioural screening

Two observers were in charge of recording behaviour. The dividing wall was gently removed and fish were allowed to interact during 5 minutes. The number of bites, escapes, approaches to the stimulus fish, moves (swimming after being frozen) and chases were recorded. The sum of all the aggressive acts given towards the stimulus fish was used as a measure of agonistic behaviour. Any attack received from the stimulus fish was also recorded (attacks against). After the behavioural observations, fish were netted out, euthanized with an overdose of anaesthesia, weight and length measured and then frozen individually. All fish were kept frozen (-70C) until further dissection for sexing.

Sexing fish

Fish were defrosted and individually identified. Sex in juvenile sticklebacks cannot be distinguished externally; however, the gonads in males and females are different. The ovaries are bigger than the testes and the visceral peritoneum that covers the testes contains many melanophores with their black pigment (Wootton, 1984). Gonads were weighed and signs of pigmentation recorded. The sex of the fish was therefore determined based on those characteristics. Figure 3.1 shows that females were distinguished from males because they indeed have bigger gonads (t=14.82 p=0.0001 DF=60) and also males' smaller gonads are pigmented (t=13.70 p=0.0001 DF=60). Early and late hatching samples consisted in exactly the same sex distribution: 12 males and 23 females in both hatching dates).

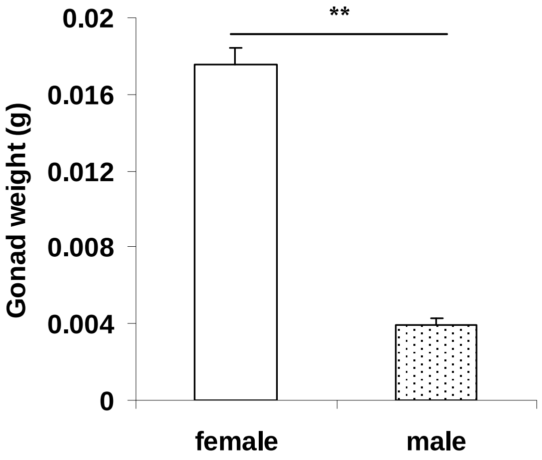


Figure 3.2. Mean (± SE) of the weight of the gonads of female and male sticklebacks. ** p<0.001

Statistical analyses

Differences between early, intermediate and late hatched fish in the morphological variables, weight, length and condition factor and the relationship of boldness and aggression between early and late hatched fish were assessed with a General Linear Model ANOVA. Significant results were then analysed with a *Tukey post-hoc* analysis. Differences in the proportion of risk-taking phenotypes in the 3 hatching dates were evaluated with a Pearson Chi-square analysis. Differences in aggression between early and late and between males and females were measured with two sample t-tests.

Results

Distribution of the risk-taking phenotypes by hatching date

Overall, there were differences in the proportion of risk-taking phenotypes among the hatching dates (Pearson Chi-Square=10.158, DF=4 p=0.038). Figure 3.3 shows the proportion of fish in each risk-taking phenotype for early, intermediate and late hatching date. There were more bold fish in the early hatching date than in the intermediate and shy sample. The late and intermediate samples contained a higher proportion of shy than the early hatching sample.

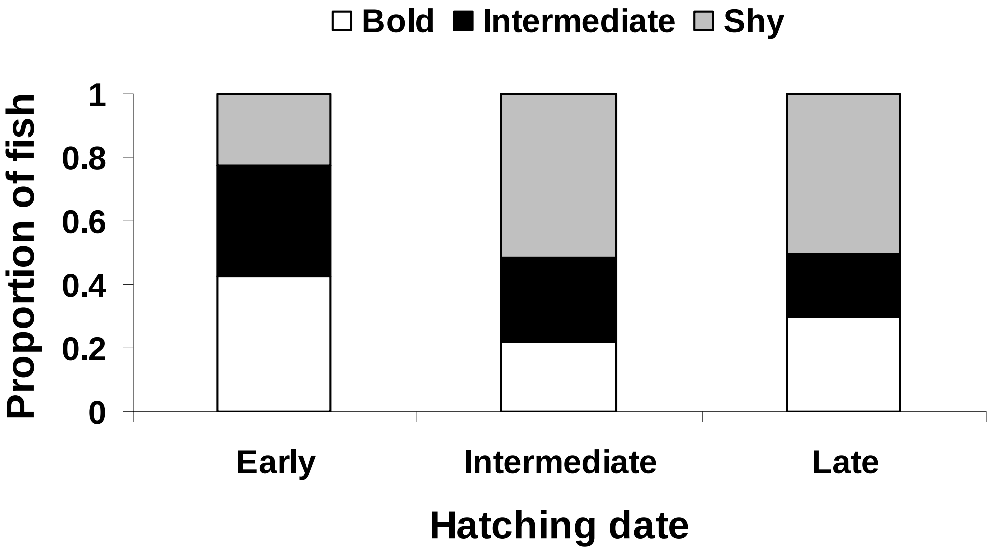


Figure 3.3. Proportion of fish in each risk-taking phenotype for early, intermediate and late hatching sticklebacks.

Response to a standard conspecific of sticklebacks that hatched early and late during the breeding season

Table 3.1 shows the mean ± S.E. of the number of aggressive acts given by a focal stickleback towards a standard conspecific. In general there were no differences in aggression between early and late hatching sticklebacks (t=0.49 p=0.624 DF=43). Male and female fish did not show differences in the number of aggressive acts performed (t=0.49 p=0.627 DF=47).

Table 3.1. Mean (± SE) of the number of aggressive acts given by sticklebacks, hatched on different dates, to a standard conspecific.

	Category	**Mean**	**S.E.**
Hatching date (t=0.49 p=0.624 DF=43)	Early	4.72	1.52
	Late	3.80	1.07
Sex t=0.49 p=0.627 DF=47)	Female	4.60	1.38
	Male	3.75	1.06
Risk-taking/hatching date ($F_{3,46}$=0.15 p=0.699)	Bold early	5.86	2.62
	Bold late	5.57	1.74
	Shy early	3.27	0.973
	Shy late	1.54	0.545

Differences in aggression between early and late hatching fish

When behavioural style was allowed for, there were no differences in the number of agonistic acts when early and late hatching fish were confronted each other (t=0.41 p=0.684 DF=17). There were no overall differences in aggressive behaviour between males and females either (t=1.18 p=0.267 DF=9).

Relationship between boldness and aggression in sticklebacks of different hatching dates

Figure 3.4 shows that overall, bold fish tended to be more aggressive than shy fish (t=2.00 p=0.05 DF=34). However, there were no differences in the number of agonistic acts among bold and shy fish from the early and late hatching dates ($F_{3,46}$=1.20 p=0.321).

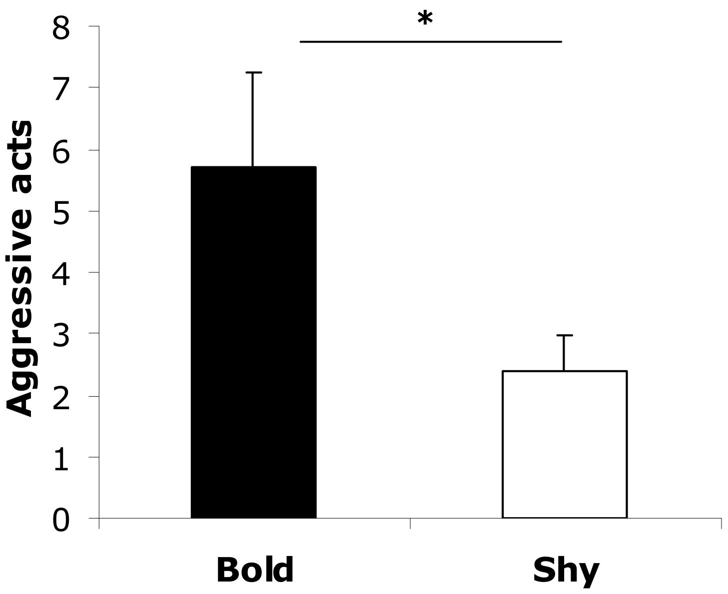


Figure 3.4. Mean (± SE) of the number of aggressive acts given by bold and shy sticklebacks to a standard conspecific during 5 minutes; * = p<0.05

Discussion

Differences in boldness between early and late hatched sticklebacks

The main aim of this study was to compare the behavioural profiles (boldness and aggression) of sticklebacks that hatch early and late during the breeding season. The most striking result was the differences in boldness between early and late hatched fish. Early individuals were in proportion bolder than intermediate and late fish, whereas most of the shy individuals were found among the intermediate and late bred fish.

In a previous chapter (chapter 2), using the same set-up to test boldness, it was found that bold sticklebacks tended to be either smaller or in poorer condition than shy fish. The same result has been also found in the poeciliid *Brachiopsis episcope* when boldness was measured in the wild (Brown & Braithwaite, 2004). The fish used in the present study were caught at very small sizes and kept under laboratory conditions in order to have individuals of identical body condition when behavioural observations took place. There were no differences in size or body condition between early and late hatched fish; however, for some reason, intermediate hatching fish were longer and heavier than early and late fish but in equal body condition. The fact that early hatched fish were bolder than late bred fish in this study was therefore not due to differences in size or body condition, so other mechanism must be involved.

One reason why early hatched fish were bolder could be the time the fish spent under laboratory conditions before being tested. Since early hatched fish were the first fish captured and brought to the aquaria facilities, they had one month more to settle to the laboratory conditions than intermediate fish and two months more than late fish. In studies where boldness has been assessed in sticklebacks, it seems that time in captivity does not affect their behaviour. Fish have been tested either the day after they have been brought from the wild (Dingemanse *et al.*, 2007) or after being held under controlled conditions (Bell & Sih, 2007) and some studies do not mention this information for example Brydges *et al.* (2008). Although the possibility existed that differences between early and late hatched fish in the time they spent in captivity influenced their performance, it seems unlikely, so this explanation will be tentatively rejected.

Environmental effects may also be among the reasons why early bred fish were bolder than late bred fish, since there may be differences in the experiences of early and late hatched fish. For example, it could be that more fry predators, such as piscivorous fish and birds are around later in the season, which might cause late bred fry to become more timid than bigger early hatched fish. Boldness is in part determined by the individual's experiences during ontogeny; for example, sticklebacks can modulate their behaviour in response to predation pressure (Bell & Sih, 2007). However, the fry in this study spent very little time in the wild before being brought to the lab, so this explanation too is unlikely.

Evidence in various species such as great tits (van Oers *et al.*, 2004), sticklebacks (Bell, 2004) and poeciliids (Brown *et al.*, 2007) suggest that boldness has an underlying heritable component. Therefore, with this background for the present study, I assumed a degree of heritability of personalities in sticklebacks. Another assumption was that early and late caught fry are the offspring of sticklebacks that bred early and late during the season. However, Candolin (1998) showed that male sticklebacks might also show trade-off between current and future reproduction, taking more risks later in the season if their possibilities of future reproduction are low. Evidence also suggests that female sticklebacks tend to lay eggs either early or late in the season and tend to die at the end of the breeding period (Wootton, 1984; Saito & Nagano, 1999). Since sticklebacks reproduce during summer, the reward of reproducing early in the breeding season may not be direct for the parents. Early hatched fish could have an advantage over individuals that are born late in the season since they can grow more before winter, when food availability and cold temperatures can constrain their development. Therefore, early born fish would have more possibilities of overwinter survival and increased chance of reproducing in the following breeding season.

It is also possible that some sort of social interaction with parents plays a role in the development of boldness in early hatched fish. Personality in general has been associated with differences in parental behaviour that affect offspring fitness; for example, growth and survival. Female house mice (*Mus domesticus*) selected for high aggression have been found to nurse and groom their pups more than females selected for low aggression (Benus & Rondigs, 1996). The personality of red squirrel mothers was correlated with growth rates and survival of their offspring both in the nest and later through their first winter (Boon *et al.*, 2008). In sticklebacks, males are in charge of attending the eggs from lying up to beyond hatching. By taking care of the spawn, the male could therefore guarantee a higher hatching success. However, parental care has also several associated costs such as high energy expenditure and reduced survival (Östlund-Nilsson *et al.*, 2007). It is not known whether sticklebacks' parental care is directly related to the offspring fitness however, different parental requirements for territorial defence during the breeding period, such as protection of the clutch against predators, may be also involved, since boldness has been associated with territoriality and aggression (Sih *et al.*, 2004; Kortet & Hedrick, 2007; Reaney & Backwell, 2007). It has been described that stickleback fathers retrieve their fry to their nests when the fry start to explore their surroundings for the first time (Rowland, 1994). Early interactions soon after hatching such as a retrieving adult chasing them, may make fry more aware of predators and allow them to avoid attacks later in life in sticklebacks (Tulley & Huntingford 1987) and guppies (Chapman *et al.*, 2008).

There is growing evidence that personalities have implications for reproductive success and that boldness can be advantageous. Rèale and collaborators (2000) found that in ewes bold animals reproduced earlier and that boldness was positively correlated to weaning success. In a recent review Smith & Blumstein (2008) showed that in fact, the general trend is that bolder individuals have greater reproductive success than shyer ones. However, the costs associated to boldness are reduced lifespan and survival (Smith & Blumstein, 2008). In the present study, I related hatching date of stickleback fry (used as an indirect measure of parental fitness) to their personalities (boldness and aggression). Perhaps in the population studied here, bold fish grow better and breed earlier; early hatched fish may be bolder than late hatched fish because the risk-taking phenotype has been inherited from one or both of their parents. If this is the case, the differences in boldness between early and late hatching sticklebacks could be used as an evidence for a fitness advantage for boldness in this river.

Differences in aggression between early and late hatched sticklebacks

Aggressive behaviour has been well studied in sticklebacks (Bakker, 1986). In the present study I did not find differences in aggression between early and late hatched fish. This result may be due the age of the fish I used in my tests. Fish were tested outside the breeding season and the levels of aggression in juveniles may be lower than those for adults, because juveniles do not need to defend a breeding territory or to engage in aggressive interactions for mates. Another explanation could be that the fish in my experiments probably did not interact aggressively for food since they were fed in excess, food availability was predictable and fish were held in small groups.

There were no differences in aggression between males and females and this may be attributed to the fish’s age as well. Bakker (1986) found that the levels of aggression in sticklebacks are the same for male and female fish in the juvenile stages. Levels increase for males and decrease for females when they start to become sexually mature. In other species, great tits for example, variation in aggression has been found to be related to personality and gender. In bluebirds the costs of nest defence shown as reduced reproductive success were specific to males; therefore, the authors suggest that aggression in a male context is the true source of the cost of aggression (Duckworth, 2006).

The fitness consequences of aggression show that the general tendency for a number of species is a small positive effect in reproductive success and a non-significant effect in survival (Smith & Blumsteim, 2008). Plasticity in aggression might be limited because it could have a heritable component. It is known that aggression is a costly trait because of high energy expenditure and because aggressive animals tend to suffer injuries and even death, with consequences for their overall fitness. The fitness costs of aggression in blue birds for example were shown when males who engaged more frequently in aggressive behaviour over the defence of their nests fed less their chicks and had the lowest reproductive success (Duckworth, 2006). Adult survival and reproductive success depended on an interaction between exploration speed, sex and winter resources availability, whereas offspring survival was related to the personality of the mother (Dingemanse *et al.*, 2004).

The relationship between boldness and aggression

There was overall variation in boldness and aggression in my study. I also found a relationship between boldness and aggression. This was independent of environmental background such as predation pressure, because fish were raised under laboratory conditions. It has been found that in stickleback that boldness and aggression correlate in sites with high predation pressure but this syndrome is not present in fish from predator free sites (Bell & Sih, 2007; Dingemanse *et al.*, 2007). In my study, bold fish were more aggressive than shy fish with no effect of hatching date. This relationship between boldness and aggression could be beneficial to individuals in this population, in the sense that the advantages of being bold for example shown as early reproduction could be increased with the benefits of being aggressive such as the ability to establish territories, gain mates and food.

In conclusion, if personality has a significant heritable component then the offspring of aggressive but bold females may survive better because their personality would allow them to compete more effectively. In the present study, this was reflected in differences in the rate of achieving a reproductive state in parents. Thus, I suggest that fish that are bold and aggressive in my study population could have more fitness advantages than fish showing any other behavioural profile.

Chapter 4 Behavioural plasticity in rainbow trout (*Oncorhynchus mykiss*) with divergent coping strategies

Introduction

Coping strategies

Within the last decade, it has been clearly established that individual animals of various species (vertebrate and invertebrate) differ in the nature of their response to stress, with a combination of behavioural, neuroendocrine, and autonomic changes that reduce the adverse effect of the challenge (Korte *et al.*, 2005). The behavioural responses allow the animal either to escape or counter the challenge, while the neuroendocrine responses provide the animal with the necessary resources to meet the demands of the altered behaviour as well as trying to maintain physiological homeostasis during the aversive situation (Koolhaas *et al.*, 1999).

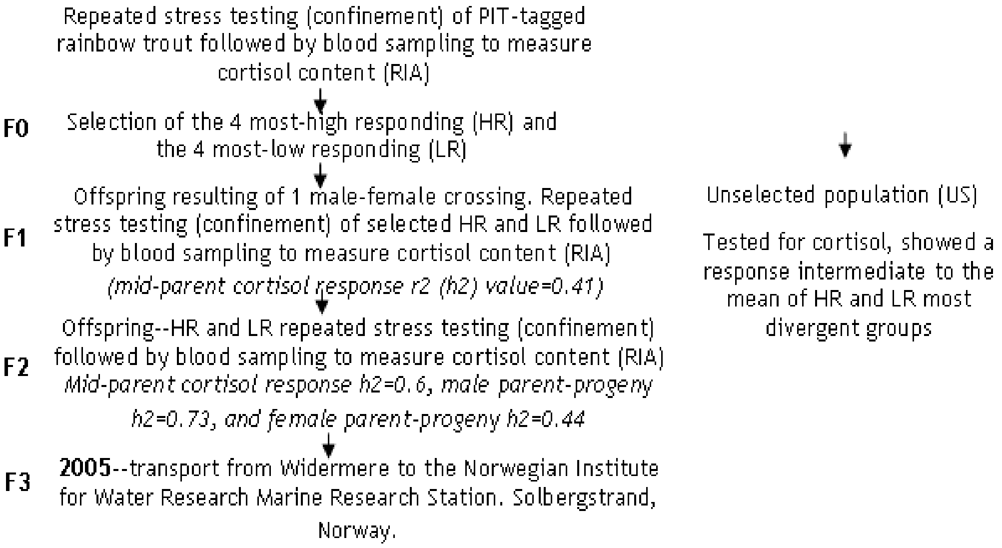
Where responses are consistent over time and across situations, they can be characterised as behavioural syndromes (Sih *et al.*, 2004; Bell, 2007) or coping strategies (Koolhaas *et al.*, 1999; Korte *et al.*, 2005), involving suites of correlated physiological and behavioural traits. In general, from the best documented cases (mice and rats among mammals and great tits among birds) two distinct coping strategies can be distinguished. At one extreme, animals with a proactive coping strategy tend to show a fight-flight response, to be more aggressive and bold (in the sense of taking risks in a variety of dangerous situations) and to have low levels of plasma corticosteroids. In contrast, at the other extreme, reactive animals show a freeze-hide response, tend to be shy and less aggressive with a more flexible behaviour than proactive individuals. The physiological response to stress in reactive individuals involves relatively higher plasma cortisol levels, as well as differences in a number of other neuroendocrine systems (Koolhaas *et al.*, 1999; Korte *et al.*, 2005).

In fish, a number of studies have documented the existence of covarying behavioural traits, or behavioural syndromes. For example, a positive correlation between aggression towards conspecifics and risk-taking in various potentially dangerous situations has been described in sticklebacks (Huntingford, 1976; though see Bell, 2005), brown trout (Sundström *et al.*, 2004) and grayling (Salonen & Peuhkuri, 2006).

The HR and LR rainbow trout system

Bidirectional selection of animals for either physiological or behavioural responses can result in the establishment of lines that show the selected features consistently. To a great extent, what is know about stress coping styles on animals has been found out through the study of different selection lines (reviewed in Korte *et al.*, 2005). There is relatively little information on physiological correlates of such behavioural variability in fish, although Bell and collaborators (2007) found that an unfamiliar conspecific as well as the presence of a predator elicited behavioural responses that were related to brain physiology in sticklebacks. However, the rainbow trout (*Oncorhynchus mykiss*) seems to be an exception.

Stress in fish is characterized by the release of catecholamines and cortisol. Both these hormones are concerned with energy reallocation from anabolic activities such as growth toward activities to restore homeostasis. The blood concentration of cortisol is therefore a major index of stress in fish and has been found to be a causal factor of the deleterious effects of stress as well. Cortisol measurements could be an instantaneous reflection or “snapshot” of the fish state at a particular sampling time and therefore, it could be hard to interpret them at a long term. In the rainbow trout it has been demonstrated through selection for cortisol responsiveness that this is an individual characteristic heritable to a certain degree (Fevolden *et al.*, 1999; Pottinger & Carrick, 1999). In this respect, two strains of rainbow trout have been selected for differences in stress responsiveness to a standardized stressor for over 3 generations. Figure 4.1 describes the selective breeding programme followed from 1996 at the Windermere Laboratory of the UK Centre for Ecology and Hydrology to create two strains of rainbow trout that diverge in their post-stress plasma cortisol response.



Repeated stress testing (confinement) of PIT-tagged rainbow trout followed by blood sampling to measure cortisol content (RIA)

F0 Selection of the 4 most-high responding (HR) and the 4 most-low responding (LR)

F1 Offspring resulting of 1 male-female crossing. Repeated stress testing (confinement) of selected HR and LR followed by blood sampling to measure cortisol content (RIA) *(mid-parent cortisol response r2 (h2) value=0.41)*

F2 Offspring--HR and LR repeated stress testing (confinement) followed by blood sampling to measure cortisol content (RIA) *Mid-parent cortisol response h2=0.6, male parent-progeny h2=0.73, and female parent-progeny h2=0.44*

F3 **2005**--transport from Widermere to the Norwegian Institute for Water Research Marine Research Station. Solbergstrand, Norway.

Unselected population (US)

Tested for cortisol, showed a response intermediate to the mean of HR and LR most divergent groups

Figure 4.1. Summary of the selection protocol followed to create the HR and LR rainbow trout strains and the results of an unselected population tested simultaneously (From Øverli et al., 2005).

As shown in figure 4.2, the high responsive strain (hereafter referred as the HR rainbow trout) and the low responsive strain (referred as to the LR rainbow trout) have differed in the plasma cortisol response over time and across generations (Pottinger & Carrick, 1999). Simultaneously, an unselected group of fish was also tested for differences in post-stress plasma cortisol response; an immediate divergent response was found suggesting that both phenotypes can be naturally found.

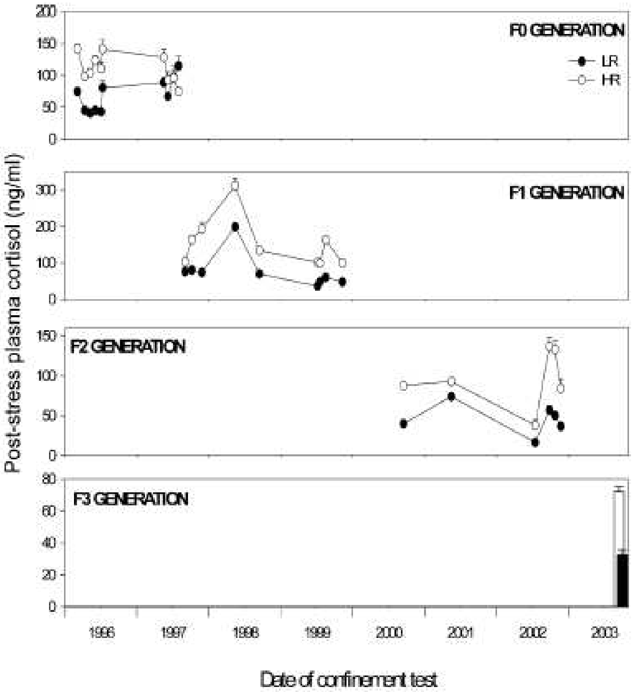
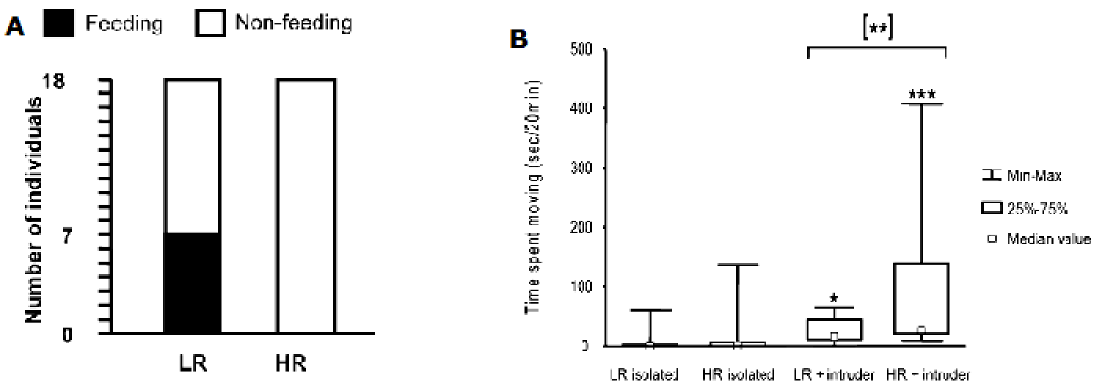


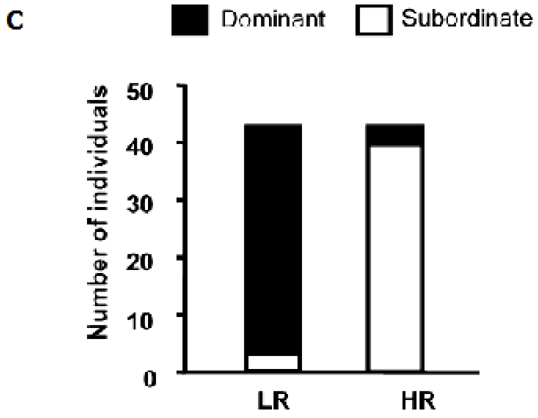
Figure 4.2. Post-stress blood plasma cortisol levels (mean ± SE) of LR and HR rainbow trout (mixed sex samples) from the establishment of the parental generation (F0) up to F3 (Reprinted from Øverli *et al.*, 2005).

The cortisol response to stress in HR and LR rainbow trout has been found to correlate consistently with some behavioural traits (figure 4.3). Fish from the LR strain become socially dominant over HR (Pottinger & Carrick, 2001). After being exposed to a new environment LR fish start to eat sooner than HR (Pottinger & Carrick, 2001; Øverli *et al.*, 2005; Schjolden *et al.*, 2005). When fish from both strains are confronted to an intruder, HR fish tended to show significantly higher locomotor activity levels than LR rainbow trout (Øverli *et al.*, 2002). Both strains also differ in their cognitive ability; LR seems to retain longer a conditioned response than HR fish (Moreira *et al.*, 2004).



The number of LR-HR rainbow trout regaining feed intake within 1 week of transfer to a new environment. LR feeding more than HR p=0.003 X^2 test (data from Øverli et al 2002).

Locomotor activity in HR and LR rainbow trout during an intruder test (data from Øverli et al 2002).



The number of LR-HR rainbow trout identified as either dominant or subordinate during paired contests. LR became dominant over HR p<0.001. X^2 test (data from Pottinger and Carrick 2001).

Figure 4.3. The post stress cortisol response is associated with behaviour in LR and HR rainbow trout. A). LR fish tend to feed earlier than HR fish after exposure to a novel environment; B). HR fish show higher locomotor activity than LR when confronted with an intruder; and C). LR fish become socially dominant over HR fish in pairwise contests (Reviewed in Øverli *et al*., 2005).

The physiological mechanisms underlying the HR line behaviour (reduced appetite, reduced ability to gain social encounters, enhanced locomotion during acute stressful challenges) are consistent with previously reported effects of the steroid hormone cortisol in non-mammalian vertebrates (DiBattista *et al.*, 2005; Gregory & Wood, 1999; Øverli *et al.*, 2002). For example, in non-selected rainbow trout, negative correlations have been reported between the magnitude of the plasma cortisol response to stress and the level of aggression shown towards subordinate fish (Øverli *et al.*, 2004), as well as between cortisol responsiveness and bold feeding behaviour in novel environments (Øverli *et al.*, 2006).

A range of other hormones, neuropeptides, and transmitters are also involved in the regulation of these behaviours and the behavioural effects of cortisol on behaviour depend on dose, duration of exposure and context. It is therefore unlikely that cortisol alone is responsible for controlling all behavioural aspects of stress coping style, in fish or other animal groups. The characteristics expressed by HR and LR rainbow trout thus resemble those for proactive and reactive coping styles reported for birds and mammals. Given the heritability of cortisol responsiveness (Pottinger & Carrick, 2001) with which the behavioural traits are associated, the indication is that these are consistent, genetically linked traits.

The data presented in this chapter form part of a series of experiments, the results of which suggest that differences in cortisol responsiveness are not sufficient to maintain coherent behavioural profiles. After 3 generations showing the distinctive behaviour described above, a batch of HR and LR rainbow trout were transported from their native rearing site (Windermere, UK) to Oslo, Norway. Unexpectedly while the divergence in post-stress plasma cortisol concentrations remained unchanged, immediately after transport the two strains switched behavioural profiles. Specifically, HR fish showed bolder feeding behaviour and became socially dominant over LR fish.

Results from tests carried out immediately after transport suggested that the switch in behaviour could be attributed mainly to stress and starvation. However, an alternative (or additional) possibility arisen from the fact that before being transported to Norway fish were held solely in fresh water, but on arrival to Norway some salt water was added to the holding tank in order to avoid fungus infections. Cortisol is one of the main regulators of hydromineral balance in fish (Wendelar-Bonga, 1997). When moving from fresh water to salt water, fish have to maintain an osmotic concentration of about one third of that of seawater. In freshwater, fish actively take salts across the gills and release the excess of water through urine. In salt water, fish drink water to replace water losses and excrete the excess of salts via gills and kidney. Cortisol has a dual job helping to the activation of the Na^+, K^+-ATPase which is involved in ion uptake and salt secretion by the gill of teleost fish (Gilmour, 2005; McCormick, 2001; McCormick & Bradshaw, 2006). It has been found that the change of media elicits a physiological responses to stress (specifically an increase on the plasma cortisol levels) and evidence also suggests that moving from freshwater to saltwater can modify feed intake (Arnesen *et al.*, 1993) and growth (Boeuf & Payan, 2001). The physiological background of HR and LR rainbow trout makes them a suitable model to study the effects of cortisol in the adaptation of fish to different salinities.

Therefore, the first aim of the studies described was to investigate whether transport had a long-term effect on the behaviour of the HR and LR strains by examining whether the switch in behaviour was still present a year after transport and on the offspring of the transported fish. The results found during 2005 were made available by Dr. Øyvind Øverli from the Norwegian University of Life Sciences and will be shown briefly here to compare with and to discuss the findings during the 2006 tests. Secondly, I examined whether changes in water chemistry (salinity) enhanced the effects of transport on behaviour, cortisol and electrolytes (sodium, chloride) and glucose in HR and LR adult fish.

Materials and methods

Subjects and their transport

In the summer of 2005, adult 3rd generation HR (n = 150, weight 493 ± 12 g) and LR fish (n = 150, weight 477 ± 9 g) were fitted with Passive Integrated Transponder (PIT) tags and transported from the Windermere Laboratory of the UK Centre for Ecology and Hydrology (Windermere, UK) to the Norwegian Institute of Water Research Marine Research Station (Solbergstrand, Akershus County, Norway). Fish were deprived of food for 5 days prior to transport and then loaded in a tank truck equipped to transport fish (Donslund Special Transport, Hejnsvig, Denmark). Transported fish arrived two days later and no mortalities occurred during transport.

Switch in HR and LR behavioural profiles after transport

Upon arrival in Norway all PIT-tags were registered, fish were weighed and screened for behavioural and physiological traits to test immediate effects of transport. Individual identification of fish with PIT-tags also permitted assessment of weight loss during the 7 day period of transport since energetic status is a variable that may strongly influence risk-taking and social behaviour (Damsgård & Dill, 1998; Johnsson *et al.*, 1996).

Immediately after transport a batch of LR and HR fish were tested for boldness, measured as the percentage of fish resuming eating and their feeding scores in a 7 day period in isolation (see below for methodology details). After the feeding period HR and LR fish were allowed to interact to determine their subordinate or dominant position in pairwise contests. The results showed that unexpectedly and opposite to the usual behaviour, HR fish resumed eating earlier and became bolder, getting higher feeding scores than LR fish. HR fish also became socially dominant over LR fish in most of the dyadic contests. However, their plasma cortisol response to stress was not altered by transport with HR fish showing higher levels than LR fish.

The results found during 2005 by Dr. Øverli and the 2006 results, which are the main part of this chapter, have been published together as a paper in Hormones and Behaviour: Ruiz-Gomez *et al.*, 2008 (see accompanying material).

Assessing long-term effects of transport on behaviour

In the summer 2006 of a set of HR (n = 18 weight: 1113 ± 65 g) and LR (n = 18 weight: 1108 ± 61 g) fish, transported the year before, were tested for risk-taking (boldness) and social dominance following the same protocols used to test fish immediately after transport (see details below). Simultaneously, 8 months old 4th generation offspring (n = 16 weight, HR 16.2 ± 0.9 g, n = 16; LR 16.4 ± 0.9

g,) generated from transported F3 fish were screened for the same behavioural traits as well as for post-stress plasma cortisol.

Screening for boldness

As shown in figure 4.4, prior to screening fish were transferred from communal rearing tanks to 250 l (adult fish) or 12 l (offspring) glass aquaria filled with fresh water, where they were held in isolation. Each observation aquarium was lined with black plastic on three sides and divided in two compartments by a removable opaque PVC wall, each section holding one HR or one LR fish in weight matched pairs. Prior to transfer from group rearing to social isolation each fish was anaesthetised, weighed, and for adult fish the PIT-tag was read. Offspring were marked to distinguish between strains by a small incision in the upper or lower section of the tail fin. This is a standard procedure to mark fish and it lasted less than one minute per fish to avoid unnecessary stress.

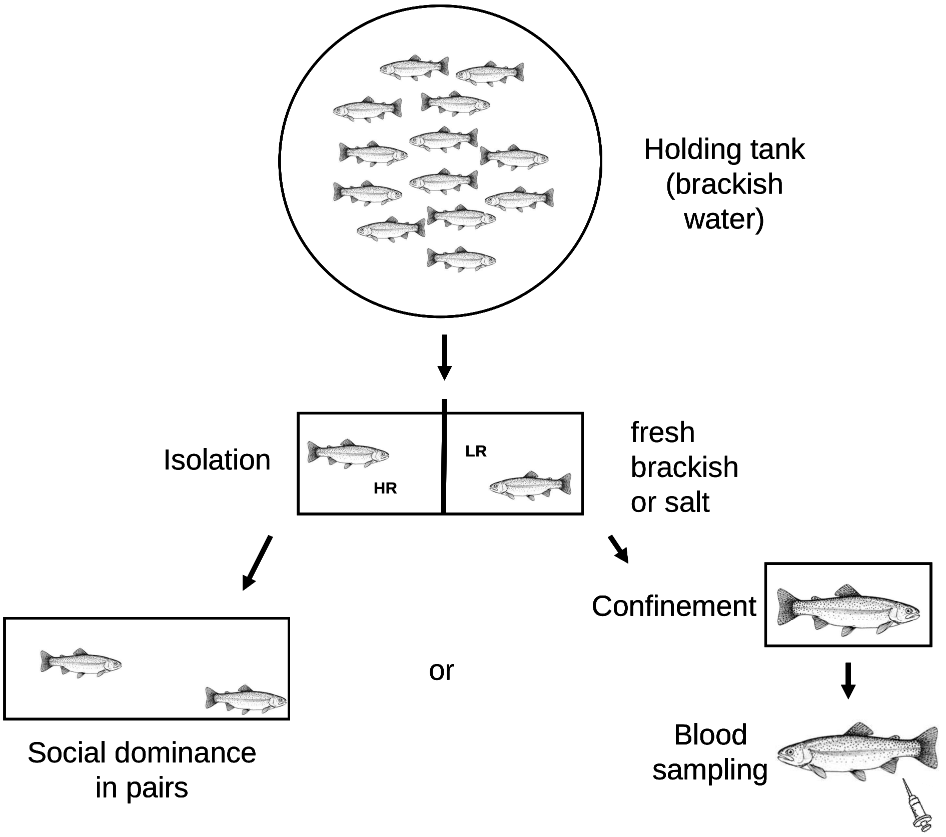


Figure 4.4 Experimental set-up followed to test boldness, social dominance and plasma cortisol in adults and offspring HR and LR rainbow trout during 2005 and 2006 studies.

Fish were allowed to recover in the test tanks overnight and testing started on the next day. For seven consecutive days, feeding was performed by dropping appropriate sized pellets where the fish was able to perceive them. Feeding was stopped either when the fish had refused to eat three pellets in a row or had consumed 3.0% of its body mass. Uneaten food was removed immediately afterwards. Feeding behaviour was graded on each occasion according to the criteria listed in table 4.1; there is a high degree of individual variation in the speed of progress from low to high scores on this scale. Accumulated scores during the course of an experiment thus reflect how quickly fish resume normal feeding, and have previously been used as a measurement of risk-taking or boldness in rainbow trout (see Øverli *et al.*, 2006b and 2007).

Table 4.1. Corresponding point scores to grad feeding behaviour in fish tested individually (reprinted from Øverli *et al.*, 2006b).

Points	Behaviour
0	Fish does not respond to food
1	Fish eats only pellets that fall directly in front, and does not move to take food
2	Fish moves more than one body length to take food, but returns to its original position in the aquarium between each food item
3	Fish moves continuously between food items and consumes all food presented

Assessing social dominance

After the 7th day of feeding, the dividing screen in each aquarium was removed and fish were allowed to interact until the conflict was resolved and dominant and subordinate fish could be clearly distinguished. The conflict lasted less than 3 hours and fish were removed immediately after to avoid unnecessary distress. After initial fights, subordinate fish showed little or no aggression towards the dominant individual and usually took up a position facing a corner of the tank, while dominant fish normally moved around the whole territory frequently chasing the subordinate fish (Øverli *et al.*, 1999; Pottinger and Carrick, 2001). After a stable dominance-subordination relationship was confirmed, fish were netted, anesthetized, and the final weight and strain of each winner and loser was recorded. A similar procedure was also carried out on F4 offspring.

Testing effects of water chemistry (salinity) on boldness

To test if the addition of salt water to the holding tanks had an effect on behaviour, after behavioural screening in 2006, a batch of G3 HR and LR adult fish from the same holding tank were tested in different water salinities: fresh water (salt concentration 0 mg/l); HR: n = 7, weight = 1058.93 ± 47.36 g, LR: n = 7, weight = 1275.9 ± 86.71 g. Brackish water (salt concentration 17.5 mg/l) resembling the communal holding tanks; HR: n = 7, weight = 1076 ± 118.59 g, LR: n = 8, weight = 1173.3 ± 97.14 g. Salt water (salt concentration 30 mg/l); HR: n = 6, weight = 9.58 ± 47.27 g, LR: n = 7, weight = 1183 ± 56.4 g and screened for boldness (7 days feeding starting the following day after being placed in isolation), following the method and using the same tanks described above, followed by blood sampling to determine plasma cortisol electrolyte (sodium, chloride and glucose) levels.

Hormone and plasma electrolytes assays

Post-stress plasma cortisol was assayed for 2005 adults immediately after transport. A year after transport only the basal plasma cortisol was assayed in the three water conditions (brackish, fresh and salt water) at day 7 after eating. In order to test acute and acclimatizing effects of changes in salinity for adult fish on plasma ion concentrations sodium (mmol/l), chloride (mmol/l), and glucose (mg/dl) were measured for fish held in either fresh or salt water conditions at day 1 after being placed in isolation and at day 7th. After eating, fish were anesthetized in 0.5 g/l MS-222 and a blood sample was collected from the caudal vasculature. Stress testing of offspring was assessed by individual confinement for 30min in 0.45 l of running water (HR n = 10, LR n = 10). After separation of plasma, cortisol levels (for adult fish in 2005 and their offspring) and electrolytes (for adult fish tested in different water salinities) at day 1 and 7 after transfer to isolation were quantified using a commercial enzyme linked immunosorbent assay kit (Neogen Corporation, Lexington, USA).

Statistical analysis

Permanence of the switch in behaviour

For adult fish and their offspring, strain differences in feeding scores as well as weight loss were assessed with two-sample t-tests. Differences in social dominance and the percentage of fish eating by day 7 were analysed by a sign test, calculating the likelihood that observed frequencies of eating-not eating, and dominant and subordinate fish in each strain represent a 1:1 ratio. Plasma cortisol concentrations in LR and HR fish were compared using a two-sample t-test for each sample.

Water chemistry effects

Differences between strains in resumption of feeding in brackish, fresh, and salt water were evaluated with Chi-square tests. General Linear Model analysis (GLM), followed by Tukey *post-hoc* tests were used to test strain and water (BW, FW, SW) effects and their interaction on the feeding scores, basal plasma cortisol and differences between strains in fresh and salt water conditions and their interactions for sodium, chloride and glucose.

All the procedures were carried out in accordance with the welfare regulations for each country and experimental site; in general, unnecessary distress was avoided.

RESULTS

Effects of transport

Table 4.2 summarizes the behavioural profiles in all the tests performed. As mentioned above, immediately after transport unexpectedly HR and LR rainbow trout switched their typical behavioural profiles so that HR fish became bolder and socially dominant over LR fish.

Table 4.2. Summary of the feeding behavioural profiles of LR and HR fish before, immediately after transport, a year after transport and in offspring of transported fish.

	Data made available by	**Resumption of feeding (higher percentage of fish eating after 7 days of isolation)**	**Boldness (highest feeding score)**
Native rearing site (Windermere, UK).	T. Pottinger	LR	Data not available
Immediately after transport (Oslo, Norway 2005) adult fish.	Ø. Øverli	No significant difference	HR
One year after transport (Oslo, Norway 2006) adult fish.	M. Ruiz	HR	HR
F4 Offspring, (Oslo, Norway 2006) juvenile fish.	M. Ruiz	No significant difference	No significant difference

As shown in figure 4.5, an immediate effect of transport on the fish was found in the observation that HR fish lost in average more body mass than LR fish as a result of the transport-starvation period the fish were subject to (t = 3.43 p = 0.003). Weight loss was also less severe among those few HR fish that lost pairwise contests (n = 4) than among HR winners (n = 13; t = 4.17, p = 0.014). This pattern was not evident in the LR strain, as LR winners and losers did not differ in weight loss (n = 19; t = 0.74 p > 0.05).

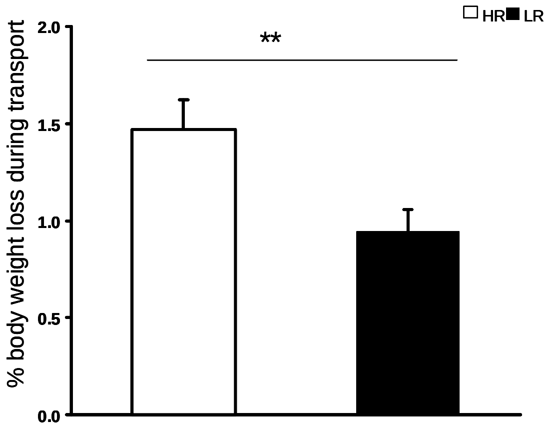


Figure 4.5. % Body mass lost per day of transport/starvation in HR and LR fish transported to Norway; ** = p<0.01.

Boldness of adult fish a year after transport and their offspring

Figure 4.6 shows that a year after transport, more HR than LR fish had started feeding by day 7 (LR: 55% feeding n=11, HR: 94% feeding n=11; p=0.018). For the 4th generation of offspring HR and LR fish did not differ significantly, most fish in both strains had started feeding by day 7 (LR: 100% feeding n=16HR: 87% feeding n=16 p=1.00).

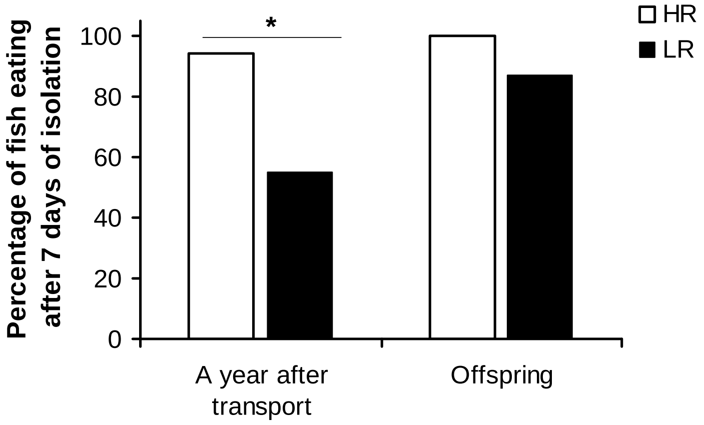


Figure 4.6. Percentage of HR and LR fish eating in a 7 days period of isolation, adult fish a year after transport and offspring of the transported fish. * p<0.05

Figure 4.7 shows the sum of the feeding behaviour scores after 7 days of isolation in the LR and HR fish a year after being transported to Norway. HR fish were bolder, getting higher feeding scores than LR fish (t=4.24 p=0.001). In the 4th generation of offspring, LR and HR fish did not differ in boldness (t=0.50, p=0.62) during the experimental period.

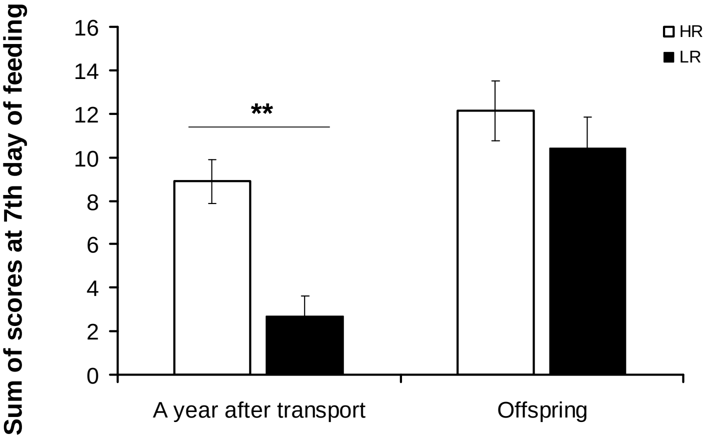


Figure 4.7. Mean (± SE) of the sum of scores at the 7th day of feeding in the HR and LR rainbow trout for adult fish a year after transport and their offspring. ** p<0.01

Social dominance in adult fish a year after transport and their offspring

LR typically become dominant over HR however after transport a majority of HR fish dominate the pairwise contests. Figure 4.8 shows that a year after transport there was no significant difference in the proportion of LR and HR becoming dominant (LR: 54% dominant n=11, HR: 45% dominant n=11 p=1.00). However, in the 4th generation of offspring the typical situation was reinstated, with LR fish becoming dominant in all encounters.

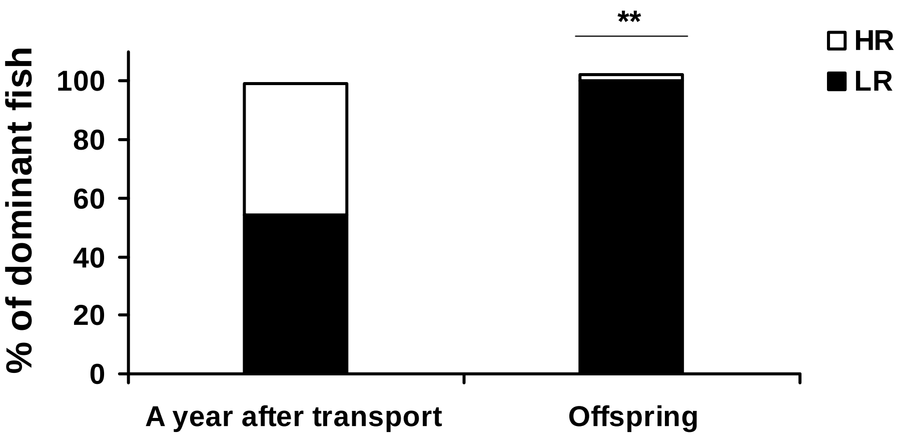


Figure 4.8. Percentage of HR and LR fish becoming dominant in size matched pairs for adult fish a year after their transport to Norway and their offspring. ** p<0.01

Post-stress plasma cortisol assays

Table 4.3 shows mean (± S E) plasma cortisol concentrations in LR and HR fish for all the sample points. Highly significant differences in post-stress plasma cortisol in non-transported fish were found, with LR fish showing lower levels than HR fish. Immediately after transport fish showed the typical divergence in post-stress plasma cortisol, with HR fish showing almost double the amount than LR fish. The offspring of the transported fish maintained their distinctive post-stress plasma cortisol concentrations when they were transported to and held in Norway as well.

Table 4.3. Mean (± SE) plasma cortisol concentrations of LR and HR rainbow trout on all the sampling sites, p-value from the two-sample t test.

	Plasma cortisol (ng/ml)		p-value
	LR	HR	
Native rearing site (Windermere, UK)	58.3±9.5	153.1±25.0	0.003
Immediately after transport (Oslo, Norway 2005) adult fish	60.6±7.3	128.0±16.0	0.001
F4 Offspring, (Oslo, Norway 2006) juvenile fish	37.4±6.7	70.1±12.0	0.034

Effects of water chemistry (salinity) on boldness

Figure 4.9 shows that HR and LR fish did not differ significantly in the percentage of fish that had started feeding by day 7 when tested in brackish water (LR: 37.5% feeding n=8, HR: 57.1% feeding n=7; X^2=0.582; p=0.446), fresh water (LR: 42.8% feeding n=7, HR: 85.71% feeding n=7; X^2=2.947; p=0.086), and salt water (LR: 16.66% feeding n=6, HR: 16.66% feeding n=7; X^2=0.014; p=1.00).

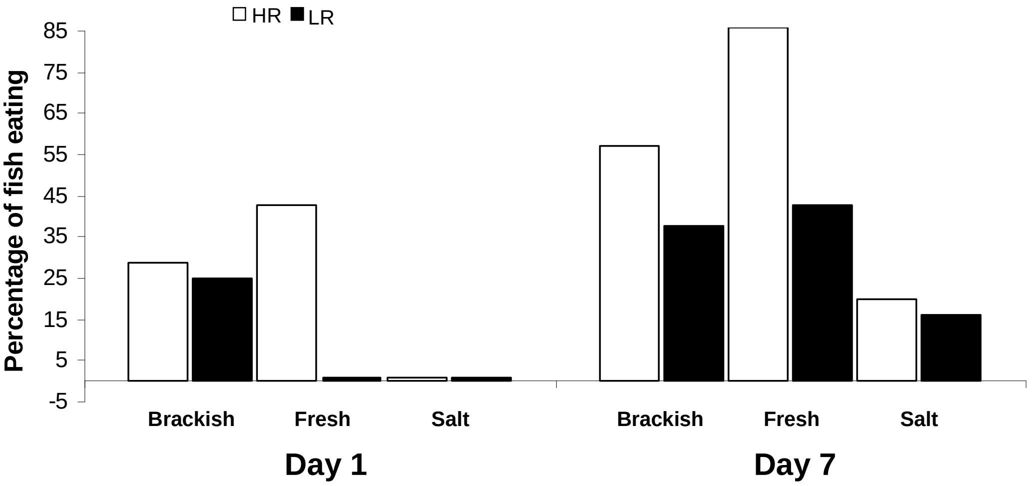


Figure 4.9. Percentage of adult HR and LR fish eating at days 1 and 7 after transfer to isolation in three different water salinities.

As shown in figure 4.10, boldness (measured as the sum of the feeding scores of the fish) after 7 days of isolation did differ between strains ($F_{1,36}$=5.87, p=0.021 n=42) and water chemistry ($F_{2,36}$=6.92, p=0.003), but there was no interaction between variables ($F_{2,36}$=1.11, p=0.340). In brackish water HR and LR did not differ in their scores ($t_{1,11}$=1.11 p=0.290); however when the test was carried out in fresh water HR fish had significantly higher feeding scores than LR fish ($t_{1,11}$=3.37 p=0.006). Finally in salt water there were no differences in their feeding scores ($t_{1,5}$=0.64 p=0.549).

Salinity in general had an effect on the feeding behaviour of the fish independent of their strains. Fish tested in brackish water did not differ in their feeding scores from fish tested in fresh water ($t_{1,23}$=1.22, p=0.23). However, fish tested in salt water had lower scores than fish in fresh water ($t_{1,15}$=3.12 p=0.007) as well as in brackish water ($t_{1,15}$=3.47 p=0.003), suggesting an effect of salinity on the behaviour of the fish.

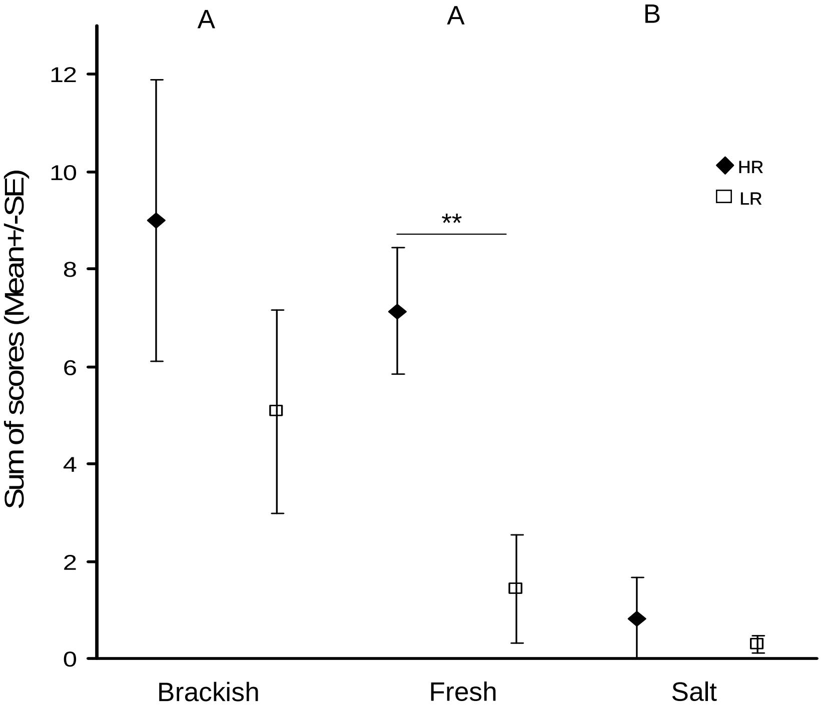


Figure 4.10. Mean (± SE) of HR and LR feeding scores tested in different water salinities. Different letters stand for overall differences between water conditions. By strain, ** and denotes a p value below 0.01 whereas different uppercase letters denote a p value below 0.01 for water salinities.

Effects of water chemistry (salinity) on ions and cortisol

Figure 4.11 shows the basal plasma cortisol concentrations for HR and LR fish in brackish, fresh and salt water after 7 days of feeding in isolation. GLM revealed that there were overall significant differences between strains, with a tendency for HR fish to have higher levels of cortisol than LR fish ($F_{1,23}$=5.52; p=0.029). Fish in the 3 water conditions did not differ significantly in plasma cortisol ($F_{2,23}$=1.42; p=0.25), nor did their strain/water salinity interactions ($F_{1,23}$=0.80; p=0.45).

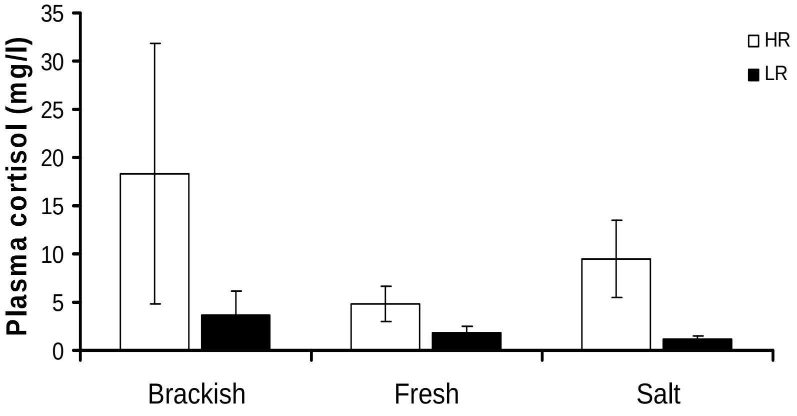


Figure 4.11. Mean (± SE) of basal plasma cortisol concentrations in HR and LR fish after 7 days of feeding in isolation in either brackish, fresh or salt water.

As shown in figure 4.12, there was an effect of water salinity on plasma sodium concentrations in fish left overnight in the new environment and water condition (or day 1, $F_{1,24}$=15.27; p=0.001). But there were no significant differences in sodium concentrations between strains ($F_{1,24}$=3.28; p=0.081) and no significant interaction between water salinity and strain ($F_{1,24}$=0.001; p=0.977). After 7 days of feeding, the differences in sodium concentrations between fresh and salt water become non significant ($F_{1,24}$=2.85; p=0.105) and there were no significant differences between strains ($F_{1,24}$=0.001; p=0.985) and interactions ($F_{1,24}$=0.001; p=0.913) were found.

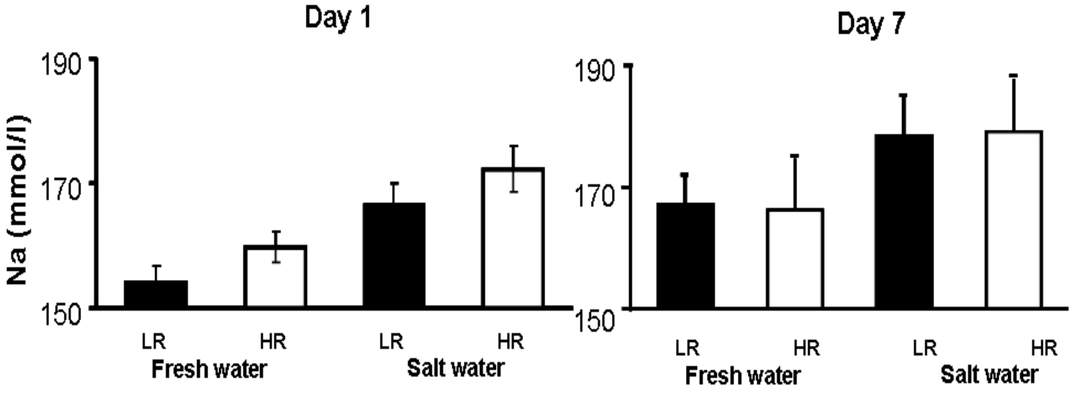


Figure 4.12. Mean (± SE) of sodium (mmol/l) concentrations in HR and LR rainbow trout after 1 day of transfer to isolation and after 7 days of feeding in isolation in either fresh or salt water.

After one day of exposure to the new environment and water salinity, as shown in figure 4.13, there were significant differences in plasma chlorine concentrations between fresh and salt water ($F_{1,24}$=5.04; p=0.034). There was a difference between strains, with HR showing higher concentrations that LR fish ($F_{1,24}$=26.90; p=0.001), but there was no interaction between factors ($F_{1,24}$=0.001; p=0.944). By the 7th day of feeding, the differences between water conditions ($F_{1,24}$=2.04; p=0.167) and between strains ($F_{1,24}$=0.39; p=0.538) and their interaction ($F_{1,24}$=0.20, p=0.659) were not significant.

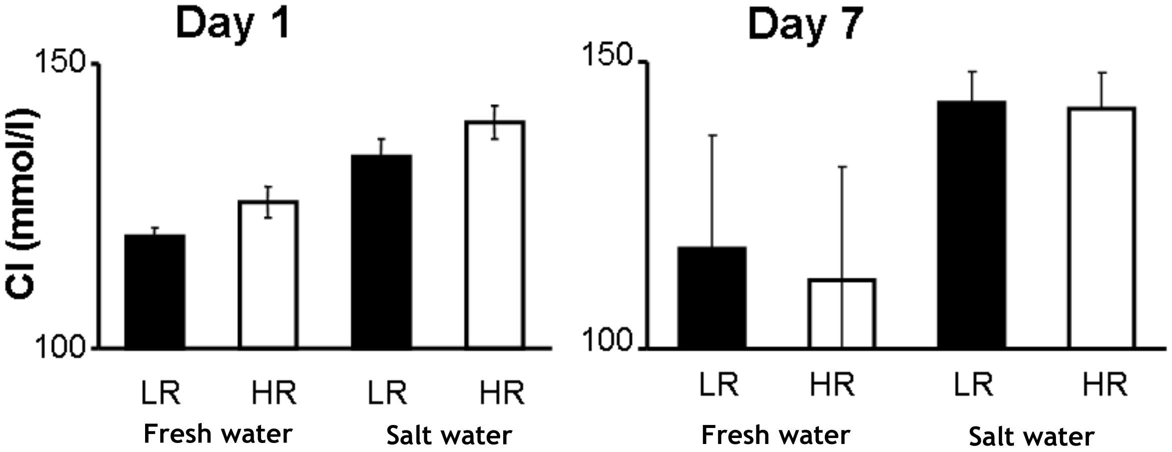


Figure 4.13. Mean (± SE) of Chlorine concentrations in HR and LR rainbow trout after 1 day of exposure to and 7 days of feeding in isolation in wither fresh or salt water.

As shown in figure 4.14, plasma glucose concentrations (mmol/l) one day after fish were transferred to the experimental tanks did not differ between strains ($F_{1,24}$=1.03; p=0.319) or water salinities ($F_{1,24}$=1.00; p=0.325) and there were no significant interactions ($F_{1,24}$=0.86; p=0.361). After 7 days of feeding there were strong differences in overall plasma glucose concentrations between fresh and salt water ($F=_{1,24}$15.57; p=0.001), but no significant strain effects ($F_{1,24}$=1.19; p=0.288) or interactions ($F_{1,24}$= 0.001; p=0.975) were detected.

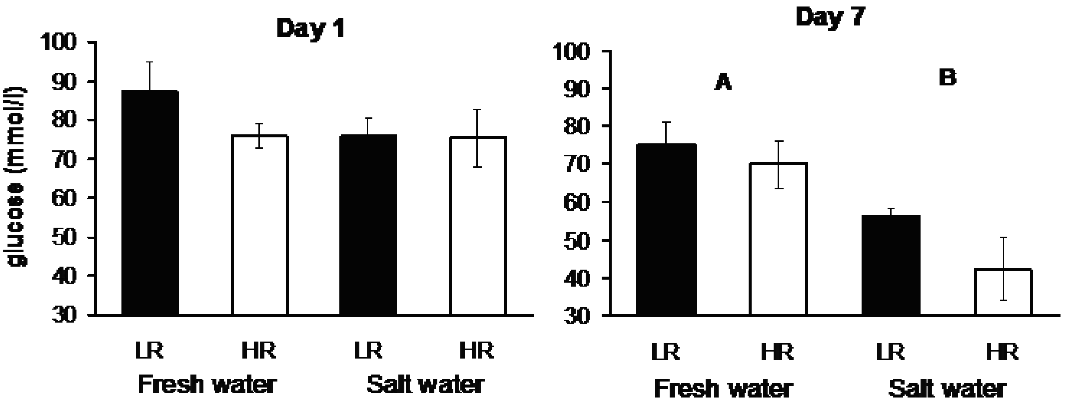


Figure 4.14. Mean (± SE) of plasma glucose concentrations in HR and LR rainbow trout after 1 and 7 days of isolation in either fresh or salt water.

Discussion

The results presented here show that an acutely stressful experience namely transport, had a long-term effect on risk-taking in two strains of rainbow trout selected for their post-stress plasma cortisol responsiveness. Transport from the UK to Norway had an immediate effect on behaviour in LR and HR rainbow trout. According to the existing literature, the strains have distinct behavioural profiles consistent not only over time, but also across different contexts, similar to those of proactive and reactive mammals (Øverli *et al.*, 2005; Schjolden *et al.*, 2005a and b; Schjolden & Winberg, 2007). These physiological and behavioural characteristics of low stress responsive (LR) and high stress responsive (HR) rainbow trout have been conserved over at least three generations (Pottinger & Carrick 2001; Øverli *et al.*, 2005). Thus, low responding trout are characterized by a faster resumption of feeding in a novel, potentially-dangerous environment and a tendency to become dominant in competitive dyadic interactions. These differences were apparent before transport to Norway, but reversed immediately afterwards. After transport, HR fish not only resumed feeding sooner in a novel environment, but also won more pairwise fights than did LR fish.

On the one hand, a clue as to a possible mechanistic explanation for the switch in traits may be found in the observation that HR fish lost a greater proportion of their body mass during the transport period than did LR fish. Furthermore, the few HR fish that became subordinate after transport were characterised by having lost relatively little weight during transport. It is well established that individuals become bolder and more aggressive in competition for resources when their need for such resources is higher (Dugatkin & Ohlsen, 1990; Morrell *et al.*, 2005; Frost *et al.*, 2007). Thus the atypical behaviour of HR fish immediately after transport could be due to them experiencing a high degree of hunger. Comparable results to those reported here were described by Carere and collaborators (2005), who found that food deprivation modulates the expression of phenotypic traits (namely begging and aggression) in lines of great tit (*Parus major*) selected for high and low exploration speed and aggression.

By one year after transport, when all the fish had recovered from transport stress and feeding was normally reinstated, inversion of the established behavioural profiles of the two lines was still evident. HR fish still took greater risks to feed in a novel environment, although individuals of the two strains were equally likely to win pairwise contests. It is possible that a long-term effect associated with the weight loss that occurred during transport continued to influence the feeding behaviour of the HR fish. Having won dominance encounters in the period straight after transfer, this may have given HR fish sufficient advantage in the intervening period (all the fish were housed in the same tanks) to still counteract the natural tendency of LR fish to win pairwise fights.

Another possible explanation for those results was that water chemistry (salinity) enhanced the effect of transport, because when arriving to Norway HR and LR fish were held in brackish water in order to avoid fungus infections. This might have altered their osmoregulatory function and thence changed the motivation to feed and/or competitive ability of the fish. The fact that no fish were eating by day 1 after transfer to isolation in salt water and that the overall feeding scores were lower independently of strain in the same water condition suggest an effect of salinity on the motivation of the fish to feed independently of their physiological background. However, there is a tendency for HR fish to eat sooner and hence to get higher scores in all water conditions. Only in fresh water were there significant differences between strains in feeding scores. At the 7th day, more fish were eating, showing a certain degree of acclimatizing to the water salinity. However, the tendency was still for more HR than LR fish to have started to eat. At the hormonal level, HR maintained higher levels of basal plasma cortisol. Although not significantly different from LR fish in the three water conditions, these results are in line with previous reports in these strains (Pottinger & Carrick, 1999). In steelhead for example it was found that food intake did not decrease as a direct result of stress, but was in fact suppressed by changes in salinity (Liebert & Schreck, 2006).

Regarding electrolytes, the results showed strain differences in chloride only for fish tested after one day of being exposed to the new environment and water salinity, with HR fish showing higher levels of chloride than LR fish. However, this effect disappeared after 7 days of isolation and so could be interpreted as an acclimation response of the fish to the water salinity as well as to the new environment. It is known that a decrease in sodium and chloride levels is characteristic of the stress response in fish. The increase in chloride ions seen in HR fish in both fresh and salt water might be the result of HR fish having higher cortisol levels prior the salinity exposure. As a consequence, the osmoregulatory pressure they had to deal with may have been lower than that for LR fish. The remaining measured electrolytes did not show significant differences between strains.

The results show that water salinity has some effect on behaviour and, although HR fish were less affected by salinity changes than LR fish, both strains performed poorly while held in salt water. Indeed changes in salinity were not sufficient to change the cortisol response of the individuals, so the possible explanation for the permanence on the switch of behaviour due to the addition of salt water into the holding tanks can be ruled out at least in its simplest form.

Regarding the 4th generation of offspring, there were no evident differences between strains in risk-taking during feeding, but LR fish now won all the paired contests. It seems that the ability to win pairwise fights is an inherited trait in rainbow trout. The situation with respect to risk-taking is more complicated and harder to explain. Although there is a tendency for LR fish to feed more and sooner than HR fish in generation 4, this difference is not significant. It is possible that some sort of non-genetic maternal effect was involved, such that the 3rd generation LR mothers were still somewhat stressed and at breeding transferred cortisol into their eggs and as a consequence, the risk-taking ability of their offspring was reduced. An effect of maternal stress on offspring cortisol levels has been described in specific by McCormick (1998). However post-stress plasma cortisol levels in parents as well as in offspring remained unchanged.

It is worth pointing out that these changes in behaviour occurred without any associated change in a key physiological component of the coping styles, divergent post-stress plasma cortisol levels, which were maintained throughout the study period. This suggests a degree of plasticity in the behavioural aspects of coping style in fish that is independent of hypothalamus-pituitary-interrenal function. A range of other elements of the neuroendocrine system (hormones, neuropeptides and transmitters) are also involved in the regulation of these behaviours (Winberg & Nilsson, 1993; Johnsson & Björnsson, 1994; Johansson *et al.*, 2005; Clements *et al.*, 2003; Volkoff, 2006; Carpenter *et al.*, 2007) but further work will be needed to assess their role in the reversal of behavioural traits observed here.

In conclusion, this study suggests that in rainbow trout genetic differences limit behavioural plasticity and determine social position early in life. However, superimposed on this template, some behavioural components of stress coping style can subsequently be modified by experience. The behavioural polarisation observed in LR and HR rainbow trout, with the former being bold and aggressive and the later shy and non aggressive, was reversed immediately after transport to Norway, the explanation might be mainly due to the stress-starvation period the fish were subject to during transport and not to changes in water salinity. Strain-typical behaviour however, was not reinstated 1 year after transport. In the offspring of the transported fish, LR fish once again defeated HR fish in pairwise fights, but no differences in boldness (measured by rate of feeding following solitary transfer to a novel environment). Whatever the explanation, these unexpected results show that what looked like a tightly defined syndrome, with clearly integrated physiological and behavioural traits, is more complex than this. Both behavioural traits, boldness and aggression, are plastic with individual levels changing, and changing independently, in response to environmental conditions.

Chapter 5 Coping strategies in rainbow trout: flexibility and reaction to novelty

Introduction

When an animal is confronted by aversive or stressful stimuli, several physiological, behavioural and metabolic changes take place. Such changes allow the animal to cope with the changing environment and to reduce the adverse effects of the challenge. The ability of an organism to cope in a given situation depends on factors such as the predictability, controllability, frequency, duration and intensity of the stimuli (Koolhaas *et al.*, 2006). Animals tend to respond to stress with a characteristic set of responses. Koolhaas and collaborators (1999) have coined the term coping styles as *"A coherent set of behavioural and physiological responses which is consistent over time and which is characteristic to a certain group of individuals"*. From the best studied cases among mammals (including humans) and birds, two broad strategies have been described.

On one hand, the proactive coping strategy refers to a response that is characterised by low hypothalamus-pituitary-adrenal activity and reactivity (HPA) and low parasympathetic reactivity, whereas sympathetic reactivity and testosterone activity is high. Behaviourally, proactive animals tend to show a fight-flight response, to be aggressive and bold, to form and follow routines and to flourish when food is stable and abundant (Koolhaas *et al.*, 1999; Korte *et al.*, 2005). On the other hand, animals with a reactive coping style show high HPA activity and reactivity, high parasympathetic reactivity and low sympathetic reactivity and testosterone activity. The behavioural strategy of reactive animals tends to be freeze and hide and they avoid risks and aggressive encounters. Reactive copers show more flexible behaviour than proactive animals and prosper when food is scarce (Koolhaas *et al.*, 1999; Korte *et al.*, 2005).

There is evidence for the presence of some aspects of coping strategies that have been previously described in higher vertebrates in other animal groups. Some of the organisms on which those responses have been studied to more detail are the squid *Euprymna tasmanica* (Sinn *et al.*, 2008), the lizard *Anolis carolinensis* (Øverli *et al.*, 2007) and several species of fish, for example *Gasterosteus aculeatus* (Huntingford, 1976; Bell, 2005; Bell *et al.*, 2007) and the rainbow trout *Oncorhynchus mykiss* (Sneddon, 2003a; Øverli *et al.*, 2005 and

2007). Therefore the presence of the constellation of traits that characterize the proactive and reactive phenotypes seems to be a widespread phenomenon.

In fish, knowledge of the physiological and behavioural changes that accompany the response to stress is growing. The most complete example of this is the rainbow trout *Oncorhynchus mykiss*. In a selection programme started in 1996 in the UK Centre for Ecology and Hydrology, Windermere, UK by T. Pottinger (Pottinger & Carrick, 1999), adult fish from a commercial strain were selected for differences in their responsiveness to a standardized stressor (confinement). This was measured as levels of post-stress plasma cortisol. This selection programme has created a high and a low responsive strain, hereafter referred to as the HR and LR strains respectively. The method used to generate those strains is described elsewhere (chapter 4 of this thesis and Pottinger & Carrick, 1999 and 2001; Øverli *et al.*, 2005). The response to stress in those fish has been found to be a heritable trait (Pottinger & Carrick, 2001) and to be associated to distinctive behavioural patterns. For example, LR fish tend to become dominant over HR fish in dyadic contests, to be bolder than HR fish, in the sense of faster resumption of feeding after transfer to a novel environment (Øverli *et al.*, 2002 and 2005; Schjolden *et al.*, 2005). LR and HR fish also show patterns of brain biochemistry that are typical of the proactive and reactive animals (Schjolden *et al.*, 2006). Therefore, this fish model resembles the proactive and reactive strategies found in mammals and birds. However, these behavioural characteristics show a degree of plasticity and can be modified by experience (Ruiz-Gomez *et al.*, 2008).

The main aim of the study presented here was to give further support to the characterisation of this model as showing the proactive and reactive coping strategies. This was done by comparing behavioural flexibility and reactivity (two other traits that differentiate proactive and reactive animals) in fish from the 3rd generation of LR and HR rainbow trout.

Materials and methods

Fish and set-up

Eggs from the 3rd generation of the high and low responding rainbow trout were transported from the Centre for Ecology and Hydrology, Windermere, UK to the Danish Institute of Fisheries Research, Hirtshals Denmark, where they where incubated, hatched and reared. We assumed these fish did not suffer the effects of transport as happened with adult fish transported to Norway (see previous chapter and Ruiz-Gomez *et al.*, 2008), since the fish themselves were not subjected to transportation as free-living individuals.

An experiment was designed to test routine formation and behavioural flexibility in individual juvenile HR and LR rainbow trout. The experimental set-up was adapted from a design that has been previously used in rats (Benus *et al.*, 1990) and pigs (Bolhuis *et al.*, 2004) to test those traits.

The test arena (figure 1a and b) consisted of an opaque plastic tank with a flow-through water supply. The tank was divided in three sections. At one end a division was made with a sliding wall, this became a darkened area which provided the fish with a shelter during the tests. The fish were housed in the darkened area between meals, see below. The opposite end of the tank comprised a T-maze with two entrances. The inside of the maze was not visible for the fish from the outside and the two arms were completely separated each other. Water flowed into each arm through two separated tubes, the ends of which had perforated plastic containers. These containers were filled each morning with food in order to control for smells in both arms, but without giving the fish access to food. The containers were removed and cleaned at the end of the day. Each arm had a clear plastic container (a petri dish) on the bottom in which food was placed at the start of every meal. The rest of the tank consisted in a bare open area.

Twelve tanks were available, so equal amount of individuals from each strain were screened simultaneously. 0+ juvenile HR (n = 10, mean weight = 17.3 ± 0.19 g) and LR (n = 10, mean weight = 17.2 ± 0.2 g) rainbow trout were used.

The same fish was subjected to all the tests and stayed in the experimental tank for the full length of the experiment. Behaviour was video recorded from above each tank through a camera attached to a laptop. In order to record the exact number of and time pellets that were eaten, a webcam showed the arm of the maze that contained food. Fish behaviour was recorded from a distance, so they were not disturbed by the observer and the cameras were installed before the fish were let out.

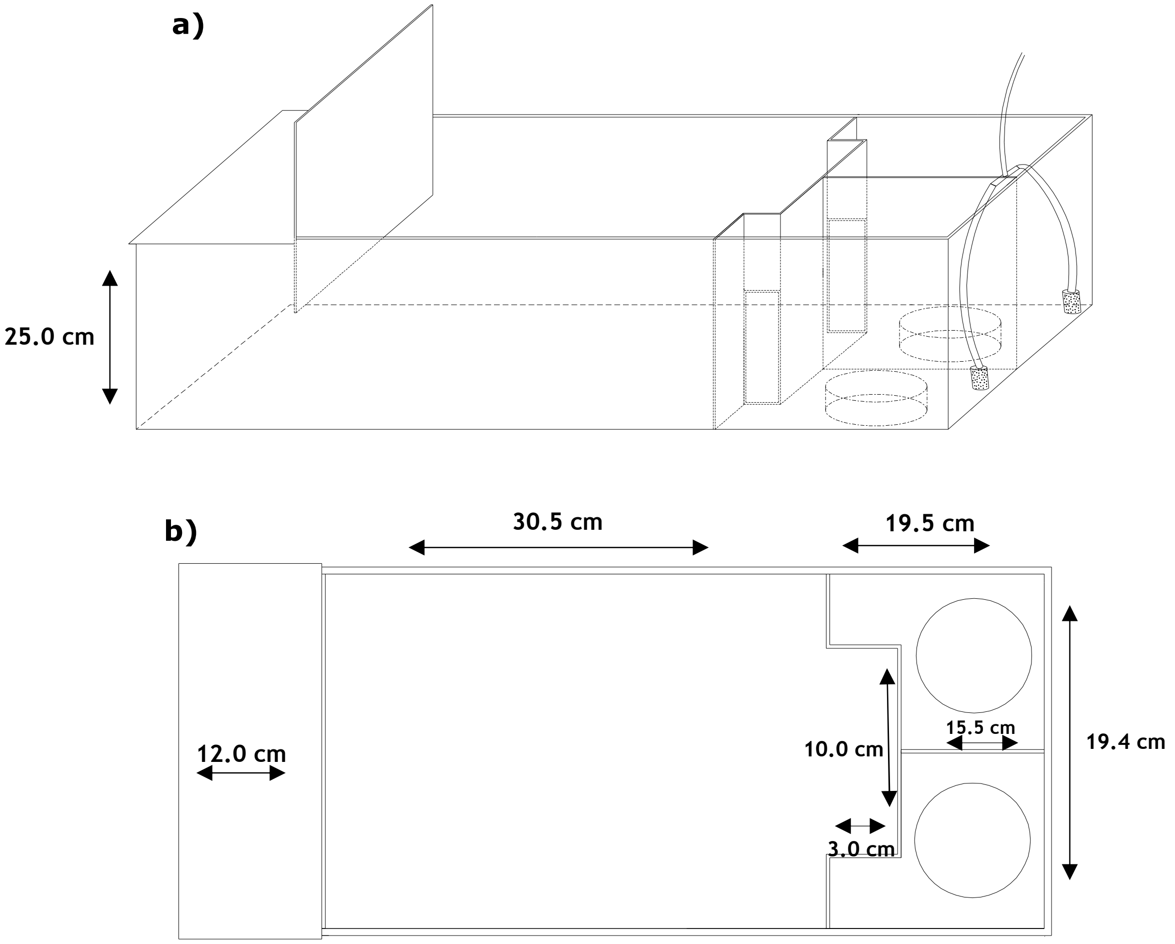


Figure 5.1. The test arena [a) a 3D view b) a view from above] consisted of an opaque plastic tank with a flow-through water supply. The tank was divided in three sections. In one extreme a division was made with a sliding wall and a cover giving a darkened safe area, or shelter. The opposite end of the tank comprised a T-maze with two entrances. The inside of the maze was not visible to the fish from the outside and the two arms were completely separated each other. Water flowed into each arm through two separated tubes, which ends had perforated plastic containers. Each arm had a clear plastic container (a *petri dish*) on the bottom in which food was placed at the start of every meal. The rest of the tank consisted in a bare open area.

Fish were placed into the shelter and allowed to settle overnight and feeding started on the following day. Commercial trout food (appropriate for the size of the fish) was placed into the plastic dish within one side of the maze. For half of the fish from each strain, food was assigned to the right and for the other half to the left arm of the maze. Fish were fed 1.3% their body weight per day, portioned into three meals: morning (8:00-10:00 am), lunch (12:00–14:00), and evening (16:00-18:00). Food was added to the plastic container, or placed on the runaway for the food outside test, before fish were let out of the shelter. At each meal the sliding door of the safe refuge was removed, letting the fish explore the open area as well as both sides of the maze. Fish were allowed to explore the tank and/or to eat during an hour and every half an hour the amount of food eaten was monitored. After 30 minutes, of exploring/feeding, the fish was gently pushed back to the shelter and the door was closed and fish were held there until the next meal. Uneaten food was removed immediately and new food was added before the start of the next meal.

The same individuals were subjected to a series of behavioural observations that as follows:

Response to a novel environment: fish were filmed during the first meal when they were presented with the new potentially dangerous environment. The time spent moving, freezing and swimming fast (presumably attempting to get away from the new environment) were recorded.

Training period: food was placed in one arm of the maze. After the fish had eaten at least 80% of its food ration within the time limit 5 out of 6 meals in a row, it was deemed to have learnt the task of finding food in the maze. All the 20 fish tested had learnt the foraging task before the challenges were performed. During the next 3 meals, behaviour was filmed for 30 minutes and the time taken by the fish to find food from the moment it came out of the shelter for first time was recorded (time to find food).

Challenges: fish were filmed for 30 minutes firstly after food was moved to the other arm of the maze and then after the food was moved to the centre of the tank in the open area. After this, two training reinforcements were carried out, in which fish were subjected to the same conditions used during the training period for two meals before the next change. For the last meal food was placed in the arm the fish were trained to find it, but with an unfamiliar object (blue rubber stopper, standard commercial size No. 7) located in the middle of the open area.

Confinement: The following morning, fish were individually confined in a clear plastic bottle (1 litre volume) and their behaviour video recorded for 30 minutes. The time spent moving, freezing and showing panic behaviour was recorded.

After the confinement test fish were killed by an anaesthetic overdose (MS-222), weighed and immediately frozen in solid carbon dioxide. Fish were then maintained at -80C until used for analysis of body cortisol. All the procedures were carried out according to local ethical requirements and unnecessary suffering was avoided.

Statistical analysis

Comparisons between strains in behaviour during the first 30 minutes after being presented with a new environment and the time to start eating after transfer to isolation were assessed with two-sample t-tests. As the rest of the data were non-parametric, I used the appropriate tests for these data as follows: to test comparisons between strains and tests of the time to find food during training, I used Mann-Whitney tests. Differences in the time to find food over time were evaluated with Friedman tests. The frequency with which fish visited a specific arm of the maze was compared with Chi-square tests. Finally comparisons between strains in the time to find food when changes were applied were studied with Wilcoxon tests for censored data as proposed by Budaev (1997).

Results

Response to a novel environment

All the times shown in this section are in seconds unless otherwise stated. Table 5.1 in next page shows means (± SE) of the time spent by the fish in various activities when first placed in the novel tank. HR and LR rainbow trout did not differ in any of their responses during the first 30 minutes of exposure to a new environment.

Table 5.1. Mean, S.E. and comparisons between HR and LR rainbow in the behaviour of individual fish during the first 30 minutes after being presented to a novel, potentially dangerous environment.

Behaviour	Strain	Mean (sec)	SE	t-student	p-value	DF
Total time swimming fast	HR	30.20	6.32	t = 0.63	0.558	17
	LR	24.10	7.36			
Total time moving	HR	341.3	87.2	t = 0.65	0.524	16
	LR	244.0	121.0			
Total time freezing	HR	1458.7	87.2	t = 0.65	0.525	16
	LR	1556.0	122.0			
Total time in the shelter	HR	605.0	164.0	t = 1.24	0.231	17
	LR	330.0	149.0			

Training period

LR fish took longer to start eating compared to HR fish (mean in days ± SE HR= 9.8 ± 1.3; LR= 19.50 ± 1.9; t=4.13, p=0.001, DF=15). Figure 5.2 shows that overall, the time to find food decreased from the full 30 minutes for both strains. Friedman analysis revealed that HR fish tended to find food faster as time went by, whereas LR fish did not show this tendency (HR: S=7.40, DF=2, p=0.025; LR: S=1.80, DF=2, p=0.407).

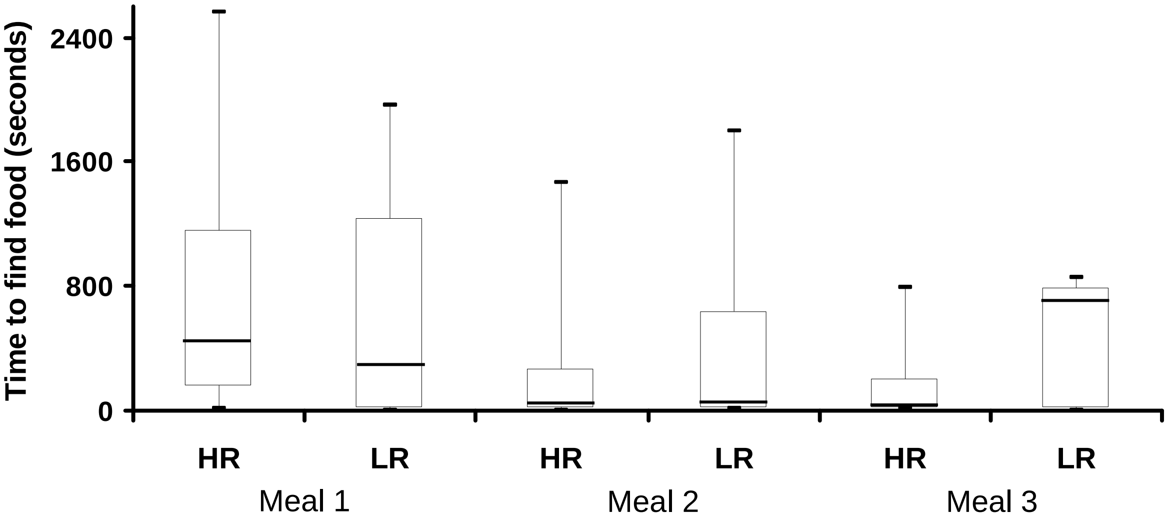


Figure 5.2. Median (solid bars), interquartile range (box) and minimum and maximum of the time to find food for HR (n=10) and LR (n=10) fish for the 3 meals recorded during the training period. The arrow shows that the time to find food decreased with time.

As shown in table 5.2, there were no between strain differences in the time taken to find food. There were no differences between strains in the frequency of fish visiting the arm of the maze containing food (X^2=0.267, p=0.606, DF=1).

Table 5.2. Comparison of the time to find food between HR (n=10) and LR (n=10) fish for the 3 meals recorded during the training period.

Meal	Strain	Median	Mann-Whitney test	p-value
1	HR	449	W = 84.5	0.130, DF=1
	LR	279		
2	HR	41	W = 99.0	0.6776, DF=1
	LR	50		
3	HR	39	W = 114.0	0.520, DF=1
	LR	695		

Challenges

Change of food arm: there were no differences between strains in the time taken to find food when it was changed to the other arm (Median: HR=516, LR=565; Wilcoxon for censored data: W=0.02908 DF=1 p=0.865). There were no differences in the number of fish visiting the arm without food (where fish had previously learnt to find food) between HR and LR fish (number of fish visiting arm without food HR=6 and LR=8; X^2=0.952, p=0.329, DF=1).

Movement of food to the open area: as figure 5.3a shows, when food was placed outside the maze, it took LR fish longer to find food compared to HR fish (Median: HR=46, LR=976; Wilcoxon for censored data, W=4.3954 DF=1 p=0.036). The low responding fish swam over the food in order to look for food into the maze (number of fish swimming over food HR=1 and LR=10; X^2=9.744, p=0.002, DF=1).

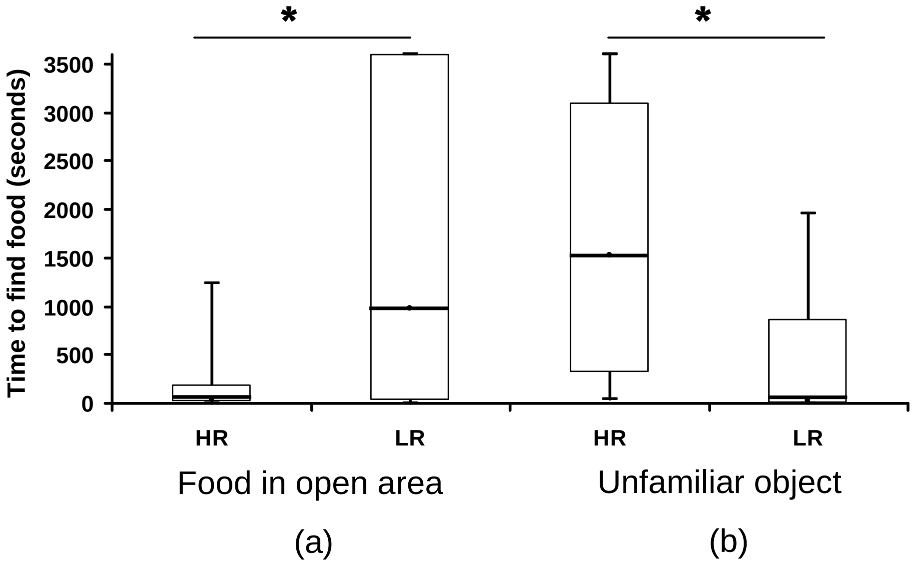


Figure 5.3. Median (solid bars) and interquartile range (box) of the time to find food for HR and LR fish when food was placed in the open area and when an unfamiliar object was placed on their way to get food; * denotes a p value<0.05.

Training reinforcement: during the training reinforcement, there were no differences between strains in the time taken to find food either during the first (Median: HR=508, LR=553; W=110.0, p=0.677, DF=1) or the second meal (Median: HR=215, LR=16; W=120.5, p=0.256, DF=1). HR fish found food faster in the second compared to the first meal (S=6.40, p=0.011, DF=1), but LR fish did not show this tendency (S=1.60, p=0.206, DF=1).

Unfamiliar object: figure 5.3b in previous page shows that HR fish took longer to find food than LR fish when an unfamiliar object, a conventional blue rubber stopper, was placed on their way since HR fish hesitated to come out of the shelter in the presence of a new feature in the environment (Median: HR=1532, LR=23; Wilcoxon for censored data W=6.6809 DF=1 p=0.010). The response of HR fish to the new object was reflected in the fact that they subsequently went to visit the arm with no food first, compared to LR fish, instead of performing as they had previously done, to go to the arm containing food (number of fish visiting arm without food HR=7 and LR=2; X^2=5.445, p=0.020, DF=1).

Confinement

As shown in figure 5.4, the time fish spent showing panic behaviour was higher for HR than for LR fish (t=2.20, p=0.05, DF=11). LR fish tended to spend more time moving in the container than HR fish did (t=2.23, p=0.052, DF=9). The time spent freezing was not different between HR and LR fish (t=0.70, p=0.493, DF=16).

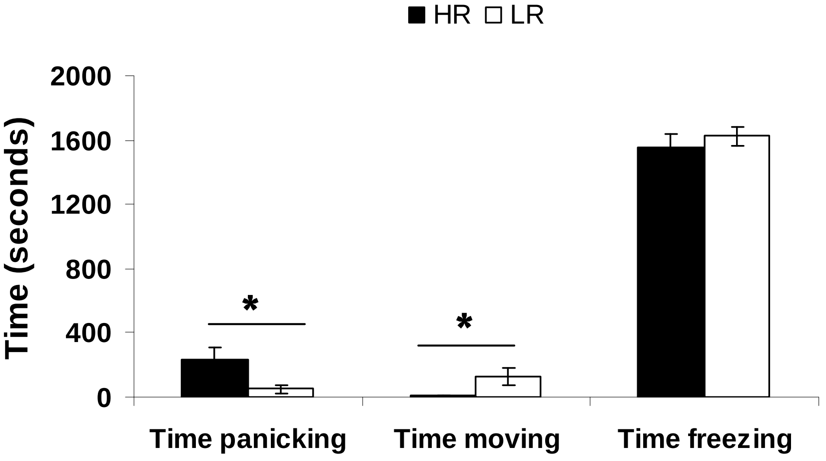


Figure 5.4. Mean and S.E. of the time panicking, moving and freezing of the HR and LR rainbow trout during 30 minutes in confinement.

Discussion

The main aim of the present study was to compare behavioural flexibility and response to novelty in two strains of rainbow trout, a system that has become something of a model for testing coping strategies in fish. The most striking results found here were that HR fish found food faster than LR trout when the resource was moved to a different position, so were clearly more flexible. In contrast, HR fish took much longer to find food in the presence of a novel object. This was probably; in part at least, a fear response, since in some cases they avoided the object by staying in shelter or by keeping out of the area in which the object was placed.

The high responding strain shares characteristics of the reactive coping strategy. Thus, individual HR trout tend to be shy and subordinate (Øverli *et al.*, 2005) and to show higher levels of locomotor activity when an intruder is present (Øverli *et al.*, 2002). They also respond to stress with high levels of plasma cortisol (Pottinger & Carrick, 1999; Øverli *et al.*, 2005). In contrast, LR fish are bold and dominant (Øverli *et al.*, 2005). When an intruder is present they respond with low levels of locomotor activity (Øverli *et al.*, 2002) and their biochemical response to stress is to show lower levels of post-stress cortisol (Pottinger & Carrick, 1999; Øverli *et al.*, 2005).

One reason why HR fish found food faster than LR fish when it was placed in a different place (into the open area of the maze) could be due to the fact that HR fish are more aware of changes in the environment, as has been suggested for reactive mice and rats (Koolhaas *et al.*, 1999; Korte *et al.*, 2005). LR fish did eventually found the displaced food, but even when the resource was on their way, they swam it over and went to the maze first. LR fish probably had formed a routine during training "swim to the maze arm to get food", so when food was outside the maze they did what they had previously learnt. This pattern has been found in pigs. Animals were selected for their resistance to a back test into reactive animals that showed low resistance when they were manually restrained in a supine position (low resistant) and proactive animals that showed high resistance during the back test (high resistance). Pigs from both groups were trained to find food in one arm of a Y maze, after which food was changed to the other arm. Proactive pigs found it harder to find food than reactive animals, because they persistently looked for food in the previously correct arm of the maze. The involvement of a degree of routine formation in the proactive animals was suggested as a plausible reason for this finding (Bolhius *et al.*, 2004). In the present study, there were no differences between strains in the time taken to find food when moved to the other arm of the maze. Probably this was not a significantly strong change, since fish did not have cues to indicate the side contained food since both arms were controlled for food smells and from training both arms of the maze were open and fish were free to visit both arms.

On the other hand, HR fish seemed less likely to form routines and to be attentive to all details of the environment and to be distracted by a change. For example, during the unfamiliar object test, some fish did not leave the shelter since they detected a change, whereas the majority of HR fish that visited the maze swam directly to the arm without food possibly in an attempt to hide from the unfamiliar object.

The possible reasons why reactive and proactive animals differ in the extent to which they are distracted by changes and in their behavioural flexibility have not been deeply studied. In pigs and rodents differences in the learning ability of reactive and proactive animals is not considered as a likely explanation, since animals showing either strategy seem equally capable of learning (Benus *et al.*, 1990; Bolhius *et al.*, 2004). In the present study all fish learnt to find food in the maze during the training period. This was supported by the fact that, when food was changed to a different arm, there was no difference between strains in the time to find food. During the training reinforcement, it took both strains longer time to find food during the first reinforcement after the changes; this could be expected since fish were subjected to modifications that altered their feeding regime. However, for the second reinforcement all fish found food as fast as in previous tests, suggesting that fish from both strains were equally capable of learning to find food in the maze. Moreira and collaborators (2004) used HR and LR rainbow trout to test whether the strains differ in their cognitive ability. They found that both types of fish could be conditioned to show an elevation in their plasma cortisol concentrations, but LR fish retained the conditioned response longer than HR fish, suggesting divergence in their cognitive function. The paradigm used by Moreira and collaborators (2004) was different from the used in the present study so it is difficult to associate the results from both studies; therefore, I tentatively reject differences in the learning capacity between HR and LR rainbow trout as a reason for the divergence in response to novelty and flexibility I found here.

In the present study, performance was easily influenced by modification of previously familiar conditions of HR fish, whereas LR fish possibly did not perceive the changes. Since this divergence seems not to reflect a difference in learning ability, another explanation could be differences in the ability of the fish to perceive and react to external stimuli. The constant execution of the learned task, visiting the maze to get food, even in the presence of an unfamiliar potentially dangerous object and the relative insensitivity to changes such as failing to perceive food on their way are likely to reflect an intrinsic behavioural control in LR trout. This reflects a routine-like behaviour in LR fish. HR fish on the other hand behaved as if they were aware of and distracted by the unfamiliar object and found the food on their way before visiting the maze. This could reflect an extrinsic behavioural control. This pattern of behavioural control has been suggested to likely reflect the divergence in behavioural flexibility and distractability between the proactive (SAL) and reactive (LAL) mice (Benus *et al.*, 1991).

When fish were exposed to the novel environment for first time, I did not detect any differences in behaviour between the HR and LR strains. The fact that HR fish initially resumed feeding faster than LR fish seems to contradict previous findings. It was known that LR fish resumed feeding faster than HR fish after transfer to isolation (Øverli *et al.*, 2005). However, the conditions in which this was measured are different. In the study by Øverli and collaborators (2005), HR and LR trout were isolated in glass tanks without any refuge, and fed by hand with pellets falling down in front of the fish. In the present study fish had a shelter in which to hide, they were isolated in opaque tanks and undisturbed by the observer and they had to learn to find the food. LR fish spent most of the time in the shelter before starting to eat for the first time. It may be that HR fish, who respond to stress with high levels of plasma cortisol, have therefore a higher metabolic rate and consume more energy than LR fish, so needed to look for food sooner than LR fish. I suggest that the differences in response to novelty and flexibility in HR and LR rainbow trout were likely to be due to differences in the way the two strains react to the environment.

During the confinement test I also found that HR and LR trout differ in how they were affected by such a stressful condition. Whereas HR fish spent more time showing panic behaviour, LR fish tended to spend more time moving around the tank and the time both strains spent immobile did not differ. Those results may also illustrate how HR and LR rainbow trout respond differently to novelty.

The success of the two coping styles in nature thus can depend on the variability of the environment. Benus and collaborators (1991) suggested that non-aggressive mice and rats can succeed in establishing territories in a new area where the environmental conditions will be different, whereas the routine-like aggressive animals will fail to do so. Another study showing the implications of routine-formation in the wild is that carried out by Dingemanse and collaborators (2004). Great tits (*Parus major)* were selected in two strains for their speed to explore new environments. It was found that fast exploring individuals, who are also the most aggressive, did better when the environment was rich and stable, whereas slow exploring, less aggressive individuals flourished in a variable, relatively poor environment (Dingemanse *et al.*, 2004).

Previous studies have shown that proactive animals develop and follow routines more strictly than do reactive animals, while the later are more aware of changes in their environment (Koolhaas *et al.*, 1999; Korte *et al.*, 2005; Koolhaas, 2006) and that this has implications in nature (Benus *et al.*, 1991; Dingemanse *et al.*, 2004). The pattern of behavioural, physiological and neurobiological responses shown by the high (HR) and low (LR) responding rainbow trout model is consistent with the characteristics that define the proactive and reactive coping styles found in mammals and birds (Koohlaas *et al.*, 1999; Korte *et al.*, 2005). Our results show that HR fish are more distracted by changes in the environment while LR fish show a routine-like behaviour and give further support to the characterisation of LR and HR rainbow trout as showing proactive and reactive coping strategies.

Chapter 6 General discussion

General discussion

This thesis describes patterns of variation of risk-taking and aggression and the relationship between them in two species of freshwater fish, the three spine stickleback (*Gasterosteus aculeatus*) and the rainbow trout (*Oncorhynchus mykiss*). The general aims of the studies described here are listed in table 6.1 and discussed hereafter.

Table 6.1. List of the general aims covered in this thesis.

GENERAL AIMS	ADDRESSED IN
Quantify risk-taking and its relationship to morphometric variables.	Chapters 2-5
Examine consistency in risk-taking	Chapters 4 and 5
Quantify aggression	Chapters 2-4
Examine the relationship between boldness and aggression	Chapters 2-4
Examine the relationship between personalities and stress physiology	Chapters 4 and 5
Quantify behavioural flexibility	Chapters 4 and 5
Examine the fitness consequences of differences in risk-taking and aggression	Chapters 2 and 3

Individual variability in risk-taking and aggression

Variability in behaviour is extensively recognized; for example, one animal may inspect boldly a predator whereas a conspecific may be hiding. Several mechanisms such as differences in the environment (Bell, 2005), social interactions (Frost *et al.*, 2007) or underlying genetic mechanisms (Benus *et al.*, 1991) have been suggested to produce individual differences in behaviour. However, it is yet open to argument whether behavioural phenotypes are consistent across contexts and situations, or whether behavioural responses can be flexible. There is also argument about the mechanisms that allow maintenance of variability in behaviour between populations (Sih *et al.*, 2004). When behavioural differences are consistent over time and across situations, several terms have been used to describe this phenomena such as personality (Goslin, 2001), temperament, individuality (Rèale *et al.*, 2007).

One of the most studied behavioural axes in several kinds of animal, including birds (Dingemanse *et al.*, 2004), fish (Brown & Braithwaite 2007; Bell, 2005), insects (Kortet & Hedrick, 2007) and spiders (Johnson & Sih, 2007) is the bold-shy continuum, defined as the willingness of an individual to take risks in potentially dangerous situations. Aggression is another commonly studied trait in species as different as mice, rats (Benus *et al.*, 1991) and fish (Bakker, 1986). Therefore, in the present study these two behavioural traits will be explored. In this chapter I will give some examples of the circumstances in which variability in behaviour (risk-taking and aggression) can be expressed, the degree to which that variability is consistent and the conditions under which risk-taking and aggression are correlated, as well as giving examples of the extent to which this relationship can be decoupled. Some of the potential fitness consequences of the expression of personalities are also explored. Those results will be discussed in the light of the current research on animal personalities, behavioural syndromes and coping strategies. Their implications for evolutionary biology and aquaculture will be also covered. These different topics involve common themes, so the same terms and concepts are inevitable discussed in several places.

The relationship between personality and morphological variables.

Morphological variables that contribute to individual productivity, in the sense of generation of new biomass via growth or reproduction, have been suggested as potential causes of individual variation in behaviour (Stamps, 2007; Biro & Stamps, 2008). For example, some studies have shown that bold individuals take risks in order to get more resources than shy individuals (Wilson et al., 1994) and various morphological measurements such as body weight, length, body condition have been found to correlate positively with risk-taking (Ward *et al.*, 2004; Stamps, 2007; Biro & Stamps, 2008). However, this seems to be more complicated because studies in fish and lizards have shown that risk-taking and body size can also be negatively related (Brown & Braithwaite, 2004; Lopez *et al.*, 2005).

In **chapter 2** for example, I found a relationship between risk-taking and some morphological variables in fish directly caught from the wild. Shy fish were in general, heavier and longer than bold and behaviourally intermediate fish. However, this result was independent of body condition. After fish were held under controlled conditions and exposed to different feeding regimes, shy fish were not only heavier and longer than bold fish but they were also in better condition. One possible explanation for those findings is that bold individuals show lower body weights because they are also more explorative and more aggressive than shy fish (Bell, 2005) and those behaviours are costly, requiring time and energy. The differences in body condition seen at the end of my experiment could have an effect on risk-taking, because an individual with lower reserves would have higher levels of hunger and might be willing to take risks in order to compensate for its state. In this context, Rands and collaborators (2003, see figure 6.1) have modelled how the social relationship between of a pair individuals that vary in body condition could work. When the body reserves of one individual (in this case an individual with low reserves) fall low enough to compromise its survival, it will leave a safe area in order to forage, even if this compromises its safety.

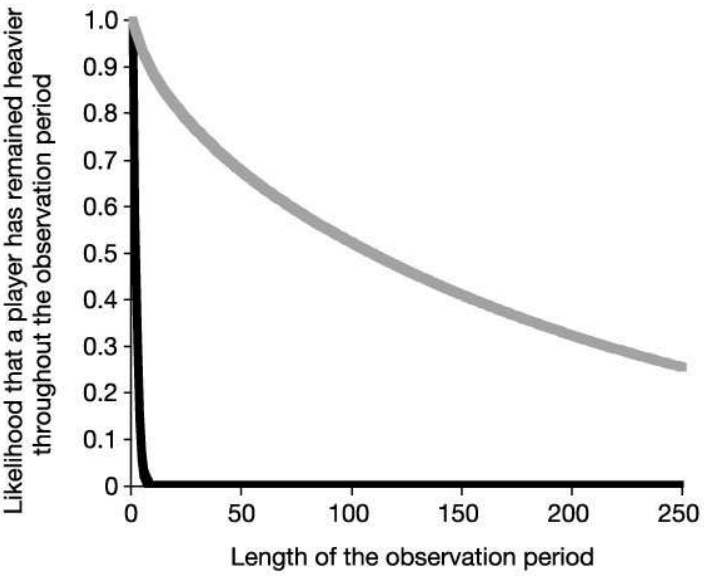


Figure 6.1. Taking into account a pair of individuals that differ in their body reserves, the probability that an individual will still have higher energy reserves than its partner at a known point in time and after any given length of time can be calculated. The black line shows the results when foraging in pairs is not affordable whereas the grey line shows results when foraging together yields an advantage (from Rands *et al*., 2003).

Consequently, the second individual, which is in a better state, will follow because it will be safer to forage accompanied than alone. The individual in better state will stop foraging sooner and therefore will go back to the refuge. Hence, the risk-taker will have to go back to the shelter. Although it has already acquired some food, it still has lower reserves, but now the risks of foraging alone are bigger (Rands *et al.*, 2003) and the same pattern will be repeated so, that a low-state individual would take most of the risks.

In those cases, boldness may result in better access to resources, possibly allowing individuals to grow big enough in order to escape size-selective predation, to deal with conspecifics competition and/or to increase their chances of reproduction. However, there is some debate about whether boldness has negative or positive effects on status. For example, the asset protection principle states that individuals with many assets (e.g. large size, in good condition) should not take risks, whereas individuals with fewer assets (smaller, in a poorer condition) should be more likely to take risks in order to get food to get big. The asset protection principle states that there could be negative feedback from behaviour (boldness) onto state, because, once individuals with low assets (in poor condition) obtain food via their risky behaviour, such individuals should then become risk-averse in order to maintain their assets (McElreath *et al.*, 2007; Wolf *et al.*, 2007 a and b). According to this mechanism, we would not expect to see individuals behave consistently, implying a degree of plasticity in behavioural responses. In **chapter 4** for example, I showed that differences in weight loss in rainbow trout could be a reason why fish that had shown consistent variability in their behavioural profiles modified the behaviour.

I designed an experiment to examine whether sticklebacks differ in their behavioural phenotypes when they grow at different rates in two competitive environments (**Chapter 2**). The experimental set up consisted of 6 groups of fish, half of which were exposed to a high interaction feeding environment and half to a low interaction regime, in the sense how food was distributed in the tanks; for both conditions food was made available in excess. After 10 weeks of feeding under those regimes, 10 fish from both extremes of the weight distribution on each tank, the so-called fast and slow growers respectively, were screened for individual behaviour. I did not detect differences in individual levels of boldness, activity and shoaling between groups. However, fish from the low interaction condition were more aggressive than fish from the high interaction feeding regime.

It has been suggested that competition over food is determined by the distribution of the resources in time and space. If resources are evenly distributed, competitive interactions are less likely, because dispersed food may not be economically defendable. When food is clumped, this would increase the chances of contact between individuals. Levels of aggression will rise through an increase of immediate competition over food, because it would be beneficial to fight for and defend a profitable food clump (Grant & Guha, 1993; Ward *et al.*, 2006). Since size can be also a predictor of competitive ability (Ward & Krause, 2001), one could think that more competitive fish would be those that monopolize the resources and hence, grow faster.

In a previous study, Ruzzante and Doyle (1991) selected medaka fish (*Oryzias latipes*) for fast growth, from groups held under two feeding conditions: food given clumped (high interaction) and food given dispersed (low interaction) with food given in excess. They found that fast growing fish from the low interaction regime were more aggressive than fish from the high interaction condition. Grant and collaborators (2002) found that juvenile convict cichlid fish (*Archocentrus nigrofasciatum*) showed decreased aggression in response to clumped food only when it was given in excess. It could be that fast growing fish did not need to behave aggressively in order to stand competition, i.e. being big was enough to display their competitive advantage. Another possibility could be that they no longer had to be aggressive, because there would be a negative feedback of aggression on growth. The interpretation could be that when food is clumped, defence of resources becomes more costly because of an increase in competitor density; it becomes uneconomic becoming ineffective to establish and defend a territory, because the benefits of territorial defence are low. On the other hand, if food is dispersed there are fewer individuals eating in the same patch at the same time, so aggression levels may increase because it is easier to defend a territory. Vahl and collaborators (2005) found the same trend in birds (turnstones, *Arenaria interpres*) and suggest that familiarity between birds would influence their levels of aggression. Turnstones may already know the dominance hierarchies in their group as well as their dominance status, so engaging in agonistic interactions would be useless. In my study this possibility seems unlikely, because I screened individual fish for aggression against a non familiar conspecific in a different set up. Therefore, in the context used in my study, being large, non-aggressive and shy is good for growth and possibly fitness, regardless of whether food is dispersed or clumped.

Are behavioural syndromes fixed or can they be uncoupled?

The relationship between boldness and aggression has been documented in several species (Huntingford, 1976; Bell & Stamps 2005; Moretz *et al.*, 2007; Sundström *et al.*, 2004; Sih *et al.*, 2004). However, the extent to which this relationship can be decoupled is poorly understood. If behavioural traits in a syndrome are tightly correlated because they are driven by underlying mechanisms, behavioural syndromes should be difficult to break. A growing body of research is suggesting that experiences could shape an individual's behavioural tendencies across a range of contexts (Dingemanse *et al.*, 2007; Bell & Sih, 2007). Some components of the neuroendocrine machinery, such as hormonal expression, are also modifiable by experience (Burmeister *et al.*, 2005; Burmeister, 2007; Watt *et al.*, 2007), so it is likely to reflect some degree of plasticity in associated behavioural traits.

Relationship between two behaviours, if tightly correlated, would have implications for survival and could even constrain the evolution of individual behaviours. For example, in environments where aggression against conspecifics is advantageous, boldness against predators could be maladaptive. Recent evidence suggests that boldness and aggression are positively correlated only under certain environmental circumstances such as under predation pressure. In this sense, Dingemanse and collaborators (2007) explored behavioural correlations in sticklebacks from 12 populations that differed in their levels of predation. In that study, behaviours were only correlated in populations where predators were present. The authors suggest that intra-population variation in behavioural syndromes may be the result of natural selection favouring a causal link between behaviours, resulting in optimal trait combinations for particular environments. One study that has unravelled some of the processes behind the expression of a correlation between boldness and aggression in sticklebacks was carried out by Bell & Sih (2007). Sticklebacks from a population with low levels of predation, where the correlation between boldness and aggression was not present, were exposed to real predation. After predation, a positive relationship between boldness and aggression was evident. The study revealed that predation was more intense for fish with specific behavioural phenotypes, namely shy-aggressive and bold-unaggressive fish. The surviving fish also changed their behavioural outputs. Therefore, predation pressure caused boldness and aggression to correlate by two mechanisms: selective mortality and behavioural flexibility.

In the study detailed in **Chapter 2**, I looked for correlations between behaviours, or behavioural syndromes, at the end of the experimental growth period. Overall, the only significant correlation I found was a weak but positive association between boldness and aggression (R^2=0.278, p=0.003). At group level, only in the fast growth, high interaction group, boldness and aggression were correlated (R^2=0.464, p=0.015). In my study, fish were caught in a site at which predators were present; therefore, it would be expected boldness and aggression to correlate. However, the fish that I used were caught at very small sizes and subjected to different competitive regimes for 10 weeks, with plenty of food and in the absence of predators. The fact that the only correlation that I found was for fast growing fish from the high interaction feeding regime could be attributed mainly to behavioural plasticity as a response to the interaction; interestingly, in that group aggressive-shy individuals were scarce. The results shown here have revealed that individual variation in risk-taking and aggressive behaviour in sticklebacks may not be fixed. However, aggression levels can vary across experimental groups and manipulation of the feeding environment could alter the degrees of boldness and aggression in a subset of fish, generating a behavioural syndrome.

The data presented in **chapter 4** suggest that consistent and inherited differences in cortisol responsiveness are not sufficient to maintain coherent behavioural profiles. After 3 generations showing the distinctive behaviour described above, a batch of HR and LR rainbow trout were transported from their native rearing site (Windermere, UK) to Oslo, Norway. Results from tests carried out immediately after transport showed a switch in behaviour which could be attributed mainly to stress and starvation as a result of transport, since HR fish lost significantly more body mass during the starvation-transport period. The changes in behaviour occurred without any associated change in a key physiological component of the coping styles, divergent post-stress plasma cortisol levels, which were maintained throughout the study period. This suggests a degree of plasticity in the behavioural aspects of coping style in fish that is independent of hypothalamus-pituitary-interrenal function. These results show that what looked like a tightly syndrome, with clearly integrated physiological and behavioural traits, is a more complex relationship. In this particular case, risk-taking and aggression were plastic, with individual levels changing in response to environmental conditions independently of its main underlying physiological mechanisms. Therefore, the results shown in this thesis extend current understanding on the sources of variation in the expression of behavioural syndromes by showing that social interactions (in particular competition for localized food), stress and variation in environmental conditions can have a similar effect to that shown by predation in natural populations (Bell and Sih 2007).

Fitness consequences of variability in behaviour

It has been suggested that variability in behaviour can be maintained if trade-offs between life-history strategies produce equal fitness for each behavioural trait (Wilson *et al.*, 1994; Stamps, 2007; Wolf *et al.*, 2007a) through mechanisms such as frequency-dependent selection (Dall *et al.*, 2004); however, this is still largely unknown. Few studies have looked at the fitness consequences of consistent behavioural traits, but it has been shown that personality traits relate to fitness components such as survival, growth and reproduction (Rèale & Festa-Bianchet, 2003; Dingemanse *et al.*, 2004; Dingemanse & Rèale, 2005; Duckworth, 2006; Smith & Blumstein., 2008).

For example in bighorn sheep ewes (*Ovis canadensis*) individual variation in boldness correlates positively with survival during seasons of high predation pressure (Rèale & Festa-Bianchet, 2003). In great tits (*Parus major*), speed of exploration is related positively to aggressiveness and competitive ability (Verbeek *et al.*, 1999). Dingemanse and collaborators (2004) found that the fitness consequences of personality reflected in annual adult survival. However, the results were sex-dependent and varied between years. In this case, fluctuating selection related to temporal variation in the competitive regime (food abundance) could be the factor that explains the maintenance of behavioural phenotypes in great tits. Another factor that plays a role here is assortative mating between animals of fast and slow exploration speed, with pairs that mated assortatively producing more surviving offspring (Both *et al.*, 2005). Recently Cote and collaborators (2008) showed that common lizards (*Lacerta vivipara*) that differ in sociality (the willingness to be close to conspecifics) displayed different levels of fitness, but this was related to population density. Asocial lizards survived better in low-density populations whereas in high density populations sociability did not affect survival. Social females had also more possibilities of reproduction than asocial lizards (Cote *et al.*, 2008).

In fish, a few studies have looked at the fitness consequences of personality traits and behavioural syndromes. For example Dugatkin (1992) found that guppies that show higher levels of predator inspection had a higher mortality rate than the more cautious individuals. Godin & Davis (1995) found that the same trait also had positive fitness consequences, because predators were significantly less attentive to, and less likely to attack, guppies that inspected them. However, in rainbow trout predators were more likely to select against risk-taking behaviour (Biro *et al.*, 2004).

Since variation in the timing of hatching has been found to have major fitness consequences for offspring in various vertebrate species (Daan *et al.*, 1996; Warner & Shine, 2007), in **chapter 3** I looked at the behavioural phenotypes of sticklebacks hatched at different times during the breeding season. I found that early hatched individuals were in proportion bolder than intermediate and late hatched fish, whereas most of the shy individuals were found among the late bred fish. If boldness has an underlying heritable component (van Oers *et al.*, 2004; Bell, 2004; Brown *et al.*, 2007), one could propose that bold parents bred early and gave birth bolder offspring, which in turn enjoyed more benefits that shyer later-born fish. Possible explanations for this are that early hatching may provide offspring with more favourable environmental conditions and also reduced competition from larger conspecifics permitting faster growth and higher probabilities of survival (Olsson & Shine, 1997; Qualls & Shine, 2000). Therefore, the conditions early in the season could favour boldness. It could also be that more fry predators, such as piscivorous fish and birds, are around later in the season, which might cause late bred fry to become more timid than bigger early hatched fish. This has been shown in lizards for which time of hatching contributed to variation in offspring growth and survival under natural conditions (Warner & Shine, 2007).

It is also possible that some sort of social information with parents plays a role in the development of boldness in early hatched fish. Personality in general has been associated with differences in parental behaviour that affect offspring fitness through growth and survival. Female house mice (*Mus domesticus*) selected for high aggression have been found to nurse and groom their pups more than females selected for low aggression (Benus & Rondigs, 1996). Personality in red squirrel mothers was correlated with growth rates and survival of their offspring both in the nest and later through their first winter (Boon *et al.*, 2007). Aggressive lizards (*Egernia whitii*) were more successful at defending kin from conspecifics attacks, which was reflected in higher survival of their offspring (Sinn *et al.*, 2008). In sticklebacks, males are in charge of attending the eggs from laying to after hatching and this behaviour could guarantee a higher hatching success. Different parental behaviour such as protection of the clutch against predators may be also involved in fitness, since boldness has been associated with territoriality and aggression (Sih *et al.*, 2004; Kortet & Hedrick, 2007; Reaney & Backwell, 2007). Stickleback fathers retrieve their fry and return them to their nests when they start to inspect their surroundings for first time (Rowland, 1994). Early interactions soon after being hatched, such as an adult chasing them to take them back, may make fry more aware of predators and allow them to avoid attacks later in life (Tulley & Huntingford, 1986).

In the study described in **chapter 3** I also found a relationship at the group level between boldness and aggression, independent of hatching date. This boldness and aggression behavioural syndrome could be beneficial to individuals in the studied population. The advantages of being bold could be favoured by behaving aggressively. If personality has a significant heritable component, then the offspring of aggressive but bold females may survive better because their personality would allow them to compete over conspecifics. In the present study, this was reflected as differences in the rate of achieving reproductive state in parents. Thus, I suggest that fish that are bold and aggressive in my study population could have fitness advantages over fish showing any other personality profile. Being bold for early-hatched individuals could provide higher fitness returns than shyness for late-hatched individuals; this pattern could therefore impose selection pressure in favour of hatching early in the season.

Coping strategies in fish

The proactive-reactive axis is another dimension of variability that involves a consistent correlation between risk-taking, aggression, dominance, response to new environments, and aspects of metabolic and stress physiology. The presence of the configuration of traits that characterize the proactive and reactive phenotypes in various animal species suggests it is a widespread phenomenon. The success of the two coping styles in nature could depend on the variability of the environment. Benus and collaborators (1991) suggested that non-aggressive mice and rats can succeed establishing territories in a new area where the environmental conditions will be different, whereas the routine-like aggressive animals will fail to do so. Great tits (*Parus major*) selected in two strains for their speed to explore new environments also show divergence in the way they react to changes and environmental stability. It was found that fast exploring individuals, who are also the most aggressive, did better when the environment was rich and stable, whereas slow exploring, less aggressive individuals flourished in a variable, relatively poor environment (Dingemanse *et al.*, 2004). Table 6.2 shows the behavioural and physiological traits found in mammals and birds that characterize the proactive and reactive coping strategies (Korte *et al.*,2005).

Table 6.2. The behavioural and physiological characteristics of proactive and reactive coping strategies from Korte *et al.* (2005).

	Proactive coping strategy	**Reactive coping strategy**
Behavioural strategy	Fight-flight	Freeze-hide
Emotional state	Aggressive and risk-takers	Non-aggressive and risk-avoiders
Exploration	Fast and superficial	Cautious and thorough
Behavioural flexibility	Rigid and routine-like	Flexible
Distractability	Hardly distracted	Easily distracted
Corticosterone /cortisol levels	Low	High
Parasympathetic reactivity	Low	High
Sympathetic reactivity	High	Low
Ecology	Flourish when food is stable and abundant and at high densities	Prosper during food scarcity and when density is low

As far as fish are concerned, a number of studies have documented the existence of covarying behavioural traits, or behavioural syndromes. For example, a positive correlation between aggression towards conspecifics and risk-taking in various potentially dangerous situations has been described in sticklebacks (Huntingford, 1976), brown trout (Sundström *et al.*, 2004) and grayling (Salonen & Peuhkuri, 2006). Physiological correlates of such behavioural variability were shown in sticklebacks where an unfamiliar conspecific and the presence of a predator elicited behavioural responses that were related to brain physiology (Bell *et al.*, 2007). In the rainbow trout *Oncorhynchus mykiss* fish have been selected for differences in their responsiveness to a standardized stressor (confinement) measured as levels of post-stress plasma cortisol. This has created a high and a low responsive strain, or the HR and LR strains respectively (Pottinger & Carrick, 1999 and 2001; Øverli *et al.*, 2002 and 2005). The individual response to stress in those fish has been found to be a heritable trait (Pottinger & Carrick, 2001) and to be associated to a distinctive behaviour. LR fish tend to become dominant over HR fish in dyadic contests, to become bolder than HR fish in the sense of faster resumption of feeding after transfer to a novel environment (Øverli *et al.*, 2002 and 2005; Schjolden *et al.*, 2005a). LR and HR fish also show patterns of brain biochemistry that are typical of the proactive and reactive animals (Schjolden *et al.*, 2006). Therefore, the physiological and behavioural profiles of the LR and HR rainbow trout had resembled those suggested for the proactive and reactive coping strategies respectively found in mammals and birds (Table 6.2 and Koolhaas *et al.*, 1999; Korte *et al.*, 2005). Studies in rats, mice and pigs show that one of the main differences between proactive and reactive animals is the degree of behavioural flexibility and distractability (in terms of how changes are perceived) between phenotypes.

The work described in **chapter 5** shows that in fact, the high and low responding rainbow trout also show the profiles of flexibility and response to novelty that characterise the reactive and proactive coping styles respectively. HR fish were able to find food faster than LR trout when the resource was moved to a different position. In contrast, LR fish were much less distracted by the presence of an unfamiliar object. In the studies described in **chapters 4 and 5** I have shown that performance was easily influenced by modification of previously familiar conditions of HR fish: change in position of food (**Chapter 5**) and transport (**Chapter 4**), whereas LR fish possibly did not perceive (or did not react) to the changes. Therefore, I could suggest that the behavioural plasticity shown by rainbow trout during the maze task and after transport in adults and in the next generation of offspring could be explained by differences in the ability of the fish to perceive and react to external stimuli.

Reactive animals tend to show flexible behaviour, perhaps because they are more aware of and reactive to any change in the environment. They are also easily distracted by change, whereas proactive animals are characterized by a rigid behaviour and the ready formation of routines (Benus *et al.*, 1991; Bolhius *et al.*, 2002). It has been suggested that it is not due to learning differences between proactive and reactive animals, but may be affected by the way proactive and reactive animals respond to changes in the environment (Benus *et al.*, 1987 and 1991; Bolhius *et al.*, 2004). The constant execution of the learned task, visiting the maze to get food, even in the presence of an unfamiliar potentially dangerous object, and the relative insensitivity to changes such as failing to perceive food on their way are likely to reflect an intrinsic behavioural control in LR trout reflecting a routine-like behaviour. HR fish on the other hand, behaved as if they were aware of and distracted by the unfamiliar object and they also found the food on their way before visiting the maze. This could reflect an extrinsic behavioural control. This pattern of behavioural control has been suggested to likely reflect the divergence in behavioural flexibility and distractability between the proactive (SAL) and reactive (LAL) mice as well (Benus *et al.*, 1991).

The pattern of behavioural, physiological and neurobiological responses shown by the high (HR) and low (LR) responding rainbow trout model is consistent with the characteristics that define the proactive and reactive coping styles found in mammals and birds (Koohlaas *et al.*, 1999; Korte *et al.*, 2005). The results in **chapter 5** show that HR fish are more distracted by changes in the environment while LR fish show a routine-like behaviour and give further support to the characterisation of LR and HR rainbow trout as showing proactive and reactive coping strategies.

Implications for aquaculture of individual variability in behaviour and behavioural syndromes

Research on fish behaviour, physiology and neurobiology is influencing the way fish are viewed (Ashley, 2007; Sneddon, 2003b; Portavella *et al.*, 2003). Therefore, there has been an increase in research related to fish welfare and the introduction of legislation and guidelines to safeguard fish wellbeing. Apart from obvious signs of distress, the concept of exactly what constitutes good welfare for a farmed fish is unclear and still under debate. Although there is no agreement on how to assess fish welfare, there is a general consensus that a number of different indicators must be selected to assess fish's welfare (Huntingford *et al.*, 2006).

Studies on wild animals have shown how individual variability in behaviour, and their possible correlations, influence characteristics that are important for aquaculture, such as growth rates (Ward *et al.*, 2004; Biro & Stamps, 2008), resumption of feeding after disturbance (Øverli *et al.*, 2002) and reaction to novelty (Brown *et al.*, 2007). Studies in domesticated strains have also revealed that farming has the potential to modulate behaviour, especially some aspects of risk-taking (Sundström *et al.*, 2004). This may indicate that under aquaculture conditions, if individual behaviours such as risk-taking and aggression are tightly correlated, domestication and selection for desirable characteristics for aquaculture, such as fast growth rates, would also inadvertently select for behaviours that can compromise welfare (for example higher levels of aggression) implying that fish showing an appropriate combination of behavioural traits (risk takers and non-aggressive) are likely to be scarce in production systems.

The consequences of aggression and boldness in aquaculture are various. For example, during feeding, agonistic interactions between fish increase. This may result in heterogeneous growth, since bold-aggressive fish could potentially get most of the food. It also could lead to the development of infectious diseases as a result of injury, the product of biting (Ashley, 2007). In production systems, food is commonly delivered clumped either in time (food delivered at specific times) or in space (through demand feeders); this could potentially promote fierce competition because fish have to interact closely when the resource is presented (Robb & Grant, 1998). In **chapter 2,** I showed that the levels of aggression in sticklebacks exposed during 10 weeks to a dispersed feeding condition were higher than those for fish for which food was presented clumped. Therefore, knowing how fish respond to food distribution may help to reduce aggression in production systems through changes of how food is delivered.

Variation in behaviour can also be correlated to how individuals respond to stress, as previously shown in the coping styles section. The importance of stress in fish welfare is well known. Stress is an adaptive response to changes but, the detrimental effects of stress are only evident when the sources of stress are unavoidable, prolonged and repeated. Therefore, differences in how animals respond to stress are in aquaculture important because proactive and reactive animals are susceptible to different diseases (Korte *et al.*, 2005; Koolhaas, 2007; Koolhaas, 2008). Many of the aquaculture practices such as transport, handling, feeding techniques, human presence, stocking densities can compromise fish's welfare because they potentially elicit chronic stress responses. Chronic stress has implications in several individual conditions such as the resistance to disease because it has been found that stress can suppress immune function (Koolhaas, 2008); stress also leads to reduction in growth rates and consequently good production (Strand *et al.*, 2007).

In **chapter 4** I showed that a common practice in aquaculture such as transport, coupled with exposure to a wholly novel environment affected differently fish with divergent behavioural and physiological profiles. Fish that normally responded to stress with high levels of cortisol lost almost twice the weight during transport than fish that show low levels of cortisol after stress. Interestingly the effect of transport lasted for more than a year. This could clearly have an effect on fish welfare as well as in production. For example as shown in figure 6.2, Øverli and collaborators (2006) showed in the same batch of fish, there was significantly more feed waste from rearing units containing high responding fish, and these fish also showed lower feed efficiency (growth per unit feed consumed). Size was more variable and growth was slower in HR rearing units.

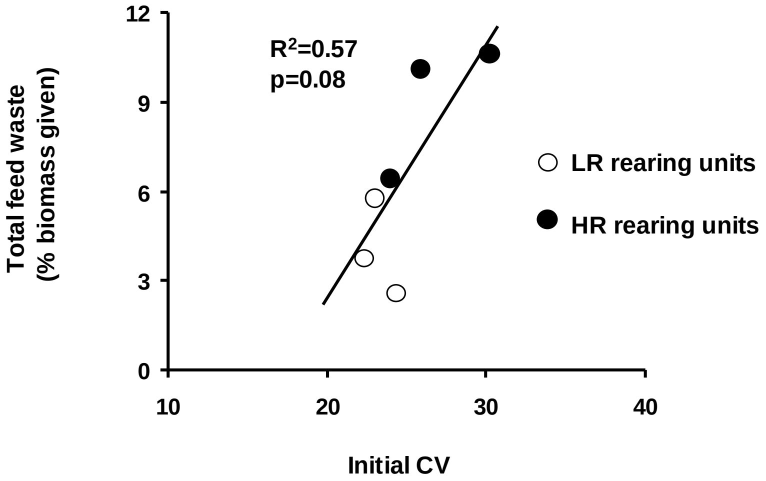


Figure 6.2. The relationship between total feed waste and initial size variation in HR and LR rearing units (From Øverli et al. 2006).

In conclusion, the results of this thesis have shown that individual variability in behaviour can have fitness consequences. However, modification of the environmental conditions can alter individual levels of behaviour, as well as being a potential source of variation on the expression of correlations between behaviours and that flexibility on behaviour may be independent of underlying physiological mechanisms.

List of references

Allen, J. R. M. & Wootton, R. J. (1982). The effect of ration and temperature on the growth of the three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology* **20**, 409-422.

Arnesen, A. M., Jorgensen, E. H., & Jobling, M. (1993). Feed-intake, growth and osmoregulation in Arctic charr, *Salvelinus alpinus* (L), following abrupt transfer from freshwater to more saline water. *Aquaculture* **114**, 327-338.

Ashley, P. J. (2007). Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science* **104**, 199-235.

Baker, J. A. (1994). Life history variation in female threespine stickleback. *The evolutionary biology of the threespine stickleback.*

Bakker, T. C. M. (1986). Aggressiveness in sticklebacks (*Gasterosteus aculeatus* L) - a behavior-genetic study. *Behaviour* **98**, 1-144.

Beauchamp, G. (2000). Individual differences in activity and exploration influence leadership in pairs of foraging zebra finches. *Behaviour* **137**, 301-314.

Bell, A. M. (2005). Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology* **18**, 464-473.

Bell, A. M. (2007). Future directions in behavioural syndromes research. *Proceedings of the Royal Society B-Biological Sciences* **274**, 755-761.

Bell, A. M., Backström, T., Huntingford, F. A., Pottinger, T. G., & Winberg, S. (2007). Variable neuroendocrine responses to ecologically-relevant challenges in sticklebacks. *Physiology & Behavior* **91**, 15-25.

Bell, A. M. & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters* **10**, 828-834.

Bell, A. M. & Stamps, J. A. (2004). Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. *Animal Behaviour* **68**, 1339-1348.

Benus, R. F., Bohus, B., Koolhaas, J. M., & Vanoortmerssen, G. A. (1991). Heritable variation for aggression as a reflection of individual coping strategies. *Experientia* **47**, 1008-1019.

Benus, R. F., Koolhaas, J. M., & Vanoortmerssen, G. A. (1987). Individual-differences in behavioral reaction to a changing environment in mice and rats. *Behaviour* **100**, 105-122.

Benus, R. F. & Rondigs, M. (1996). Patterns of maternal effort in mouse lines bidirectionally selected for aggression. *Animal Behaviour* **51**, 67-75.

Biro, P. A., Abrahams, M. V., Post, J. R., & Parkinson, E. A. (2004). Predators select against high growth rates and risk-taking behaviour in domestic trout populations. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 2233-2237.

Biro, P. A., Post, J. R., & Parkinson, E. A. (2003). From individuals to populations: Prey fish risk-taking mediates mortality in whole-system experiments. *Ecology* **84**, 2419-2431.

Biro, P. A. & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution* **23**, 361-368.

Boeuf, G. & Payan, P. (2001). How should salinity influence fish growth? *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* **130**, 411-423.

Bolhuis, J. E., Schouten, W. G. P., de Leeuw, J. A., Schrama, J. W., & Wiegant, V. A. (2004). Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. *Behavioural Brain Research* **152**, 351-360.

Boon, A. K., Rèale, D., & Boutin, S. (2008). Personality, habitat use, and their consequences for survival in North American red squirrels Tamiasciurus hudsonicus. *Oikos* **117**, 1321-1328.

Both, C., Dingemanse, N. J., Drent, P. J., & Tinbergen, J. M. (2005). Pairs of extreme avian personalities have highest reproductive success. *Journal of Animal Ecology* **74**, 667-674.

Bremner-Harrison, S., Prodohl, P. A., & Elwood, R. W. (2004). Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). *Animal Conservation* **7**, 313-320.

Briffa, M. & Sneddon, L. U. (2007). Physiological constraints on contest behaviour. *Functional Ecology* **21**, 627-637.

Brown, C. & Braithwaite, V. A. (2004). Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour* **68**, 1325-1329.

Brown, C., Jones, F., & Braithwaite, V. A. (2007). Correlation between boldness and body mass in natural populations of the poeciliid *Brachyrhaphis episcopi*. *Journal of Fish Biology* **71**, 1590-1601.

Brown, C. & Laland, K. N. (2003). Social learning in fishes: a review. *Fish and Fisheries* 4, 280-288.

Brydges, N. M., Colegrave, N., Heathcote, R. J. P., & Braithwaite, V. A. (2008). Habitat stability and predation pressure affect temperament behaviours in populations of three-spined sticklebacks. *Journal of Animal Ecology* **77**, 229-235.

Budaev, S. V. (1997). The dimensions of personality in animals: The comparative and evolutionary framework. *Advances in Ethology; Contributions to the XXV International Ethological Conference* 90.

Burmeister, S. S., Jarvis, E. D., & Fernald, R. D. (2005). Rapid behavioral and genomic responses to social opportunity. *Plos Biology* **3**, 1996-2004.

Burmeister, S. S., Kailasanath, V., & Fernald, R. D. (2007). Social dominance regulates androgen and estrogen receptor gene expression. *Hormones and Behavior* **51**, 164-170.

Candolin, U. (1998). Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 1171-1175.

Carere, C., Drent, P. J., Privitera, L., Koolhaas, J. M., & Groothuis, T. G. G. (2005). Personalities in great tits, Parus major: stability and consistency. *Animal Behaviour* **70**, 795-805.

Carpenter, R. E., Watt, M. J., Forster, G. L., Overli, O., Bockholt, C., Renner, K. I., & Summers, C. H. (2007). Corticotropin releasing factor induces anxiogenic locomotion in trout and alters serotonergic and dopaminergic activity. *Hormones and Behavior* **52**, 600-611.

Chapman, B. B., Morrell, L. J., Benton, T. G., & Krause, J. (2008). Early interactions with adults mediate the development of predator defenses in guppies. *Behavioral Ecology* **19**, 87-93.

Clements, S., Moore, F. L., & Schreck, C. B. (2003). Evidence that acute serotonergic activation potentiates the locomotor-stimulating effects of corticotropin-releasing hormone in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Hormones and Behavior* **43**, 214-221.

Cote, J., Dreiss, A., & Clobert, J. (2008). Social personality trait and fitness. *Proceedings of the Royal Society B-Biological Sciences* **275**, 2851-2858.

Daan, S., Dijkstra, C., & Weissing, F. J. (1996). An evolutionary explanation for seasonal trends in avian sex ratios. *Behavioral Ecology* **7**, 426-430.

Dall, S. R. X., Houston, A. I., & McNamara, J. M. (2004). The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters* **7**, 734-739.

Damsgard, B. & Dill, L. M. (1998). Risk-taking behavior in weight-compensating coho salmon, *Oncorhynchus kisutch*. *Behavioral Ecology* **9**, 26-32.

DiBattista, J. D., Anisman, H., Whitehead, M., & Gilmour, K. M. (2005). The effects of cortisol administration on social status and brain monoaminergic activity in rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology* **208**, 2707-2718.

Dingemanse, N. J., Both, C., Drent, P. J., & Tinbergen, J. M. (2004). Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 847-852.

Dingemanse, N. J., Both, C., Drent, P. J., van Oers, K., & van Noordwijk, A. J. (2002). Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour* **64**, 929-938.

Dingemanse, N. J. & de Goede, P. (2004). The relation between dominance and exploratory behavior is context-dependent in wild great tits. *Behavioral Ecology* **15**, 1023-1030.

Dingemanse, N. J. & Rèale, D. (2005). Natural selection and animal personality. *Behaviour* **142**, 1159-1184.

Dingemanse, N. J., Wright, J., Kazem, A. J. N., Thomas, D. K., Hickling, R., & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology* **76**, 1128-1138.

Drent, P. J., van Oers, K., & van Noordwijk, A. J. (2003). Realized heritability of personalities in the great tit (*Parus major*). *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 45-51.

Duckworth, R. A. (2006). Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. *Behavioral Ecology* **17**, 1011-1019.

Duckworth, R. A. & Badyaev, A. V. (2007). Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 15017-15022.

Dugatkin, L. A. (1992). Tendency to inspect predators predicts mortality risk in the guppy (Poecilia *reticulata*). *Behavioral Ecology* **3**, 124-127.

Dugatkin, L. A. & Ohlsen, S. R. (1990). Contrasting asymmetries in value expectation and resource holding power: Effects on attack behaviour and dominance in the pumpkinseed sunfish, Lepomis gibbosus. *Animal Behaviour* **39**, 802-804.

Einum, S. & Fleming, I. A. (1997). Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. *Journal of Fish Biology* **50**, 634-651.

FAO. (2007). The state of world fisheries and aquaculture 2006. Fisheries and aquaculture department. *Food and Agriculture Organization of the United Nations*. Rome. ISSN 1020-5489

Fevolden, S. E., Roed, K. T., Fjalestad, K. T., & Stien, J. (1999). Poststress levels of lysozyme and cortisol in adult rainbow trout: Heritabilities and genetic correlations. *Journal of Fish Biology* **59**, 900-910.

Frost, A., Winrow-Giffen, A., Ashley, P., & Sneddon, L. (2007). Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of the Royal Society B: Biological Sciences* **274**, 333-339.

Gilmour, K. M. (2005). Mineralocorticoid receptors and hormones: Fishing for answers. *Endocrinology* **146**, 44-46.

Godin, J. G. J. & Davis, S. A. (1995). Who dares, benefits - predator approach behavior in the guppy (*Poecilia reticulata*) deters predator pursuit. *Proceedings of the Royal Society of London Series B-Biological Sciences* **259**, 193-200.

Goede, R. W., Barton, B. A., & see, B. T. (1990). Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. *American Fisheries Society Symposium, Bethesda, Maryland, USA.*

Gosling, S. D. (2001). From mice to men: What can we learn about personality from animal research? *Psychological Bulletin* **127**, 45-86.

Gosling, S. D. & John, O. P. (1999). Personality dimensions in nonhuman animals: A cross-species review. *Current Directions in Psychological Science* **8**, 69-75.

Grant, J. W. A., Girard, I. L., Breau, C., & Weir, L. K. (2002). Influence of food abundance on competitive aggression in juvenile convict cichlids. *Animal Behaviour* **63**, 323-330.

Grant, J. W. A. & Guha, R. T. (1993). Spatial clumping of food increases its monopolization and defence by convict cichlids, *Cichlasoma nigrofasciatum*. *Behavioral Ecology* **4**, 293-296.

Gregory, T. R. & Wood, C. M. (1999). The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* **72**, 286-295.

Hedenskog, M., Petersson, E., & Jarvi, T. (2002). Agonistic behavior and growth in newly emerged brown trout (*Salmo trutta* L) of sea-ranched and wild origin. *Aggressive Behavior* **28**, 145-153.

Huntingford, F. (1976). The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. *Animal Behaviour* **24**, 245-260.

Huntingford, F. & Adams, C. (2005). Behavioural syndromes in farmed fish: implications for production and welfare. *Behaviour* **142**, 1207-1221.

Huntingford, F., Turner, A. (1987). Animal conflict. *Oxford University Press*. Pp. 448.

Huntingford, F. A. (2004). Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology* **65**, 122-142.

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandoe, P., & Turnbull, J. F. (2006). Current issues in fish welfare. *Journal of Fish Biology* **68**, 332-372.

Johansson, V., Winberg, S., & Bjornsson, B. T. (2005). Growth hormone-induced stimulation of swimming and feeding behaviour of rainbow trout is abolished by the D-1 dopamine antagonist SCH23390. *General and Comparative Endocrinology* **141**, 58-65.

Johnson, J. C. & Sih, A. (2007). Fear, food, sex and parental care: a syndrome of boldness in the fishing spider, *Dolomedes triton*. *Animal Behaviour* **74**, 1131-1138.

Johnsson, J. I. & Bjornsson, B. T. (1994). Growth-hormone increases growth-rate, appetite and dominance in juvenile rainbow trout, *Oncorhynchus mykiss*. *Animal Behaviour* **48**, 177-186.

Johnsson, J. I., Jonsson, E., & Bjornsson, B. T. (1996). Dominance, nutritional state, and growth hormone levels in rainbow trout (*Oncorhynchus mykiss*). *Hormones and Behavior* **30**, 13-21.

Koolhaas, J. M. (2008). Coping style and immunity in animals: Making sense of individual variation. *Brain Behavior and Immunity* **22**, 662-667.

Koolhaas, J. M., de Boer, S. F., & Buwalda, B. (2006). Stress and adaptation - Toward ecologically relevant animal models. *Current Directions in Psychological Science* **15**, 109-112.

Koolhaas, J. M., de Boer, S. F., Buwalda, B., & van Reenen, K. (2007). Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. *Brain Behavior and Evolution* **70**, 218-226.

Koolhaas, J. M., Korte, S. M., de Boer, S. F., Van Der Vegt, B. J., van Reenen, C. G., Hopster, H., de Jong, I. C., Ruis, M. A. W., & Blokhuis, H. J. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews* **23**, 925-935.

Korte, S. M., Koolhaas, J. M., Wingfield, J. C., & Mcewen, B. S. (2005). The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. *Neuroscience and Biobehavioral Reviews* **29**, 3-38.

Kortet, R. & Hedrick, A. (2007). A behavioural syndrome in the field cricket *Gryllus integer*: intrasexual aggression is correlated with activity in a novel environment. *Biological Journal of the Linnean Society* **91**, 475-482.

Liebert, A. M. & Schreck, C. B. (2006). Effects of acute stress on osmoregulation, feed intake, IGF-1, and cortisol in yearling steelhead trout (*Oncorhynchus mykiss*) during seawater adaptation. *General and Comparative Endocrinology* **148**, 195-202.

Lopez, P., Hawlena, D., Polo, V., Amo, L., & Martin, J. (2005). Sources of individual shy-bold variations in antipredator behaviour of male Iberian rock lizards. *Animal Behaviour* **69**, 1-9.

Magnhagen, C. (2007). Social influence on the correlation between behaviours in young-of-the-year perch. *Behavioral Ecology and Sociobiology* **61**, 525-531.

Magnhagen, C. & Borcherding, J. (2008). Risk-taking behaviour in foraging perch: does predation pressure influence age-specific boldness? *Animal Behaviour* **75**, 509-517.

Magnhagen, C. & Staffan, F. (2003). Social learning in young-of-the-year perch encountering a novel food type. *Journal of Fish Biology* **63**, 824-829.

Marchetti, C. & Drent, P. J. (2000). Individual differences in the use of social information in foraging by captive great tits. *Animal Behaviour* **60**, 131-140.

McCormick, M. I. (1998). Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. *Ecology* **79**, 1873-1883.

McCormick, S. D. (2001). Endocrine control of osmoregulation in teleost fish. *American Zoologist* **41**, 781-794.

McCormick, S. D. & Bradshaw, D. (2006). Hormonal control of salt and water balance in vertebrates. *General and Comparative Endocrinology* **147**, 3-8.

McElreath, R., Luttbeg, B., Fogarty, S. P., Brodin, T., & Sih, A. (2007). Evolution of animal personalities. *Nature* **450**, E5.

Metcalfe, N. B., Valdimarsson, S. K., & Morgan, I. J. (2003). The relative roles of domestication, rearing environment, prior residence and body size in deciding territorial contests between hatchery and wild juvenile salmon. *Journal of Applied Ecology* **40**, 535-544.

Moreira, P. S. A., Pulman, K. G. T., & Pottinger, T. G. (2004). Extinction of a conditioned response in rainbow trout selected for high or low responsiveness to stress. *Hormones and Behavior* **46**, 450-457.

Moretz, J. A., Martins, E. P., & Robison, B. D. (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral Ecology* **18**, 556-562.

Morrell, L. J., Lindstrom, J., & Ruxton, G. D. (2005). Why are small males aggressive? *Proceedings of the Royal Society B-Biological Sciences* **272**, 1235-1241.

Olsson, M. & Shine, R. (1997). The seasonal timing of oviposition in sand lizards (Lacerta agilis): Why early clutches are better. *Journal of Evolutionary Biology* **10**, 369-381.

Östlund-Nilsson, S., Mayer, I., Huntingford, F. A. (2007). Biology of the three-spined stickleback. [Marine Biology Series]. *CRC Press. UK*. 392 pp.

Øverli, Ø., Harris, C. A., & Winberg, S. (1999). Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain Behavior and Evolution* **54**, 263-275.

Øverli, Ø., Korzan, W. J., Larson, E. T., Winberg, S., Lepage, O., Pottinger, T. G., Renner, K. J., & Summers, C. H. (2004). Behavioral and neuroendocrine correlates of displaced aggression in trout. *Hormones and Behavior* **45**, 324-329.

Øverli, Ø., Pottinger, T. G., Carrick, T. R., Øverli, E., & Winberg, S. (2002). Differences in behaviour between rainbow trout selected for high- and low-stress responsiveness. *Journal of Experimental Biology* **205**, 391-395.

Øverli, Ø., Sorensen, C., Kiessling, A., Pottinger, T. G., & Gjoen, H. M. (2006a). Selection for improved stress tolerance in rainbow trout (*Oncorhynchus mykiss*) leads to reduced feed waste. *Aquaculture* **261**, 776-781.

Øverli, Ø., Sorensen, C., & Nilsson, G. E. (2006b). Behavioral indicators of stress-coping style in rainbow trout: Do males and females react differently to novelty? *Physiology & Behavior* **87**, 506-512.

Øverli, Ø., Sorensen, C., Pulman, K. G. T., Pottinger, T. G., Korzan, W., Summers, C. H., & Nilsson, G. E. (2007). Evolutionary background for stress-coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neuroscience and Biobehavioral Reviews* **31**, 396-412.

Øverli, Ø., Winberg, S., & Pottinger, T. G. (2005). Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout - a review. *Integrative and Comparative Biology* **45**, 463-474.

Portavella, M., Salas, C., Vargas, J. P., & Papini, M. R. (2003). Involvement of the telencephalon in spaced-trial avoidance learning in the goldfish (*Carassius auratus*). *Physiology & Behavior* **80**, 49-56.

Pottinger, T. G. & Carrick, T. R. (1999). Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. *General and Comparative Endocrinology* **116**, 122-132.

Pottinger, T. G. & Carrick, T. R. (2001). Stress responsiveness affects dominant-subordinate relationships in rainbow trout. *Hormones and Behavior* **40**, 419-427.

Qualls, F. J. & Shine, R. (2000). Post-hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink, *Lampropholis guichenoti*. *Biological Journal of the Linnean Society* **71**, 315-341.

Rands, S. A., Cowlishaw, G., Pettifor, R. A., Rowcliffe, J. M., & Johnstone, R. A. (2003). Spontaneous emergence of leaders and followers in foraging pairs. *Nature* **423**, 432-434.

Rèale, D. & Festa-Bianchet, M. (2003). Predator-induced natural selection on temperament in bighorn ewes. *Animal Behaviour* **65**, 463-470.

Rèale, D., Gallant, B. Y., Leblanc, M., & Festa-Bianchet, M. (2000). Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Animal Behaviour* **60**, 589-597.

Rèale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews* **82**, 291-318.

Reaney, L. T. & Backwell, P. R. Y. (2007). Risk-taking behavior predicts aggression and mating success in a fiddler crab. *Behavioral Ecology* **18**, 521-525.

Robb, S. E. & Grant, J. W. A. (1998). Interactions between the spatial and temporal clumping of food affect the intensity of aggression in Japanese medaka. *Animal Behaviour* **56**, 29-34.

Rose, J. D. (2002). The Neurobehavioral nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science* **10**, 1-38.

Rowland, W. J., Bell, M. A., & Foster, S. A. (1994). Proximate determinants of stickleback behaviour: an evolutionary perspective. *The evolutionary biology of the threespine stickleback.* 297-344.

Ruiz-Gomez, M. D., Kittilsen, S., Hoglund, E., Huntingford, F. A., Sorensen, C., Pottinger, T. G., Bakken, M., Winberg, S., Korzan, W. J., & Øverli, Ø. (2008). Behavioral plasticity in rainbow trout (*Oncorhynchus mykiss*) with divergent coping styles: When doves become hawks. *Hormones and Behavior* **54**, 534-538.

Ruzzante, D. E. (1994). Domestication effects on aggressive and schooling behavior in fish. *Aquaculture* **120**, 1-24.

Ruzzante, D. E. & Doyle, R. W. (1991). Rapid behavioural changes in Medaka (*Oryzias latipes*) caused by selection for competitive and noncompetitive growth. *Evolution* **45**, 1936-1946.

Saito, T. & Nakano, S. (1999). Reproductive-timing-dependent alternation of offspring life histories in female threespine sticklebacks. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* **77**, 1314-1321.

Salonen, A. & Peuhkuri, N. (2006). The effect of captive breeding on aggressive behaviour of European grayling, *Thymallus thymallus*, in different contexts. *Animal Behaviour* **72**, 819-825.

Schjolden, J., Backstrom, T., Pulman, K. G. T., Pottinger, T. G., & Winberg, S. (2005a). Divergence in behavioural responses to stress in two strains of rainbow trout (*Oncorhynchus mykiss*) with contrasting stress responsiveness. *Hormones and Behavior* **48**, 537-544.

Schjolden, J., Stoskhus, A., & Winberg, S. (2005b). Does individual variation in stress responses and agonistic behavior reflect divergent stress coping strategies in juvenile rainbow trout? *Physiological and Biochemical Zoology* **78**, 715-723.

Schjolden, J., Pulman, K. G. T., Pottinger, T. G., Tottmar, O., & Winberg, S. (2006). Serotonergic characteristics of rainbow trout divergent in stress responsiveness. *Physiology & Behavior* **87**, 938-947.

Schjolden, J. & Winberg, S. (2007). Genetically determined variation in stress responsiveness in rainbow trout: Behavior and neurobiology. *Brain Behavior and Evolution* **70**, 227-238.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: An integrative overview. *Quarterly Review of Biology* **79**, 241-277.

Sinn, D. L., Gosling, S. D., & Moltschaniwskyj, N. A. (2008). Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. *Animal Behaviour* **75**, 433-442.

Sinn, D. L. & Moltschaniwskyj, N. A. (2005). Personality traits in dumpling squid (*Euprymna tasmanica*): Context-specific traits and their correlation with biological characteristics. *Journal of Comparative Psychology* **119**, 99-110.

Smith, B. R. & Blumstein, D. T. (2008). Fitness consequences of personality: a meta-analysis. *Behavioral Ecology* **19**, 448-455.

Sneddon, L. U. (2003a). The bold and the shy: individual differences in rainbow trout. *Journal of Fish Biology* **62**, 971-975.

Sneddon, L. U. (2003b). The evidence for pain in fish: the use of morphine as an analgesic. *Applied Animal Behaviour Science* **83**, 153-162.

Stamps, J. A. (2007). Growth-mortality tradeoffs and 'personality traits' in animals. *Ecology Letters* **10**, 355-363.

Strand, A., Magnhagen, C., & Alanara, A. (2007). Effects of repeated disturbances on feed intake, growth rates and energy expenditures of juvenile perch, *Perca fluviatilis*. *Aquaculture* **265**, 163-168.

Sundström, L. F., Petersson, E., Hojesjo, J., Johnsson, J. I., & Jarvi, T. (2004). Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. *Behavioral Ecology* **15**, 192-198.

Templeton, J. J. & Giraldeau, L. A. (1996). Vicarious sampling: The use of personal and public information by starlings foraging in a simple patchy environment. *Behavioral Ecology and Sociobiology* **38**, 105-114.

Tulley, T. & Huntingford, F. (1987). Paternal care and the development of adaptive variation in anti-predator responses in sticklebacks. *Animal Behaviour* **35**, 1570-1572.

Vahl, W. K., Lok, T., van der Meer, J., Piersma, T., & Weissing, F. J. (2005). Spatial clumping of food and social dominance affect interference competition among ruddy turnstones. *Behavioral Ecology* **16**, 834-844.

van Oers, K., Drent, P. J., de Goede, P., & van Noordwijk, A. J. (2004). Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 65-73.

Vanoortmerssen, G. A. & Bakker, T. C. M. (1981). Artificial selection for short and long attack latencies in wild *Mus musculus domesticus*. *Behavior Genetics* **11**, 115-126.

Verbeek, M. E. M., de Goede, P., Drent, P. J., & Wiepkema, P. R. (1999). Individual behavioural characteristics and dominance in aviary groups of great tits. *Behaviour* **136**, 23-48.

Volkoff, H. (2006). From goldfish to cod: The neuroendocrine control of food intake in fish. *Journal of Experimental Zoology Part A-Comparative Experimental Biology* **305A**, 190.

Ward, A. J. W. & Krause, J. (2001). Body length assortative shoaling in the European minnow, *Phoxinus phoxinus*. *Animal Behaviour* **62**, 617-621.

Ward, A. J. W., Thomas, P., Hart, P. J. B., & Krause, J. (2004). Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology* **55**, 561-568.

Ward, A. J. W., Webster, M. M., & Hart, P. J. B. (2006). Intraspecific food competition in fishes. *Fish and Fisheries* **7**, 231-261.

Warner, D. A. & Shine, R. (2007). Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia* **154**, 65-73.

Watt, M. J., Forster, G. L., Korzan, W. J., Renner, K. J., & Summers, C. H. (2007). Rapid neuroendocrine responses evoked at the onset of social challenge. *Physiology & Behavior* **90**, 567-575.

Wendelar Bonga, S. E. (1997). The stress response in fish. *Physiological reviews* **77**, 591-625.

Wilson, D. S., Clark, A. B., Coleman, K., & Dearstyne, T. (1994). Shyness and boldness in humans and other animals. *Trends in Ecology & Evolution* **9**, 442-446.

Winberg, S. & Nilsson, G. E. (1993). Roles of Brain Monoamine neurotransmitters in agonistic behavior and stress reactions, with particular reference to fish. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology* **106**, 597-614.

Wolf, M., van Doorn, G. S., Leimar, O., & Weissing, F. J. (2007a). Evolution of animal personalities - Reply. *Nature* **450**, E5-E6.

Wolf, M., van Doorn, G. S., Leimar, O., & Weissing, F. J. (2007b). Life-history trade-offs favour the evolution of animal personalities. *Nature* **447**, 581-584.

Wootton, R. J. (1984). *A functional biology of sticklebacks. Croom Helm.* London. pp. 1-265.

Yamamoto, T. & Reinhardt, U. G. (2003). Dominance and predator avoidance in domesticated and wild masu salmon Oncorhynchus masou. *Fisheries Science* **69**, 88-94.

Accompanying material

Hormones and Behavior 54 (2008) 534–538

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Behavioral plasticity in rainbow trout (*Oncorhynchus mykiss*) with divergent coping styles: When doves become hawks

Maria de Lourdes Ruiz-Gomez [a,*], Silje Kittilsen [b], Erik Höglund [c], Felicity A. Huntingford [a], Christina Sørensen [d], Thomas G. Pottinger [e], Morten Bakken [b], Svante Winberg [f], Wayne J. Korzan [g], Øyvind Øverli [b]

[a] *Fish Biology Group, Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK*
[b] *Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Aas, Norway*
[c] *Danish Institute for Fisheries Research, Department of Marine Ecology and Aquaculture, North Sea Center, Postbox 101, DK-9850 Hirtshals, Denmark*
[d] *Department of Molecular Biosciences, University of Oslo, POBox 1041, N-0316 Oslo, Norway*
[e] *Centre for Ecology and Hydrology, Lancaster Environment Centre, Bailrigg, Lancaster LA1 4AP, UK*
[f] *Evolutionary Biology Centre, Department Comparative Physiology, Uppsala University, Norbyvägen 18A, SE-752 36 Uppsala, Sweden*
[g] *Deptartment of Biology, Stanford University. Stanford, CA 94305, USA*

ARTICLE INFO

Article history:
Received 10 March 2008
Revised 7 May 2008
Accepted 11 May 2008
Available online 22 May 2008

Keywords:
Physiology
Limited plasticity
Coping strategy
Social dominance

ABSTRACT

Consistent and heritable individual differences in reaction to challenges, often referred to as stress coping styles, have been extensively documented in vertebrates. In fish, selection for divergent post-stress plasma cortisol levels in rainbow trout (*Oncorhynchus mykiss*) has yielded a low (LR) and a high responsive (HR) strain. A suite of behavioural traits is associated with this physiological difference, with LR (proactive) fish feeding more rapidly after transfer to a new environment and being socially dominant over HR (reactive) fish. Following transport from the UK to Norway, a switch in behavioural profile occurred in trout from the 3rd generation; HR fish regained feeding sooner than LR fish in a novel environment and became dominant in size-matched HR–LR pairs. One year after transport, HR fish still fed sooner, but no difference in social dominance was found. Among offspring of transported fish, no differences in feeding were observed, but as in pre-transported 3rd generation fish, HR fish lost fights for social dominance against size-matched LR opponents. Transported fish and their offspring retained their distinctive physiological profile throughout the study; HR fish showed consistently higher post-stress cortisol levels at all sampling points. Altered risk-taking and social dominance immediately after transport may be explained by the fact that HR fish lost more body mass during transport than did LR fish. These data demonstrate that some behavioural components of stress coping styles can be modified by experience, whereas behavioural plasticity is limited by genetic effects determining social position early in life story.

Introduction

Within the last decade, it has been clearly established that individual animals of various species (vertebrate and invertebrate) differ in the nature of their response to challenges. Such differences may involve suites of correlated physiological and behavioural traits and are often referred to as “coping strategies” (Koolhaas et al., 1999). In mammals and birds, two distinct coping strategies can be distinguished. At one extreme, animals with a proactive coping strategy tend to show a fight-flight response, to be more aggressive and bold (in the sense of taking risks in a variety of dangerous situations), and to have low levels of plasma corticosteroids. In contrast, at the other extreme, reactive animals show a freeze-hide response; tend to be shy and less aggressive with a more flexible behaviour than proactive individuals. The physiological response to stress in reactive individuals involves relatively higher plasma cortisol levels, as well as differences in a number of other neuro-endocrine systems (Koolhaas et al., 1999; Korte et al., 2005).

In several species it has been shown additionally that such differences in physiological and behavioral stress responses are heritable (van Oers et al., 2005; Øverli et al., 2005; Koolhaas et al., 2007), which raise a question about how such variability is maintained within populations. The emerging consensus is that proactive and reactive animals flourish in different selective environments, possibly in a frequency-dependent manner. Some authors have likened proactive and reactive animals to the hawks and doves of classical game theory (see for example Korte et al., 2005). The existence of such adaptive individual differences within a population has evoked considerable scientific interest and has important consequences for disciplines as diverse as evolutionary ecology (Bolnick et al., 2003; Sih et al., 2004), animal husbandry (Cavigelli, 2005; Huntingford and Adams, 2005) and biomedicine (Korte et al., 2005).

---

* Corresponding author. Fax: +44 141 330 5971.
E-mail address: m.ruiz.1@research.gla.ac.uk (M.L. Ruiz-Gomez).
URL: http://www.gla.ac.uk/departments/eeb/researchinterests/pgs/mariaruizgomez/ (M.L. Ruiz-Gomez).

0018-506X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2008.05.005

One outstanding question is the relationship between behavioural plasticity and underlying physiological mechanisms (Øverli et al., 2007). On the one hand, if behaviour is closely linked to strongly heritable physiological traits (as in Miller et al., 2004; Uhart et al., 2004; Serretti et al., 2006; Poirier et al., 2007), this may limit behavioural plasticity. On the other hand, some components of the neuro-endocrine machinery are rapidly modifiable by experience (e.g. Burmeister et al., 2005; Burmeister, 2007; Watt et al., 2007) and this is likely to be reflected in plasticity in associated behavioural traits. The results presented in this paper provide an example of flexible dissociation of behavioural and physiological components of the coping strategy, using an established fish model, the rainbow trout (*Onchorhynchus mykiss,* Walbaum, 1792).

A number of studies have documented the existence of co-varying behavioural traits in teleost fish (e.g. Bell and Sih, 2007; Wilson and McLaughlin, 2007). For example, a positive correlation between aggression towards conspecifics and risk-taking in various potentially dangerous situations has been described in three-spined sticklebacks (*Gasterosteus aculeatus*, Huntingford 1976, though see Bell and Stamps 2004), brown trout (*Salmo trutta*, Sundstrom et al., 2004), and grayling (*Thymallus thymallus*, Salonen and Peuhkuri, 2006). There is relatively little information on physiological correlates of such behavioural variability in fish, though Bell et al. (2007) report a correlation between individual risk-taking behaviour and brain biochemistry in sticklebacks. The rainbow trout provides an exception, in that a number of behavioural differences have been reported in strains of rainbow trout selected for high (high responsive, or HR-trout) and low (low responsive, LR) cortisol responsiveness to a standardized stressor (see reviews by Øverli et al., 2005; Schjolden and Winberg, 2007).

The first behavioural study conducted on these strains documented that fish from the LR strain become socially dominant over HR fish (Pottinger and Carrick, 2001). In addition, following transfer from group rearing to isolation in an unfamiliar tank, trout from the LR strain resumed feeding earlier than did fish from the HR strain (Øverli et al., 2002a). It was later shown that rapid resumption of feeding following transfer to a novel environment also predicts social dominance and level of aggression towards territorial intruders in non-selected aquaculture strains of rainbow trout (Øverli et al., 2004; Schjolden et al., 2005a). Most of the behavioural characteristics of the HR line are consistent with previously reported effects of the steroid hormone cortisol in non-mammalian vertebrates (Gregory and Wood, 1999; Øverli et al., 2002b; DiBattista et al., 2005). However, it seems unlikely that cortisol alone is responsible for controlling all behavioural aspects of stress coping style (Koolhaas et al., 2007; Øverli et al., 2007).

In this paper we present data indicating that cortisol responsiveness and behavioural profiles may be uncoupled. After 3 generations showing distinctive behavioural profiles, a batch of HR and LR rainbow trout were transported from their original rearing site (Windermere, UK) to Oslo, Norway. Unexpectedly, immediately after transport both strains switched behavioural profiles, with HR fish now being bolder in terms of rapid resumption of feeding behaviour after transfer to isolation and exhibiting social dominance over LR fish. The divergence in post-stress plasma cortisol concentrations between strains remained unchanged in transported as well as non-transported fish from the same generation. These data suggest a degree of plasticity in the behavioural aspects of coping style in fish that is independent of hypothalamus-pituitary-interrenal function.

Materials and methods

Outline of screening regime

Results presented here come from studies carried out on the third and fourth generations of LR and HR rainbow trout. Observations were made both at the original rearing site (CEH Windermere, UK) and after transport between this site and an experimental facility in Norway. Weights below are given as mean ± S.E.M. Details of the selection programme used to generate the HR and LR strains have been described previously (Pottinger and Carrick, 1999, 2001), as has their typical behaviour (Pottinger and Carrick, 2001; Øverli et al., 2002a; Schjolden et al., 2005a; Schjolden and Winberg, 2007).

In the summer of 2005, adult 3rd generation HR (n = 150, weight 493 ± 12 g) and LR fish (n = 150, weight 477 ± 9 g) were fitted with Passive Integrated Transponder (PIT) tags and transported from the Windermere Laboratory of the UK Centre for Ecology and Hydrology (Windermere, UK) to the Norwegian Institute of Water Research Marine Research Station (Solbergstrand, Akershus County, Norway). Fish were deprived of food for 5 days prior to transport and then loaded in a tank truck equipped to transport fish (Donslund Special Transport, Hejnsvig, Denmark). Transported fish arrived 2 days later and no mortalities occurred during transport. Upon arrival in Norway all PIT-tags were registered, fish were weighed and screening for behavioural and physiological traits initiated. Individual identification of fish with PIT-tags permitted body mass loss during the 7 day period of transport to be assessed, since energetic status is a variable that may strongly influence risk-taking and social behaviour (e.g Johnsson et al., 1996; Damsgard and Dill, 1998); however, due to logistic reasons the body mass of some fish could not be measured.

Four sessions of behavioural testing were carried out. Immediately after transport adult HR (n = 23 weight: 448 ± 21 g) and LR fish (n = 23 weight: 457 ± 16 g) were tested for risk-taking (boldness). Data for this trait are not available for non-transported fish, but previous studies reported that adult LR fish resume feeding more rapidly than HR fish at the original rearing site (Øverli et al., 2002a). Social dominance and plasma cortisol following an acute stressor were also assayed in Norway. Simultaneously in Windermere UK, non-transported 3rd generation adult fish (n = 16 weight: HR 742 ± 41 g, n = 16; LR 645 ± 28 g) were tested for social dominance and post-stress plasma cortisol. In Norway, 1 year later in the summer 2006 a set of HR (n = 18 weight: 1113 ± 65 g) and LR (n = 18 weight: 1108 ± 61 g) fish, transported the year before, were screened for the same behavioural traits as in 2005. Finally, 8 months old 4th generation offspring (n = 16 weight, HR 16.2 ± 0.9 g, n = 16; LR 16.4 ± 0.9 g,) generated from transported F3 fish were screened for the same behavioural and physiological traits in August 2006.

Screening for boldness and social dominance

Prior to screening, fish were transferred from communal rearing tanks to 250 l (adult fish) or 12 l (offspring) glass aquaria, where they were held in isolation. Each observation aquarium was lined with black plastic on three sides and divided in two compartments by a removable opaque PVC wall, each section holding one HR or one LR fish in weight matched pairs. Prior to transfer from group rearing to social isolation each fish was anaesthetised, weighed, and for adult fish the PIT-tag was read. Offspring were fin clipped to distinguish between strains, by a small incision in the upper or lower section of the tail fin.

Fish were allowed to recover overnight and testing started the next day. For seven consecutive days, feeding was performed by dropping appropriate sized pellets where the fish was able to perceive them. Feeding was stopped either when the fish had refused to eat three

Table 1
Point scores to grade feeding behaviour in fish after transfer to social isolation (reprinted from Physiology and Behaviour)

Points	Behaviour
0	Fish does not respond to food
1	Fish eats only pellets that falls directly in front, and does not move to take food
2	Fish moves more than one body length to take food, but returns to original position in aquarium between each food item
3	Fish moves continuously between food items and consumes all food presented

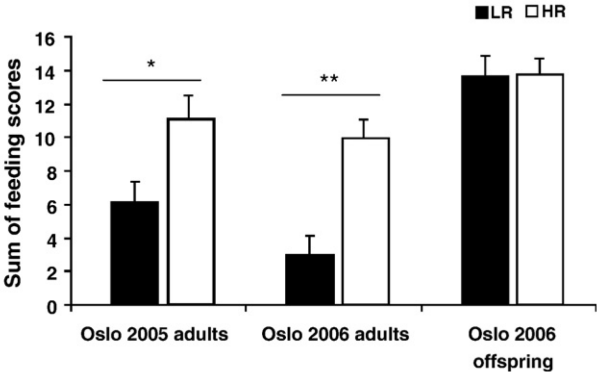


Fig. 1. Sum of feeding scores by 7th day following transfer to isolation in HR and LR fish. *$p<0.05$; **$p<0.01$. Data not available for non-transported fish.

pellets in a row or had consumed 3.0% of its body mass. Uneaten food was removed immediately after. Feeding behaviour was graded on each occasion according to the criteria listed in Table 1, in accordance with the method presented by Øverli et al. (2006). There is a high degree of individual variation in the speed of progress from low to high scores on this scale. Accumulated scores during the course of an experiment thus reflect how quickly fish resume normal feeding, and have previously been used as a measurement of risk-taking or boldness in rainbow trout (see Øverli et al., 2006, 2007).

After the 7th day of feeding, the dividing screen in each aquarium was removed and fish were allowed to interact until the conflict was resolved and dominant and subordinate fish could be clearly distinguished. For all the experimental sites the conflict did not last more than 3 h, fish were removed from the experimental tanks immediately after to avoid unnecessary distress. After initial fights, subordinate fish showed little or no aggression towards the dominant individual and usually took up a position facing a corner of the tank, while dominant fish normally moved around the whole territory frequently chasing the subordinate fish (Øverli et al., 1999; Pottinger and Carrick, 2001). After a stable dominance-subordination relationship was confirmed, fish were netted, anesthetized, and the final weight and strain of each winner and loser was recorded. A similar procedure was also carried out on F4 offspring in summer 2006. These fish were transferred from group rearing in 150 l holding tanks to feeding and dominance tests in 12 l observation aquaria (HR: $n=15$, LR: $n=15$), or subjected to an acute stress test (see below).

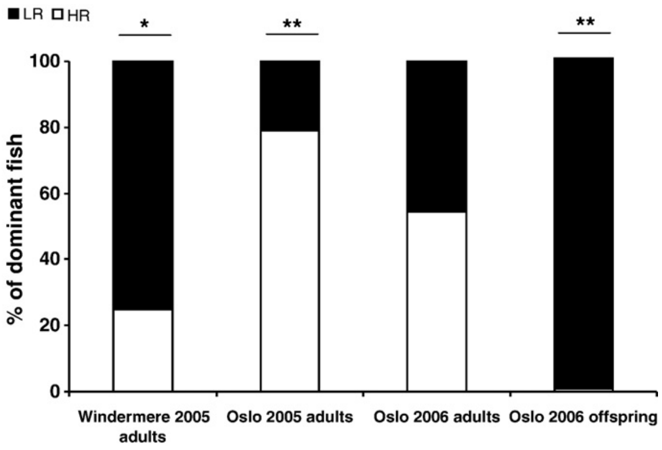


Fig. 2. Percentage of LR and HR fish becoming dominant in size-matched pairs on the 4 sampling points. *$p<0.05$, **$p<0.01$.

Table 2
Mean and S.E.M. post-stress plasma cortisol concentrations of LR and HR rainbow trout, p-value from the two-sample test

	Plasma cortisol (ng/ml)		*p*-value
	LR	HR	
Original rearing site (Windermere, UK)	58.3±9.5	153.1±25.0	0.003
Immediately after transport (Oslo, Norway 2005) adult fish	60.6±7.3	128.0±16.0	0.001
G4 Offspring, (Oslo, Norway 2006) juvenile fish	37.4±6.7	70.1±12.0	0.034

The above procedures applied to tests carried out in Norway. In 2005 the Windermere laboratory was not equipped to carry out feeding tests in glass aquaria, so at this location adult F3 fish were assessed for the ability to gain dominance over a size-matched competitor, using 50 l PVC tanks. Size-matched fish from the two lines (16 pairs) were held in 50 l tanks for 7 days by which time all fish were feeding normally. They were then paired (HR, LR) by transfer to a new 50 l tank and the positions of the fish were noted after 3 h of interaction. Within each pair the identity of each fish was denoted by a panjetted alcian blue dye mark. The latter test was carried out to exclude the possibility that the ability to gain social dominance in HR fish is an age-dependent phenomenon, as previous studies were carried out on juvenile fish (Pottinger and Carrick, 2001).

Stress testing and hormone assays

For analysis of the plasma cortisol levels for transported fish, in July 2005 adults of the 3rd generation (HR $n=20$, LR $n=15$) were subjected to a confinement stress test (confinement in 12.5 l of water for 0.5 h) after 7 days of rearing in isolation. Upon sampling fish were anesthetized in 0.5 g/l MS-222, and a blood sample was collected from the caudal vasculature. At CEH Windermere, in October 2005, fifteen fish of each line were confined for 2 h in groups of three in a 25 l volume in polyproylene tanks receiving a constant flow of lake water. The fish were sedated in 2-phenoxyethanol (1:2000) and a 1.0 ml blood sample was collected from the Cuverian duct. Stress testing of offspring by individual confinement for 30 min in 0.45 l of running water (HR $n=10$, LR $n=10$) was carried out in August 2006. After separation of plasma, cortisol levels were quantified using a previously validated radioimmunoassay (Pottinger and Carrick, 2001) or (for fish sampled in Norway) a commercial enzyme linked immunosorbent assay kit (Neogen Corporation, Lexington, USA).

Statistical analysis

For the fish transported to Norway, strain differences in feeding scores as well as body mass loss were assessed with two-sample t-tests. Differences in social dominance were analysed by a sign test, calculating the likelihood that observed frequencies of dominant and subordinate fish in each strain represent a 1:1 ratio. Plasma cortisol concentrations in LR and HR fish were compared using a two-sample t-test for each sampling point.

All the procedures were carried out in accordance with the welfare regulations for each country and experimental site; in general, unnecessary distress was avoided.

Results

Risk-taking

As shown in Fig. 1, HR fish gained higher feeding scores than LR fish immediately after transport, ($t=2.59$, $p=0.013$) and 1 year later ($t=4.24$, $p=0.001$). In 4th generation offspring of transported fish, LR and HR fish did not differ with respect to this trait ($t=0.50$, $p=0.62$).

Social dominance

As expected from previous reports (Pottinger and Carrick, 2001), see Fig. 2; in Windermere LR fish typically became dominant over HR fish

(LR dominant in 12 of 16 pairs, $p=0.03$). Immediately after transport, HR fish tended to dominate over LR fish (LR dominant in 4 of 19 pairs, $p=0.001$). The following year, there was no significant difference in the proportion of transported LR and HR becoming dominant (LR dominant in 5 of 11 pairs, $p=1.00$). In offspring of transported fish the original pattern was reinstated, with LR fish becoming dominant in all pairwise encounters (15 of 15 pairs, $p<0.001$).

Body mass loss in transported fish

Among adult fish tested in Norway, on average, HR fish lost almost twice as much body mass due to stress and food deprivation in connection with transport than did LR fish. ($t=3.43$ $p=0.003$). Mean body mass of the HR group fell from 458.38±27.91 g before transport to 403.57±23.33 g after transport to Norway. The equivalent figures for LR fish were 426.39±18.74 g and 401.37±19.34 g respectively. Body mass loss was also less severe among those few HR fish that lost pairwise contests ($n=4$) than among HR winners ($n=13$; $t=4.17$, $p=0.014$). This pattern was not evident in the LR strain, as LR winners and losers did not differ in body mass loss ($n=19$; $t=0.74$ $p>0.05$).

Post-stress plasma cortisol concentrations

Table 2 shows post-stress plasma cortisol concentrations (mean±S.E.M.) in transported and non-transported adult LR and HR fish, and in 4th generation offspring. There was a highly significant difference in post-stress plasma cortisol in non-transported fish, with LR fish showing significantly lower levels ($t=3.52$; $p=0.003$). Transported fish maintained this typical divergence in post-stress plasma cortisol ($t=3.74$; $p=0.001$). The offspring of transported fish also showed strain distinctive post-stress plasma cortisol concentrations (c.f. Table 2, $t=2.34$; $p=0.03$) as well.

Discussion

These results demonstrate a hitherto undisclosed level of behavioural flexibility in selected lines of rainbow trout that serve as a comparative model for the study of coping strategies. Previous studies on these lines and on non-selected strains of rainbow trout have revealed distinct behavioural profiles consistent not only over time, but also across different contexts, similar to the results of studies on proactive and reactive mammals (Øverli et al., 2005; Schjolden et al., 2005a and b; Schjolden and Winberg, 2007). These physiological and behavioural characteristics of low stress responsive (LR) and high stress responsive (HR) rainbow trout have been conserved over at least three generations (Pottinger and Carrick 2001; Øverli et al., 2005).

An unexpected change in behaviour occurred in the HR and LR strains following a prolonged stressful experience (transfer of the fish from the UK to Norway). Previously, LR-trout trout have typically been characterized as taking greater risks when feeding in a novel environment (equates to boldness, but see Øverli et al., 2007) and becoming dominant in competitive pairwise interactions with size-matched HR-trout (Pottinger and Carrick, 2001, Øverli et al., 2002a and 2005, Schjolden et al., 2005a). This set of behavioural differences was present in adult fish of the third generation kept at the original rearing site. However, these traits were reversed immediately after transport to Norway, in which fish experienced a 7 day period of starvation. After transport HR fish not only resumed feeding sooner after transfer to the novel environment, but also won more pairwise fights than did LR fish.

A clue as to a possible mechanistic explanation for the switch in traits may be found in the observation that HR fish lost a greater proportion of their body mass during the transport period than did LR fish. Furthermore, the few HR fish that became subordinate after transport were characterised by having lost relatively little body mass during transport. It is well established that individuals become bolder and more aggressive in competition for resources when their need for such resources is high (Dugatkin and Ohlsen 1990; Morrell et al., 2005; Frost et al., 2007). Thus the atypical behaviour of HR fish immediately after transport could be due to experiencing a high degree of hunger. Comparable results to those reported here were described by Carere et al. (2005), who found that food deprivation modulates the expression of phenotypic traits (namely begging and aggression) in lines of great tit (*Parus major*) selected for high and low exploration speed and aggression.

By 1 year after transport when all the fish had recovered from transport stress, inversion of the established behavioural profiles of the two lines was still evident. HR fish still took greater risks to feed in a novel environment, although individuals of the two strains were equally likely to win pairwise contests. It is possible that a long-term effect associated with the body mass loss that occurred during transport continued to influence the behaviour of the HR fish in pairwise fights. Growth rates between 2005 and 2006 (unpublished data) were similar in the two strains but perhaps HR fish retained a metabolic “debt” that they were unable to discharge in their group holding tanks. In the 4th generation of the two lines no differences between lines were evident in risk-taking during feeding, but LR fish now won all the paired contests. It seems that the ability to win pairwise fights is an inherited trait in rainbow trout. The situation with respect to risk-taking is more complicated and harder to explain; although there is a tendency for LR fish to feed more and sooner than HR fish in generation 4, this difference is not significant.

These changes in behaviour occurred without any associated change in a key physiological component of the coping styles, divergent post-stress plasma cortisol levels, which were maintained throughout the study period. This suggests a degree of plasticity in the behavioural aspects of coping style in fish that is independent of hypothalamus-pituitary-interrenal function. A range of other elements of the neuro-endocrine system (hormones, neuropeptides and transmitters) are also involved in the regulation of these behaviours (Winberg and Nilsson 1993; Johnsson and Bjornsson, 1994; Johansson et al., 2005; Clements et al., 2003; Volkoff, 2006; Carpenter et al., 2007) but further work will be needed to assess their role in the reversal of behavioural traits observed in the present study. In conclusion, data presented in this paper suggest that in rainbow trout genetic differences determine social position only in early life. Superimposed on this template, some behavioural components of stress coping style, including resource holding potential and risk-taking, can subsequently be modified by experience.

References

Bell, A.M., Stamps, J.A., 2004. Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. Anim. Behav. 68, 1339–1348.

Bell, A.M., Sih, A., 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecol. Lett. 10, 828–834.

Bell, A.M., Backstrom, T., Huntingford, F.A., Pottinger, T.G., Winberg, S., 2007. Variable neuroendocrine responses to ecologically-relevant challenges in sticklebacks. Physiol. Behav. 91, 15–25.

Bolnick, D.I., Svanback, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., Forister, M.L., 2003. The ecology of individuals: incidence and implications of individual specialization. Am. Nat. 161, 1–28.

Burmeister, S.S., 2007. Genomic responses to behavioral interactions in an African cichlid fish: mechanisms and evolutionary implications. Brain Behav. Evol. 70, 247–256.

Burmeister, S.S., Jarvis, E.D., Fernald, R.D., 2005. Rapid behavioral and genomic responses to social opportunity. Plos Biol. 3, 1996–2004.

Carere, C., Drent, P.J., Koolhaas, J.M., Groothuis, T.G.G., 2005. Epigenetic effects on personality traits: early food provisioning and sibling competition. Behaviour 142, 1329–1355.

Carpenter, R.E., Watt, M.J., Forster, G.L., Øverli, Ø., Bockholt, C., Renner, K.I., Summers, C.H., 2007. Corticotropin releasing factor induces anxiogenic locomotion in trout and alters serotonergic and dopaminergic activity. Horm. Behav. 52, 600–611.

Cavigelli, S.A., 2005. Animal personality and health. Behaviour 142, 1223–1244.

Clements, S., Moore, F.L., Schreck, C.B., 2003. Evidence that acute serotonergic activation potentiates the locomotor-stimulating effects of corticotropin-releasing hormone in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Horm. Behav. 43, 214–221.

Damsgard, B., Dill, L.M., 1998. Risk-taking behavior in weight-compensating coho salmon, *Oncorhynchus kisutch*. Behav. Ecol. 9, 26–32.

DiBattista, J.D., Anisman, H., Whitehead, M., Gilmour, K.M., 2005. The effects of cortisol administration on social status and brain monoaminergic activity in rainbow trout *Oncorhynchus mykiss*. J. Exp. Biol. 208, 2707–2718.

Dugatkin, L.A., Ohlsen, S.R., 1990. Contrasting asymmetries in value expectation and resource holding power: effects on attack behaviour and dominance in the pumpkinseed sunfish, *Lepomis gibbosus*. Anim. Behav. 39, 802–804.

Frost, A., Winrow-Giffen, A., Ashley, P., Sneddon, L., 2007. Plasticity in animal personality traits: does prior experience alter the degree of boldness? P. Roy Soc. B. Bio. 274, 333–339.

Gregory, T.R., Wood, C.M., 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. Physiol. Biochem. Zool. 72, 286–295.

Huntingford, F., 1976. The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. Anim. Behav. 24, 245–260.

Huntingford, F., Adams, C., 2005. Behavioural syndromes in farmed fish: implications for production and welfare. Behaviour 142, 1207–1221.

Johnsson, J.I., Bjornsson, B.T., 1994. Growth hormone increases growth rate, appetite and dominance in juvenile Rainbow trout, *Oncorhynchus mykiss*. Anim. Behav. 48, 177–186.

Johnsson, J.I., Jonsson, E., Bjornsson, B.T., 1996. Dominance, nutritional state, and growth hormone levels in Rainbow trout (*Oncorhynchus mykiss*). Horm. Behav. 30, 13–21.

Johansson, V., Winberg, S., Bjornsson, B.T., 2005. Growth hormone-induced stimulation of swimming and feeding behaviour of rainbow trout is abolished by the D-1 dopamine antagonist SCH23390. Gen. Comp. Endocrinol. 141, 58–65.

Koolhaas, J.M., Korte, S.M., de Boer, S.F., Van Der Vegt, B.J., van Reenen, C.G., Hopster, H., de Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935.

Koolhaas, J.M., de Boer, S.F., Buwalda, B., van Reenen, K., 2007. Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. Brain Behav. Evol. 70, 218–226.

Korte, S.M., Koolhaas, J.M., Wingfield, J.C., Mcewen, B.S., 2005. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. Neurosci. Biobehav. Rev. 29, 3–38.

Miller, G.M., Bendor, J., Tiefenbacher, S., Yang, H., Novak, M.A., Madras, B.K., 2004. A mu-opioid receptor single nucleotide polymorphism in Rhesus monkey: association with stress response and aggression. Mol. Psychiatry 9, 99–108.

Morrell, L.J., Lindstrom, J., Ruxton, G.D., 2005. Why are small males aggressive? P. Roy Soc. B. Bio. 272, 1235–1241.

Øverli, Ø., Harris, C.A., Winberg, S., 1999. Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in Rainbow trout. Brain Behav. Evol. 54, 263–275.

Øverli, Ø., Pottinger, T.G., Carrick, T.R., Øverli, E., Winberg, S., 2002a. Differences in behaviour between Rainbow trout selected for high-and low-stress responsiveness. J. Exp. Biol. 205, 391–395.

Øverli, Ø., Kotzian, S., Winberg, S., 2002b. Effects of cortisol on aggression and locomotor activity in Rainbow trout. Horm. Behav. 42, 53–61.

Øverli, Ø., Korzan, W.J., Larson, E.T., Winberg, S., Lepage, O., Pottinger, T.G., Renner, K.J., Summers, C.H., 2004. Behavioral and neuroendocrine correlates of displaced aggression in trout. Horm. Behav. 45, 324–329.

Øverli, Ø., Winberg, S., Pottinger, T.G., 2005. Behavioral and neuroendocrine correlates of selection for stress responsiveness in Rainbow trout-a review. Integr. Comp. Biol. 45, 463–474.

Øverli, Ø., Sørensen, C., Nilsson, G.E., 2006. Behavioral indicators of stress-coping style in Rainbow trout: do males and females react differently to novelty? Physiol. Behav. 87, 506–512.

Øverli, Ø., Sørensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W., Summers, C.H., Nilsson, G.E., 2007. Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. Neurosci. Biobehav. Rev. 31, 396–412.

Poirier, R., Jacquot, S., Vaillend, C., Soutthiphong, A.A., Libbey, M., Davis, S., Laroche, S., Hanauer, A., Welzl, H., Lipp, H.P., Wolfer, D.P., 2007. Deletion of the Coffin-Lowry syndrome gene RSK2 in mice is associated with impaired spatial learning and reduced control of exploratory behavior. Behav. Genet. 37, 31–50.

Pottinger, T.G., Carrick, T.R., 1999. Modification of the plasma cortisol response to stress in Rainbow trout by selective breeding. Gen. Comp. Endocr. 116, 122–132.

Pottinger, T.G., Carrick, T.R., 2001. Stress responsiveness affects dominant-subordinate relationships in Rainbow trout. Horm. Behav. 40, 419–427.

Salonen, A., Peuhkuri, N., 2006. The effect of captive breeding on aggressive behaviour of European grayling, *Thymallus thymallus*, in different contexts. Anim. Behav. 72, 819–825.

Schjolden, J., Backstrom, T., Pulman, K.G.T., Pottinger, T.G., Winberg, S., 2005a. Divergence in behavioural responses to stress in two strains of Rainbow trout (*Oncorhynchus mykiss*) with contrasting stress responsiveness. Horm. Behav. 48, 537–544.

Schjolden, J., Stoskhus, A., Winberg, S., 2005b. Does individual variation in stress responses and agonistic behavior reflect divergent stress coping strategies in juvenile Rainbow trout? Physiol. Biochem. Zool. 78, 715–723.

Schjolden, J., Winberg, S., 2007. Genetically determined variation in stress responsiveness in Rainbow trout: behavior and neurobiology. Brain Behav. Evol. 70, 227–238.

Serretti, A., Calati, R., Mandelli, L., De Ronchi, D., 2006. Serotonin transporter gene variants and behavior: a comprehensive review. Curr. Drug Targets 7, 1659–1669.

Sih, A., Bell, A.M., Johnson, J.C., Ziemba, R.E., 2004. Behavioral syndromes: an integrative overview. Q. Rev. Biol. 79, 241–277.

Sundstrom, L.F., Petersson, E., Hojesjo, J., Johnsson, J.I., Jarvi, T., 2004. Hatchery selection promotes boldness in newly hatched brown trout (Salmo trutta): implications for dominance. Behav. Ecol. 15, 192–198.

Uhart, M., Mccaul, M.E., Oswald, L.M., Choi, L., Wand, G.S., 2004. GABRA6 gene polymorphism and an attenuated stress response. Mol. Psychiatry 9, 998–1006.

van Oers, K., de Jong, G., van Noordwijk, A.J., Kempenaers, B., Drent, P.J., 2005. Contribution of genetics to the study of animal personalities: a review of case studies. Behaviour 142, 1185–1206.

Volkoff, H., 2006. From goldfish to cod: The neuroendocrine control of food intake in fish. J. Exp. Zool. A. Comp. Exp. Biol. 305A, 190.

Watt, M.J., Forster, G.L., Korzan, W.J., Renner, K.J., Summers, C.H., 2007. Rapid neuroendocrine responses evoked at the onset of social challenge. Physiol. Behav. 90, 567–575.

Wilson, A.D.M., McLaughlin, R.L., 2007. Behavioural syndromes in brook charr, Salvelinus fontinalis: prey-search in the field corresponds with space use in novel laboratory situations. Anim. Behav. 74, 689–698.

Winberg, S., Nilsson, G.E., 1993. Roles of brain monoamine neurotransmitters in agonistic behavior and stress reactions, with particular reference to fish. Comp. Biochem. Physiol. C, Pharmacol. Toxicol. Endocrinol. 106, 597–614.