Stress Coping Strategies in Brown Trout (Salmo Trutta): Ecological Significance and Effects of Sea-Ranching

DANIEL BRELIN
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Abstract

Two distinct stress coping strategies, proactive and reactive, have been stated in various animal studies, each associated with a set of behavioural and physiological characteristics. In a given challenging situation, proactive animals show more aggression, a higher general activity and a predominant sympathetic reaction. In contrast, the reactive copers respond more with immobility and avoidance, and a predominant parasympathetic/hypothalamic activation. This divergence in coping has also been indicated in salmonid fish. Interestingly, many of the differences reported between sea-ranched and wild fish resembles characteristics that differentiate proactive and reactive copers. In the present thesis it is shown that individuals with divergent stress coping styles are identifiable in several brown trout (Salmo trutta) populations. Further, the results show that the distribution of individuals displaying these distinct stress coping strategies differs between populations. This strongly indicates that these traits are heritable and that the variation in selection regime in the native rivers influences these traits. In addition, the results show that populations with hatchery origin are biased towards having higher frequencies of trout displaying a proactive style than populations having wild origin. Also, even though the frequency of early sexual maturation, known as a viable alternative life history in salmonids, differs between populations of brown trout, no link between stress coping strategy and early sexual maturation were found. However, this thesis show that maternal contribution, in the form of egg size, is of major importance whether the progeny will sexually mature early and that it also might be of importance for stress coping strategy. Further, correlations of traits commonly associated with stress coping strategies and behavioural syndromes across context and over time is investigated. The results show that individuals with a strong sympathetic reactivity are more prone to change their behaviour than others.

Keywords: Stress-coping-strategies, Animal-personalities, Behavioral-syndromes, Brown trout, Salmo trutta, Catecholamines, Sexual maturation, Hatchery, Phenotype, Learning

Daniel Brelin, Department of Physiology and Developmental Biology, Comparative Physiology, Norbyvägen 18A, Uppsala University, SE-75236 Uppsala, Sweden

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</tr>
<tr>
<td>A</td>
<td>Adrenaline</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>CA</td>
<td>Catecholamines</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-release hormone</td>
</tr>
<tr>
<td>CS</td>
<td>Conditioned stimuli</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DHBA</td>
<td>3, 4 Dihydroxybenzylamine</td>
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<tr>
<td>EC</td>
<td>Electrochemical</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>HCat</td>
<td>High catecholaminergic activity</td>
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<tr>
<td>HR</td>
<td>High responding</td>
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<tr>
<td>HPA</td>
<td>Hypothalamus-pituitary-adrenocortical</td>
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<tr>
<td>HPI</td>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>HSC</td>
<td>Hypothalimic-sympathetic-chromaffin</td>
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<tr>
<td>LCat</td>
<td>Low catecholaminergic activity</td>
</tr>
<tr>
<td>LR</td>
<td>Low responding</td>
</tr>
<tr>
<td>NA</td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>PCA</td>
<td>Perchloric acid</td>
</tr>
<tr>
<td>PIT</td>
<td>Passive integrated transponder</td>
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<tr>
<td>SGI</td>
<td>Somato gonadal index</td>
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<tr>
<td>SGR</td>
<td>Specific growth rate</td>
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<td>SOS</td>
<td>Sodium octyl sulphate</td>
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<td>US</td>
<td>Unconditioned stimuli</td>
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INTRODUCTION

STRESS, STRESSORS AND STRESS RESPONSE

The word “stress” entered the English language in the 14th century. It is short for 
(destresse) dating back to the 13th century. One meaning of 
destresse is pain or suffering affecting the body, a bodily part, or the mind 
(Merriam-Webster Online). The word ”stress” is commonly used in our daily 
lives, but its meaning can be interpreted in various ways. The concept of 
stress in a wider sense could be described as an animal’s physiological re-
sponse to an unfavourable change in its environment (Henry and Stephens, 
1977) but many, including myself, favours one of Selye’s original defini-
tions of stress formulated as “a non-specific response of the body to any 
demand made on it” (Selye, 1952). However, this definition is only, at its 
best, correct for the autonomic part of the stress response since behavioural 
stress responses are known to be context-specific (Benus et al., 1987). Even 
though the concept of stress is quite old it is only until recently one started to 
distinguish between the stress response itself and what causes the stress 
response (Gold et al., 1988; Selye, 1936; von Holst, 1998). Today, all causes 
of stress are normally described as stressors, namely as any biotic or abiotic 
stimuli, whether real or not, that disturb the homeostatic equilibrium of an 
individual. Another conception of stress is one associated with individual 
costs. Allostasis is a term used for the maintenance of stability through 
changes within an organism. Allostatic load refers to the cost and allostatic 
overload to when the cost becomes too high and needs serious attention 
(Korte et al., 2005; McEwen and Wingfield, 2003). However, the concept of 
a physiological homeostasis is much older; it was coined by the “father of 
physiology” Claude Bernard (Bernard, 1865). Even though the definition of 
stress has been debated for almost a century it remains a current issue. It 
might be as Levine (1989) stated; ”I am not certain whether one who undertakes 
this task (of defining the concept of stress) either has an enormous ego, 
is immeasurably stupid, or is totally mad.”. In order to keep things as un-
complicated as possible the definition of stress used in this thesis is as 
physiological and behavioural responses induced by stressors. In the follow-
ing paragraphs I will describe and discuss the stress response in terms of 
physiological stress responses and behavioural stress responses. There is an
immense amount of literature written on stress and only issues of high general interest (in my personal point of view) or with specific relevance to attached papers will be addressed here in greater detail.

The physiological stress response is mainly regulated by two endocrine systems. Both systems are of major importance with specific purposes. When a fish is challenged it leads to the immediate activation of the hypothalamic-sympathetic-chromaffin-cell (HSC) axis, and this response are essentially similar in all vertebrate taxa (Bonga, 1997; Henry, 1993). The mechanisms of this axis, described in Figure 1, lead to a rapid increase in circulatory...
catecholamines (CA)(Box 1)(Perry and Bernier, 1999). The HSC axis is commonly referred to as the system handling the acute stress response, but a more correct term is the acute humoral adrenergic stress response (Perry and Bernier, 1999). In short, CA enhances oxygen transport and mobilizes energy substrates to appropriate locations in the body (Bonga, 1997; Thomas and Perry, 1992). Quantifying levels of CA in the blood of challenged animals gives a useful measure of the individual sympathetic reactivity (Sapolsky, 1994).

The fast activation of the HSC-axis is also connected to the shut down of several, long term, life sustaining processes (e.g. feeding, digestion, tissue repair, immune functionality etc) and as such it must be regulated (Pickering, 1990; Sapolsky et al., 2000). The slower (still within minutes) onset of the hypothalamic-pituitary-adrenocortical (HPA) axis is considered one of the main regulatory functions for restoring homeostasis in vertebrates (Bonga, 1997). In fish, this axis is referred to as the hypothalamus-pituitary-interrenal (HPI) axis and is also primarily controlled by the hypothalamus with hormonal secretion, at least partly, from the head kidneys (Bonga, 1997; Carragher and Sumpter, 1990; Young et al., 1982). Actually, the control of cortisol secretion in fish is very complex (the main mechanism is described in Figure 1) and many neuroendocrine peptides are involved (Balment et al., 2006; Lovejoy and Balment, 1999). The roles of cortisol are diverse but mainly include mineralocorticoid and glucocorticoid functions (c.f. Box 1). The elevation of circulatory cortisol is probably the most commonly used indicator of stress in fish (Barton et al., 1987; Mommsen et al., 1999; Pickering, 1993; Pottinger, 2006; Winberg et al., 2007; Øverli et al., 2002a). Consequently, quantifying circulatory cortisol levels in fish who have been exposed to a stressor gives an estimate of the activity in the HPI axis (Barry et al., 1995; Mommsen et al., 1999). Typically, after acute stress, it takes at least one hour before the cortisol level returns to ordinary (<5 ng/ml in rainbow trout (Oncorhyncus mykiss)) (Bonga, 1997; Ruane et al., 1999). However, there are also circadian and circannual rhythms in circulatory levels of cortisol to consider when sampling fish. It has even been shown that daily peaks in cortisol are in the same range as typically stressed fish (Laidley and Leatherland, 1988). So while the prime functions of the HSC axis is short term and aims to aid individual survival the HPA axis primary inhibits several functions of the HSC axis and restores homeostasis. However, chronic elevation of cortisol is in many aspects also lethal and increased circulatory levels of cortisol have been linked to behavioural changes in fish, e.g. inhibitory on feeding, activity and aggression (Øverli et al., 2002a). When using fish, or any other ectothermic animal, in experimental set-ups one have to consider that the time-course of the physiological stress response is also affected by the surrounding temperature, as well as if the fish has been fed or not and certainly the nature of the stressor applied (Bonga, 1997; Mommsen
et al., 1999). Thus, great considerations should be taken when choosing methods for quantifying both physiological and behavioural stress responses (Beitinger and McCauley, 1990).

Box 1. Information about Catecholamines and Cortisol.

**Catecholamines** – are all amine derivates of catechol (2-hydroxyphenol), which act as hormones or neurotransmitters. They include;

<> **Adrenaline** (US; epinephrine) – which acts on specific adrenergic receptors in different tissues and has many effects e.g. speeding up heart beat and speeding up break down of glycogen to glucose in muscle tissue. It is also an important neurotransmitter and is secreted in nerve endings through out the sympathetic nervous system.

<> **Noradrenaline** (US; norepinephrine) – which is closely related to adrenaline with similar structure and effects. However, it also acts as an important neurotransmitter secreted by sympathetic nerve endings in various internal organs.

<> **Dopamine** – which is mainly secreted by the adrenal medulla and works as a neurotransmitter in the central nervous system.

**Corticoids** – Steroid hormones secreted by the cortex of the adrenal glands, some of which are hormones. One such glucocorticosteroid is;

<> **Cortisol** – Or hydrocortisone which is very similar to cortisone in both structure and properties. As such, it is an immunosuppressant and has effects on carbohydrate metabolism. Specifically it is known to be inhibitory to both aggression and activity.

Information on hormones from Bonga, (1997), Lawrence, (2000) and pictures from Wikipedia Commons.
Before I describe and discuss the behavioural stress responses I believe it to be of importance to point out that all behaviours are preceded by neurological and neuroendocrine processes (Iwama, 1997; Øverli et al., 2007). Thus, the variations in behavioural stress responses are, to some extent, derived from the variation in neuroendocrine activity. A wide variety of biological, chemical and even physical factors have been identified as stressors causing alterations in behaviours of animals (Iwama, 1997). Consequently, fish respond with various behaviours when being challenged and many of them are context specific (Beitinger, 1990; Beitinger and McCauley, 1990). Looking from a pure functional perspective the main purpose of the behaviour is to reduce the amount of stress. However, there are almost always alternative choices in the behavioural response. As an example, if your territory is intruded upon by a conspecific one can either attack or escape. In either case the cause of stress is effectively removed. There is also the option of doing nothing or, as it probably should be interpreted, to wait and see what happens. The prior is referred to as a flight/fright response and the latter a freeze/hide response (Cannon, 1915; Cannon, 1936). Like most other vertebrates fish possess a set of adaptive behavioural strategies which have evolved in order for them to cope with adverse conditions, or stressors. For example, changed patterns of swimming speed, direction and activity are a common response to numerous stressors (Juell and Fosseidengen, 2004; Schjolden et al., 2005a). A change in swimming behaviour has also been shown in rainbow trout when transferred to an unfamiliar/novel environment (Schjolden et al., 2005a) and in crusian carp (Carrasius carrasius) when the oxygen saturation decreases (hypoxia) in the current environment (Nilsson et al., 1993). When attacked by a con-specific fish common behavioural responses include fleeing, hiding or taking up submissive postures or even changing body/eye colour (Höglund et al., 2000; O'Connor et al., 2000; Suter and Huntingford, 2002). Further, fish may, after successfully escaping an attack from a predator, respond with freezing, hiding, changing colour or shoaling (Bannerot, 1983; Brown and Warburton, 1999a; Brown and Warburton, 1999b; Goodey and Liley, 1986; Parrish, 1992). Stressors, such as forced transfer and predator encounters, are also known to inhibit feeding behaviour (Blake and Hart, 1993; Carr, 2002; Øverli et al., 2002b). Fish may also avoid areas where they were earlier attacked and it has been shown that northern pike (Esox lucius) and carp (Cyprinus caprio) avoid bait that is associated with traumatic experiences (Beukema, 1970; Beukema and Devos, 1974; Lima, 1998). Another more specific behavioural response, evoked by tissue damage, can be how rainbow trout, injected with acid, rub the injected area against firm surrounding objects (Sneddon et al., 2003).

Another important aspect to consider is that intra- and inter individual differences in the physiological and behavioural stress responses are indispensable pieces that build up “individuality”. Challenges really pin point the essence
of personality by bringing individual qualities to their peaks. With that in mind, it is important to understand the mechanisms behind behavioural and physiological stress responses to grasp the meaning of everything involving individuality in animals. The mechanisms of the stress response are also the origins of stress coping strategies which will be discussed together will other aspects of animal personality in the next chapter.

INTER INDIVIDUAL VARIATION IN PHYSIOLOGY AND BEHAVIOUR

In this chapter I describe and discuss inter individual or intra population variation in terms of stress coping strategies, animal personalities and behavioural syndromes. Even though similar in many aspects there are also certain distinctions between these subjects of inter-individual variation. Finally I describe and discuss life history phenotypes in terms of early sexual maturation in salmonids.

STRESS COPING STRATEGIES

Stress coping strategies describe the phenomena of variation in how individuals in a group/population respond, physiologically and behaviorally, to specific challenges. Coping has been defined as the behavioural and physiological efforts to master a situation and it may become a strategy when the response is consistent over time and/or across context (Koolhaas et al., 1999). It should be noted that Henry (1977) probably was the first researcher to make the distinction between active and passive stress coping (Henry and Stephens, 1977). The coping strategies, called proactive and reactive, are associated with a set of physiological and behavioural characteristics (Table 1.). In general, the proactive copers are active, aggressive and bold (a response often referred to as a fight/flight reaction (Cannon, 1915; Koolhaas et al., 1999)) compared to reactive copers who often respond with avoidance, immobility, shyness and non aggressive behavior when challenged (a response often referred to as a conservation/withdrawal reaction (Koolhaas et al., 1999)). On the physiological side proactive copers show high HSC axis activity and low HPA/HPI axis activity compared to reactive copers (Koolhaas et al., 1999). This differences are typically demonstrated with the proactive copers showing higher plasma levels of catecholamines (noradrenaline, adrenaline, and dopamine) and lower levels of plasma cortisol compared to reactive copers following upon a challenge (Koolhaas et al., 1999). Further, it has been shown that active and aggressive individuals have higher plasma concentrations of testosterone (Deruiter et al., 1993; Koolhaas...
et al., 1980). A characteristic which later also has been ascribed to proactive copers (Koolhaas et al., 1999).

Table 1. Summary of the behavioural and physiological differences between proactive and reactive male rats and mice (modified from Koolhaas et al., 1999).

<table>
<thead>
<tr>
<th>Reactive</th>
<th>Behavioural Characteristics</th>
<th>Proactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Attack latency</td>
<td>Low</td>
</tr>
<tr>
<td>Low</td>
<td>Active avoidance</td>
<td>High</td>
</tr>
<tr>
<td>Low</td>
<td>Routine formation</td>
<td>High</td>
</tr>
<tr>
<td>High</td>
<td>Conditioned immobility</td>
<td>Low</td>
</tr>
<tr>
<td>High</td>
<td>Flexibility</td>
<td>Low</td>
</tr>
</tbody>
</table>

**Physiological Characteristics**

<table>
<thead>
<tr>
<th>Reactive</th>
<th>Physiological Characteristics</th>
<th>Proactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>HPA axis reactivity</td>
<td>Low</td>
</tr>
<tr>
<td>Low</td>
<td>Sympathetic reactivity</td>
<td>High</td>
</tr>
<tr>
<td>High</td>
<td>Parasympathetic reactivity</td>
<td>Low</td>
</tr>
<tr>
<td>Low</td>
<td>Testosterone activity</td>
<td>High</td>
</tr>
</tbody>
</table>

HPA; hypothalamic-pituitary-adrenocortical axis, the neuroendocrine axis regulating adrenocortical glucocorticoid production and release. Teleost fish have the homologue HPI axis; hypothalamic-pituitary-interrenal axis.

This kind of intra-population divergence in behavioural and physiological traits has been observed in various animal studies, predominantly in mammals (Benus et al., 1991, Bohus et al., 1987, Bolhuis et al., 2005, Dingemanse et al., 2002, Koolhaas et al., 1999, Korte et al., 2005). Sometimes quantified variables follow a continuum whereas in other studies a bimodal distribution is apparent. Several of these traits have shown to be heritable and even to affect fitness (Dingemanse et al., 2002, Drent et al., 2003, Koolhaas et al., 1999). A fundamental difference between proactive and reactive copers in birds and mammals seems to be to what extent the behaviour is guided by environmental stimuli (Benus et al., 1990, Benus et al., 1987). In general, proactive, aggressive animals easily develop routines and a rather rigid type of behaviour, whereas reactive, non-aggressive animals, in contrast are more flexible (Benus et al., 1990, Benus et al., 1987, Koolhaas et al., 1999). In fact, in feral populations of birds the fitness of different coping styles appears to depend on the stability of the environment in terms of social structure and food availability (Koolhaas et al., 1999).

Some information on whether different individual stress coping strategies have evolved in vertebrates other than mammals and birds is available. Among teleost fish consistent behavioural patterns have been observed in cichlids (Budaev et al., 1999, Francis, 1990), and recent results suggest that a similar intra-population divergence in both behavioural and physiological traits, i.e. coping strategies, also exists in salmonids. Rainbow trout (Onkorhynchus mykiss) strains selectively bread on high (HR trout) and low (LR trout) post stress plasma cortisol levels, show differences in behaviour and physiology that appear to reflect divergent stress coping strategies, the LR
strain displaying a more proactive and the HR strain a more reactive stress coping strategy (Pottinger and Carrick, 1999; Schjolden et al., 2005a; Øverli et al., 2002b). Divergent stress coping strategies have also been identified in unselected strains of juvenile rainbow trout (Larson et al., 2004; Schjolden et al., 2005b; Øverli et al., 2004; Øverli et al., 2002a).

ANIMAL PERSONALITIES AND BEHAVIOURAL SYNDROMES

The concept of stress coping strategies focuses mainly on stress and displays the prospect of a bimodal pattern of animal personalities. However, there are many other studies with a similar approach which do not refer to it as stress coping strategies but animal personalities, behavioural types, individualities or temperament (Koolhaas et al., 1999; Reale et al., 2007; Sih et al., 2004b; Øverli et al., 2007). Other studies which look into a more specific trait often refer to this phenomenon as continuums or gradients. One such famous continuum is the bold and shy continuum (Alvarez and Bell, 2007; Brown et al., 2005; Coleman and Wilson, 1998; Dall, 2004; Frost et al., 2007; Huntingford, 1976; Korte et al., 2005; Sneddon, 2003; Wilson et al., 1994a). Further, what has come to be called behavioural syndromes also work with correlated behavioural traits. Even though the concepts of animal personalities and behavioural syndromes are similar in many respects, they approach the problem of correlated traits from fundamentally different perspectives (Fig. 2).

The research on behavioural syndromes has made a significant effort to understand the evolution of correlated sets of traits across widely divergent situations (Sih et al., 2004b). Thus, in some aspects it is important to make a distinction between behavioural syndromes and animal personalities (Bell, 2007). Nevertheless, evolutionary theoreticians find positively correlated traits peculiar since fixed traits would limit behavioural plasticity and are ultimately maladaptive in most evolutionary models (Dall et al., 2004; Ernande and Dieckmann, 2004; Neff and Sherman, 2004; Sih et al., 2004a).
However it seems that this variation in traits is important because it has evolved and more importantly it is somehow maintained in all these different animal taxa. Thus, selection does not form all individuals in a population to one homogenous group. Frequency dependent selection plays a major role in many evolutionary models, such as the maintenance and evolution of sex, host-parasite co-evolution, male reproductive strategies, polymorphisms, interspecific competition and speciation and it might also be important for maintaining variation in personal traits. However, examples of frequency-dependent selection are rare, especially when attempting to describe the genetic basis of the phenotype under selection (Fitzpatrick et al., 2007). Bürger et al. (2006) proposed a model and argued that very strong competition requires extremely strong assortment to induce population splitting. Individuals being average in studied characteristics do not suffer a fitness reduction from competition. Höjesjö et al. (2002) performed a combined laboratory–field experiment and noted that wild-caught juvenile brown trout could be split into three categories: dominants, subordinates and non-aggressive individuals of indeterminate status. All tested fish were released back into the stream and recaptured after 3 and 8 weeks. They found that dominant fish
grew faster than subordinates, but non-aggressive fish grew as fast as dominants. These results indicated that less aggressive individuals could be successful in heterogeneous natural habitats. Later Höjesjö et al. (2004) showed in a laboratory study that the growth rate of aggressive dominant individuals of newly emerged brown trout fry was reduced in relation to subordinates in a heterogeneous habitat. Thus, variation in environmental complexity may induce fluctuating selective pressures, maintaining behavioural variation in natural populations, allowing subordinate and dominant strategies to coexist. Consequently, mean differences between populations, or groups of animals, in quantified variables might imply that the frequency of animal personalities differs. One explanation for maintaining animal personality might be a simple positive feedback mechanism. Individuals possessing good prerequisites for being bold be-come better and better at the skills associated with boldness. Eventually it becomes relatively costly to act different then you are used to (Andrew Sih, personal communication). Therefore, bold individuals evolve more and more skills to be bold and shy individuals evolve their skills to be shy. Sih and Bell (2004) also suggests common neurological and/or neuroendocrine mechanisms for several different behaviours. Thus, a common neuroendocrine characteristic such as high sympathetic reactivity might predispose individuals to be more active, more aggressive and bolder, that way linking these different behaviours to each other.

**PRECOCIOUS SEXUAL MATURATION**

Early ("precocious") sexual maturation in males is considered a viable alternative reproductive tactic in many salmonid species (Fleming, 1996; Mecalfe, 1998; Thorpe, 1994; Thorpe et al., 1998). Instead of migrating out to the sea, as their anadromous conspecifics, precocious sexual mature males (also called sexually mature parr) remain in the river, undergo sexual maturation, and in the autumn compete with the larger anadromous males for access to females by adopting a “sneaker” spawning behaviour (Fleming, 1996). During spawning the precocious males (at least in Atlantic salmon, Salmo salar) hide both from anadromous males and the female, and upon egg release dart to the mating pair and releases their sperm (Fleming, 1996). The spawning success of precocious males is commonly quantified as number of matings and ultimately as percent of fertilized eggs (Fleming, 1996). It is well established that they can have considerable genetic contribution in natural populations (Fleming, 1996; Garant et al., 2003). For example, in brown trout (Salmo trutta) it has been shown that 10-57% of the males sexually mature as parr (Hutchings and Jones, 1998) and that they can fertilize up to 40% of the eggs in a population (Thomaz et al., 1997).

There are still exist major knowledge gaps in understanding the mechanisms controlling precocious sexual maturation in teleost fishes. In salmonids at least, the general consensus is that early sexual maturation results from an
interplay between both endogenous (genetic) and exogenous factors such as food availability and temperature. One common hypothesis is that the “decision” whether to mature precociously or not is determined at an early age, being a function of growth rate. This hypothesis proposes that those males having a higher growth rate during this critical window are more likely to undergo early sexual maturation, while those males showing a lower growth rate will undergo smoltification the following spring (Dellefors and Faremo, 1988; Fleming, 1996; Metcalfe, 1998; Metcalfe et al., 1995; Morgan and Metcalfe, 2001; Thorpe, 1994). Support for this hypothesis comes from the observation that the probability of precocious sexual maturation in brown trout is clearly related to individual condition one year prior to the actual spawning (Bohlin et al., 1994). Also, further evidence supporting the concept that early male sexual maturation is closely associated with fast growth at an early age comes from hatchery data. It is now well established that under favourable hatchery conditions, characterized by high growth rates, the incidence of precocious male maturation in both Atlantic salmon and brown trout is much higher than observed in the wild (Dellefors and Faremo, 1988; Thorpe, 1991; Thorpe, 1994). On the contrary, reduction in the proportion of sexually mature males is achieved by restricting food availability during the winter and spring before expected maturation (Reimers et al., 1993; Rowe and Thorpe, 1990; Thorpe et al., 1990). Additionally, other variables, for example photoperiod (Berrill et al., 2006), also affect the probability to sexually mature as parr, but it is hard to exclude that such factors indirectly influence specific growth rate. Thus, it seems that genetic thresholds, sensitive for status-dependent cues, decide when/if to sexually mature (Garant et al., 2003). At an individual level that means that sexual maturation starts when, and only when, the right conditions are reached at the right time. This threshold level in conditions may, due to genetic differences, vary between individuals and populations.

Salmonid species show differences in egg size, with semelparous species usually having larger eggs than iteroparous species (Einum and Fleming, 2007). Furthermore, anadromous salmonid females experiencing poor growth as juveniles, in their home river, will later, during sexual maturation, produce less and larger eggs whereas juvenile females growing well will later produce more but smaller eggs (Einum and Fleming, 1999; Jonsson et al., 1996; Roff, 2000). Sea-ranched Atlantic salmon females produce smaller eggs than wild females in response to their higher juvenile growth rate (Fleming et al., 1997), and Atlantic salmon juveniles originating from larger eggs tend to outgrow siblings from smaller eggs (Einum, 2003). Also, it is known that an increase in number of eggs produced will be compensated with a decrease in egg size (Brown, 1946; Einum and Fleming, 2000; Olofsson and Mosegaard, 1999). It has also been concluded that survival is higher in brown trout progeny derived from large eggs during natural conditions (Bagenal, 1969). The relation between egg size and juvenile size at
different ages seems, however, to be more complicated than previously presumed. In chinook salmon (*Onchorynchus tshawytscha*) egg size has no effect on alevin survival and appears to have little effect on metabolic rate (Rombough, 2007). Another study on the same species found no relation between egg size and offspring body length (Pitcher and Neff, 2007). Studies on other fish species indicate that smaller larvae with larger yolk sacs have a greater growth rate during the period from hatching until two weeks after hatching (Kennedy et al., 2007). However, newly hatched juveniles originating from larger eggs might be better at withstanding periods of starvation (Higashitani et al., 2007; Rideout et al., 2005). In addition, previous growth history might be more closely linked to the probability of maturing than body size (Morita and Fukuwaka, 2006). Thus, individuals of the same size, but having different growth history might mature with different probabilities. Since growth rate is indisputably associated with precocious sexual maturing (Hutchings and Jones, 1998; Thorpe, 2004), it is clear that precocious sexual maturation is also linked to egg size. However, the direction might be harder to predict, especially in benign hatchery conditions, where growth rate is higher than in the natural environment.

**INTER POPULATION VARIATION IN PHYSIOLOGY AND BEHAVIOUR**

Differences between populations have to some extent already been discussed in the previous chapter under animal personalities. However, this chapter reviews some of the literature on differences between hatchery reared and wild fish.

**THE EFFECTS OF SEA-RANCHING**

The extremely high survival rate in hatcheries represents a huge relaxation of the selection that would normally occur in the wild. Therefore, hatchery rearing of fish is in many regards considered an unselective process, and as such most certainly has consequences.

Artificial rearing of fish destined for release into the natural environment as juveniles (sea-ranching) is frequently practiced in an attempt to restore anadromous salmonid populations. Indeed, in some areas, the numbers of deliberately released cultured salmon and trout is now approaching or even exceeding the numbers naturally produced fish. In the Baltic, the proportion of salmon having wild origin in the late 1980’s made up only 10% of the total smolt production (Eriksson and Eriksson, 1993), but have increased since then to about 25% (Swedish Board of Fisheries, unpublished data). For fish there are apparent differences in selection regimes between the wild and
hatchery environment. Most traits are influenced both by genetic and environmental factors. Genetic factors are important early in life, but with time, experience becomes increasingly important (Futuyma, 1998). In the hatchery, food is provided and predators are not a factor (Johnsson, 1993). In addition, rearing density, substrate and the scope for interactions with conspecifics differ from natural conditions (Einum and Fleming, 2001; Fleming, 2001). Consequently, the prerequisite for development of traits differs between the hatchery environment and the wild, which has led to a range of behavioural alterations in hatchery-reared fish (Brelin et al., 2008; Brown and Laland, 2001; Olla et al., 1998). Through generations, these alterations may become incorporated into the genome through the process of domestication (Kohane and Parsons, 1988). For example, a number of studies support the hypothesis that domestication imposes alterations in fish behaviour (Einum and Fleming, 2001; Fleming and Einum, 1997; Huntingford and Adams, 2005; Huntingford, 2004).

There are also studies showing differences in both physiological and behavioural traits between strains of brown trout having wild and sea-ranched origin. Even though these strains both originate from the River Dalälven they have been kept separated in the management for the last nine generations (Lepage et al., 2000; Petersson and Järvi, 2003). In the River Dalälven there are still some spawning sites available downstream the hydro electric power plant dams making a gene flow from the sea-ranched strain to the wild strain possible. More recent studies have shown that the level of gene flow from sea-ranched to wild trout to be about 80% per generation, suggesting that the hatchery and naturally produced trout may be quite similar in most respects. Apparently the phenotypic stock divergence reported in previous laboratory studies reflects genetic differences between fish born in the wild and in the hatchery, a difference that recurrently develops within a single year-classes, but is counteracted by strong gene flow preventing cumulative differentiation over generations. Assuming additive genetic variance for the traits under study and a marked difference in selective regimes between wild and hatchery environments, some degree of genetic divergence may be generated from the egg to adult stage within a year class, also when a majority of the wild trout have parents born in the hatchery (Palm et al., 2003).

Thus, obviously the differences between the hatchery and wild environment are extreme, resulting in very different selection regimes. Interestingly, many of the differences reported between sea-ranched and wild fish resemble characteristics that differentiate proactive and reactive copers, such as boldness (Sundström et al., 2004), anti-predator behaviour (Fernö and Järvi, 1998; Petersson and Järvi, 2006), growth (Hedenskog et al., 2002), activity (Alvarez and Nicieza, 2003), the endocrine stress response (Lepage et al., 2000), and aggression (Einum and Fleming, 2001), even if the results on aggression might be contradictory (Hedenskog et al., 2002; Ruzzante, 1994).
Different strategies and behaviours adopted by proactive and reactive stress copers, respectively, are illustrated in Tab 2. (Korte et al., 2005).

Table 2. Summary of behaviours and environmental cues correlated to proactive and reactive coping (modified from (Korte et al., 2005).

<table>
<thead>
<tr>
<th>Reactive Behaviour</th>
<th>Coping strategy</th>
<th>Proactive Behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-hide</td>
<td>Behavioural strategy</td>
<td>Fight-flight</td>
</tr>
<tr>
<td>Shy/non-aggressive</td>
<td>Emotional state</td>
<td>Bold and aggressive</td>
</tr>
<tr>
<td>Avoid danger</td>
<td>Biological role</td>
<td>Establish/Defend territory</td>
</tr>
<tr>
<td>Cautious and thorough</td>
<td>Exploration</td>
<td>Fast and superficial</td>
</tr>
<tr>
<td>Flexible</td>
<td>Behavioural flexibility</td>
<td>Rigid and routine-like</td>
</tr>
<tr>
<td>Energy conservation</td>
<td>Energy metabolism</td>
<td>High energy consumption</td>
</tr>
<tr>
<td>Low risk</td>
<td>Body damage</td>
<td>High risk</td>
</tr>
<tr>
<td>During food scarcity</td>
<td>Advantage in a food context</td>
<td>When stable and abundant</td>
</tr>
<tr>
<td>When density is low</td>
<td>Advantage during life cycle</td>
<td>When density is high</td>
</tr>
</tbody>
</table>

Eggs of individual female brown trout tend to be very uniform in size, but eggs vary in size when comparing different parent fish even if they are of the same length, weight and stock (Bagenal, 1969). Sea-ranching is known to inadvertently select for a smaller egg size in salmonids (Einum and Fleming, 1999; Heath et al., 2003; Jonsson, 1997; Thorpe, 2004; Thorpe et al., 1984), which in turn might lead to increased proportion of precocious sexual maturation in both Atlantic salmon and brown trout (Einum and Fleming, 1999; Heath et al., 2003; Jonsson, 1997; Thorpe, 2004). However, there is significant difference to compare hatchery reared fish in the hatchery against wild fish in the wild, than to compare hatchery derived fish against wild fish on mutual ground. This can be either in the wild, laboratory or in a hatchery environment. Because in the former example it is not possible to separate environmental influence from inherited traits. As an example it is known that the hatchery environment normally leads to an increase in precocious sexual maturation due to the faster growth rate in that environment compared to the wild.

INTRA INDIVIDUAL VARIATION IN PHYSIOLOGY AND BEHAVIOUR

Individuals change or adjust their behaviours over time, and there are two explanations for this: development and learning. Even though fish are very capable of learning and a lot of scientific literature shows this, most people seem to live with the apprehension of fish and their 3-second memory. This is, of course, not true and I will therefore start this chapter with a somewhat anecdotal story about catfish.
When H.N. Maier (1909) investigated hearing capabilities of fish he could detect no reaction to a loud ringing of a bell which was rung in the water in the immediate vicinity of his fish. He therefore became convinced that fish have no sense of hearing. However, later he observed the following; In a large aquarium there was among other fish a catfish (*Ameiurus nebulosus*) which was quite shy, and which, if anyone approached hid itself among the stones. However, if one remained quiet in front of the aquarium the fish came out of its hole a few minutes later and swam about in the aquarium. On one such occasion Maier accidentally whistled once. Immediately the catfish turned around and hurried back to its hiding place as though frightened. Within two hours Maier repeated the test about fifteen times, and each time his whistling caused the fish to flee at once. If he held his hand before his mouth so that the fish could not see the movement of his mouth the result was the same. Still sceptical, Maier in further experiments placed himself about five meters away from the aquarium so that he could not be seen by the fish and vice versa, but instead the open and inverted hands of an observer placed before the aquarium. This observer gave the signal to whistle by closing his hands as soon as the catfish had left his hiding place and the response was still immediate. Maier therefore concluded that among all the fish investigated by him only the catfish was capable of hearing sounds and this in a very pronounced degree (Maier, 1909). Later von Frisch (1923) made experiments with the same species of fish. He blinded them and placed them in an aquarium. The fish preferred to swim in a hollow pipe. He fed the fish daily with raw sliced meat, at the same time as he whistled. He repeated this procedure daily and after six days the fish started to move when he just whistled and no food was placed in the aquarium (Von Frisch, 1923). This is the first example of conditioned learning in fish that I know of.

It has long been known that fish are capable of several different kinds of learning, e.g. conditioning, habituation, sensitization, imprinting etc (Laland et al., 2003). Some species of fish are even known to learn simple responses as quickly as, at least, some mammals (Flood et al., 1976; Warren, 1965). Fish can also use past experience for longer or shorter periods of time i.e. they are capable of memory (Brown and Laland, 2003; Brown, 2003; Dodson, 1988; Giske et al., 1998; Hasler et al., 1978; Hughes et al., 1992; Warren, 1965). Several recent studies have demonstrated further evidence of associative learning in several species of fish (Mizusawa et al., 2007; Sneddon, 2003; Wisenden et al., 2008). In most of these studies variants of Pavlovian learning are used, which is a conditioned learning paradigm (Pavlov, 1927). In which the conditioned stimulus (CS) are linked to an unconditioned stimuli (US). The CS in a neutral stimuli but the US does have an adaptive value. The cues (CS) might be changes in light or water inflow to the aquaria before feeding (Sneddon, 2003), anti-predator behaviour (Ferrari et al., 2005), foraging behaviour (Hughes and Blight, 2000) which are all examples of US with adaptive values. Conditioned olfactory stimulus has
also been shown to work in Salmon (Salmo salar) during natural conditions (Leduc et al., 2007). Another method used is self feeding by means of a trigger system controlled by the fish itself (Alanärä, 1996; Boujard and Leatherland, 1992; Mizusawa et al., 2007). The number of times until the experimental animals learn to associate food (US) with the given cue (CS) are quantified and considered a good indicator of the learning capabilities (Alanärä, 1996; Pavlov, 1927). On the other hand, studies demonstrating non-associative learning in fish are less common.

There is great variability in learning how to feed on new prey between two species of sunfish (Kieffer and Colgan, 1992), but also between individuals of the same species (Brown et al., 2003; Brown and Laland, 2001; Huntingford, 2004; Sundström and Johnsson, 2001). Further, Magnhagen and Staffan (2005) have shown that European perch (Perca fluviatilis) displayed individual variation in boldness when transferred to a new environment. They explain at least part of this variation with individual differences in habituation to a new environment (Magnhagen and Staffan, 2005). Some of these studies also emphasize variation in learning capabilities and associate them with variation in a bold-shy continuum (Frost et al., 2007; Magnhagen and Staffan, 2005; Sneddon, 2003; Wilson et al., 1994b). Thus, it is clear that learning or even willingness to form routines is of major relevance when it comes to “animal personalities”. Recent studies have demonstrated variation in behavioural plasticity in threespined sticklebacks (Gasterosteus aculeatus) and rainbow trout (Onchorhyncus mykiss) (Bell and Sih, 2007; Frost et al., 2007). More specific, Frost et al. (2007) showed that rainbow trout being classified as “bold” became more “shy”, when experiencing lost dyadic fights. However, if bold individuals won or watched other bold demonstrators, they did not change. Further, they have showed that rainbow trout classified as shy when winning a dyadic fight became bolder. Bell et al. (2007) have shown that predation produced a positive correlation (a behavioural syndrome, see (Sih et al., 2004b) for review) between boldness and aggression in a group of threespined sticklebacks that during earlier experiments showed no such correlation. Bell et al. (2007) explains the altered behaviour with both predation and behavioural plasticity. Thus, in fish, at least some behavioural traits associated with animal personality are plastic. Learning and memory in animals (fish included) are key functions which together leads to durable behavioural modifications i.e. adaptive behavioural changes.

In previous studies, it has been shown that sympathetic reactivity is typical for fast responding, active and/or aggressive individuals in mammals (Koolhaas et al., 1999; Korte et al., 2005), lizards (Korzan et al., 2000; Korzan et al., 2002; Summers and Greenberg, 1994) and fish (Brelin et al., 2008; Brelin et al., 2005; Schjolden et al., 2006b; Van Raaij et al., 1996). However, it has not properly been examined how this key feature of endocrinology is
linked to learning or behavioural change. Moreover, the stress response is considered to be of great importance for understanding individuality in animals but at the same time consistency in stress response is not well known (Schjolden and Winberg, 2007; Winberg et al., 2007).
OBJECTIVES

The first objective (A) of this thesis has been to reveal if divergent stress coping strategies exist in a sea-ranched strain of juvenile brown trout. To that end an experiment was setup to quantify variation in different behaviours and physiological stress responses. All tests were chosen with emphasis on quantifiable characteristics associated with stress coping strategies. The experimental design was also evaluated with regards to the results with the purpose of gaining a faster and more precise experimental design for future experiments.

The second objective (B) was to test if the frequency distribution of stress coping strategies differed between juvenile brown trout originating from four different populations which were brought up and reared under the same conditions. To that end we used an improved experimental setup refined from Paper I on both sea-ranched and wild strains of fish from different regions in Sweden. The aim was to draw conclusions about effects of sea-ranching programmes and the role of stress coping strategies in natural environments.

The third objective (C) was to examine to what extent precocious sexual maturation is associated with personality traits, specifically, high sympathetic reactivity earlier in life. This was achieved by using individual brown trout originating from four different Swedish brown trout populations, one with a known history of sea-ranching and three from the wild, which were reared together under identical conditions.

The last objective (D) was to look at the consistency of personality traits over time. To that end repeated series of tests on brown trout were performed. The main purpose of the chosen experimental design was to check for possible correlations between the quantified variables (also key characteristics in the theory of stress coping strategies) as well as consistency in those variables over time. The results are discussed in terms of consistency of stress coping strategies and if sympathetic reactivity is linked to behavioural plasticity in fish.
MATERIALS & METHODS

The experiments were performed at the Laboratory of Stream water Ecology, Swedish Board of Fisheries, Älvkarleby, Sweden. Biochemical analyses were performed at the Department of Comparative Physiology, Evolutionary Biology Centre, Uppsala University in Uppsala, Sweden. Whereas biomolecular analyses were performed at the Institute of Freshwater Research, Swedish board of Fisheries, Drottningholm, Sweden.

EXPERIMENTAL FISH

The fish used in Paper I and paper IV were juvenile brown trout (age: 1+; body mass, 79.3±20.0 g; total length 196.4±16.5 mm, mean±SD, n=23) originating from the sea-ranched strain of brown trout from the River Dalälven. The fish were a mix of offspring from 57 females and approximately the same number of males. They were reared at the hatchery in Älvkarleby until the start of the experiment. During rearing the fish were graded once and the fish used in the present experiment represent the smallest fish from seven different tanks. These fish were reared together in one tank and from that tank experimental fish (n=99) were randomly selected, anaesthetized (200 mg/l, ethyl 4-aminobenzoat), PIT-tagged, weighed, measured and transferred to a separate 300 l holding tank. The water supply to this tank was then gradually exchanged from River Dalälven water to groundwater (6.5±0.2°C) which was used for the remainder of the experiment. The fish were fed daily (Aqua Aller S3) at approximately 0.5% of their body mass. Following acclimation for 90 days experimental fish (N=23) were selected at random, anaesthetized, weighed, measured and transferred to isolation in individual 20l aquaria supplied with groundwater at 1l/min.
The fish used in paper II were also juvenile brown trout (age: 1+; body mass, 64.2±17.3 g; total length 180.7±16.5 mm, mean±SD, at the start of the trial, n=120) but originating from four different rivers in Sweden. Eggs and milt were collected (autumn year 2002) from two different strains of brown trout from the River Dalälven, one sea-ranched strain (F9), one wild strain, and from River Åvaån (both located at the Swedish east-coast), River Jörlandaån and River Norumån (at the Swedish west-coast). The fish were hatched and raised in half-sibling groups, originating from, in total, 46 females and the same number of males, and later transferred to a single tank in the hatchery at Älvkarleby where they were reared until the start of the experiment. From this tank fish (n=300) were randomly selected, anaesthetized (100 mg/l, ethyl 4-aminobenzoate), PIT-tagged, weighed, measured, sampled (for DNA
analyzes) and later transferred to a separate 300 l holding tank. The water supply to the holding tank was then gradually exchanged from River Dalälven water to ground water (6.8±0.3°C) that was used in all experimental tanks and aquaria for the remainder of the experiment. The fish were fed daily (Aqua Aller S3) at approximately 0.5% of their body mass. Following acclimation in holding tank experimental fish (n=120) were selected at random, anaesthetized, weighed, measured and transferred to isolation in individual 20 l aquaria supplied with groundwater at 1 l/min. To standardise recovery time between the tests (see below) eight fishes were transferred on each of three consecutive days, giving a total of 24 fishes in each of five similar batches. The experimental fish were not fed during isolation. Following the tests the fish were sexed and the stage of sexual maturation checked. Finally, origin of individual test fish was checked using micro satellite analysis comparing DNA samples from experimental fish with DNA samples from their parents. The result of this analysis showed that the number of fish from each population that were tested in the experiment was; River Dalälven sea ranching strain (Dal S) n=30, River Dalälven wild strain (Dal W) n=33, River Åvaån n=23, River Norumån n=16 and River Jörlandaån n=18.

Figure 3. Brown trout inhabit very different waters, small stream and larger rivers. On the left hand side is the River Dalälven and on the right is River Jörlandaån (Left photograph provided by Erik Petersson and right Daniel Brelin).

In the autumn of 2003, mature adults from the Rivers Dalälven, Åvaån, Jörlandaån and Norumån were used to produce experimental fish for paper III. 12 males and 12 females from each of the four populations were sampled, except for River Åvaån where only 10 males and 10 females were caught. A half-sib mating design was used to be able to carry out quantitative genetic analyses not included in this paper. Eggs of two females were fertilised with milt from two males to create six 2×2 breeding sets for each population, except for River Åvaån where only four complete 2×2 breeding sets and two additional full-sib families could be produced. Hence, the total number of unique crosses (full-sib families) was 90 (24 from each population, except for River Åvaån with 18 unique crosses). The mature adults were stripped in
the field, and eggs and milt were transported directly on ice to the River Dalälven hatchery in Älvkarleby where crossings were made. Two females were crossed with two males from the same population, resulting in half-sib families that were incubated in individual trays in the hatchery. After hatching and yolk sac absorption in spring 2004 fry from each family were pooled and reared in a 1×1 m² rearing tank in the hatchery until the experiments were initiated. In autumn 2004, all fish were marked with PIT-tags (Passive Integrated Transponders, BTS Scandinavia AB) and tissue samples were taken for microsatellite analysis to determine family- and population-origin. On the 31st of August 2005 280 trout (70 from each population, age 1+) were randomly selected and transferred to a separate 300 l holding tank. Following acclimation in the holding tank the trout exposed to confinement test (n=216) were selected at random, anaesthetized, weighed, measured and transferred to isolation in individual 20 l aquaria supplied with groundwater at 1 l/min. Since only 24 aquaria were available this procedure was repeated nine times. After sampling the trout were transferred back to their aquarium and later, after recovery, allocated back to the common holding tank. The experimental fish were not fed during isolation. In October the following year, when sexual maturation was expected, the trout were anaesthetized, sampled for blood, measured, the incidence of precocious maturation recorded (gonads weighed) and the experiment terminated. Later, from PIT-tag information and microsatellite analyses it was possible to determine population origin of all sampled trout (see ASSAYS below for further details).

EXPERIMENTAL PROTOCOLS

The light regime was 12L/12D (light on at 06.00, light off at 18.00) and all the experimental work and sampling was performed during the period 09.00-15.00. The experimental fish were exposed to at least one of five different tests:

Recovery - This test was designed in order to observe the motivation of individual fish to resume feeding after transfer to isolation in an unfamiliar environment. The fish were hand fed (Aqua Aller S3) daily for ten days. Feed intake was quantified by counting the number of pellets consumed by each individual fish. The feeding stopped if three pellets in a row were rejected by the fish. The test was used in paper I and III.

Resident-intruder - Ten days following transfer to social isolation a small con-specific intruder (approximately 50% of the body mass of the resident fish) was introduced to the aquaria. The behaviour of such pairs was recorded on video during 1.5 hours after which the intruder was removed. From video recordings, the latency to first attack and the number of attacks performed by the resident fish during 30 minutes, starting from the time of
the first attack was recorded. This test was used in paper I, II and IV. However, in paper II the test was conducted six days following transfer to social isolation.

**Novel Environment** - This test was only used in paper II and was used to observe how individual trout respond behaviourally when introduced to an unfamiliar environment. Five days after recovery from the resident-intruder test the fish were moved to a 20l aquarium and their behaviour were recorded on video the following 1.5 hours. During the first time period, 45 minutes, the fish were allowed to acclimate and the activity during this time period is hereafter referred to as activity in a novel environment. After one hour the fish were subjected to a reduction in O$_2$ saturation i.e. hypoxia (see below). The behaviour during the 15 minutes between these two tests was used to generate a value for normal/baseline activity of the fish. Escape attempts and mean movement were quantified using the software EthoVision 3.0 (Noldus).

![Figure 4. The setup used for novel environment and hypoxia test. On the left a trout that is panicking and trying to escape, while on the right a trout that is very calm and residing on the bottom of the aquaria (photograph provided by Daniel Brelin).](image)

**Hypoxia** - After five days of recovery from the resident intruder test the fish were moved to a 20l aquarium and allowed to acclimate for one hour after
which they were subjected to this test during which the behaviour was observed during an inescapable challenge. The challenge applied was a gradual reduction of the O\textsubscript{2} saturation in the water from 100 to 10 % over a period of 30 min (Fig. 4). Escape attempts and mean movement were quantified in paper I and IV while percent swimming per minute (referred to as activity) was quantified in paper II using the software EthoVision 3.0 (Noldus).

**Confinement** - After five days (three days in paper II and III) of recovery from the hypoxia test the fish were subjected to confinement stress in grey PVC boxes (volume 2.7 l, water flow 0.4 l/min) with a transparent lid. After 45 min (20 min in paper III) the fish were anaesthetized (200 mg/l, ethyl 4-aminobenzoate) while still in the confinement box and sampled for blood. The blood samples were immediately centrifuged (9000 rpm, 6 min) and the separated plasma was kept on ice (<5 min) until being stored at -80°C. Later the blood samples were used to quantify plasma levels of noradrenaline, adrenaline, dopamine and cortisol (see ASSAYS below for more details).

In paper I and paper IV the complete set of tests was repeated twice for each individual fish, the first replicate performed during November 2003 and the second during January 2004. During the time between the replicates the fish were kept in the 300l holding tank (described above). In Paper II the experimental tests started 9th of August and ended 28th of November in 2004. In paper III only the confinement test was used but the experimental part of that study started on the 19\textsuperscript{th} of September 2005 and the experiment was terminated on the 20\textsuperscript{th} of October 2006.

**ASSAYS**

In paper I, II and IV post stress blood plasma concentration of cortisol was analysed using a validated radioimmunooassay (RIA) (Olsen et al., 1992) but with the modifications provided by (Winberg and Lepage, 1998). In short the frozen blood plasma and standards prepared from hydrocortisone were diluted threefold in a phosphate buffer (0.1M pH 7.5, containing 1 % bovine serum albumin). Control plasma was diluted three times. Samples were analyzed in duplicates. Protein binding was prevented by adding a solution of trichloroacetic acid (7.5 g/l) and sodium hydroxide (2.25 g/l). \[^{3}H\]-cortisol was added to use as the tracer. The samples were incubated overnight at 4°C. Free and bound tracer was separated by 30 minutes incubation with a charcoal dextran solution on ice followed by centrifugation for ten minutes in 4400 rpm. Samples were counted in Aqua Safe scintillation fluid (Zinser Analytic, Frankfurt, Germany). The concentration of plasma cortisol in paper III were analysed using a commercial enzyme linked immunosorbent assay (ELISA) kit (product # 402710, Neogen Corporation, Lexington, USA, delivered by Skafte Medlab, Onsala, Sweden). In brief, plasma samples were
extracted in ethyl acetate, the liquid phase evaporated and sample dissolved in buffer. The samples were then placed in wells together with enzyme-conjugate and substrate. After stopping the competitive binding with 1M HCl the absorbance was read at 450N.

Catecholamine concentrations (noradrenaline, adrenaline and dopamine) in blood plasma were analysed using high performance liquid chromatography with electrochemical detection (HPLC-EC) (Montpetit et al., 2001) following alumina extraction of the catecholamines (Winberg et al., 1997). The HPLC-EC apparatus consisted of a solvent delivery system (Coulotech, W/Multiole, P’STATS, USA), an auto injector (Midas, Spark, Holland) with a cooler keeping the samples in queue at 4°C, a reverse phase column (4.6 x 100 mm, Reprocil, C18, 3.5 μm) kept at 40°C, and an ESA 5200 Coulotech II EC-detector (ESA, Bedford, Ma., USA) with two electrodes at an oxidizing potential of -40 mV and +320 mV. A guard electrode with a potential of +400 mV was employed before the analytical electrodes to oxidize potential contaminants. The mobile phase was delivered at 1 ml/min and contained 10.35 g/l NaH₂PO₄, 0.33 g/l Sodium Octyl Sulphate (SOS), 0.0037 g/l EDTA, 70 ml/l Acetonitril and brought to a pH of 3.0. Concentrations were calculated relative to corresponding standards and with 3, 4 dihydroxybenzylamine hydrobromide (DHAB) as an internal standard in all determinants.

In the blood samples from the end of the experiment in paper III plasma levels of testosterone, 11-ketotestosterone and 17β-estradiol were measured by radioimmunoassay (RIA) according to (Mayer, 1990). The suitability of this RIA for the measurement of steroids in brown trout has previously been validated (Petersson et al., 1999). In short, individual plasma samples (200μl) were mixed with RIA buffer in a ration of 1:2 and heat-treated for 1 hour at 80°C. After centrifugation, the supernatant was extracted and stored at 4°C during the period of steroid determination. Non-detectable values have been taken as zero.

Assessment of family and stock origin of experimental fish in paper II and III was performed using eleven microsatellite loci. Locus designations, DNA extractions, polymerase chain reactions (PCR) and allele scoring are described in (Dannewitz et al., 2003) and (Dannewitz et al., 2004). Parentage was assessed by comparing the alleles at a given locus from each experimental fish with the alleles in each of the potential parental crosses using the software WHICHPARENTS (available on http://www-bml.ucdavis.edu/whichparents.htm). Potential parental crosses with alleles incompatible with those of a particular offspring were excluded from the set of possible crosses (Dannewitz et al., 2004).The markers used gave an extremely high discriminatory power, and 100% of the experimental fish could be assigned unambiguously to a single parental pair.
STATISTICS

All statistical analysis in the included papers was performed using SYSTAT 8.0 or SAS 9.13. All quantified variables that did not fulfil the assumption of normal distribution were log transformed. If normal distribution could not be achieved, a non-parametric test was used. All individuals with quantified variables are included if nothing else is stated. All data are presented as means ± standard error of the mean (SEM) if nothing else is stated. For further readings on specific details regarding statistical methods see separate papers.

CLUSTER ANALYSIS

In paper I, II and III a clustering method was used to group (cluster) brown trout based on their levels of plasma noradrenaline. Noradrenaline is an important catecholamine which is typically secreted during sympathetic activation and high post stress plasma levels of NA is characteristic of animals displaying a proactive stress response (Koolhaas et al., 1999), (Korte et al., 2005)(Box 2). Therefore, one could use plasma levels of noradrenaline as a marker for individual sympathetic (re)activity. Further, plasma levels of noradrenaline were one of the best correlated variables between trial replicates. Thus, indicating consistency over time. Nevertheless, in the mentioned papers two groups were identified using a clustering method (Euclidean K-Means clustering, 1000 iterations, 2 groups, based on residuals of log transformed values of noradrenaline against starting weights of the trout) based on their post stress blood plasma noradrenaline (NA) levels after a standardised confinement test. Trout of these two groups (clusters), will hereafter be referred to as high sympathetic reactivity (HCat) and low sympathetic reactivity (LCat, low in regards to the response induced by confinement but still higher than baseline levels). For further details about this procedure see papers I, II and III.
RESULTS & DISCUSSION

In this section I have chosen to mainly include results related to the specific aims of this thesis for further results and a detailed discussion see specific papers.

OBJECTIVE A – IDENTIFICATION OF DIVERGENT COPING STYLES

Results from paper I, II and III all contributed to the identification of divergent stress coping strategies in brown trout. First, in all these papers two groups were successfully identified using a clustering method (see M&M for further information) based on plasma levels of noradrenalin (NA) following confinement stress. As may be predicted the two clusters differed significantly in blood plasma concentrations of NA but also in plasma levels of adrenalin (A) and dopamine (DA) (Fig. 5). High post stress plasma levels of A and DA are characteristic of animals displaying a proactive stress response (Koolhaas et al., 1999); (Korte et al., 2005). Second, in paper I fish in the group with high levels of post stress plasma noradrenaline concentrations displayed a much stronger behavioural response during the hypoxia test then fish in the other group, both with regard to more escape attempts and greater mean movement. A higher degree of active avoiding behaviour is also a characteristic of animals displaying a proactive stress response (Koolhaas et al., 1999); (Korte et al., 2005). Further, there was also a trend towards a shorter latency to the first attack during the resident-intruder test. However, there were no significant differences between the two clusters with regards to how fast they started feeding or how much they fed during the ten day recovery period. We expected to see a gradient in feeding ranging from proactive resuming feeding early and more to reactive resume feeding later and less, due to the fact that strong stress responses inhibit feeding behaviour. Other studies show positive correlation between time to resume feeding following transfer to an unfamiliar environment and dominance (Larson et al., 2004).
Figure 5. Significant differences in post stress plasma levels of adrenaline (A), noradrenaline (NA) and dopamine (DA) in the identified clusters HCat (high levels of catecholamines indicate high sympathetic reactivity) and LCat (low sympathetic reactivity, low in regards to the response induced by confinement but still higher than baseline levels) after a confinement test (from paper II).

In paper II the two clusters clearly differed in their initial response (10 min) to the novel environment. This can be explained by the fact that trout with high sympathetic reactivity (HCat) spent more time in the upper part of the aquaria exploring and trying to escape. However, fish in the two clusters from paper I, II or III did not differ in post-stress plasma cortisol levels. Still, even though HPA/HPI axis reactivity is a key characteristic in the concept of stress coping strategies divergent post-stress plasma levels of glucocorticoids are not always reported (Schjolden et al., 2006b). The stress-induced activation of the HPI axis is a highly dynamic process (Wendelaar Bonga, 1997) and the time-course of this response is likely to vary between proactive and reactive animals (Pottinger and Carrick, 1999). Thus, even though we were not able to detect any significant difference in plasma cortisol concentrations between fish classified as HCat and LCat following confinement these fish may still differ in HPI axis reactivity. In fact, in a study on stress coping strategies in the HR and LR rainbow trout (Schjolden et al., 2006b) were unable to detect any difference in post-stress plasma cortisol between HR and LR trout, even though these strains were selectively bred for high and low post-stress plasma cortisol, respectively, and differences in post-stress plasma cortisol between these two strains had been repeatedly reported (Pottinger and Carrick, 1999; Schjolden et al., 2005a; Øverli et al., 2005).

Thus, trout with a high sympathetic reactivity were also shown to be more active during novel environment and during reduction in oxygen saturation. Further, in paper I these trout also showed a tendency towards being more aggressive. Taken together, these results support the suggestion that phenotypes similar to mammals displaying proactive and reactive stress coping exists in fish.
OBJECTIVE B – FREQUENCY DISTRIBUTION OF STRESS COPING STRATEGIES

In both paper II and III brown trout derived from several wild populations as well as trout from sea-ranched populations were used. Genetic analyses of parental fish (used to produce the experimental fish) and experimental fish were used to determine which population the experimental fish came from and allele frequency differences between populations.

Using clustering analysis, two clusters that clearly differed in their sympathetic reactivity, as indexed by post-stress plasma catecholamine concentrations, were identified. In paper II there was a significant effect of population on the frequency of HCat and LCat individuals. The River Dalälven populations had a higher frequency of HCat trout than did the other populations. The number of HCat trout ranges from 14-48% in the different populations (Fig. 6). In paper III the number of HCat trout ranges from 24-56% in the different populations. Further, the distribution of HCat and LCat differ significantly in frequency between populations. The River Dalälven and River Åvaån populations have higher frequency of HCat trout than did the other populations. The rivers River Dalälven and River Åvaån are both situated on the Swedish east coast, and they are both relatively large and stable environments as compared to the rivers River Norumån and River Jörlandaån which are small streams on the Swedish west coast (Fig. 3). Also, the rivers River Dalälven and River Åvaån both have a history of hatchery rearing which is not the case for River Norumán and River Jörlandaån. Thus, the higher frequency of HCat trout in the River Dalälven and River Åvaån rivers appear to support the hypothesis that individuals that display a more proactive stress coping strategy are favoured in a stable environment; HCat trout shows higher sympathetic reactivity and behaviourally a more active avoidance when subjected to stressful challenges than the LCat fish. However, even though it seems clear that HCat and LCat trout represent two distinct phenotypes, showing divergent stress coping, it is still ambiguous to what extent HCat and LCat trout display proactive and reactive stress coping strategies, as defined by Koolhaas et al. (1999).

In paper II several behavioural tests where used and in the resident-intruder test there was a significant effect of population origin on attack rate against the smaller con-specific intruder. This can be observed by the higher attack rates shown by brown trout from River Åvaån, River Norumán and River Jörlandaån, taken together, compared to the combined values from the two strains from River Dalälven. The River Dalälven populations displayed a higher basal activity than fish of the populations originating from River Norumán and River Jörlandaån. All fish, irrespective of origin, responded to the novel environment with a high initial activity that gradually declined during the first ten minutes. However, there were also effects of population
origin on the temporal pattern of the fish in the novel environment. Similarly, all fish, irrespective of origin, responded to the gradual decline in oxygen saturation. Further, the response to hypoxia of fish from the different populations differed. The River Norumån and River Jörlandaån populations displayed generally lower activity and significantly less escape attempts than fish of the other populations until the oxygen saturation declines below 20% when they instead showed an increase in the number of escape attempts exceeding that of the other populations. This was also reflected in their mean movement. However, the clusters, called HCat and LCat, did not differ significantly in either attack latency or number of attacks during the resident-intruder test. Further, HCat and LCat did not differ in their basal activity or in behaviour during reduction in oxygen saturation. But they clearly differ in their initial response (10 min) to the novel environment which can be explained by HCat trout spending more time in the upper part of the aquaria exploring and trying to escape.

Figure 6. The frequency distribution of juvenile brown trout identified as HCat originating from five different populations.
There was no perfect match between the behavioural profiles of HCat and LCat trout and the behavioural profiles described for proactive and reactive animals. When subjected to novel environment HCat trout showed higher activity than LCat trout, a result suggesting that HCat trout displayed a proactive and LCat a reactive response. On the other hand, the fact that HCat and LCat trout did not differ in aggression appears to contradict that conclusion. One reason for not detecting differences in aggression between HCat and LCat trout may have been that each fish was tested in its home aquaria. In rodents proactive individuals appear to show exclusively proactive coping, whereas reactive individuals may respond either reactively or proactively to stress depending on context (Benus et al., 1989). Reactive individuals may respond proactively if challenged in familiar environment but reactively if challenged in an unfamiliar environment (Sluyter et al., 1996). Similar results have been reported from studies on rainbow trout (Schjolden et al., 2006b). In the present study HCat trout seemed to respond proactively and LCat trout reactively in the novel environment test, whereas they did not differ in aggression, as determined by the resident/intruder test in the home aquaria of the resident fish. In addition, the intruder may not have imposed much of a threat to the resident fish since it was only half the size of the resident fish.

The observation that River Dalälven trout are less aggressive than trout from River Åvaån, River Norumån and River Jörlandaån stands in opposition to earlier studies showing that the hatchery rearing promotes aggression as only River Dalälven trout have a history of hatchery rearing (Huntingford, 2004; Ruzzante, 1994). However, the effects of domestication on aggressive behaviour are ambiguous, and both stimulatory (Mesa, 1991; Moyle, 1969; Swain and Riddell, 1990) and inhibitory effects (Hedenskog et al., 2002; Holm and Fernö, 1986; Robinson and Doyle, 1990; Ruzzante and Doyle, 1991) have been reported. Game theory predicts that at extremely high densities (such as those found in hatcheries) there should be strong selection pressure for less aggressive individuals because the number of interactions with conspecifics involved will make it almost impossible for them to maintain territories (Doyle and Talbot, 1986).

If HCat and LCat trout represent distinct phenotypes it implies that distinct phenotypes play a role in maintaining genetic, physiological and behavioural variation. Even though there could be multiple factors affecting the behavioural and physiological profile of the fish, the fact that the fish were reared under identical conditions clearly suggests that inter population differences in the frequency of HCat and LCat individuals have genetic background. The results raise the important question of how multiple distinct phenotypes, such as stress coping strategies, can be maintained within populations. There are at least three possible explanations. First, frequency dependent selection may operate on these strategies. If for example proactive individuals become
numerous, the relative fitness of reactive individuals may increase because they do not spend time and energy in aggressive interactions. On the other hand, if reactive individuals become numerous, proactive individuals will be favoured. Differences in the frequency distribution of HCat and LCat individuals among populations could be explained by differences in the environment, yielding different evolutionary stable proportions where fitness of the strategies on average are equal. Second, the coexistence within populations of individuals adopting different coping styles could be explained by a variable environment favouring different alternatives under different environmental conditions. Finally, multiple coping styles could be a result of individual “choices” early in life. The “best of a bad job” hypothesis has been used to explain the phenomenon of multiple mating tactics in salmonid fish (Lee, 2005). Individuals who for some reason are less successful chose one particular tactic based on some cue like growth or body condition, whereas more successful individuals chose another tactic. Differences in frequency distributions of alternative tactics between populations could then be explained by population specific, genetically based threshold values separating the different alternatives. This hypothesis does not require equal fitness of the alternative behaviours.

OBJECTIVE C – EARLY SEXUAL MATURATION AND COPING STRATEGIES

As the title implies, the aim here was to investigate if a specific stress coping strategy coincides with early sexual maturation in male brown trout. This was mainly investigated in paper III but some of the results from paper II are also of interest.

In paper II the number of identified precocious sexually mature males was 15 out of 55 males (27.3%) and no sexually mature female were identified. There was a significant effect of population on the frequency of precocious males. The River Dalälven populations had a lower frequency of precocious males than the others. In paper III the number of identified precocious sexually mature males was 20 out of 81 males (25%) the second autumn. The third autumn 59 out of totally 115 identified males (51%) were sexually mature and 19 of those were sexually mature at both occasions. Also in this study there was an effect of population origin on the frequency of precocious males. Interestingly, once again with the lowest proportion of precocious males in the River Dalälven population. Also, six out of 114 females reached sexual maturity the third autumn. One of them originated from River Ävaån, three from River Jörlandaån, two from River Kävlingeån but none from River Dalälven. Additionally, in paper II out of 27 male brown trout classified as HCat 11 sexually matured as parr (41%) and out of the 40 male trout
classified as LCat 18 sexually matured as parr (45%). Thus, individuals classified as HCat were not more prone to sexually mature either the second or the third autumn compared to LCat individuals.

When comparing male trout that sexually matured the third autumn with those that did not in regards of specific growth rate it turned out that there was no difference when considering the entire time span of the experiment. However, the male trout that matured grew faster in the beginning and slower towards the end of the experiment. This difference in specific growth rate also turned out to be significantly affected by time. This difference was also reflected in condition index factor. Generally, trout that sexually matured precociously had higher condition than those who did not but there was a tendency towards the opposite their first autumn. Also, trout that sexually matured precociously came from females with relatively small eggs and maternal egg size was negatively correlated with the gonadosomatic index of the male offspring. Further, when probability to sexually mature as parr was tested in a logistic regression, fast growth early in life and hatching from a small egg (or having a mother with small eggs) was the major explanations (Fig 7). As expected, levels of plasma testosterone were also positively correlated with the probability of precocious sexual maturation in male brown trout. Further, sexually mature males (the third autumn) being HCat or LCat did not differ in plasma concentration of testosterone, 11-ketotestosterone or GSI. However, male brown trout identified as LCat had both higher specific growth rate (SGR) and were in better condition during the second spring but overall there were no difference between the two clusters in either SGR or condition. Interestingly, there is also a tendency for trout hatched from large eggs to have a higher probability of becoming HCat (Fig. 7).
Thus, our data shows that males that sexually mature precociously have a more rapid initial growth compared to immature trout. This is to a large extent logical as the potential for rapid growth among progeny from small eggs are bigger than among progeny from large eggs (Thorpe et al., 1984). Nevertheless, these results imply that early growth is involved in the regulation of precocious sexual maturation. This result is well in line with earlier studies which show that precocious sexual maturation is linked to fast growth and good condition (Bohlin et al., 1990; Bohlin et al., 1994; Dellefors and Faremo, 1988; Fleming, 1996; Metcalfe, 1998; Metcalfe et al., 1995; Morgan and Metcalfe, 2001; Thorpe, 1994). However, in the present study all fish have been reared under identical conditions and the differences seen between populations in frequency of precocious males have a genetic background. In Atlantic salmon precocious males and age matched immature males (future large anadromous males) differed consistently in gene expression (Aubin-Horth et al., 2005). This suggests a variance in genetically de-
termined thresholds triggered by different environmental cues. The ability to gain high social status (leads to more food in the wild and in the hatchery) or the genetic thresholds for what is a “good” growth rate at a certain time might then differ between the populations. The latter seems like a reasonable explanation to why trout originating from the small rivers have higher frequencies of precocious sexual mature males, based on the assumption that scarce food resources in the wild compared to the hatchery environment give rise to a lower growth rate. While the River Dalälven populations during decades of hatchery influence have been accustomed to higher growth rates and their threshold might then have been increased.

Because we find a negative correlation between the probability to sexually mature as a parr and egg size, our data support the hypothesis that small egg size increases the probability to sexually mature as parr. However, the brown trout that originate from the sea-ranched population (River Dalälven) had the lowest proportion of sexually mature parr males and was the only population with no sexually mature females in the third autumn. These results were the opposite from results reported from most earlier studies (Einum and Fleming, 1999; Heath et al., 2003; Jonsson, 1997; Thorpe, 2004), although Fleming and Einum (1997) reported similar results from a study on Atlantic salmon where the cultured fish displayed a lower proportion (21.6%) of parr maturity compared to the wild founder population (44.6%). These authors suggested that this observation might have been because of deliberate selection for delayed maturity (Fleming et al., 1997). The River Dalälven brown trout have been sea-ranched for approximately nine generations. Normally the hatchery environment is considered to lack selecting factors (Gippoliti and Carpaneto, 1997; Reisenbichler and Rubin, 1999). However, our results strongly imply that there is a selection against precocious sexual maturation in the hatchery environment. When it comes to these results we can think of two possible explanations. Firstly, only migrating parents are used in the breeding program of the hatchery in River Dalälven. Thus, a direct selection favouring a migratory phenotype and, as such, an indirect selection against precocious sexual maturation is built into the system. This, however, should be customary in other hatcheries as well. Secondly, there might be a difference in genetic thresholds determining the conditions when to sexually mature and not. Thus, it is easy to imagine an evolutionary response in the sea-ranched trout. When juvenile salmonids are introduced for the first time to a hatchery environment, characterised by optimum food availability and high growth rates, the portion of sexually mature parr increase to maladaptive proportions. Thus, this leads to selection towards an increase of the threshold separating these two life history strategies. Thus, sea-ranched strains might become accustomed to abundance of food and scarcity of predators, compared to wild trout which are adapted to harsher environments, and this leads to a difference in genetic thresholds deciding when and where to sexually mature. Thus, what is to be considered good condition is a matter of great
subjectivity. Or, in other words, River Dalälven trout might have a skewed threshold on what is a good state for early sexual maturation through both selection and adaptation to the hatchery environment.

**OBJECTIVE D – COPING AND BEHAVIOURAL PLASTICITY**

Recent studies have shown that learning capabilities in fish might be connected to certain behavioural traits. Interestingly, these behavioural traits are often similar to behavioural traits associated to one of the previously described coping strategies. Therefore, in paper IV we decided to examine correlations within and between experimental replicates. In addition we look at the absolute differences i.e. the change in the behavioural and physiological responses between replicates.

During the first trial the latency to resume feeding was strongly negatively correlated with the amount of consumed feed. This basically shows that both measures are useful indicators of individual willingness to resume feeding after stress induced anorexia, i.e. the earlier they resume eating the more they eat during this test. Further, attack latency and number of attacks were also negatively correlated, strongly indicating that both variables are measures of the same thing, the willingness to attack an intruder in ones home aquaria. Mean activity (percent of time spent swimming per minute) during the hypoxia test were positively correlated with plasma noradrenaline levels following the confinement test. Unexpectedly, plasma adrenaline were positively correlated with plasma cortisol following confinement. Normally, high catecholaminergic reactivity is associated with low levels of plasma cortisol in fish (Bonga, 1997; Van Raaij et al., 1996).

When the same individual trout was transferred to isolation the second time the amount of feed and starting time of eating was, once again, negatively correlated. However, the starting time of feeding was also positively correlated with plasma cortisol levels following confinement. Additionally, this time activity during the hypoxia test was positively correlated with the amount of feed and negatively correlated with the starting time for feeding. As in the earlier replicate number of attacks and attack latency was negatively correlated. However, number of attacks was also positively correlated with activity during the second hypoxia test.

When comparing the behavioural and physiological responses between trials the experimental trout both resumed feeding sooner and consumed more feed the second trial compared to the first trial. Also, during the second trial experimental trout did not attack an intruder as quickly nor as many times
when compared to the first exposure to an intruder. Even more, experimental trout had lower levels of circulatory noradrenalin, following a standardized confinement test, the second time when compared against the levels, after the exact same test, five weeks earlier. However, no significant difference was found in plasma cortisol between the two replicates, even though distinct increases in cortisol were noticed in response to confinement. Besides the post stress plasma levels of noradrenaline and the post test individual condition no other quantified variables were found to be significantly correlated between trial replicates.

Plasma levels of noradrenaline from the second replicate were positively related with the change in the behavioural response during hypoxia (activity first trial–activity in the second trial). Thus, this result shows that sympathetic reactivity is associated with the actual change in a specific behavioural stress response. Further, it suggests that individuals with higher sympathetic reactivity are more prone to change their behavioural responses compared to individuals with low sympathetic reactivity.

Figure 8. This figure shows the change in response between the first and second replicate in paper IV. (A) Food intake after transfer to social isolation. (B) Number of attacks towards a conspecific during a resident–intruder test. (C) Plasma noradrenaline levels after a standardized confinement test. (D) Difference in Fultons condition index after the first and second test series.
Thus if one compares individual data from the two separate replicates it is apparent that almost all individuals change their behaviour in the same manner. In general, they eat more, are less aggressive and tend to be calmer through the hypoxia test. Additionally, experimental fish even lowered their sympathetic reactivity after a confinement test, as shown by a significant reduction in circulatory noradrenaline. One presumable reason for the current results might be that the experimental trout somehow became generally more acclimated to stress during the first series of tests and therefore became less reactive during the second test. However, if that is the case, the difference between replicates would then normally be explained in terms of habituation. However, habituation is considered a simple (even though the mechanisms are still not fully understood) form of non-associative learning and could be defined as a reduction in a response to a novel neutral stimuli with no apparent adaptive value (Leussis and Bolivar, 2006). But the behavioural and physiological responses in the experimental trout are to no extent non-adaptive and some of the stimuli are to be considered as potent stressors rather than neutral. Instead, starting to eat earlier, not engaging in fights, saving oxygen during hypoxia and reduced sympathetic reactivity during confinement should all be conceived as potentially adaptive.

Another explanation for the changes in response to the different tests is the decline in condition factor. Meaning that the experimental trout, for example, resume feeding sooner and eat more because they are in worse shape the second time compared to the first. However, we find this highly unlikely due to the fact that condition factor was one of the few variables that were positively correlated between trial replicates and this reduction in condition factor was not significantly correlated with the difference in any of the other quantified behavioural and physiological responses.

The only other variable that was significantly correlated between trial replicates were the plasma concentrations of noradrenaline. Remarkably, the concentration of noradrenaline was positively correlated with the magnitude of change in activity between the two replicates during the hypoxia test. The level of circulatory noradrenaline is known to give a good reflection of the sympathetic activity (Perry and Bernier, 1999). High sympathetic activity has earlier been suggested to be an important characteristic for proactive copers (an active/aggressive/bold behavioural type) who are normally associated with a rather rigid type of behaviour. For example, proactive male mice and rats are known to easily form routines and for being inflexible in their behaviour (Koolhaas et al., 1999). As such, our result clearly contradicts this part of the mammalian model of stress coping strategies. However, if we accept that the noticeable change in responses to applied tests is based on the fact that the experimental fish recognise the repeated procedure, then we have several examples of similar results in fish. For example it has been shown that more active and less exploratory guppies (Poecilia reticulata)
performed better in a shuttlebox avoidance paradigm (Budaev and Zhuikov, 1998). Further, Sneddon et al. (2003) showed that bold rainbow trout, that also were more active than shy individuals, learned a conditioned task quicker than trout identified as shy. Also, it has been shown that two lines of rainbow trout selectively bred for divergence in stress response (post stress plasma cortisol) differ in capability to maintain a conditioned response (Moreira et al., 2004). Indeed, results from an experiment following up the results provided from Moreira et al. (2004) suggest that high plasma cortisol impair the memory process in rainbow trout (Barreto et al., 2006). These two strains selected on HPI-axis reactivity have opposite endocrine stress responses. Following stress the high responders have high levels of plasma cortisol and low levels of catecholamines compared to the other strain (Pottinger and Carrick, 1999). Considering these examples together, they suggest that individuals with high catecholaminergic and low HPI-axis activity learn quicker because they have a better memory. However, plasma cortisol seems to be variable and it has been shown that the mentioned strains of rainbow trout, even though selected on this specific trait, do not always differ in plasma levels of cortisol following confinement (Schjolden et al., 2006b).

One peculiar finding in this study is the fact that no other significant correlations in quantified variables were seen between trial replicates. However, the experimental trout used in the present study were from a hatchery-reared strain of brown trout and the hatchery environment lacks certain selective factors that are present in the wild, such as predators. Also, food is often presented in abundance and parasites are effectively removed. This generally leads to very high survival rates in hatcheries compared to wild environments (Fleming, 1996; Huntingford and Adams, 2005; Huntingford, 2004; Huntingford et al., 2006). Recent studies have suggested that a certain selection is needed for behaviours to be consistent over time (Alvarez and Bell, 2007; Bell and Sih, 2007; Bell and Stamps, 2004). Thus, a correlation similar to that shown in threespined sticklebacks by Huntingford (Huntingford, 1976) that aggression towards conspecifics is positively correlated with boldness towards predators, would only occur in certain selection regimes. Thus, other behavioural syndromes may also need a certain selection to remain consistent over time. Alternatively, certain conditions are needed for innate behavioural plasticity to lead to explicit changes in behaviour. However, Salonen (Salonen and Peuhkuri, 2006) have shown that hatchery rearing of grayling might alter single behavioural traits but the correlation between repeated trials remains significant positive, i.e. the behavioural syndrome itself is not affected by hatchery rearing. Clearly, there are some contradictions in this issue and likely there are differences between species and even populations within species.
During the first trial mean activity in the hypoxia test was positively correlated with plasma levels of noradrenaline following a confinement test. Thus sympathetic reactivity reflects the behavioural activity during stress in two separate tests. We know from earlier studies on mammals that individuals with high sympathetic reactivity are also often more behaviourally active. Thus, these results are well in line with studies on other vertebrates but also to some extent with earlier studies on fish. For example, the two lines of rainbow trout mentioned earlier differ in activity during a novel environment test (Schjolden et al., 2005a; Schjolden et al., 2006a). Also, brown trout from several different populations, both from the wild and hatchery derived, with high sympathetic activity are more active during a novel environment test (Brelin et al., 2008). But the hypoxia test applied in the present study must be considered a severe stressor compared to a novel environment test. However, during a similar hypoxia test as the one in the present study Van Raaij et al. (Van Raaij et al., 1996) showed that rainbow trout displaying the most active behaviour during the test also had higher levels of plasma catecholamines and lower levels of plasma cortisol compared to less active trout. Results such as these, linking physiological variables to behavioural traits are of key importance when investigating behavioural types. Especially so when showing that behavioural and physiological stress responses are correlated. Stress brings out the essence of personality traits and is therefore very important when investigating animal personalities (Øverli, 2007; Øverli et al., 2007).

During the second test willingness to resume feeding after transfer was positively correlated with plasma cortisol following the confinement test. It is known that low levels of cortisol act stimulatory whereas high levels of cortisol, as a consequence of acute or chronic stress, act inhibitory on appetite in fish (Bernier, 2006; Bernier and Peter, 2001). Further, it has been shown that cortisol stimulates appetite in rainbow trout as well as having other time and context specific behavioural effects (Øverli et al., 2002a). For example Øverli et al. (2002) has shown that locomotor activity was stimulated by short term treatment with cortisol. Also, our results show that there is a positive correlation between willingness to resume feeding and activity during the hypoxia test which was also positively correlated to the number of attacks against a conspecific intruder. Thus, individuals that resume feeding quicker are prone to be more active during hypoxia as well as more aggressive. This is typical for proactive rodents described by Koolhas et al. (1999). Further it has been shown that LR trout adjust faster to a new environment and as such resume feeding faster than HR trout (Øverli et al., 2001; Øverli et al., 2005). Thus, the results from this study show that there are similarities but also differences between stress coping strategies described in mammals and those in fish.
CONCLUSIONS & FUTURE OBJECTIVES

In conclusion the results of this thesis show that there are differences in both behavioural and neuroendocrine stress response between the examined brown trout populations, likely reflecting different selection regimes in the different streams. Further, our data suggests that sea-ranching promotes more active individuals with a stronger sympathetic reactivity. However, it is not clear to what extent inter individual differences in behaviour and physiology reflects divergent coping strategies, as described in mammals (Koolhaas et al., 1999). The result in this thesis further strengthens the idea that differences in sympathetic reactivity are a more robust characteristic than difference in HPA/HPI axis reactivity in stress coping strategies. Most interestingly, the frequency distribution of brown trout displaying distinct phenotypes differs among examined populations.

Further, the results of this thesis show that precocious sexual maturation varies between brown trout populations and is less prevalent in trout from the population used for sea-ranching purposes, as compared to trout from the other wild populations. However, we find no link between sympathetic reactivity and the probability to sexually mature as parr. Thus, it seems that sympathetic reactivity only has a minor influence on precocious sexual maturation and vice versa. But instead maternal contribution (relative egg size) is of major importance for at least precocious sexual maturation. Consequently, our results strengthen the hypothesis that males coming from females with small egg size will be more prone to sexually mature as parr compared to males hatching from large eggs.

Also, we show that juvenile brown trout change their behavioural and physiological stress responses when going through the same test paradigm a second time. When exposed to the repeated stressors the experimental fish display reduced behavioural and physiological stress responses. Further, a number of significant correlations between behavioural and physiological variables within trials are evident, but only plasma noradrenaline levels, following a confinement test, were correlated between trials. Further, sympathetic reactivity is positively correlated with the magnitude of reduction in activity between trials. This clearly suggests that fish with a high sympathetic reactivity are more prone to change their behaviour, at least after exposure to stressors. Thus, our results strengthen the hypothesis that variation
in learning capabilities in fish, and other vertebrates, is linked to the concept of animal personalities.

It has been shown that personality plays a major role in the probability of becoming dominant. This is seen for example in rainbow trout selected on their post stress cortisol response individuals with a low response more often wins dyadic fights. However, certain aspects of this setup are corrupt because we know that social status has a major influence on both behaviour and physiology (including post stress plasma cortisol). This means that individual fish might be predisposed to win not because their personality but because of their prior social status. Thus, the time has come to turn this issue around and approach it from the totally opposite direction. Consequently, we need to investigate how social status affects specific personality traits.

It seems that animal personality traits might be plastic. Relyea (2002) found that, depending on the trait examined and the environment experienced, increased plasticity had either positive effects, negative effects, or no effects on tadpole mass, development, and survivorship. He found no relationship between increased plasticity and greater developmental instability. This suggests that costs of plasticity may be pervasive in nature and may substantially impact the evolution of optimal phenotypes in organisms that live in heterogeneous environments (Relyea, 2002). However, most studies refer to morphological plasticity, and behavioural plasticity might need more attention. Obviously, we need to investigate and understand the neuroendocrine mechanisms behind animal personality better, as well as the genetics (heritabilities) of the traits. It is also important to disentangle plasticity and local adaptation (Weitere et al., 2004). Also, more studies are needed that investigate personality traits in new species while at the same time comparing populations within species. We need theories on how and why this bimodal variation is maintained. How important are these variations in personality traits regarding rapid environmental changes? The latter might be a highly important question during this era of an ongoing global climate change. Thus, further work is needed in this interesting field.
SVENSK SAMMANFATTNING


Detta arbete (1) verifierar att dessa två stressrespons strategier förekommer hos öring, (2) undersöker om olika öringpopulationer skiljer sig med avseenden på andelen individer med aktiv och passiv stressrespons, (3) undersöker om kompensationsodling påverkar fördelningen av individer med olika typer av stressrespons inom populationen, (4) samt undersöker om tidig könsmognad hos öringhannar är kopplad till någon av ovanstående stresshanterings-
strategier. Vidare, (5) undersöker den om det är någon skillnad i hur öring som utrycker respektive stresshanteringsstil förändrar sitt beteende när de är med om upprepade försök.


Sammantaget så styrker resultaten i den här avhandlingen hypotesen om att olika stresshanteringsstrategier existerar hos fisk. Dessutom visar den på skillnader i frekvens av stresshanteringsstilar mellan olika populationer av öring, vilket indikerar att stresshanteringsstilar är kopplat till den adaptiva repertoaren av fenotypiskplasticitet.
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“I can’t put my finger on it but there is definitely something fishy about this thesis” / Daniel, 2008
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