The Pace of Life of Juvenile Brown Trout

Inter- and Intra-individual Variation in Growth and Behaviour

Joacim Näslund

UNIVERSITY OF GOTHENBURG

Department of Biological and Environmental Sciences
Faculty of Science

Gothenburg 2015

Akademisk avhandling för filosofie doktorsexamen i naturvetenskap, inriktning biologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 30 oktober 2015 kl. 10:00 i Föreläsningssalen, Institutionen för biologi och miljövetenskap, Medicinaregatan 18A (Zoologihuset), Göteborg

Opponent är Dr. Robert L. McLaughlin, Department of Integrative Biology, University of Guelph, Guelph, ON, Kanada

Cover illustration: Brown trout fry (Joacim Näslund)

The Pace of Life of Juvenile Brown Trout - Inter- and Intra-individual Variation in Growth and Behaviour

Joacim Näslund

Department of Biological and Environmental Sciences
University of Gothenburg
Box 463
SE-405 30 Gothenburg, Sweden

joacim.naslund@gmail.com

ISBN 978-91-628-9606-5 (PDF)
ISBN 978-91-628-9605-8 (Print)
Electronic version: http://hdl.handle.net/2077/40167

Printed by Kompendiet (Aidla Trading AB), Gothenburg, Sweden 2015
(http://www.kompendiet.se/)

Till minne av Sone Göransson och Tore Näslund

"Science does not rest upon solid bedrock. The bold structure of its theories rises, as it were, above a swamp. It is like a building erected on piles. The piles are driven down from above into the swamp, but not down to any natural or 'given' base; and if we stop driving the piles deeper, it is not because we have reached firm ground. We simply stop when we are satisfied that the piles are firm enough to carry the structure, at least for the time being."

- Karl R. Popper
The Logic of Scientific Discovery, 1968 [p 111, rev. ed.]

This page intentionally left (almost) blank

Abstract

The pace-of-life (POL) syndrome hypothesis is the prevailing model used for explaining the differences in how animals live their lives. The POL syndrome is a framework connecting life-history traits (traits describing the characteristics of the life cycle of an organism) with behavioural and physiological traits, which can be used to describe differences between species, individuals and even within single individuals at different energetic states. A fast POL reflects attributes such as increased risk-taking, high metabolism, fast growth, low cellular maintenance, proactive stress handling and shorter life expectancy, whereas a slow POL is attributed with the opposite traits. This thesis investigates both inter- and intra-individual differences in a range of POL traits in juvenile brown trout (*Salmo trutta*).

In a stream experiment, one-year-old (1+) trout were shown to elicit faster than normal growth rates (i.e. increasing their pace-of-life) following starvation. This phenomenon, commonly known as compensatory growth (CG), was observed over summer and autumn before diminishing in winter. Despite CG, a hypothesised decrease in cellular maintenance was not detected and survival was not significantly affected.

A subsequent stream experiment investigating the effects of food-restriction on trout in late autumn also showed that CG was not elicited in the winter. Nevertheless, food-restricted fish performed equally well in terms of survival and condition (length-mass relationship), when compared to well-fed fish in the following spring. However, seaward migration in spring appeared to be delayed as a consequence of this achievement.

Given that 1+ trout could elicit CG, the focus of the study switched towards trout fry (0+). Fry normally grow much faster than 1+ trout, which may affect their scope to further increase growth rates. The experiments showed that CG in fry could occur in the laboratory environment but not in the wild, suggesting that fry growth rates are environmentally constrained in nature. Investigation of the behaviour in fry following different food treatments indicated that their behaviour was not influenced by CG, which may be adaptive if higher than normal growth is impossible to achieve in nature at this life-stage.

Behavioural syndromes (associations between different behavioural POL traits) were found, generally reflecting different activity levels. More active 0+ fish had higher survival rates than less active ones, however no such difference was observed in 1+ fish. Instead, the more active 1+ fish appeared to grow better in high-quality habitats whilst in habitats of lower quality a more passive strategy was more beneficial. Thus, different behavioural strategies appear to be advantageous in different environments.

This thesis presents several results contradictory to the general POLS hypothesis, which may be attributed to the territorial life-style of trout. This highlights the importance of not assuming trait correlations or ecological consequences of single traits only because the POLS hypothesis predicts such associations.

Keywords: Behavioural syndrome, Compensatory growth, Growth rate, Mortality, *Salmo trutta*, Pace-of-life syndrome, State-dependent behaviour, Trade-off

The author collecting fish in Jörlandaån using electrofishing (Paper 5).
Photo: Malin Rosengren

Supervisor: Prof. Jörgen I. Johnsson, Department of Biological and Environmental Sciences, University of Gothenburg

Co-supervisor: Dr. Johan Höjesjö, Department of Biological and Environmental Sciences, University of Gothenburg

Co-supervisor: Dr. Angela Pauliny, Department of Biological and Environmental Sciences, University of Gothenburg

Examiner: Prof. Staffan Andersson, Department of Biological and Environmental Sciences, University of Gothenburg

Contents

Definitions

Compensatory growth: A state-dependent growth response, characterised by faster-than-normal growth rates, elicited to regain 'lost' growth following a transient period of growth depression.

Fitness: A property of a class of individuals (e.g. with a specific genotype), describing their success in producing new individuals within the same class, typically through reproduction.

Genotype: The genetic characterisation of an individual.

Life-history: A set of strategies that influence death rate and birth rate in organisms, such as life-span (longevity), age at maturation (developmental rate), adult size, growth rate, and reproductive output (fecundity).

Phenotype: The expression of the genotype after interactions with environmental factors.

State: A phenotypic trait which changes over time.

Syndrome: Covariation among different traits (physiological, behavioural, cognitive, etc.) within an organismal group.

Trade-off: Negative functional interaction between traits.

Scope and outline of the thesis

This thesis is divided into five major parts:

1. A general introduction which is itself divided into three main sections: The first covers the general background of pace-of-life theory and mainly concerns among-species and among-population variance in life-history traits. The next two sections mainly concern the relationships between life-history traits (growth and mortality) and behaviour. The second section details the differences among individuals in the context of the pace-of-life syndrome hypothesis, while the third section deals with effects of energy status and size on the growth and behaviour of single individuals. This section introduces major concepts, on which the later discussion of the findings of this thesis will be based.

2. A part on salmonid biology, which starts with general information, followed by a more detailed description of inter- and intra-individual differences in growth and behaviour of salmonids. This section also present the aims and discuss the major findings of the publications and manuscripts on which this thesis is based.

3. A part on methodology, which is focused on explaining the main methodological differences among the experiments, and the consequences of these differences for interpretations of the results. For detailed description of materials and methods for each study, I refer to the original articles, which are included at the end of this thesis.

4. A general conclusion and future perspectives.

5. The six original works on which this thesis is based.

1. Introduction

1.1 Life-history and pace-of-life theory

1.1.1 Life-history differences among species - the *r*-*K* continuum

Life-history theory is based on a set of discrete demographic traits which is used to describe life cycle adaptations of organisms in an evolutionary framework (Roff 1992; Stearns 1992; Ricklefs and Wikelski 2002). Different species differ widely when it comes to life-history traits, which include size at birth/hatching, growth patterns, age and size at maturity, number and size of offspring, reproductive investments, mortality schedules, and longevity (Roff 1992; Stearns 1992). Given the continuous scales of the life-history traits, there is an endless list of possible trait combinations, but many of these combinations do in fact not exist in nature (Ricklefs and Wikelski 2002). Instead, certain life-history traits seem to be related to each other in suites. Fast growing species tend to have early maturation, short life-span, small adult size, and high fecundity (Pianka 1970). In classical ecological theory, these species are referred to as r-selected species, with the r stemming from standard ecological algebra, more specifically the equation for logistic population growth (MacArthur and Wilson 1967):

$$\frac{dN}{dt} = rN(1 - \frac{N}{K}) \tag{1}$$

In this equation, r is the maximal intrinsic growth rate of the population (N), K is the carrying capacity and dN/dt is the derivative of N with respect to time t. In other words, the r-selected species have evolved to have a high productivity per time unit (short generation time), at the expense of the quality (survivability) of the products (bodies and offspring). In contrast, slow growing species generally mature later, live longer, have a larger adult size, and a lower fecundity. These are classically referred to as K-selected species (Pianka 1970), with K referring to the carrying

capacity parameter (K) in Eqn. 1 (i.e. the maximum population size of the species that can be sustained indefinitely given the resources available in the environment). Thus, K-selected species are selected to produce highly competitive bodies and offspring, at the expense of productivity per unit time.

r-selection refers to density-independent natural selection, while K-selection refers to density-dependent natural selection (MacArthur and Wilson 1967; Pianka 1972). However, no species are completely r- or K-selected, but instead reach some trade-off between the extremes (Pianka 1970). Particularly ectotherms, like fish, deviate largely from the predicted trait combinations, e.g. with large bodies often being associated with high fecundity (Roff 1992). Thus, an organism should only be considered as r- or K-strategists relative to other organisms (Pianka 1974), and these organisms need to be relatively closely related to make the comparison relevant (Roff 1992).

While the idea of the $r \rightarrow K$ continuum is the original ecological basis for the hypothesis being discussed in this thesis, the present general view of the r-K selection hypothesis is that it is too simplistic, particularly since it does not take into account extrinsic mortality or different selective pressures on different life-stages (see e.g. Stearns 1992; Reznick et al. 2002). Therefore, we now leave this idea for a more comprehensive hypothesis.

1.1.2 Living fast, or living slow? The pace-of-life syndrome

Instead of using the $r \rightarrow K$ continuum, we can describe life-history traits as following a fast $\rightarrow$ slow continuum, which leads to the concept of pace-of-life syndromes (POLS) (Ricklefs 2000; Ricklefs and Wikelski 2002; Sih et al. 2004a; Réale et al. 2010). This newer concept has clear parallels to the $r \rightarrow K$ continuum, but also incorporates components which the organism uses to cope with extrinsic risk factors, both biotic (e.g. parasites, pathogens, and predators) and abiotic (e.g. extreme environmental deviations) (Ricklefs and Wikelski 2002; Réale et al. 2010). Henceforth, 'syndrome' will indicate that we discuss suites of correlated traits in organismal groups (e.g. species or populations) (Sih et al. 2004a,b).

The POLS hypothesis is a holistic model, integrating physiological mechanisms, as well as behavioural characteristics, to explain life-history variation among species (and within species; see section 1.2) in greater detail (Ricklefs and Wikelski 2002). The hypothesis suggests that life-history and behavioural traits are connected in suites because of physiological control mechanisms restricting life-history- and behavioural types to a key axis of

variation (i.e. the fast → slow life-history continuum), which limits the plasticity of organisms (Ricklefs and Wikelski 2002; Sih et al. 2004a,b) (see Table 1 for the general POLS model). For instance, when observing amphibian tadpoles swimming in a safe environment, some species are moving more actively than others (Richardson 2001). This trait probably leads to a higher foraging efficiency and higher growth rate (i.e. fast POL traits[1]), given a good food supply. However, the more active tadpoles are also more active when predators are present, which leads to higher mortality rates in these situations (Richardson 2001). It would be better to have relatively lower activity when predators are present, as activity increases the exposure to predators (Werner and Anholt 1993). These apparently non-adaptive trait combinations signal that behaviour is not completely plastic, probably due to carry-over effects among different traits (Sih et al. 2004a). For instance, physiological traits making an individual active in a context where being active is beneficial, may also affect activity similarly in another context where it is not beneficial.

One can view the POLS from two complementary perspectives, proximate causation and ultimate causation (Mayr 1961). Proximate causation explains *how* variation in genes and development affect physiological, morphological and behavioural traits that form the phenotype. Ultimate causation explains *why* traits have evolved, i.e. the mechanism through which traits have been selected through evolution. Put in a context of growth rate, we can either say that species that grow fast do so due to e.g. high expression of growth promoting hormones (proximate explanation), or that it is due to natural selection, where faster growers historically have had higher fitness, e.g. by reducing the time to first reproduction (ultimate explanation). The POLS is largely an ultimate explanation of life-history diversity among organisms. A similar proximate hypothesis is the rate-of-living hypothesis[2], which is older (Rubner 1908; Pearl 1928) but not as extensive in its predictions, as it relates mainly to energetic trade-offs determining life-span of organisms.

The proximate view – physiological trade-offs and limitations

Several physiological traits are considered to be key traits behind the POL of organisms, e.g. metabolic rate (Wiersma et al. 2007), immune system

[1]Note the difference between the abbreviations POL (used when discussing specific pace-of-life traits and strategies) and POLS (used for the general pace-of-life syndrome, describing the whole suite of different POL strategies (or 'POL types')). Also see Fig. 1.

[2]If the $r \rightarrow K$ continuum hypothesis is the ultimate predecessor to the POLS hypothesis, the rate-of-living hypothesis is its proximate counterpart

Table 1: Schematic illustration of the *pace-of-life syndrome* hypothesis. Basic structure modified from Réale et al. (2010), with additions from Koolhaas et al. (1999), Korte et al. (2005), Mittelbach et al. (2014), and Castanheira et al. (2015).

Pace-of-Life continuum		
Fast	⟷	**Slow**
	Life history	
Short life expectancy	⟷	Long life expectancy
Early reproduction	⟷	Late reproduction
High growth rate	⟷	Low growth rate
High fecundity	⟷	Low fecundity
	Behaviour	
Bold	⟷	Shy
Aggressive	⟷	Timid
Active	⟷	Passive
Superficial explorer	⟷	Extensive explorer
Extensive dispersal	⟷	Limited dispersal
Asocial/Territorial	⟷	Social
Low behavioural flexibility	⟷	High behavioural flexibility
(Intrinsic factors important)		(Extrinsic factors important)
	Physiology	
Low HPI/HPA axis* reactivity	⟷	High HPI/HPA axis* reactivity
High sympathetic reactivity	⟷	Low sympathetic reactivity
Low parasympathetic reactivity	⟷	High parasympathetic reactivity
High metabolic rate	⟷	Low metabolic rate
Low cellular maintenance	⟷	High cellular maintenance
Low immune response	⟷	High immune response
Large metabolically costly organs	⟷	Small metabolically costly organs
Low neural plasticity	⟷	High neural plasticity

* HPI/HPA axis: hypothalamus-pituitary-interrenal/adrenal axis (HPI in fishes)
- controlling the physiological stress response

capacity (Lochmiller and Deerenberg 2000), stress reactivity (Korte et al. 2005), cellular maintenance capacity (Mangel 2008), and endocrine regulation of growth and reproductive behaviour (Ricklefs and Wikelski 2002) (see also Table 1). Naturally, all these traits are related to energy costs and an organism cannot sustain perfect functioning of all these traits with a limited energy intake. Instead, energy has to be allocated in some optimal way (Zera and Harshman 2001). Energy allocation can be described using the balanced energy equation (e.g. Jobling 1985a, Nelson 2011):

$$C = F + A = F + U + A' = F + U + R + W + S + B + G \tag{2}$$

In this equation, C is the energy consumption, F is energy lost in faeces, A is energy absorbed, A' is energy assimilated, U is energy lost in nitrogen excretion, R is energy spent in maintenance (standard metabolism), W is energy spent in non-maintenance functions (e.g. motion, stress response, etc.), S is the energy spent in assimilation food molecules (specific dynamic action), B is energy allocated to growth and energy storage, and G is energy allocated to reproduction. Several of these terms can be broken down into even finer detail. For instance, R can be divided into different types of

maintenance (e.g. immune function, chromosomal integrity, anti-oxidation, etc.), and B can be divided into growth of different tissues (muscles, skeletal structures, neural tissue, fat tissue, etc.). The point made by this equation is that energy expended must equal energy intake, or else the body itself will be broken down to supply the energy need - which ultimately will lead to starvation and death. An increase in one term on the right-hand side of the equation must, by necessity, be balanced by a decrease in another right-hand term, or an increase of the left-hand term C.

Generally, when looking at trade-offs from a multi-trait perspective, we should recognise that two traits can be positively correlated, even though all traits are costly – simply because both of them can trade off with a third trait (Roff 1992; Zera and Harshmann 2001). Given that all physiological processes are costly in terms of energy, there is a multitude of possible trade-offs, and even if the POLS hypothesis gives a general picture of the expectations, deviations from the general model should perhaps be expected.

Based on empirical studies the original rate-of-living hypothesis suggested that the basal metabolic rate is negatively related with life-span of an organism (Rubner 1908; Pearl 1928). Radical oxygen species (ROS) are by-products of the metabolic processes causing damage to cellular macromolecules, which in turn could be a potential cause of ageing (Harman 1956; Finkel and Holbrook 2000). Furthermore, fast metabolism is costly and the energy spent on metabolism may be traded off against other bodily functions, like the cellular maintenance system which repair damage and errors caused by e.g. ROS, transcription associated mutations, and environmental toxins (e.g. Arendt 1997; Mangel and Munch 2005; Mangel 2008).

Results from studies investigating the direct trade-off between metabolic rate and life-history traits are mixed (Speakman 2005). However, this may be due to a multitude of factors being involved in the trade-off, leading single cases to diverge from general patterns (Zera and Harshman 2001). Evidence for a general connection between metabolic rate and several life-history traits has been found in birds, where tropical birds both show lower basal metabolism and life-history traits connected to a slower POL, as compared to temperate species (Wiersma et al. 2007). In endotherms (as opposed to ectotherms), a warmer climate leads to reduced activity and lower need for heat generation (Speakman 2005). This can explain how tropical birds can spend less energy on metabolism than birds in colder climates (Speakman 2005; Wiersma et al. 2007). They also tend to have lower mass (and thereby lower energetic costs) of several central organs (Wiersma et al. 2012). Furthermore, following the POLS, the tropical

birds tend to have high immune system capacity – a trait which helps to obtain a longer life-span (Tieleman et al. 2005).

There is general evidence that smaller animals expend more energy per gram tissue than larger animals, supporting the general view that smaller animals have a faster POL than larger animals (Speakman 2005). Increased size will come together with higher absolute metabolic rate, leading to a demand for more food (Speakman 2005). The larger body, however, also leads to more energy storage capacity, so while the demand for food increases, the animal may not need to eat as often. Smaller size comes along with higher mass specific metabolic rate and less storage space, so while the absolute energy demand decreases, the animal instead needs to eat more often. The relationships between body size and energy demand affect the behaviour in more or less predictable directions. A small animal can sustain itself on smaller absolute amounts of food than a larger animal, but needs to spend more time foraging and may take higher risks by doing so (Arendt 1997). A larger animal can be more risk averse due to its energy stores, but may also need to migrate to find enough quantities of food. An illustrative example is the humpback whale *Megaptera novaeangliae*, a species with a typical slow POL, which migrates long distances between tropical and high latitude environments (Clapham 2000). The humpback whale breed and give birth in winter in relatively safe tropical areas but, in contrast to smaller marine mammals like dolphins, it cannot find enough food to sustain its life there indefinitely and therefore has to find foraging grounds with sufficient amounts of food at higher latitudes in summer (Clapham 2000). The breeding migration in this species is allowed by the immense capacity to store energy in the humpback whale body.

However, not all species follow the typical patterns predicted by the POLS hypothesis. For instance, some animals have both high metabolic rate and a long life-span - traits which reflect conflicting POL strategies (examples include some bats and birds; Munshi-South and Wilkinson 2010). This is likely because many other physiological traits in addition to metabolic rate also affect life-span (Speakman et al. 2002). Still, the proximate mechanism governing longevity, and other life-history traits, are likely influenced by energetic trade-offs (Zera and Harshman 2001).

The proximate reason why we see syndromes of correlated traits in organisms, and not all possible combinations of trait trade-offs, is thought to depend to a large extent on regulatory endocrinology (Ricklefs and Wikelski 2002). Hormone levels (e.g. glucocorticoids and catecholamines) are, for instance, linked to many behavioural traits (Oliveira and Gonçalves 2008). The same is true for growth-regulating peptide hormones (e.g. growth hor-

mone, ghrelin, and leptin) and sex steroids (e.g. androgens, oestrogens, and progestogens) (Oliveira and Gonçalves 2008). Expression levels of hormones are generally genetically heritable (Zera et al. 2007; Øverli et al. 2007; Carlson and Seamons 2008), and thus also provide a base for natural selection to act on.

There may also be genetic constraints on phenotypic plasticity, such as pleiotropy and linkage disequilibrium (Arnold 1994). Pleiotropy refers to single genes affecting several traits, and linkage disequilibrium means that alleles at different loci are associated to each other in some way, e.g. being located closely together in the genome (Futuyma 2009). Both these effects limit variability of the traits involved in the plasticity.

The ultimate view – natural selection

Any given environment provides certain stressors and risks, as well as beneficial qualities, to which the inhabiting organisms adapt through natural selection to maximise their relative fitness. Relative fitness is here defined as relative life-time reproductive success (maximizing $\sum l_x m_x$ in Eqn. 3), which is assessed in relation to the other individuals in the population in question (Futuyma 2009).

Returning to the example concerning tropical versus temperate birds, the difference in POL was seen to be associated to physiological trade-offs. A potential explanation to why tropical birds have lower basal metabolism was that heat regulation is less costly in a warmer climate (Speakman 2005). This relates to the ultimate explanation of their slower POL. The lower metabolism of tropical birds is not simply a plastic response to a warmer environment, but also has a genetic (and thus evolved) component (Wikelski et al. 2003). This suggests that the trait has evolved through natural selection, where tropical birds with lower metabolic rate have had higher reproductive output than conspecifics with higher metabolic rate. Likely, by avoiding spending energy on heat producing metabolism, they can allocate more energy to other processes, e.g. to immune defence which can allow the bird to live longer. However, this would only be a good strategy if mortality rates are generally low, otherwise a long life might not be realised anyway. In fact, tropical birds experience lower extrinsic mortality risks (Hirshfield and Tinkle 1975, Law 1979). Slow POL traits often evolve when mortality risk is relatively low, and this could be part of the ultimate explanation for why tropical birds have generally slow POL (Saether 1988; Promislow and Harvey 1990; Ricklefs 2000; Futuyma 2009). Other potential explanations could be that a short growing season in temperate areas

select for fast growth, while a long growing season allow for slower growth (e.g. Conover and Present 1990). To be able to survive winter, or to be able to migrate, animals may need to reach a certain size before a certain time-point (or time-window) of the year, thus promoting a higher growth rate (Conover and Present 1990; Metcalfe 1998).

As a more formal view of the ultimate causation of life-history strategies, and the trade-offs among different traits, we can use the equation for lifetime reproductive success:

$$\sum_{x=\alpha}^{\omega} l_x m_x = l_\alpha m_\alpha + l_{\alpha+1} m_{\alpha+1} + \cdots + l_{\omega-1} m_{\omega-1} + l_\omega m_\omega \tag{3}$$

Here, l_x is the probability of surviving from birth/hatching to beginning of time x, m is the expected number of offspring for a female at time x, α is the age at first reproduction, and ω is the maximal life span (e.g. Roff 1986; Stearns 1992).

Increasing $\sum l_x m_x$ is increasing 'fitness'. This can be done by decreasing α, increasing ω, increasing l, and increasing m. Achieving perfect values for all of these parameters at the same time is virtually impossible unless the organism in question is completely unconstrained – a case commonly referred to as the Darwinian demon (e.g. Law 1979). Both l and m are generally positively related to size, but size also increases α; i.e. larger size comes with increased developmental time and later maturation (Roff 1986). Growing fast to a large size may mitigate this effect on α, but achieving this may require a reduction in l, e.g. due to higher risk-taking associated to foraging, or energy re-allocation from maintenance to growth (Arendt 1997; Stamps 2007). Increasing m generally comes with reduction in ω or reduced l, because there are energy costs in producing offspring which could otherwise be allocated to present or future survivability (Williams 1966; Roff 1986). This illustrates the trade-offs in life-history traits. In absence of a Darwinian demon, combinations of life-history traits are optimised given biomechanical, physiological, phylogenetic, genetic, and environmental constraints (Roff 1992; Stearns 1992).

Biomechanical constraints simply mean that organisms have to obey fundamental laws of physics and chemistry. Physiological constraints relates to some physiological traits being restricted, e.g. by body size (like metabolism). Phylogenetic constraints mean that some traits have been fixed, or limited to certain intervals, during the evolutionary process. For instance, the clutch size in the bird order Procellariformes (petrels) is fixed to one egg (Lack 1947) and since there is no variation in this trait, it cannot evolve (until variation appears and can be selected upon). Genetic

constraints involve e.g. pleiotropy or linkage disequilibrium (Arnold 1994; Zera and Harshman 2001). These genetic constraints could possibly be broken through alterations of the genome, such as genome duplications (allowing two copies of pleiotropic genes to evolve in different directions), or recombination of chromosomes (which can break linkages between genes). Environmental constraints involve predators, competitors, abiotic hazards, parasites, temperature and a wide range of other factors which appear in the habitat of the organism in question. Environmental constraints commonly affect different life-stages in different ways, and organisms can adapt to these, e.g. through physiological or behavioural adaptations.

In less demanding environments with stable and/or predictable resources, little seasonal variation and relatively low extrinsic mortality risk we would expect evolution of larger body size (and associated traits such as small clutch sizes; i.e. a slow POL), since this environment is relatively safe (Promislow and Harvey 1990; Robinson et al. 2010). In more stressful environments, with unpredictably fluctuating resources and high extrinsic mortality risk, we would instead expect smaller body size, along with fast POL traits (Promislow and Harvey 1990; Robinson et al. 2010). Deviations from general patterns of POLS can also be found. For instance, Darwin's finches *Geospiza* spp. live in fluctuating environments with low predation mortality on Daphne Island (Galápagos) and this environment have selected for long life (slow POL trait) and large clutch sizes (fast POL trait) (Grant and Grant 2011).

In the POLS physiological and behavioural traits are related to life-history traits. The physiological traits can safely be assumed to underlie much of the behavioural expressions, as well as their stability and covariance (Wolf and McNamara 2012), and behavioural expressions are connected to several key life-history traits, like growth rate and probability of survival (Biro and Stamps 2010). Thus, physiological and behavioural traits are being part of the whole trade-off system shaping the life-histories of animals. Genetic constraints could be linked to covariance among behavioural traits, e.g. when a single genetic locus affects a physiological factor which in turn affects several behavioural traits (e.g. Norton et al. 2011). Environmental constraints include temperature, seasonality, predation pressure, resource availability and a wide range of other factors which can set boundaries for physiological and behavioural traits with effects on life-history traits. For example, low temperatures may limit ectotherms to relatively slow POL, with slow growth rates and large size-at-maturity (Angilletta et al. 2004).

1.2 Within-population variation in pace-of-life syndrome traits

The intra-specific variation in traits is generally smaller than the inter-specific variation. Still, individuals within single population do indeed differ, and the variation can be large in many traits (Magurran 1993). The same underlying hypotheses work for intra-specific comparisons of POLS as for inter-specific comparisons (Réale et al. 2010; also see Table 1). The POLS itself can be described as an overarching syndrome being composed of several lower-level syndromes. These other syndromes are the coping style syndromes (connecting physiological and behavioural stress coping traits), metabolic rate syndromes (connecting metabolic rates with behavioural traits, as well as other physiological traits) and behavioural syndromes (connecting different behavioural traits)[3]. Coping style-, metabolic rate-, and behavioural syndromes represent personality traits within populations and are integrated into the POLS (Réale et al. 2010). These lower-level syndromes are discussed separately below[4], and are thereafter discussed together in the context of intra-specific POLS.

1.2.1 Coping style syndromes

The term coping style refers to consistent individual responses to stress or novelty challenges (Koolhaas et al. 1999; Réale et al. 2007; Øverli et al. 2007; Castanheira et al. 2015). The term is sometimes used to describe behavioural traits only, but most commonly it is used to describe physiology-behaviour syndromes (Koolhaas et al. 1999; Sih et al. 2004b; Castanheira et al. 2015). The coping style syndrome is a proximate framework to explain consistent individual differences in behaviour (i.e. individuals' personalities). Certain suites of correlated physiological and behavioural traits are generally expressed along a coping style continuum, ranging from passive to active coping, in a reactive → proactive continuum. This continuum is often dichotomised into two general clusters based on the extreme traits of the continuum (i.e. grouping into reactive and proactive individuals). Whether or not such clustering is warranted depends on the modality of the distribution of the traits along the continuum (see Fig. 1).

[3]There may be other syndromes as well, like cognition syndromes, linking cognitive ability to behaviours and life-history traits (e.g. Kotrschal et al. 2013), but I limit the scope of my introduction to discuss only the three syndromes presented here.

[4]The studies in this thesis only concern behavioural syndromes, but the other are described as well because it allows for discussion in the following section on POLS in the context of salmonid ecology

The concept of coping styles fits very well into the POLS hypothesis, likely due to the POLS hypothesis being strongly influenced by it (compare e.g. schematics of POLS in Réale et al. 2010 with schematics of coping styles in Koolhaas et al. 1999, here summarised together in Table 1).

1.2.2 Metabolic rate syndromes

The term 'metabolic rate syndrome' is not a term normally used in the literature (and should not be confused with the term 'metabolic syndrome' which describes obesity and related diseases). I use it here as a term describing the syndrome between metabolic traits and behavioural traits, which has attracted many researchers in recent years (Careau et al. 2008; Biro and Stamps 2010; Burton et al. 2011b). Typically, the metabolic rate in a resting state (RMR), allowing for some spontaneous activity, is used as the metabolic trait (Burton et al. 2011b). The inter-individual variation in RMR can vary threefold, and the variation is often consistently different among individuals (Biro and Stamps 2010; Burton et al. 2011b). Given that RMR can constitute a large proportion of the energy expenditure (up to 50% in turtles; Congdon et al. 1982) it should have a great impact on energy allocation patterns (Eqn. 2). It is also linked to many behavioural traits (Biro and Stamps 2010), suggesting that a syndrome is an appropriate term. For instance, higher metabolic rate requires higher energy intake, but can also support a greater energy output (Biro and Stamps 2010). However, the causal relationships within this syndrome are obscure, as metabolism could affect behaviour and vice versa (Careau et al. 2008; Burton et al. 2011b). Metabolic rate is also a key factor in the rate-of-living hypothesis described in a previous section (1.1.2), affecting ROS and potentially life-span.

1.2.3 Behavioural syndromes

Behavioural syndromes refer to suites of correlated behaviours across multiple contexts or situations[5] (Sih et al. 2004a,b). For instance, a population may show a behavioural syndrome involving a positive covariance between boldness, aggression, and activity (e.g. Huntingford 1976; Kortet

[5]Sih et al. 2004a,b define *context* as "a functional behavioral category; e.g. feeding, mating, anti-predator, parental care, contest or dispersal contexts", and *situation* as "a given set of conditions at one point in time. Different situations could involve different levels along an environmental gradient (e.g. different levels of predation risk) or different sets of conditions across time (e.g. the breeding season versus the non-breeding season)".

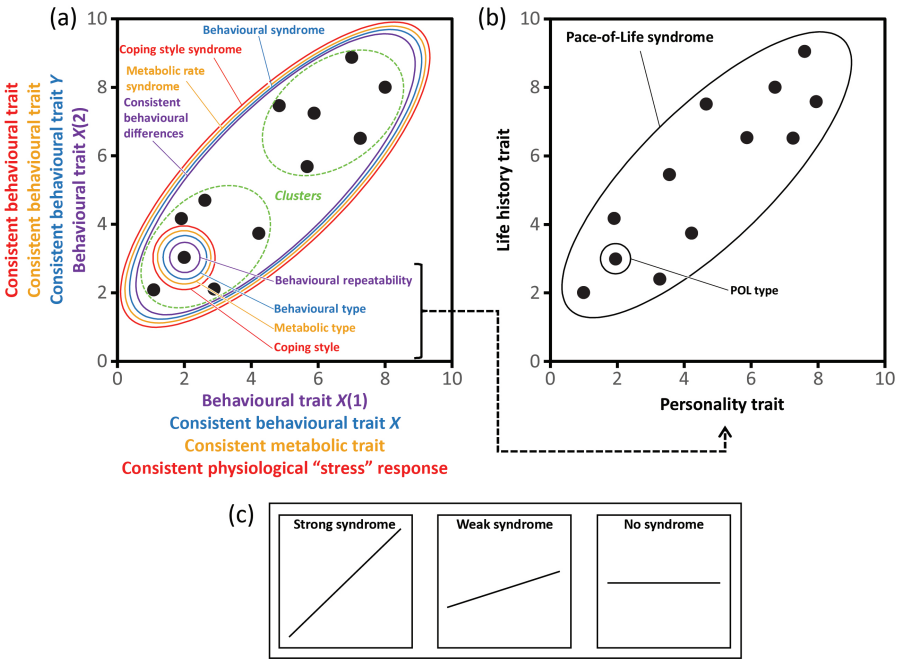


Figure 1: Graphical illustration of (a) different personality syndromes, coded in colour and (b) their link to the pace-of-life syndrome. The scale (0-10) on the axes is arbitrary and not necessarily linear. Markers in (a) can represent any of the personality traits. Clustering of behavioural types [green circles in (a)] can be formed through e.g. disruptive selection on different general strategies. Personality trait X in (b) can be the average of a behavioural trait, or a component variable of behavioural- or coping style syndromes. The figure is adapted from definitions and illustrations in Sih et al. (2004a,b), Bell (2007), McKay and Haskell (2015), and Castanheira et al. (2015). Strength of syndromes are illustrated in (c), with the x- and y-axes of all three graphs being scaled identically.

and Hedrick 2006). Individuals within a population which exhibit a behavioural syndrome show different behavioural types, e.g. bolder and more active, or less bold and less active. Variation in behavioural types can be continuously distributed along the syndrome axis, or clustered in groups along the axis (see example of clustering in Fig. 1) (Sih et al. 2010).

Whether or not behavioural syndromes have a genetic basis is relatively poorly understood at the moment (Sih et al. 2010). Some evidence suggests that there can be a genetic background to syndromes. A quantitative trait locus analysis suggests that there are sections of the nine-spined stickleback *Pungitius pungitius* genome which are correlated with the variation in cross-context activity syndrome (Laine et al. 2014). However, determination of genetic effects is hard as there are considerable environmental effects, as well as gene × environment (G × E) interactions. For

instance, behaviours which appear to be genetically linked in juvenile blue tits *Cyanistes caeruleus* disappear with age which suggests that G × E interactions affect the expression of the syndrome (Class and Brommer 2015).

Notably, a behavioural syndrome may be a part of a coping style- or a metabolic rate syndrome. There may also be several independent behavioural syndromes within populations (Budaev 1998). Behavioural syndromes may also arise from time-budget constraints, e.g. within a foraging context where there is a negative correlation between anti-predator behaviours and foraging activity.

1.2.4 Intra-specific syndromes in an ecological context

The presence of syndromes within a population suggests that individuals are not entirely plastic. This is particularly interesting when considering behaviour, which is historically treated as being almost infinitely plastic with all individuals behaving optimal in all situations (e.g. Sih et al. 2004a). Restrictions in behavioural plasticity (e.g. due to underlying physiological traits such as expression of stress hormones, or metabolic rate) can lead to carryover effects across different situations and contexts (Sih et al. 2004a,b, 2010). For instance, being aggressive in one context, e.g. showing high voracity against prey when juvenile, may promote high growth rates and a good body condition, but a carryover effect may lead the same individual into being aggressive in another context, e.g. towards potential mates as an adult, which could be negative for the fitness (Johnson and Sih 2005). Another example of non-adaptive carryover effects is that individuals which are bold and active in predator-free environments also can be more bold and active also when predators are present (Sih et al. 2003). There may also be time constraint effects. For instance, males expressing high levels of nest defence have less time to care for their offspring (Duckworth 2006). Carryover and time constraint effects associated with syndromes can lead to some individuals behaving optimally in some situation/contexts, but sub-optimally in other (Sih et al. 2010).

Syndromes may have substantial effects on ecology at the population and community levels, as the syndromes likely affect the probability that certain individuals and which species that interact in different situations and contexts (Sih et al. 2012). Syndromes may also affect dispersal patterns, and thereby gene flow and allele frequencies in sub-populations (Sih et al. 2012). Given that life-history strategies are linked to syndromes, the growth rate of a population is likely affected by the frequency of different

behavioural types (Sih et al. 2012).

If heritable, syndromes may lead to speciation in the long term due to e.g. separation in niches, if this separation also comes with assortative mating (discussed in McLaughlin 2001). In many lake dwelling fishes separation into different ecotypes with different niches are common (e.g. Schluter and Rambaut 1996); a phenomenon which may originate from syndromes in the founding populations. When such separation occurs, several differences in morphology and life-history traits also follow, which might be depending on the links within the general POLS being enhanced through directional selection.

Syndromes need not be genetically determined to have a large influence on ecology. Even if a syndrome is shaped by individual experience it will likely affect the dynamics of the population. Syndromes can affect competition levels through different dispersal among individuals. It may also affect susceptibility to disease and parasite infestation, and risk of predation.

The strength of the syndrome (see Fig. 1c) can also depend on the environment. The behavioural syndrome linking aggression and boldness/activity in three-spined sticklebacks *Gasterosteus aculeatus* populations exposed to strong predator pressure, is not seen in populations where the predator pressure is low (Bell and Sih 2007; Dingemanse et al. 2007). Sticklebacks exposed to non-lethal predation simulation early in life grow faster and mature earlier (i.e. a faster POL) than sticklebacks not being exposed to the simulated predation (Bell et al. 2011). The syndrome may therefore arise from an adaptive response to predation affecting POL. Speculatively, a general benefit of high growth rates in a population may increase the competition among individuals, favouring aggressive individuals which gain a growth advantage in this context. These superior individuals may then force less competitive individuals into alternative behavioural strategies (shy and inactive) - giving rise to the syndrome in the population.

1.2.5 The evolution of intra-population variance

The existence of syndromes implies that trait-variance is maintained in populations. One mechanism which may keep syndromes in a population is negative frequency dependent selection (Maynard Smith 1982; Wolf and McNamara 2012; Wolf et al. 2013). This means that there may be more than one viable strategy for obtaining high relative fitness, and that the frequency of individuals adopting each strategy will determine the success of these strategies (Maynard Smith 1982). For more than one strategy to exists, each strategy is required to have higher relative fitness when be-

ing rare. Frequency dependence can result from different genotypes having different strategies. This situation leads to coexistence of the alternative strategies, as a consequence of increasing fitness of each strategy when its frequency in the population decreases. However, different strategies may also arise within a single genotype (so called mixed strategy genotypes) (Wolf et al. 2013). This can be achieved in several ways, e.g. through individual behaviour mixing, where individuals change behaviour over time, or through developmental determination of strategies (Wolf et al. 2013). Developmental determination of strategies may be an effect of 'developmental coin-flipping' (i.e. more or less random assignment to a certain strategy), but can also be manipulated by the parents during development (Wolf et al. 2013). The phenomenon that a single genotype produces alternative phenotypes with different strategies is considered as a bet-hedging strategy of the genotype. Typically, this is thought to be a result of varying environments, with one phenotype having higher fitness under a particular environmental condition, while another phenotype has higher fitness in another condition (Seger and Brockmann 1987). By producing different phenotypes, a mixed strategy genotype can reduce variance in fitness over time, as compared to a genetically determined 'pure' strategy (Seger and Brockmann 1987; Bolnick et al. 2003; Wolf et al. 2013).

1.3 Within-individual variation in pace-of-life syndrome traits - state-dependent performance

The state of an individual refers to any given phenotypic trait that can change over time. This is a wide definition, including many possible properties such as condition, size, experience, immunocompetence, parasite load, etc. When discussing state-dependence of behaviour and growth rate (which is the focus of this thesis), the most typical states investigated are energetic status and body size. These two states are therefore selected to be discussed here.

1.3.1 State-dependent feedbacks on behaviour

Theoretically, the optimal behaviour of an individual should maximise total life-time fitness (Briffa and Sneddon 2010). Given that the state of an individual affects its future prospects in terms of reproductive capacity, we should therefore expect that the behaviour reflects the present state, and also projected future states, to optimise fitness (McNamara and Houston 1996; Wolf et al. 2013). Syndromes may restrict the overall flexibility

in behaviour, but behavioural flexibility may also lead to stable syndromes through state-dependent feedbacks (Luttbeg and Sih 2010; Sih et al. 2015).

The state-dependent safety feedback

Acquisition of resources (e.g. food, shelter, and territories) influences the reproductive value of an individual. Thus, competitive ability is a key factor determining fitness. Having a higher energetic state (more energy reserves) will allow an individual to spend more energy e.g. in a contest for a resource (Maynard Smith and Parker 1976; Briffa and Sneddon 2010). This, in turn, means that high energetic state individuals have advantages over lower state individuals, everything else being equal, and are more likely to obtain and successfully defend resources. Along similar lines, a relatively larger individual likely has superior strength and vigour compared to smaller competitors, and can through this advantage obtain and defend resources (e.g. Lindström 1992). A situation where a superior individual confronts an inferior competitor is an asymmetric contest, where the superior individual has a relatively higher resource holding potential (RHP) (Maynard Smith and Parker 1976). Asymmetric contests can lead to a positive feedback where superior individuals become ever more superior with time, given that these individuals take advantage of their high RHP to progressively gain more resources (Kelly 2008). State can also affect predation risk. For example, larger size can reduce the number of predators that can consume an individual by gape-size limitation (Godin 1997), or through increased defence capacity. American lobster *Homarus americanus* juveniles, for instance, hide when being small, but defend themselves actively against potential predators when reaching a larger size (Wahle 1992). Reducing the time in shelter likely increases the time available for foraging, and thereby increases the growth rate. These examples are part of the concept of 'state-dependent safety' where higher state leads to higher reproductive value (e.g. through lower mortality risk and higher resource acquisition). In these situations, the less competitive individuals may have to resort to 'best-of-a-bad situation' strategies (Maynard Smith 1982). In cases where state-dependent safety is at work, behavioural syndromes may arise as a consequence of this feedback mechanism where strong competitors adopt a behavioural strategy which differs from that of worse competitors (Luttbeg and Sih 2010).

The starvation-threshold feedback

As previously stated, reduced mortality risk can result both from lower risk of starvation because of successful competition for food, and lower risk of being predated because of successful competition for good shelters and lowered predation risks by gape-limited predators through increased size. However, having obtained a high energetic status or a large size ('high state') may decrease the motivation to obtain even more resources. Even though a high-state individual can be relatively safer than a low-state individual, there are likely still some risks (e.g. predation by non-gape-limited predators or injuries when defending resources). Thus, a high-state individual may give up in a contest against a more motivated low-state individual (Colgan 1993). Individuals with lower energetic status, or smaller size, have a higher immediate demand of food. For these individuals the food therefore has a relatively higher resource value (RV) (Dill 1983). For this reason, low-state individuals should be more willing to take risks (e.g. fight or being more active) to obtain the food (Lima 1998). A high-state individual has already obtained a high potential fitness value and may want to protect these assets (the 'asset protection principle'), while a low-state individual would need to obtain a higher potential fitness value, e.g. to avoid death by starvation (the 'starvation avoidance principle'). Together, the asset protection and the starvation avoidance principles constitute the two extreme ends of the starvation-threshold feedback (Houston and McNamara 1999). This negative feedback could reduce the population level expression of a syndrome over longer periods of time (Luttbeg and Sih 2010; Sih et al. 2015).

Is behaviour state-dependent in early ontogeny?

Motivation to perform certain behaviours is linked with ontogeny, with some life-stages having a very high innate motivation to e.g. feed (Colgan 1993). The early juvenile stage is such a stage, as survival is generally low and linked to size (e.g. through gape-size limitations of predators). In larval sprat *Sprattus sprattus*, for instance, little behavioural alterations are seen as responses to starvation, likely as a consequence of an inability to raise foraging levels above the innate levels (Peck et al. 2014).

1.3.2 Compensatory growth

A state-dependence phenomenon which has received much attention from researchers is the compensatory growth phase which follows environmen-

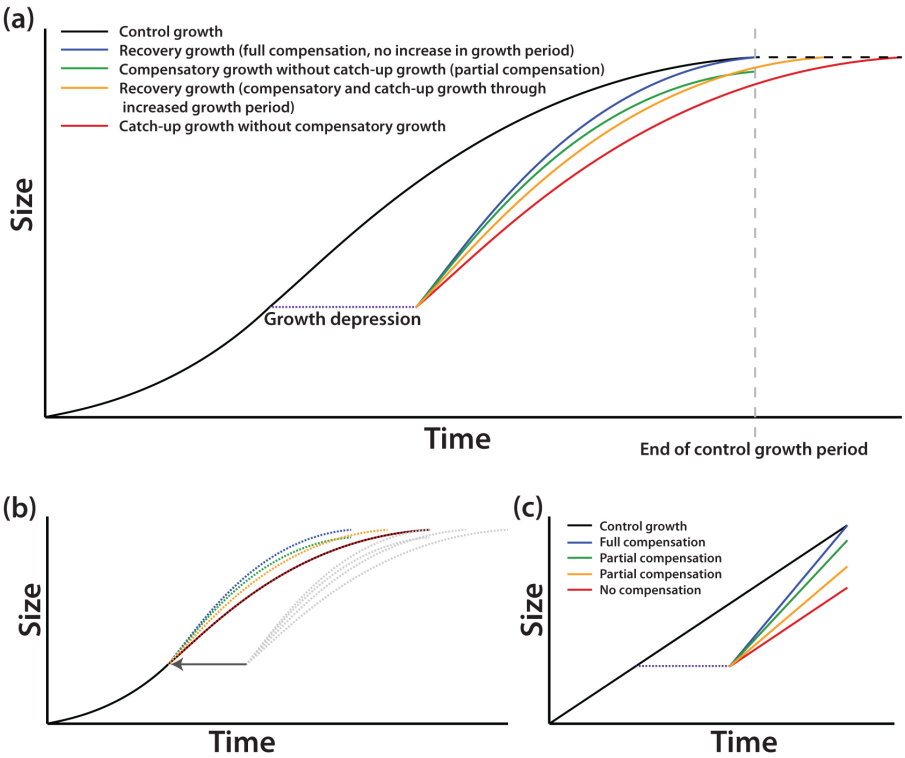


Figure 2: Illustration of compensatory growth and catch-up growth. Using a typical sigmoidal growth pattern (a) we can see compensatory growth (blue, green, yellow) and catch-up growth (blue, yellow, red) patterns; their combination is called recovery growth (blue, yellow). If we compare the growth rate (steepness of curves) (b) we can get an overview of the magnitude of the compensatory growth with respect to body size. Note that the red curve matches the control curve, i.e. no compensatory growth. No growth allometry (c) makes comparisons easier. In this case catch-up growth equals full compensation.

tally induced growth depression. A consequence of growth depression is that an individual will not achieve the state it should have had, were it not deprived of food. Compensatory growth refers to an increase in growth rate above the normal rates for the organism, which is elicited to regain 'lost' growth after a transient period of growth depression. Compensatory growth is empirically demonstrated in a wide variety of organisms, and is a good example of temporal intra-individual phenotypic plasticity, where individuals alter their pace-of-life to a faster pace (Reed 1921; Wilson and Osbourn 1960; Williams 1981; Boersma and Wit 1997; Ali et al. 2003; Dmitriew 2011; Hector and Nakagawa 2012).

There is some confusion regarding terminology which needs to be addressed. The terms 'compensatory growth' and 'catch-up growth' are often

used synonymously. A recent publication has re-defined these two terms (Jobling 2010), with catch-up growth being defined as the achievement of attaining control size following growth depression (i.e. catching up to the size an individual would have, had it not been growth depressed), and compensatory growth being defined as increased growth rate following growth depression. This increased growth rate can result in full compensation of control size, or partial compensation. Catch-up growth and compensatory growth can be combined (then termed recovery growth, following Jobling 2010), and each can be achieved without the other [6] (Jobling 2010). In some biology disciplines (e.g. botany, paediatrics, and clinical zoology) catch-up growth instead refers to increased growth rate following growth-restriction (compensation of *potential* tissue loss), while compensatory growth refers to rapid re-growth of tissue lost through direct removal (compensation of *actual* tissue loss) (McNaughton 1983; Williams 1981; Boersma and Wit 1997). To complicate things further, compensatory growth is sometimes used to describe changes in growth rate which are not following involuntary growth depression, but instead seem to be strategic changes in growth rate, like the spring growth spurt seen in salmonids prior to migration to the sea (Sigourney et al. 2013). In this thesis, I discuss compensatory growth as defined by Jobling (2010) – i.e. referring to increased growth rate – as this definition appear to have been adopted within the field of animal ecology (e.g. Hector and Nakagawa 2012; Marcil-Ferland et al. 2013).

Compensatory growth can offset effects of growth depression on development and life-history transitions, which is the reason why it is particularly interesting from an ecological perspective (Jobling 2010). Growth rate in itself is, as described in previous sections, one of the key life-history traits. Given that animals appear to be able to increase their growth rate above their normal rate, we can assume that animals normally do not push their growth rate to the physiological maximum, but instead grow at a submaximal, more optimal, rate (Arendt 1997; Gotthard 2001; Dmitriew 2011; Hector and Nakagawa 2012). Assuming that reaching a large size quickly comes with positive effects on fitness, there must be some costs to growing too quickly (Arendt 1997; Gotthard 2001; Metcalfe and Monaghan 2003). Trade-offs between e.g. growth rate and risk-taking, and growth rate and life-span, have been discussed in the previous sections on inter-specific (1.1) and intra-specific (1.2) differences in this introduction. Similar trade-offs likely occur at the individual level, which has been shown through modelling (Mangel and Munch 2005), as well as in literature reviews and in

[6] For catch-up growth, this requires that growth rate levels off with size – which is commonly the case (West et al. 2001; Nicieza and Álvarez 2009)

a meta-analysis of published results (Metcalfe and Monaghan 2001; Hector and Nakagawa 2012). For some animal taxa compensatory growth is more important than for others. In birds and mammals, which stop growing at adulthood, compensatory growth responses appear to be of large importance in the juvenile stage, as a smaller adult body size can have severe consequences for their life-time fitness (Blanckenhorn 2005; Hector and Nakagawa 2012). For fish and other animals with indeterminate growth, the pressure to grow fast before adulthood appear to be lower, as these animals can continue to grow between reproductions (Hector and Nakagawa 2012). However, some fish may also have time-horizons before which they need to achieve a certain size (Gotthard 2001). For instance, anadromous salmonids migrate to sea at a certain size, which may make adaptive growth responses to growth depression important for these species (Sigourney et al. 2013). Another time-horizon could be the onset of winter, before which an ectothermic individual needs to build large enough energy reserves to be able to survive until spring (Gotthard 2001).

One of the major constraints on growth rate appears to be the trade-off between predation risk and foraging (Lima and Dill 1990, Werner and Anholt 1993; Dmitriew 2011). Several studies suggest that hyperphagia (higher than normal food consumption rates) can be the main mechanism driving growth compensation (Ali et al. 2003). In natural environments, hyperphagia likely comes with increased exposure to predators while foraging, as higher foraging activity is likely required to increase food intake. Studies on butterfly *Parage aegeria* (Gotthard 2000) and damselfly *Lestes sponsa* larvae (Stoks et al. 2005) being manipulated to grow at different rates through induced time stress, have shown that faster growing individuals suffered higher mortality from predation. In the case of damselfly larvae, this was shown to be behaviourally mediated (Stoks et al. 2005). Similarly, faster growing populations of Atlantic silverside *Menidia menidia* fish have higher predation mortality rates (Lankford et al. 2001; Munch and Conover 2003).

The quality of a rapidly grown body may also be lower. This has been demonstrated in birds, where fast growth leads to lower quality feathers (Dawson et al. 2000), and in fish where it leads to e.g. weaker scales (Arendt et al. 2001) and increased levels of skeletal asymmetry (Robinson and Wardop 2002).

There may also be trade-offs between growth and maintenance within individuals (following Eqn. 3), so that fast growth is traded against lower maintenance and, as a consequence, a shortened life-span (e.g. Mangel and Stamps 2001; Metcalfe and Monaghan 2003; Mangel and Munch 2005; Lee

et al. 2011, 2013). If cellular maintenance is decreased, there should be signs of this in the bodies of animals growing faster than normal. One potential marker for reduced maintenance is the telomere length of the chromosomes (which is investigated in **Paper 1**). Telomeres are hexanucleotide repeats with associated proteins (e.g. Gomes et al. 2010), located distally on eukaryote chromosomes (Blackburn and Gall 1978). The telomeres cap the ends of the chromosomes and their function is to maintain the integrity and stability of the chromosomes (Gomes et al. 2010). Telomeres are shortened each cell cycle due to the '*end replication problem*', where the last repeats of the DNA sequence of the lagging strand cannot be copied (Olovnikov 1973). Telomeres are also shortened through damage caused by ROS (von Zglinicki 2002). ROS can be protected against by investment in anti-oxidative agents, but with lower energy allocation to maintenance the effectiveness of these protective mechanisms is likely reduced. Thus, if maintenance is reduced as a consequence of compensatory growth, we could expect more rapid shortening of the telomeres (e.g. Jennings et al. 1999) (for more details, see the introduction to **Paper 1**).

2. Pace-of-Life in the context of salmonid ecology, with special reference to the studies of the thesis

"The most important family of all the fishes of the world is the Salmonidae [...]"

- Barton Warren Evermann, 1924
In *Salmon of the Atlantic [McFarland 1925 (p V)]*

2.1 Salmonids - the world´s most important fishes?

The quote above, by the former museum director of California Academy of Science, B. W. Evermann, may be bold and subjective. However, there should be no doubt that the fish belonging to the taxonomic family Salmonidae are important to the world even today, ecologically, economically, scientifically, socially, and culturally (e.g. Ruckelshaus et al. 2002; NASCO 2008; Degerman et al. 2012). Native to the Holarctic region and its surrounding seas, they are nowadays also stocked into waters on the majority of the continental plates (MacCrimmon et al. 1970, 1971; MacCrimmon 1972). Furthermore, they are kept in enormous quantities in aquaculture systems (FAO 2015), used as model organisms in many areas of the life sciences, and fished by both commercial and leisure fishermen. A good indicator of the importance of salmonids for humans is their is their recurring presence in the literature, with the published record consisting of virtually all types of publications, including genres like popular science (e.g. Frost and Brown 1967), fiction (Torday 2007), history (e.g. Newton 2013), fishing (e.g. Birkesten 1963), graphic novels (Sacks 2004), biographies (e.g. Grey and Charleston 2011), art (Prosek 2003), and children's books (e.g. Hei-

derose and Fischer-Nagel 1991). Despite their importance and popularity, wild salmonid populations are commonly disadvantaged by human activities (Crisp 1993; Parrish et al. 1998). For this reason, the conservation of salmonids in their natural environments is generally considered important by politicians, fisheries managers, environmental protection organizations, and the general public (Ruckelshaus et al. 2002). Given their popularity, the biology of salmonids is an important research topic and despite the fact that large amounts of scientific efforts have already been made, further knowledge can help to conserve and manage these fish for future generations.

2.2 Notes on inter-specific comparisons

The majority of the fish in Salmonidae, and particularly the species in the subfamily Salmoninae (salmon, trout, and char; genera: *Salmo*, *Oncorhynchus*, *Salvelinus*, *Hucho*, *Parahucho*, *Salvethymus* and *Brachymystax*; Eschmeyer 2015), are similar in their general ecological niche. With a few exceptions (e.g. lake char *Salvelinus namaycush*, Lake Garda trout *Salmo carpio*, and kunimasu *Oncorhynchus kawamurae*), lotic (running water) environments are the principal spawning and juvenile habitats (Froese and Pauly 2015).

However, even though the niches of salmonid fishes are similar, they are not identical. For instance, brown trout are stronger and more aggressive competitors than many other species, which leads to differences in the outcome of competition between individuals, both when these species live in sympatry (brown trout generally dominate size-matched Atlantic salmon and brook char, e.g. Höjesjö et al. 2010; Öhlund et al. 2008; Skoglund et al. 2012), and when they live in allopatry (dominant trout are more fierce intra-specific competitors than e.g. dominant salmon, Skoglund et al. 2012). Different species also differ in movement behaviour and stream micro-habitat choice (Tunney and Steingrímsson 2012).

Salmonids are phenotypically variable and plastic (e.g. Willson 1997; Klemetsen 2013), to the extent that species delimitation is hard and full of controversy (e.g. regarding validity of species within the brown trout 'species complex'; Pustovrh et al. 2014). There are large differences in morphology and behaviour among populations which are regarded to belong to the same species, as well as large differences within populations, and even among siblings (Jonsson and Jonsson 2011). Furthermore, genetic diversity contributes less to transcriptome variation, which affects development and behaviour, than life-history type (Giger et al. 2006). Thus, salmonids from

genetically distant populations can be more similar in many biological traits than individuals from the same population.

Given that the ecology of the different salmonid species and populations differ, caution needs to be taken when generalizing results from one species across other species. In the following text I therefore note the species which is used as an example (as a subscript after the reference, see abbreviations used in Table 2).

Table 2: Abbreviations used to denote salmonid species in references cited in sections 2.3 - 2.6. References without subscript are not species specific.

Common name	Scientific name	Abbreviation
Arctic char	*Salvelinus alpinus*	AC
Atlantic salmon	*Salmo salar*	AS
Brook char	*Salvelinus fontinalis*	BC
Brown trout	*Salmo trutta*	BT
Coho salmon	*Oncorhynchus kisutch*	CoS
Chum salmon	*Oncorhynchus keta*	ChS
Rainbow trout	*Oncorhynchus mykiss*	RT
Sockeye salmon	*Oncorhynchus nerka*	SS

2.3 The salmonid life-cycle, with special reference to brown trout

Different salmonid species have similar, but not identical, life-cycles. Here, the general salmonid life-cycle is described, with special reference to brown trout - the model species of this thesis. All species spawn in freshwater and bury their eggs in the bottom substrate, often in series of nests (*redds*). After the eggs have hatched, the yolk-sac fry (called *alevins*) stay buried in the gravel until the yolk is more or less depleted. The alevins then emerges from the spawning gravel (this event is termed *swim-up*), and start exogenous feeding. From this stage until the end of the first summer they are called *fry*[7]. During the first free-swimming stage the fry generally stay close to the nest. This period is commonly referred to as the first critical period, as the majority of the cohort mortality occurs at this stage (Le Cren 1961; Elliott 1994; Degerman et al. 2001). After the first summer the fry disperse in the stream and are subsequently called *parr*. The parr stage can

[7]The terminology at this life-stage is somewhat controversial, as the definition of fry has differed among researchers. Here, I do not follow the suggested terminology by Allan and Ritter (1977), which defines fry as the transitional stage between swim-up and dispersal from the nest. I believe that 'fry' should be defined as the first critical stage of the life when most of the mortality occurs, i.e. the first summer.

last for several years [in brown trout from the Swedish west coast typically 1-3 years, depending on growth opportunities in the rivers (Degerman et al. 2001)]. When reaching a certain size some populations go through a physiological transformation and turn into a *smolt* (Jonsson and Jonsson 2011). Smolts migrate to the sea (anadromy), or to a lake (potamodromy), where the growth potential is better than in the juvenile habitat. Whether or not a population goes through this smoltification process depends on species and the population's access to the sea or larger lakes (Pavlov and Savvaitova 2008; Jonsson and Jonsson 2011). Brown trout growing up in temperate coastal streams, or streams connected to larger lakes, generally smoltify, while many inland populations do not. The better growth opportunities in marine or lake environments make it possible for the fish to grow substantially faster and reach a larger size, which is beneficial for fecundity and competitive ability (Pavlov and Savvaitova 2008; Jonsson and Jonsson 2011). However, smolt migration also has substantial mortality risks, and some individuals may reduce these risks by staying in the juvenile habitat as parr until maturation, even if ana-/potamodromy is possible (Pavlov and Savvaitova 2008; Jonsson and Jonsson 2011). In brown trout, these mature parr (the majority being males) generally stay in the river their entire life (Dellefors and Faremo 1988; Pettersson 2002). In other species, such as Atlantic salmon, the mature parr (only males) instead migrate to the sea in a following year (Jonsson and Jonsson 2011). Anadromous and stream resident individuals often spawn together; however, stream resident males are less competitive than large anadromous males and tend to use a sneaking strategy to access females (Degerman et al. 2001; Jonsson and Jonsson 2011).

2.4 Stream life

Lotic environments, in particular small streams, are interesting from an ecological perspective. On a larger spatial scale, these environments could be considered more or less one-dimensional since they are very long in relation to their depth and width. The moderate depths of streams and rivers also make the environment very variable, as small changes in precipitation can have relatively large effect on water levels. The moving water shapes the streams into variable environments on the longitudinal scale, with regular shifts between deeper pools and shallow riffles, and a continuous increase in channel size as the stream is followed downstream, accompanied by changes in the ecosystem (Allan and Castillo 2007). Salmonids typically live in higher order watercourses with relatively low water temperatures

(< 20°C), fast flowing water, and high concentrations of dissolved oxygen (Bjornn and Reiser 1991; Crisp 1993). As a general pattern, younger individuals keep to shallower areas (riffles or stream margins) while larger individuals move to deeper water (e.g. pools) where growth opportunities often are better (Bohlin 1977; Näslund et al. 1998). Larger individuals also tend to be found at higher water velocities than smaller ones (Bjornn and Reiser 1991). From the perspective of the smaller individual, the habitat choice is not necessarily by free choice, as inter-cohort competition exists (e.g. Kaspersson 2010). At low temperatures salmonids reduce their activity and seek shelter or deeper habitat - likely to avoid unnecessary energy expenditure and predators (Bjornn and Reiser 1991). Commonly, the salmonids do not utilise all of the space in a watercourse, but are aggregated in the most suitable habitats (Bjornn and Reiser 1991). Still, the best foraging stations in the stream are likely occupied by the most competitively strong individuals, and lower quality individuals can be pushed out to unfavourable habitats, or even into lakes or the sea (Degerman et al. 2001). A typical foraging station lies in front or on top of the downward-sloping rear of a submerged object, where little effort is required to hold position, and access to a nearby shelter (Bachman 1984). The foraging station is generally not shared, but the shelter may be temporarily shared among several individuals (Bachman 1984; pers. obs.).

The stream life during the fry stage differs in many ways from stream life during the parr stage. During the fry stage, the fish are generally strongly territorial, aggressively defending small territories which they do not share with other individuals (Kalleberg 1957; Héland 1999). During the parr stage, the fish utilise larger areas of the stream ('home ranges') which often overlap among individuals – usually with strong dominance hierarchies determining which individuals have first choice of holding station (Jenkins 1969; Bachman 1984; Héland 1999). The intra-specific competition also differs considerably between these stages. During the fry stage competition is very hard, most likely surpassing carrying capacity due to the high number of eggs being hatched at similar time. Over the first summer, the fish density generally decreases substantially and competition is consequently reduced (towards carrying capacity). Inter-specific competition may also occur in some streams. However, small coastal streams in Sweden (like the subject streams in this thesis[8]) contain few, if any, inter-specific competi-

[8]European minnow *Phoxinus phoxinus* in Jörlandaån is the only other potential competitor species occurring in substantial numbers. However, the minnows appear mainly in areas where trout are less common and are rare in the experimental sections (Johansson 1971; author's pers. obs.).

tors.

Predation is a very important aspect of the environment, particularly in the context of POLS. Smaller temperate streams often contain relatively few piscine predators, other than the salmonids themselves. Instead a large proportion of the predation pressure likely comes from cannibalism or terrestrial endothermic predators like birds and mammals. Importantly, the predation pressure is changing through time depending on the food preference of the predators present. For instance, smaller fish are more vulnerable to fish, and small mammals and birds, while larger fish are specifically targeted by larger mammals and birds (Alexander 1977, Sogard 1997). In the streams where the experiments for this thesis were carried out, the details on the predation are largely unknown. The information that exists is based on personal observations of potential predators and predation events. A summary of known and potential predators and the life-stage they likely target is presented here as Table 3.

2.5 Syndromes in salmonids

2.5.1 Empirical evidence

Even if research concerning different syndromes is relatively recent, differences in behavioural traits in salmonids have not gone unnoticed. For instance, Frost and Brown (1967) made notes on this in their brown trout monograph: "*Those who have kept a few trout in an aquarium over a length of time will have observed individual differences in temperament, as well as in appearance – even if all the fish are of the same age and came from eggs from the same female parent fertilised with milt from the same male*". Such individual differences in behaviour and appearance could be the result of an individuals position in the social hierarchy, but such individual consistencies can also be important as they may lead to stable syndromes through positive state-dependent feedbacks (see section 1.3.1).

Today, salmonids are used as model organisms in research on coping-style syndromes, metabolic rate syndromes, and behavioural syndromes, as well as the more holistic POLS which combine the other syndromes (Metcalfe et al. 1995; Øverli et al. 2007; Conrad et al. 2011; Mittelbach et al. 2014).

Table 3: Confirmed and potential predators of juvenile brown trout in the streams where studies of this thesis were conducted (Norumsån - NÅ, Jörlandaån - JÅ, and Stenunge å - SÅ). Vulnerable life-stage assumed to be related to the relative size of the predator and the predator foraging mode. ?? = No information available.

Species	Confirmed in experimental areas?	Eat fish?	Eat salmonids?	Vulnerable life-stage	Selected references
Fish					
Brown trout *Salmo trutta*	Direct obs., Electrofishing	Yes	Yes	Egg	Näslund et al.2015a, **Paper 5**
				Fry	Frost and Brown 1967; Pers. obs.
				Parr	Pers. obs.
European eel *Anguilla anguilla*	Electrofishing	Yes	Yes (limited)	Parr	Frost 1946; Frost and Brown 1967; Sinha and Jones 1967
Birds					
Corvids (Corvidae)	Direct obs.	Yes	??	Fry??/Parr??	Svensson et al. 2010
Dipper *Cinclus cinclus*	ArtDatabanken 2015	Yes	Yes	Fry	Fleming 2012 (Photography)
Grey heron *Ardea cinerea*	Direct obs., Tracks	Yes	Yes	Parr	Geiger 1984; Marquiss and Leitch 1990
Grey wagtail *Motacilla cinerea*	Direct obs.	Yes	Yes	Fry	Davey 2011 (Photography)
Gulls *Larus* spp.	Obs., but not in streams	Yes			Svensson et al. 2010
Kingfisher *Alcedo atthis*	Anecdotal (NÅ)	Yes	Yes	Fry	Hallet 1982; Raven 1986 Degerman et al. 2001
Mallard *Anas platyrhynchos*	Direct obs. (NÅ)	Yes	Yes	Eggs/Fry??/Parr??	Dieter et al. 2014
Owls (Strigiformes)	No, but likely	Yes	Yes	Parr??/Adults??	Obuch 2004; Obuch and Karaska 2010
Mammals					
American mink *Neovison vison*	Direct obs., Tracks	Yes	Yes	Parr	Gerell 1967; Erlinge 1969; Cuthbert 1979
Otter *Lutra lutra*	ArtDatabanken 2015*	Yes	Yes	Parr	Erlinge 1969; Cuthbert 1979; Kyne et al. 1989
Water shrew *Neomys fodiens*	No, but likely (Suitable environment)	Yes	Yes	Fry	Frost and Brown 1967; Churchfield 1985; Greenwood et al. 2002
Reptiles					
Grass snake *Natrix natrix*	Likely, Anecdotal	Yes	??	Parr??	Gregory and Isaac 2004
Invertebrates					
Crayfish (Astacidae)	Electrofishing (SÅ)	Possibly	Possibly (but no effect on trout density)	Egg/Fry	Degerman et al. 2007
Dragonfly larvae (Odonata)	Direct obs.	Yes	??	Fry	Reist 1980; Mobley et al. 2013

Piscine predators which have not been observed in experimental areas: Northern pike *Esox lucius*, Burbot *Lota lota*, Sculpins *Cottus* spp. (Degerman et al. 2001)
Avian predators which have never been observed in experimental areas, and for which the habitat is judged to be unsuitable (Svensson et al. 2010):
Great cormorant *Phalacrocorax carbo*, Goosanders *Mergus* spp., Grebes (Podicipedidae), Loons *Gavia* spp.
* Tracks observed within 10 km radius from experimental area in 2014. Likely very rare (Bisther and Roos 2006)

Coping style syndromes

Coping style syndromes, linking physiological stress-coping traits with behaviours have been investigated and found in salmonids, particularly in rainbow trout from domesticated genetic lines. Typically, the individuals are grouped into 'proactive' and 'reactive' coping styles. Proactive individuals have a high sympathetic reactivity (fight or flight reaction), while reactive individuals have a high parasympathetic/hypothalamic reactivity (withdrawal reaction) (Schjolden and Winberg 2007_{RT}). Behaviours associated with these physiological stress responses are boldness in novel environments, general activity, and aggressive and dominant behaviour in social contexts – all having higher scores in proactive individuals relative to reactive individuals (Schjolden and Winberg 2007_{RT}). Interestingly, the behaviour of fish with different coping styles mainly differ in novel environments and not in environments familiar to the fish, suggesting that the stressful situation is what enforces the differences (Schjolden et al. 2005_{RT}).

At least some components of the stress coping syndrome in rainbow trout are genetically inheritable, as shown by successful selection for trout with high and low cortisol (hypothalamic reactivity) response to stressful confinement trials (Pottinger and Carrick 1999_{RT}).

From an ecological perspective, the stress coping styles seem to be associated with different strategies in the very young stages. For instance, proactive individuals tend to have earlier swim-up than reactive conspecifics (Andersson et al. 2013_{RT}). The fact that proactive fish also are more active, aggressive and tend to become dominant, suggests that their strategy is strongly territorial.

Metabolic rate syndromes

Resting metabolic rate (RMR; also see section 1.2.2) is considered to be part of the proximate basis for behavioural and life-history variation in salmonids (Metcalfe 1998_{AS}; Thorpe et al. 1998). RMR is relatively stable over time within individuals (McCarthy 2000_{AS}; O'Connor et al. 2000_{AS}), and it is correlated to other physiological variables, like heart rate (Lucas 1994) and food processing rate (Millidine et al. 2009_{AS}) Thus, it can be described as being part of a syndrome in salmonids, and this syndrome also has behavioural components. For instance, there is a strong relationship between RMR and aggression in Atlantic salmon fry (Metcalfe 1992_{AS}; Cutts et al. 1998_{AS}).

High RMR individuals tend to have earlier swim-up and are more likely to become dominant – probably as a consequence of the combination of

prior residency and high aggression (Metcalfe 1995$_{AS}$; Cutts et al. 1999$_{AS}$). In the wild, the early life of salmonids is characterised by very high competition, so it is possible that there is an initial benefit for survival in being able to obtain a territory (Cutts et al. 1999$_{AS}$). However, high metabolism is costly in terms of energy requirement and may result in reduced growth rates if the required amounts of food cannot be obtained (Reid et al. 2012$_{AS}$). In brown trout, some populations show no, or negative, relationship between RMR and growth rate, highlighting that high RMR may be costly in certain environments (Álvarez and Nicieza 2005b$_{BT}$). High RMR individuals may also leave their nursing grounds at a smaller size to find foraging grounds with high enough food levels to sustain growth; a journey which likely is risky (Forseth et al. 1999$_{BT}$). Furthermore, high RMR individuals need to be more active in winter to avoid starvation which could increase the vulnerability to predation (Finstad et al. 2007$_{AS}$).

Many metabolism studies on salmonids have been conducted in artificial environments (hatchery tanks or laboratory aquaria). As the artificial environment may benefit certain behavioural types over others, and not necessarily the same types as would be favoured in a natural environment (Saikkonen et al. 2011$_{AS}$), the effects of metabolic rate on life-history traits may be exaggerated. In constrained artificial environments, despotic individuals can reap the benefit of their dominance by consuming food enough to grow at a very fast rate (Cutts et al. 1998$_{AS}$). In the wild, where food levels are likely lower, this may not be possible (Reid et al 2012$_{AS}$; Álvarez and Nicieza 2005b$_{BT}$).

Behavioural syndromes

Behavioural syndromes are often part of the findings in studies investigating metabolic rate- and coping style syndromes. Here, however, the focus will be on studies investigating behavioural covariance in wild populations, and in this context the brook char and the brown trout have been the main model species.

In brook char fry, found in still-water pools along the sides of streams, two clusters of behavioural types are generally found. The groups are clustered based on foraging behaviour, with one cluster containing sedentary fish foraging on crustaceans in the lower portion of the water column and the other containing more mobile fish foraging mainly on insects in the upper portion (McLaughlin et al. 1992$_{BC}$, 1994$_{BC}$, 1999$_{BC}$); intermediate types exist, but appear to be selected against (McLaughlin et al. 1999$_{BC}$). The clustering of behavioural types appears to be associated with spa-

tial separation of different prey organisms, and could potentially constitute the initial stage of resource polymorphism (McLaughlin et al. 1999_{BC}; McLaughlin 2001_{BC}).

The foraging activity is linked with other behaviours in behavioural syndromes. Char with lower foraging activity in nature also have lower activity in aquaria, as well as longer emergence latency from a dark tube; suggesting that they belong to a generally more risk averse behavioural type (Wilson and McLaughlin 2007_{BC}; Farwell et al. 2009_{BC}). Other indications of behavioural syndromes have also been shown for this species, e.g. longer emergence latency being related to further dispersal in nature, and a negative correlation between dispersal in a laboratory test and dispersal in nature (Edelsparre et al. 2013_{BC}). Furthermore, activity is negatively correlated with the cortisol stress response, suggesting that coping style could be part of a POLS in brook char (Farwell et al. 2014_{BC}; also see section 1.2.1). Metabolic rate (at least RMR) is not correlated to behavioural type (Farwell and McLaughlin 2009_{BC}). There are differences in the brain architecture of the two behavioural types (Wilson and McLaughlin 2010_{BC}), but generally no external morphological differences (McLaughlin et al. 1994_{BC}). Brook char fry also differ between fast and slow running water in streams, with fish from faster running water being more stationary foraging on drift, while fish in slower running waters are more mobile, foraging on benthos (Grant and Noakes 1987_{BC}). In zero current, the more mobile fish switch to feeding in the upper portion of the water (Grant and Noakes 1987_{BC}), suggesting that this might be the same behavioural type that is more active in still-water pools.

Like brook char, brown trout populations often show behavioural syndromes (e.g. Brännäs et al. 2004_{BT}; Höjesjö et al. 2011_{BT}; Adriaenssens and Johnsson 2010_{BT}, 2013_{BT}; Kortet et al. 2014_{BT}). In contrast to the brook char, there has generally been no explicit dichotomisation of behavioural types when describing the ecological effects of the syndromes.

Investigating behavioural types in one-year-old hatchery reared brown trout in laboratory stream tanks, Brännäs et al. (2004) found evidence for a behavioural syndrome in brown trout. Aggression was positively related to movement within the tank, particularly in the mid-section of the water column. Other studies suggest that this behaviour is typical for highly competitive individuals in salmonids (Abbott and Dill 1985_{RT}; Puckett and Dill 1985_{CoS}; Brännäs et al. 2003_{BT}). However, these supposedly competitive individuals were not necessarily the ones becoming dominant, and the syndrome did not predict growth rate. Further analyses in Brännäs et al. (2004_{BT}) showed that the benefit (in terms of growth) of being aggressive

became less important with increasing density. Using younger trout in a similar setup, Hoogenboom et al. (2012_{BT}) showed that growth increase with aggression only when the environment is predictable. Activity and aggression were positively correlated, suggesting a behavioural syndrome (Hoogenboom et al. 2012_{BT}). Overall, studies suggest complex relationships among resting metabolic rate, body size, environment, and behaviour, where alternative behavioural (and POL) strategies may be viable in different environmental contexts.

Interestingly, syndromes including positive associations between swimming activity in novel environments and active aggression (e.g. attack of a mirror image or a smaller conspecific) emerges in several studies (Adriaenssens and Johnsson 2010_{BT}, 2013_{BT}; Kortet et al. 2014_{BT}). One study also found a syndrome where mirror aggression was positively correlated with propensity to approach novel objects and food items (Höjesjö et al. 2011_{BT}). The links between behavioural syndromes and performance variables (e.g. growth and survival) in nature are, however, not clear cut. Neither growth, nor survival, were associated with behavioural syndromes in brown trout fry in one study (Höjesjö et al. 2011_{BT}), while another found that the less explorative individuals grew better than more explorative individuals (Adriaenssens and Johnsson 2010_{BT}). In the same study, aggression in itself was a poor indicator of social dominance (Adriaenssens and Johnsson 2010_{BT}). Yet another study, using young-of-the year individuals (after the initial critical period), showed that activity in an open-field test is positively associated to survival (Adriaenssens and Johnsson 2013_{BT}). In this latter study, there was also evidence for a progressive emergence of a behavioural syndrome, depending on both selective removal of individuals with low correlation between behavioural traits, and behavioural plasticity (Adriaenssens and Johnsson 2013_{BT}), which is contrasting to the study by Höjesjö et al. (2011_{BT}) where a syndrome was found already in recently emerged fry. The mixed results suggest that there is a complex relationship between behaviour and performance in the wild. Hypothetically, temporal and spatial variation in environmental factors may explain differences in results.

2.5.2 Salmonid syndromes: why and how?

Why?

In salmonids, inter-individual variation in POLS traits has commonly been explained by adaptive strategies concerning timing of major ontogenetic shifts (e.g. Metcalfe 1998_{AS}; Thorpe et al. 1998_{AS}; Rikardsen et al.

2004$_{AC}$; Schjolden and Winberg 2007$_{RT}$). The first key shift is the swim-up from the spawning gravel, at the stage when the alevin turns into a free swimming fry. Earlier emerging individuals tend to be the ones with the lowest energetic potential, i.e. the ones depleting their stored energy the fastest (a fast POL-trait) (Lelong et al. 2008$_{BT}$). Consequently, it has been hypothesised that emergence timing depends on a trade-off between energy demand and developmental stage (Lelong et al. 2008$_{BT}$; Régnier et al. 2012$_{BT}$). First of all, there is likely a limit for how early a fry can emerge, depending on e.g. climatic conditions and food abundance (Jones et al. 2015). Within the feasible emergence period, negative effects of early emergence could depend on low swimming capacity in earlier developmental stages. Positive effects of early emergence can be mediated through prior residence in salmonids, which mean that the territory owner tends to win a fight over the territory even when having a size disadvantage (Brännäs 1995$_{AS}$; Cutts et al. 1999$_{AS}$; Johnsson et al. 1999a$_{BT}$; Kvingedal and Einum 2011$_{AS}$). Earlier emerging fry also display territorial behaviour sooner than later emerging fry (Huntingford and Garcia de Leaniz 1997$_{AS}$). However, if predators are present, emerging early may instead have severe consequences due to smaller dilution effects (i.e. higher individual predation risk). These effects have been shown in laboratory experiments on emerging Atlantic salmon fry (Brännäs 1995$_{AS}$). Exactly how the predators behave during swim-up is often not known in natural systems, but it likely varies among streams. It may be that predators move in to nest areas first when there is a profitable density of fry available, but if fry emergence is predictable (e.g. due to previous experience of the predators) the predators may anticipate fry emergence and move in to these grounds before emergence starts. If early emergers claim all good territories, late emergers could potentially utilise other strategies than territoriality to obtain resources. Mortality is often very high during the first weeks post emergence, and all else being equal, body size has a positive effect on survival at this stage, which may be due to better competitive ability, better swimming performance, higher starvation resistance, or a combination of these traits (Elliott 1994$_{BT}$; Einum and Fleming 2000a$_{AS}$; Ojanguren et al. 1996$_{BT}$).

The second major ontogenetic shift, at least in migrating salmonids, is the smoltification. The growth of juveniles in the stream will influence the onset of smoltification – whether it happens at all, and if so, at which age and which time of the year (Metcalfe 1998$_{AS}$; Jonsson and Jonsson 2011$_{BT,AS}$). Growth rate is faster in the sea or in a lake, and will increase fitness of the migrating individual, provided that it survives the journey (Jonsson and Jonsson 2011$_{BT,AS}$). A larger size at smoltification increases

post-smolt survival (reviewed in Sogard 1997), but smoltifying earlier at a smaller size can reduce the time to first reproduction. Thus, early smoltification at a large size seems like a good life-history strategy, but this requires fast growth and the growth-mortality trade-off hypothesis suggests that fast growers should be at higher risk of mortality (Werner and Anholt 1993; Biro and Stamps 2010). Several different strategies may therefore exist to optimise future fitness. Typically, fish that grow fast smoltify at a younger age and a smaller size, while slower growers tend to extend their stream life and therefore smoltify later, but at larger sizes (Økland et al. $1993_{BT,AS}$). It should here be noted that smoltification at all may be seen as a fast POL strategy (high risk/high gain) when compared with the stream resident strategy. Migration to sea or a lake is likely a more profitable, but also a more risky choice than staying the whole life in the stream. Stream residency is more common among males, where fecundity is not as strongly associated with body size as it is in females, but even some females stay as stream residents. These stream residents are still participating in the spawning with the migrating adults (Jonsson and Jonsson $2011_{BT,AS}$).

POL strategies may also be affected by environmental factors. It could be possible that a fish with late swim-up has no choice but to adopt a slow growing strategy. Making the best of a poor situation (Magurran 1993), a late emerging individual may avoid as much risk as possible and feed only to maintain life. The next year, this fish will be small, but could possibly dominate the new emerging fry and adopt a fast POL. Such a strategy would require a lot of behavioural flexibility. If flexibility is limited within syndromes, this strategy may not be possible for all individuals, but limited to fish with slow POL.

How? Genetic effects

Artificial selection has shown that many behaviours have a genetic background in salmonids (Huntingford and Adams 2005). For instance, clear differences in boldness (novel object and novel food trials) can be seen when comparing wild and hatchery strains of brown trout, originating from the same river (Sundström et al. $2004c_{BT}$). Furthermore, domesticated rainbow trout selected for divergent cortisol expression during confinement show clear differences in behaviour which both points to a potential genetic inheritance of behaviour, and a coping style syndrome (Pottinger and Carrick 2001_{RT}). More evidence comes from salmonids genetically modified to grow faster. These fish deviate from wild and normal hatchery reared conspecifics in behaviours related to risk taking, such as hastiness, activity,

and risky area usage (Sundström et al. 2003_{CoS}, $2004a_{CoS}$,b_{CoS}). There is also evidence for local adaptation in several POLS traits, such as aggression, growth, and metabolic rate (Lahti et al. 2001_{BT}, 2002_{BT}; Jensen et al. 2008_{BT}).

However, while many life-history traits are shown to have significant heritability (additive genetic variance) (Carlson and Seamons 2008; Vøllestad and Lillehammer 2000_{BT}), the same is not true for behavioural traits. Fewer studies have investigated the heritability of behaviour, but those that did so give results pointing at low and insignificant heritability values (Carlson and Seamons 2008). It should be clearly noted that a trait can be genetically based without showing significant heritability, e.g. if there is no or little variance in the trait in the parents to the investigated offspring, or if environmental or parental effects mask the genetic effects (e.g. Smulders 2015). The reason why strong differences in behaviour arise between wild and artificially selected fish, can be that while hatchery selection is directional toward extreme phenotypes, which may not be able to survive in nature, the selection in the wild is likely more or less stabilizing (Kohane and Parsons 1988; Johnsson 1993_{RT}). A relaxed mortality in hatcheries could also lead to more variable phenotypes in the absence of strong selection (e.g. when keeping brood stocks for compensatory stocking) (Conrad and Sih 2009_{RT}).

How? Parental effects

Recent studies have indicated considerable intra-brood variance in several of the POLS traits (Régnier et al. $2012a_{BT}$; Burton et al. $2013a_{BT}$). Particularly it seems like high quality females (dominant, or larger sized) have higher variance in their offspring, which suggests maternal effects (Burton et al. $2013b_{AS}$). A female can potentially manipulate her offspring to become more variable through e.g. differential investment in eggs (Einum and Fleming $2000b_{AS}$), or through differential hormone exposure depending on e.g. placement in the egg sac (Burton et al. $2011a_{BT}$). Maternal effects could depend on e.g. the egg's proximity to blood vessels, which likely could affect size, oxygen supply, and hormone exposure (Kamler 1992).

In salmonids, the egg size is known to vary within and among females (Einum and Fleming $2000b_{AS}$; Rollinson and Hutchings 2011_{AS}; Burton et al. $2013b_{AS}$). Embryos in larger eggs generally have higher metabolic efficiencies and slower developmental rates (slow POL-traits) (Einum and Fleming $2000b_{AS}$; Régnier et al. 2012_{BT}; Rollinson and Hutchings 2010_{AS},

2011_{AS})[9]. Among females from the same population, egg size has been related to juvenile growth rate of the females, with females growing slowly as juveniles producing larger eggs for their size than females with faster juvenile growth (Burton et al. $2013b_{AS}$). Larger eggs produce larger young (Ojanguren et al. 1996_{BT}; Einum and Fleming $2000b_{AS}$; Vøllestad and Lillehammer 2000_{BT}; Rollinson and Hutchings 2011_{AS}), and the fry from larger eggs often have better performance in natural or semi-natural environments (but not in artificial environments such as hatcheries) (Einum and Fleming 1999_{BT}; Rollinson and Hutchings 2013_{AS}; Burton et al. $2013b_{AS}$).

Mass specific metabolic rates also vary among siblings (Régnier et al. 2010_{BT}). Generally, fry with higher metabolic rate tend to start exogenous feeding earlier and become socially dominant more often, which suggests a potential link between metabolism and life-history (i.e. a POLS) (Metcalfe et al. 1995_{AS}; Régnier et al. $2012b_{BT}$). Interestingly, also coping style syndromes appear to influence swim-up (Andersson et al. 2013).

Pre-natal hormone exposure is known to affect subsequent behaviour of the hatched juveniles. Exposing eggs to the stress hormone cortisol can, for instance, affect aggression and behavioural stress response at later life stages, although the exact effects seem to depend on the timing of the exposure (Auperin and Geslin 2008_{RT}; Espmark et al. 2008_{AS}; Sloman 2010_{BT}; Burton et al. $2011a_{BT}$). Cortisol exposure of eggs also leads to smaller size at exogenous feeding and more subordinate behaviours, but has no detectable effects on metabolic rate (Burton et al. $2011a_{BT}$). Parental effects may be most important in early life (Einum and Fleming $2000b_{AS}$), but could also have lasting effects through positive feedback mechanisms such as state-dependent safety (Luttbeg and Sih 2010; also see section 1.3.1).

How? Environmental effects

Environmental effects can by themselves result in stable syndromes, if there is a positive feedback loop in action where the adoption of a trait in an individual results in strengthening the expression of that, and associated, traits (Luttbeg and Sih 2010). This situation is related to state-dependence and will be discussed in section 3.6. Environmental effects can also affect the expression of syndromes. For instance, the link between physiological stress

[9]Developmental rate does not seem to differ among egg sizes in hatchery environments (Beacham et al. $1985_{CoS,ChS}$; Elliott 1994_{BT}; Einum and Fleming 1999_{BT}; Vøllestad and Lillehammer 2000_{BT}). It may be that relatively high temperatures in hatcheries make developmental rates more similar (Jensen et al. $1989_{AS,BT,AC}$)

response and behaviour is often only apparent in novel environments, but not in environments familiar to the fish (Schjolden et al. 2005_{RT}). Temperature and/or season may have similar effects. Given that salmonids generally become inactive at low temperatures, syndromes may not be detectable in winter due to low inter-individual variance in behaviour. It is important to note that these latter effects are examples of environment affecting the expression of syndromes, not environment affecting the existence of the syndromes.

2.5.3 Contributions of the thesis: behavioural syndromes in juvenile brown trout

Objectives

The overall aim with respect to behavioural syndromes, was to investigate whether different behavioural types existed and, if so, whether clusters of behavioural types exist within the syndrome, and whether behavioural syndromes have consequences for growth and survival in the wild.

Specific aims were to:

Explore whether behavioural consistency and behavioural syndromes can be detected in brown trout juveniles (**Papers 3**, **4**, **6**).

Investigate whether behavioural types aggregate in clusters within a behavioural syndrome, indicating discrete behavioural strategies (**Papers 2** and **3**).

Investigate if higher general activity is associated with higher mortality, higher dispersal and higher growth rates in the wild, as suggested by the general POLS hypothesis (**Papers 4** and **6**).

Main findings and discussion

A cross-context behavioural syndrome was found in brown trout fry, with swimming activity in a forced open-field test being positively associated with active aggression towards a mirror (**Paper 3**). These two behavioural scores were also generally repeatable over two trials, run within an interval of two days. Further indications of the repeatability of trout fry behaviour

was provided for open-field activity scores in **Paper 4**. In this study, the interval between trials was 17 days, suggesting that open-field activity is relatively stable over this time-interval. In **Paper 4** half of the fish were tested first at a normal energetic state, and the second time while undergoing compensatory growth, following a period of food-restriction. The repeatability of open-field activity in these fish was independent of state, which indicates a cross-situation activity syndrome. A possible cross-context behavioural syndrome in brown trout was also found in **Paper 6**, where parr being more active in a forced open-field test also dispersed longer in a stream after release (in this case, with repeatability of the behavioural traits being assumed).

Within the behavioural syndrome in **Paper 3**, the behavioural types clustered into two distinct groups, indicating two distinct behavioural strategies. In line with this finding, a separation into two behavioural clusters was also indicated in **Paper 2**, this time based on latency to enter into a novel area (interpreted as risk-taking propensity). In this study, however, only one behavioural trial was run and no explicit investigations into syndromes or behavioural consistency were made.

Size was positively associated with open-field activity and active aggression, as well as emergence latency into a novel area (**Paper 2** and **3**). The behavioural clusters represented here, may reflect the behavioural groupings which can be observed in hatcheries, with territorial fish at the bottom, and non-territorial fish being more 'pelagic' in the hatchery tanks (Pavlov et al. 2010). Similar behavioural groupings are also found in wild brook trout, where they represent different foraging strategies (e.g. McLaughlin et al. 1999).

Laboratory open-field activity of fry was not associated with growth in the wild, but positively associated with probability of recapture in fry being recaptured one month after release in the stream (**Paper 4**). This is in contrast to the predictions made by the general POLS hypothesis (Table 1), which assumes higher mortality for more active individuals, due to higher encounter rate with predators. The higher apparent survival of more active fry, is in line with a previous study on slightly older brown trout juveniles (Adriaenssens and Johnsson 2013). This previous study also suggested that there was no bias in the recapture rate of different behavioural types (Adriaenssens and Johnsson 2013). The higher apparent survival may depend on higher competitive ability of more active individuals. As indicated by the syndrome found in **Paper 3**, more active individuals are also more aggressive. Speculatively, these individuals may belong to a territorial behavioural strategy gaining benefits during the fry stage, which constitute a

critical period in the life of brown trout (Elliott 1994). A good territory is likely both safe and provide much food (Gilliam and Fraser 1984). Interestingly, at the parr stage, no significant association was detected between activity and survival. Together with previous studies from the same area, where 'explorative tendency' (a measure which included activity) did not affect recapture rate in 1+ trout (Adriaenssens and Johnsson 2010), **Paper 6** suggests that negative effects of being more passive in fry, may not be expressed after the initial critical period. In fact, Adriaenssens and Johnsson (2010) show that less explorative (more passive) individuals grow better than more explorative. Growth data from **Paper 6** show a similar picture, albeit more complicated. In this study, more active individuals were growing slower than expected (based on their size) when finding themselves in areas where they need to utilise large home-ranges, but faster than expected when being able to utilise smaller home-ranges. A large home-range may be interpreted as a poor habitat, as it signals that an individual has to move around to satisfy its resource demands. For more passive individuals, home-range size did not affect growth. Based on these findings, it is hypothesised that an active pace-of-life is beneficial in habitats with plenty of resources, while a more passive pace-of-life is benefited in lower-quality habitats (**Paper 6**: Fig. 6). Active individuals may spend more energy on finding and defending scarce resources in a poor habitat, making them disadvantaged.

It is possible that the more passive fish constitute a more flexible phenotype, being able to handle a wider range of environments, or more variable environments. It is worth to note that previous studies on salmonids finding support for the hypothesis that more active (and bold) individuals are at higher risk of predation comes from environments where territoriality is likely less expressed (i.e. lakes; Biro et al. 2004, 2006, 2007), while studies presenting evidence against this hypothesis are conducted in environments where territoriality is highly expressed (i.e. streams). Active fish may be able to claim the best fry-territories in streams, which should be both relatively safe and contain a high food supply (Gilliam and Fraser 1984), leading to relatively good chances of survival. When the energy need increases (i.e. with increased size) more active fish may have to spend more time exposed to gain enough energy and to defend territories, while more passive individuals can use more energy saving strategies, leading to an equalization of performance between the strategies, or even a reversal in performance. When (if) migrating to a more open environment (like lakes or the sea) the active strategy may be at more risk of predation, following the original growth-mortality hypothesis which is part of the general POLS

hypothesis.

In summary, behavioural consistency and behavioural syndromes were found in brown trout juveniles (already at the fry stage). However, the performance of the behavioural types did not conform to the predictions of the POLS hypothesis, where more active fish should grow faster and be at higher risk of mortality. With respect to survival, a pattern opposite to the expected was found in fry, with more active fish being the better survivors, and no significant effect of activity on survival was detected in 1+ parr. With respect to growth, no effects of activity were detected in fry. In 1+ parr, the growth performance depended on an interaction between activity and home-range size.

2.6 State-dependence in salmonid pace-of-life traits

2.6.1 Compensatory growth in salmonids

Juvenile salmonids show compensatory growth (i.e. faster than normal growth rate following environmentally induced growth depression) in both artificial environments (Weatherley and Gill 1981_{RT}; Nicieza and Metcalfe 1997_{AS}; Blake and Chan 2006_{RT}; Morris et al. 2011_{AS}), and in the wild (Álvarez and Nicieza $2005a_{BT}$; Johnsson and Bohlin 2005_{BT}, 2006_{BT}; Elliott 2009_{BT}; Sundström et al. 2013_{BT}). Following energy-restriction the fish typically start to compensate their bodily condition, followed by a mass compensation (and thus an overcompensation in body condition) relatively quickly after feeding commences (Morgan and Metcalfe 2001_{AS}; Johnsson and Bohlin 2006_{BT}). During this initial mass compensation the length (i.e. structural growth) is often not compensated. However, within a couple of months, structural growth is also compensated, at least partially (Morgan and Metcalfe 2001_{AS}; Johnsson and Bohlin 2006_{BT}). Compensatory growth appears to be dependent on season, which likely reflects temperature related activity levels and resource abundance. A study on brown trout in a natural environment, however, suggests that resource limitation can restrict the capacity for growth compensation even during the main growth season (Sundström et al. 2013_{BT}).

The main mechanism behind compensatory growth in salmonids appears to be hyperphagia, but reduced energy expenditure through lowered aggressive activity may also be a component in the increased growth rate (Nicieza and Metcalfe 1997_{AS}). There are also many physiological processes that appear to be altered during growth compensation. For instance, in grass carp *Ctenopharyngodon idella* some genes involved in muscle syn-

thesis and fatty-acid metabolism are up-regulated, and genes suppressing appetite are down-regulated (He et al. 2015). These patterns of gene-expression changes indicate how the growth compensation is executed, with increased growth of muscles made possible by stronger appetite leading to hyperphagia.

Compensatory growth is generally believed to be associated with costs, like increased risk-taking associated with hyperphagia, or delayed costs like reduced reproductive capacity, somatic development, immune function, stress resistance, and physiological maintenance (Arendt 1997; Mangel and Stamps 2001; Metcalfe and Monaghan 2001,2003; Mangel and Munch 2005). One study on brown trout found that over-winter mortality increased following compensatory growth in summer and autumn (Johnsson and Bohlin 2006$_{BT}$). However, other studies from the same geographic area have found no such effects (Johnsson and Bohlin 2005$_{BT}$; Sundström et al. 2013$_{BT}$). The study by Johnsson and Bohlin (2005$_{BT}$) used less severe food-restriction treatment than Johnsson and Bohlin (2006$_{BT}$), which could possibly have accounted for the lack of mortality effects in that study, but Sundström et al. (2013$_{BT}$) used similar restriction treatment. Thus, it may be that among-year variation in the stream environment determines whether or not compensatory growth is associated with increased over-winter mortality. Reduced growth at a later stage of life, and reduced capacity to mature (in male parr) can also be long-term costs related to earlier growth compensation (Morgan and Metcalfe 2001$_{AS}$). Furthermore, studies on GH transgenic coho salmon show that faster growing fish have higher levels of oxidative stress (Carney Almroth et al. 2012$_{CoS}$) and telomere attrition (Pauliny et al. 2015$_{CoS}$). A study on GH-implanted brown trout in a natural stream environment suggests that rapidly growing fish may grow in body size at the expense of energy storage, which could have implications when food abundance is decreased, e.g. in winter (Johnsson et al. 1999b$_{BT}$).

The compensatory response has been shown to be more strongly expressed in fish that have a possibility to smoltify the following spring, than in fish which have no possibility to reach the threshold size for smoltification (Nicieza and Metcalfe 1997$_{AS}$). This suggests that compensatory growth could be used to reach certain ontogenetic threshold sizes within a critical window in time. The 'physiological smolt window' in spring is potentially one such time-limited threshold for ana-/potamodromous populations of salmonids (Jonsson and Jonsson 2011). To be able to adjust growth rate to the 'smolt window', researchers have suggested that there are 'assessment windows' in autumn where the fish can assess its future state and

adjust its development to the optimal decision given the projected state in spring. Potentially, the fish could have an additional possibility to adjust the actual decision shortly before it is executed, in case the autumn projection of spring state was erroneous (Thorpe et al. 1998_{AS}; Rikardsen et al. 2004_{AC}).

Few studies in salmonids have investigated whether compensatory growth occurs at the youngest life-stages. Young fish have very high baseline growth rates (Pedersen 1997; Rombough 2011), and the question is whether they can increase growth rate above normal. One hatchery study has suggested that growth compensation is possible in salmonid fry (Bilton and Robins 1973_{SS}). However, hatchery environments can provide an unnaturally high food supply, which could allow for growth rates not being possible in nature. A recent study by Sundt-Hansen et al. (2012_{AS}) showed that Atlantic salmon fry implanted with growth-hormone (GH) grew faster than sham-injected fry in the hatchery, but slower in nature, indicating a potential for increased growth which cannot be achieved in natural environments. Similarly, GH-transgenic coho salmon and rainbow trout fry have lower growth rates than normal fry in food-restricted environments (Sundström et al. $2004b_{CoS}$; Crossin et al. 2015_{RT}). So, while a physiological capacity for faster growth seems to exist, it may nevertheless be impossible to realise the necessary growth rates in nature. Even aiming to reach such rates may be detrimental for the fry if the elevated energy expenditure when foraging surpass the energy intake needed for optimal growth (Sundström et al. $2004b_{CoS}$; Sundt-Hansen et al. 2012_{AS}; Crossin et al. 2015_{RT}).

2.6.2 Contributions of the thesis: compensatory growth and its costs in juvenile brown trout

Objectives

The overall aims regarding compensatory growth were to expand the knowledge about the compensatory growth response, and its costs in brown trout.

Specific aims were to:

Investigate compensatory growth of age 1+ brown trout juveniles in a natural environment during a year, following a month of food-deprivation in the laboratory (**Paper 1**).

Investigate if age 1+ brown trout show compensatory responses in growth and/or in the maintenance of condition during winter, following a month of food-restriction in the laboratory (**Paper 5**).

Investigate whether compensatory growth is elicited by food-restricted brown trout fry (**Paper 4**).

Investigate whether food-restricted individuals have increased mortality (as indicated by lower recapture rates) in the wild, as compared to non-restricted conspecifics (**Papers 1**, **4**, **5**).

Relate compensatory responses in growth to telomere dynamics, to investigate whether compensatory growth is traded off against chromosomal maintenance (**Paper 1**).

Explore whether food-restriction in late autumn affects the smoltification process in the following spring (**Paper 5**).

Main findings and discussion

Food-deprivation in spring was shown to elicit a compensatory growth response in brown trout parr (**Paper 1**). This response started with compensation of body mass over the first month, which led to complete compensation of body condition, as compared to non-deprived controls. Over the following summer months compensation in both body mass and length

was seen, resulting in partial compensation of body size until September. This general pattern of growth compensation is in line with several previous studies from the same geographic area (Johnsson and Bohlin 2005, 2006; Sundström et al. 2013). Over the winter, no compensatory growth could be detected, indicating that the fish were limited in their growth during this time. This finding was further supported in **Paper 2**, where fish being food-restricted in late autumn did not show any compensation in body size over winter. The food-restricted fish in this study, however, managed to achieve the same spring body condition as fish fed high rations. The body condition of all fish decreased over winter, but since the food-restricted fish were starting the winter with a lower condition, the achievement of ending up with similar condition at the end of winter suggests that these fish could maintain their condition at similar levels as the high-ration fish - which likely required higher feeding rates.

Food-restricted brown trout fry were able to initiate growth compensation in mass in the laboratory (**Paper 4**). However, after 11 days of re-feeding in the laboratory they were released into their native stream (supposedly in the midst of their compensatory growth phase), where growth compensation ceased. Instead, both food-restricted and non-restricted controls grew at similar average rates in the wild. This suggests that environmental conditions in the stream limit the growth rate, so that individuals are growing at the maximal allowed growth rate. This finding is in line with previous studies on Atlantic salmon fry, which are able to increase growth rate in hatchery environments, but not in the wild, when being injected with growth-hormone to stimulate appetite (Sundt-Hansen et al. 2012).

No indications of increased mortality in fish being food-deprived/restricted were found in any of the studies. In **Paper 1**, where growth compensation was observed, a similar (higher, but non-significantly so) proportion of food-deprived and non-deprived fish were recaptured after a year in the stream. In **Paper 5**, were food-restricted fish managed to keep up with non-restricted conspecifics in body condition, the food-restricted fish were also recaptured at similar rates as non-restricted fish. In the fry, no effects on recapture rates were seen being due to previous food-restriction (**Paper 4**). As the fry did not compensate growth in the wild, it was not possible to assess effects of growth compensation in natural environments in this study. However, the similar recapture rates of food-restricted and non-restricted fry at least indicate that mortality was not a consequence of fish trying to compensate in the wild, as such an effect should have led to lower recapture in the food-restricted groups.

No evidence for a trade-off between growth compensation and chro-

mosomal maintenance was found, as both food-deprived and non-deprived individuals had similar change in telomere length (**Paper 1**). Interestingly, there were no indications of general telomere-attrition on the population level, indicating that brown trout can maintain their telomeres well. However, exploration of previous growth (prior to the experiment) in relation to change in telomere length suggested that fish with previously high growth investment may pay a delayed cost in reduced chromosomal maintenance, as telomere attrition was higher in initially larger fish.

Estimation of smoltification status in spring indicated that individuals being food-restricted in late autumn had delayed the initiation of smoltification (**Paper 5**). Speculatively, this may be a consequence of having to spend more energy to maintain body condition over winter.

In summary, compensatory growth was elicited by 1+ parr in natural environments and by fry in the laboratory. Fry did not manage to compensate body size in the wild, suggesting that the natural environment put limits to the growth rates at this stage. Increased growth rates were not directly traded off against chromosomal maintenance. Neither were there any apparent costs in therms of increased mortality.

2.6.3 State-dependent behaviour

Immediate changes in behaviour related to energetic status are likely associated to changes in plasma concentrations of hormones, such as GH and ghrelin (Bar 2014). Increased GH levels decrease anti-predator behaviour, and increase food intake and aggression (Johnsson et al. 1996a$_{RT}$,b$_{BT}$; Jönsson et al. 2008$_{RT}$; Neregård et al. 2008$_{BT}$; Sundt-Hansen et al. 2009$_{BT}$). Similar effects have been observed from implants of ghrelin (Tinoco et al. 2014$_{BT}$).

Longer-term changes in behaviour and growth could be driven by the amount of stored fat in the body (Jobling and Johansen 1999). For instance, under low food conditions the decrease in body lipids in relation to the remaining stores could be used to project future starvation risk (e.g. Bull et al. 1996$_{AS}$). The fish could, based on such a projection, then adjust the behaviour to match both short- and long-term energetic needs (Bull et al. 1996$_{AS}$; Bull and Metcalfe 1997$_{AS}$; Railsback et al. 2005). For instance, when the energy content of the body is reduced, an individual may modify its behaviour and spend relatively more time foraging at risky foraging sites and being exposed during the more hazardous times of the day (Railsback et al. 2005).

Following food-restriction, behaviour is often altered towards more ac-

tive foraging to increase food intake and elevated aggression to increase the ability to compete for food. Food restricted salmonids have been shown to be more risk taking, showing higher activity in the presence of potential predators (Höjesjö et al. 1999_{RT}; Vehanen 2003_{AS}). Short-term starvation or low food ration can also increase aggression, particularly in dominant fish (Symons 1968_{AS}; Slaney and Northcote 1974_{RT}; Dill et al. 1981_{CoS}). These findings match the expectations based on the starvation-avoidance principle, where individuals in risk of starvation become more risk-taking. However, the studies are likely showing immediate hunger effects. While hunger effects are clear state-dependent alterations of behaviour, there may also be other effects of energetic state. Immediate hunger effects only last until satiation, while effects of energetic status should last until a higher energetic status is reached. Salmonid studies suggest that the increased risk-taking is not only an immediate hunger response due to an empty stomach, but lasts through periods of compensatory growth where fish actually may have higher than normal satiation levels due to their high food intake (Damsgård and Dill 1998_{CoS}). After prolonged starvation, however, metabolism and activity goes down, supposedly due to the fish entering an energy-saving mode (Beamish 1964_{BC}; Wang et al. 2006).

Size effects on behaviour may also be due to energy stores. A larger body can store more energy reserves, and demands relatively less energy per gram body mass than a smaller body. This means that it takes longer for a larger individual to reach a state of starvation. Consequently, if one larger and one smaller individual are starved for the same time, their response to the starvation is expected to differ. We would expect that larger individuals are less willing to take risks, following the starvation-threshold feedback (i.e. the combination of asset protection and starvation-avoidance). Furthermore, experience may differ between larger and smaller individuals, affecting the behaviour. An example is that larger individuals may experience a different predation threat than smaller individuals.

2.6.4 Contributions of the thesis: state-dependent behaviour in brown trout fry

Objectives

The overall aim regarding the studies of state-dependent behaviour was to investigate whether behavioural POLS-traits could be altered through manipulation of energetic state, and whether size affected the expression of these traits in brown trout fry.

The specific aims were to:

Investigate whether manipulated energetic state or body size affected behaviour in standardised laboratory trials. The behaviours investigated were: risk-taking (**Paper 2**), activity (**Paper 3**), boldness (**Paper 3**) and aggression (**Paper 3**).

Investigate whether a change in state, from a normal growth rates to compensatory growth rates, leads to a change in the open-field activity (**Paper 4**).

Key to feeding treatments:

HH - an initial period with high food ration followed by another period with high food ration

HL - an initial period with high food ration followed by a period with low food ration

LH - an initial period with low food ration followed by a period with low food ration

LL - an initial period with low food ration followed by another period with low food ration

Main findings and discussion

With respect to energetic state, the main prediction following the starvation-threshold feedback (i.e. starvation-avoidance and asset-protection; SA-AP), is that low-asset individuals with high food demand (i.e. being small or having low condition) should be relatively more active, bold, and aggressive than high-asset individuals (i.e. being large or having high condition). In contrast, the state-dependent safety feedback (SDS) predicts that high-asset individuals should be more active, bold, and aggressive. This is because the high-asset individuals are relatively safer from risks, which allows them to express these behaviours to a higher extent. These feedbacks could potentially be at work simultaneously if each feedback relates to a different state (e.g. SA-AP to body condition, and SDS to body size).

The studies in this thesis provided only weak evidence for the SA-AP feedback, but relatively strong evidence for the SDS feedback.

In **Paper 2**, no general difference in risk-taking (as indicated by the latency to enter a novel area) was found among the treatment groups. However, splitting the treatment groups based on the behavioural type (as divided by cluster analysis), suggested that HH fish from the short-latency group emerged relatively faster than the short-latency fish from the other groups (albeit only being significant for the comparison between HH and LH). This latter (ad hoc) analysis tend to support the SDS feedback over the SA-AP feedback. The SDS feedback is also supported by a significant main effect of size, where larger fish generally emerge faster, regardless of treatment-group.

The evidence for the SA-AP feedback consist of the findings of **Paper 3**, where recently food-restricted fish (HL + LL) were more aggressive than recently well-fed fish (LH + HH), and where HL tended to be more active than other groups. The HL and LL treatment groups are indeed low-asset groups, and seem to express their general behaviour in ways predicted by the SA-AP. However, the evidence is not strictly supported statistically, as the overall treatment effects in the omnibus tests (the generalised linear mixed models) were not significant at the conventional 5% level. The difference in aggression between recently food-restricted and well-fed fish is based on an ad hoc test, pooling treatments together based on their most recent experimental food-ration, and the effect on activity is based on an overall trend effect of treatment, followed up by post hoc pairwise contrasts of the estimated marginal means. Thus, these results should be considered indicative, but not conclusive. Importantly, given the indicative nature of the data, we should not exclude the SA-AP as a possible feedback mechanism in trout fry. The LH group, which were considered to be in a state of compensatory growth, did not differ from HH in contrast to the expectations based on SA-AP. Like in **Paper 2**, there was a general main effect of size, where larger fish were more active and aggressive. This provides further evidence that the SDS feedback is the more important mechanism regulating behaviour in brown trout fry.

In **Paper 4** there was no change in open-field activity due to compensatory growth. Two separate experiments both suggest that growth compensation did not affect behaviour in an open-field test.

Overall, these experiments support the SDS feedback mechanism, in particular through the size effects detected where larger fish tend to express behaviours commonly considered as more risky (although, they need not be more risky given a safety effect of larger size). LH fish undergoing

compensatory growth generally did not differ from HH fish. In **Papers 2** and **3** the LH fish may have been in the final stages of compensation (3 weeks into the re-feeding period of LH), given that their body length and mass were close to the HH when trialled. There were indications of behaviour being altered in recently food-restricted groups, which could potentially help in the onset of a compensatory response (e.g. drive the fish to seek out and obtain new foraging territories). If this is the case, the effects appear to be short-lived, as there were no differences in activity between HH and LH fish in **Paper 4**, when trialled 6-7 days into the re-feeding period of the LH fish.

The lack of support for the SA-AP feedback contrasts finding from other fish studies on e.g. three-spined sticklebacks where evidence supporting the SA-AP have been found (Krause et al. 1998, 1999). Differences in ecology between the species may explain the different results. Trout are territorial and aggressive and may take advantage of high assets to assure a good position in the social hierarchy, while low-asset individuals may adopt a more evasive foraging tactic. Sticklebacks, in contrast, are schooling and relatively non-aggressive (Wootton 1984) and low-asset individuals may have to be more active to compete for food-resources within the school.

The overall conclusion is that feeding history has relatively little effect on the average expression of behavioural traits in brown trout fry in standardised laboratory trials. This could be a consequence of fry being under general growth stress, regardless of previous feeding history. This is further supported by the fact that wild fish in **Paper 2** appear to grow faster than any experimental fish. It is also supported by the finding that compensatory growth is not detected in fry in the wild. If fry are maximising their growth in general, they may not have any mechanism to alter their basal expression of behaviour.

3. Notes on methodology

The methodology of all experiments is described in detail in each of the original articles and manuscripts on which this thesis is based. However, some notes on the methodology from a wider perspective are necessary to clarify the choice of experimental designs. This is because some methods developed through the studies, based on experience from preceding experiments.

Fish origin/experimental streams

All fish used in the experiments originated from a restricted geographical area on the Swedish west coast (Fig. 3). Three different streams were used: Norumsån, Jörlandaån, and Stenunge å. These streams are ecologically similar, particularly experimental sections which all were running through mainly deciduous forests, with the main differences being that Stenunge å is smaller than the other two. All streams house high densities of trout and a small number of European eel. In addition, Jörlandaån houses European minnow, and Stenunge å houses non-native signal crayfish. For potential predators, see Table 3.

Laboratory growth: experimental design

Trout were fed in the lab for monitoring of growth in four of the experiments presented here (**Papers 2**, **3**, **4** and **5**.

In **Papers 2** and **3**, where fish were reared individually in small compartments, the food rations were calculated based on the energy requirements of juvenile trout fed invertebrates (Elliott and Hurley 1998; procedure described in detail in **Paper 2**). Maximal intake rations were calculated based on the largest fish and from these calculations a fixed daily rations were determined. In effect, the maximal intake rations seemed to

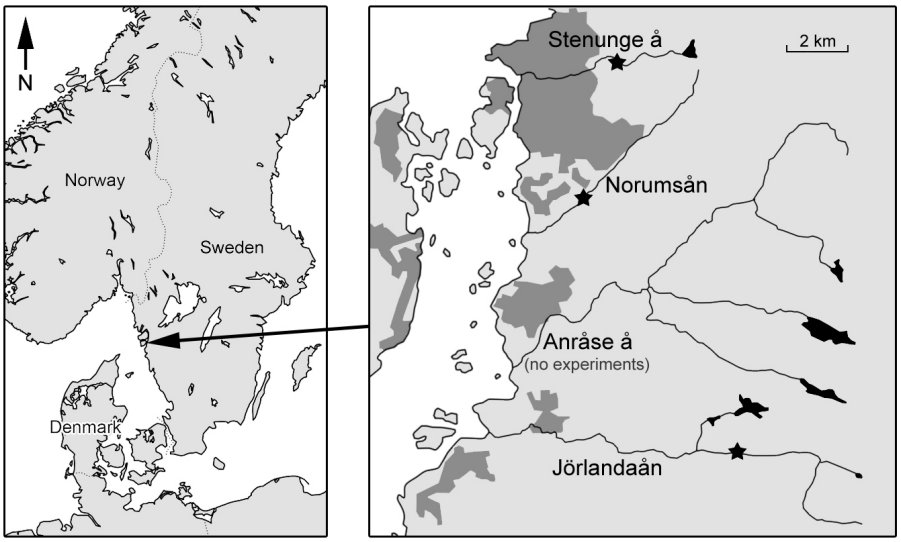


Figure 3: Location of experimental streams. Stenunge å: **Paper 4**; Norumsån: **Papers 1**, **2**, **3**, **4**; Anråse å: not used; Jörlandaån: **Papers 5**, **6**. Stars denote the location of capture/experimental areas. Dark grey areas show human population centres, with the largest most northerly population centre being Stenungsund city.

constitute ad libitum feeding at the daily feeding event, as food left-overs were observed in compartments where this ration was given. However, the fish were fed only once a day due to time constraints and this fact makes the high ration not conforming to strict ad libitum criteria (i.e. feeding at will). It seems likely that fry in nature can feed over the whole day, and thereby achieve higher growth rates than observed in **Papers 2** and **3** - and this was also indicated by a field sampling in **Paper 2**.

In **Paper 4**, the fish were group-reared in larger compartments with the expectation that competition would increase the growth rates of the fry, as compared to **Papers 2** and **3**. Using larger rearing compartments also allowed for excess feeding, without any major problems of leftover food creating a poor environment. We also fed the fish twice a day to give them two opportunities to feed over the day. This method did improve growth rates, as compared to the previous experiments.

In **Paper 5** the fish were fed high and low rations in the laboratory. In this experiment the fish were group-housed and rations based on observed ad libitum levels of the high ration group, at every feeding occasion. The high and low ration was achieved by modifying the number of feeding occasions.

Behavioural tests

Papers 2, **3**, **4**, and **6** involved laboratory scoring of behaviour.

In **Paper 2**, a start-box emergence test was used, where an animal is put in a start-box and subsequently presented a novel area into which it can enter freely. Variations of this test are commonly used in fish behaviour research (Näslund et al. 2015b) and the test is generally claimed to measure aspects of 'boldness', or risk-taking. The behavioural measure most commonly scored in this test is the latency to emerge into the novel environment. The arena design, e.g. the size of the entrance into the novel area and the environmental complexity in the novel area, can affect the behaviour of brown trout fry in this test (Näslund et al. 2015b). Compared to the arena designs tested by Näslund et al. (2015b), we used a design with large door toward a complex novel environment, which seem to be a relatively robust design promoting large behavioural variation among individuals[10].

In **Paper 3**, we utilised a suite of three tests, which were executed sequentially in the same order for all fish. Carryover effects from one test to another should thus be the same for all tested individuals (Bell 2012). The first test was a 'forced open-field' test, where an individual is put directly into a restricted area without structural complexity. The swimming activity of the individual is then measured in this 'open field' (this behavioural score is likely a compound measure of activity, boldness and exploration; as discussed by e.g. Burns 2008 and Toms et al. 2010). Following this test a novel object was introduced into the test-tank to measure boldness towards the object. This type of test is also common in fish behaviour research (e.g. Höjesjö et al. 2011; Adriaenssens and Johnsson 2013)[11]. The final test in the sequence was a mirror aggression test (Gallup 1968); again a common test for fish behaviour research (e.g. Höjesjö et al. 2011; Adriaenssens and Johnsson 2013). A mirror is presented to the fish, after which the aggression and display behaviours toward the mirror image are scored. While this test could potentially elicit behaviours and physiological responses that are not exactly the same as in a confrontation with a real conspecific (Desjardins and Fernald 2010), the high responsiveness toward

[10]The study by Näslund et al. (2015b), was partly designed to assess the test used in Paper 2.

[11]The novel object test used in **Paper 3** was judged not to work as intended, as behaviour in the presence of the novel objects was not repeatable over the two trials, and most fish appeared to be neither neophobic, nor neophilic, but instead moving in the arena more or less randomly. See supplemental material to **Paper 3**, at the end of this thesis, for further information

the mirror was interpreted to reflect aggressive behaviour in a relevant way, which is supported by Höjesjö et al. (2004). The suite of tests was repeated for all individuals two days after the first trial to measure behavioural consistency.

In both **Papers 4** and **6**, the forced open-field test was utilised. This test was specifically chosen because of the relatively high repeatability seen in the experiment described in **Paper 3**. In **Paper 4**, open-field activity was measured twice, before and after food-ration alterations. In contrast to **Paper 3**, where only the mean difference in behaviour after the treatment could be investigated, the before-after design allows for investigations of how individuals change their behaviour in response to the treatment. In **Paper 6** the open-field trials were run only once for each individual, assuming that the behaviour would be repeatable; based on Adriaenssens and Johnsson (2013) and results from **Paper 3**).

Growth rate calculations

In **Paper 1** specific growth rate for both body wet mass and fork length (i.e. the percentage increase in mass and length per day) was calculated. While mass growth of fishes is appropriately described as the percentage increase per day, length tends to increase linearly over time (Sigourney et al. 2008). Consequently, the calculations of growth rate in body length were changed to absolute increase in length per day in the following papers [**Papers 2-5** (no analysis of length growth was performed in **Paper 6**)].

Growth rate is also size specific, with smaller fish typically growing at faster rates than larger fish (Brett 1979). However, this physiological phenomenon can be over-shadowed by dominance effects, particularly in artificial environments where subordinate individuals have no possibilities to move elsewhere (e.g. Brown 1957; Jobling 1985b, Ivanova and Svirskaya 2013). For this reason, the observed specific growth rates were corrected for size effects in the statistical models when fish were reared individually or in crowded environments (which do not allow for strong dominance effects) in the laboratory (**Papers 2**, **3**, and **5**), or monitored for growth in natural systems (**Papers 1**, **5**, and **6**). When fish were reared in the laboratory in environments allowing for dominance hierarchies to form (**Paper 4**), the growth rate was corrected for body size using the mass-specific growth rate formula (Ostrovsky 1995) and the allometric mass exponent for brown trout (Elliott et al. 1995).

Recapture and estimation of survival

In the experiments conducted in natural streams (**Papers 1**, **4**, **5**, and **6**) the recapture rate was used as a proxy for survival. Given that no differences exist in recapture rate or emigration from the experimental area, this proxy should be a good indicator of actual survival. In **Papers 1** and **5** the recaptures were made using electrofishing equipment in early spring at low water temperatures. The low temperatures likely affected the catchability negatively, since electrofishing has relatively low efficiency in cold water as a consequence of hiding-behaviour and general inactivity of the fish (Heggenes et al. 1993). Even in warmer temperatures, the recapture rate is seldom 100% (Degerman and Sers 2001). Thus, the number of fish being recaptured is most likely an underestimation of the actual number of surviving fish. However, actual numbers are not needed, as survival is based on relative proportion of fish recaptured.

In **Paper 4**, recapture rates of the fry were calculated and found to be very good ($> 85\%$ of the fry being captured over three electrofishing passes). As we compared different behaviours it is important that different behavioural types do not have different recapture probability, which could actually be expected based on activity - with active fish being more likely to be recaptured. Due to time-restrictions in the field, we prioritised fishing longer buffer sections out of the main experimental area, instead of identifying all individuals each fishing-pass. Thus, we could not assess differences between more and less active fish. However, a previous study in one of the experimental streams used in **Paper 4** found that there were no differences in recapture probability between behavioural types (Adriaenssens and Johnsson 2013). It is assumed that this is also true for **Paper 4**.

While 'blind' electrofishing was used in **Papers 1**, **4** and **5** (i.e. fishing without knowledge of where the fish are positioned in the stream), in **Paper 6** portable antennas allowed for location of the electronic PIT-tags (which were implanted in the experimental fish to identify individuals) in the stream. When a tag was detected, electrofishing was used to try to capture the fish carrying the tag. In addition to obtain tagged individuals without disturbing other fish in the stream, this targeted electrofishing technique also allowed for the collection of information on fish being dead in the stream. Individuals were determined to be dead when a tag was detected but no response of any fish was seen when disturbing the tag-location by a combination of electrofishing and disturbance of the bottom substrate.

4. General conclusions and future perspectives

Behavioural types

Brown trout appear to have behavioural types. More specifically, a behavioural syndrome where activity in open-field tests correlates positively with overt aggression in a positive association was found. Both open-field activity and aggression were repeatable traits. Cluster analyses suggested, in two separate experiments, that brown trout fry were separated in two behavioural groups. This could possibly be an indication of two major behavioural strategies. The existence of two behavioural strategies could be maintained in a population through frequency dependent selection (Maynard Smith 1982; Wolf et al. 2013). The mechanism behind the behavioural expression could be directly genetic, or modified through maternal or early environmental effects. The studies in these thesis, however, suggest that behaviour is relatively insensitive to environmental factors such as food availability at the fry life-stage.

The relationship among behavioural-, coping style-, and metabolic rate syndromes was not investigated in the experiments of this thesis. However, investigations into the holistic nature of the POLS, incorporating measurements of all these syndromes within the same individuals, are recommended.

Main conclusions:

- Behavioural syndromes were found in fry
- Fry cluster into distinct behavioural strategies

State-dependent growth rate and behaviour

Compensatory growth rates were confirmed in parr during the main growth season, but not in wintertime. Compensatory growth, at least in mass, was also shown to be elicited during the fry stage, but only in laboratory environments. In the wild, environmental factors appear to limit the growth rate of fry, making compensatory growth rates impossible.

No evidence for altered behaviour due to compensatory growth was found in fry. However, indications of state-dependent alterations of open-field activity and aggression were found in fish being recently food-restricted. This could be an indication of a behavioural alteration facilitating the onset of growth compensation (e.g. helping the fish to find a new food source and attain a new territory). It is possible that behaviour was affected by the fish being tested in a stressed state and in a highly unnatural environment. In future studies, behavioural scoring in the fishes' home-tanks could further elucidate whether or not behavioural alterations follow compensatory growth in fry.

Growth compensation did not increase mortality in any of the field studies. It is possible that increased mortality will come at a later life-stage (Metcalfe and Monaghan 2001), or only under certain environmental conditions (e.g. under high predation pressure). Repetition of induced compensatory growth experiments within the same stream system over several years, with monitoring of environmental conditions and demographic traits could possibly give insights into environmental costs of faster than normal growth rates.

Main conclusions:

- Compensatory growth can be elicited by parr in the wild
- Compensatory growth can be elicited by fry in the laboratory, but not in the wild
- Compensatory growth does not alter the general activity of fry
- No indications of increased mortality due to faster growth rate were detected

Activity and performance in the wild

The studies in this thesis show that behaviour is variable among individuals both at the fry and at the parr stages. It is, however, not known whether or not the variation is stable over the whole life. The behaviour likely

depends on both innate physiology and the experience of the individual (Senner et al. 2015). Open-field activity predicted performance in the wild in both fry and parr. In fry, high activity had positive effects on survival in two different streams, a finding which is in accordance with previous studies (Adriaenssens and Johnsson 2013). At the parr stage, no difference in survival was detected. This difference in effects could depend on high initial competition after swim-up favouring active (and actively aggressive) fish, which then disappear at the parr stage when fish spread out in the stream. Studies have shown that more aggressive, active, and bold individuals could be more efficient invaders in novel environments (Sih et al. 2012; Thorlacius et al. 2015), and swim-up basically constitute an invasion of a novel environment for the fry.

Open-field activity measurements likely contains aspects of boldness and exploration as well as general activity (Burns 2008; Toms et al. 2010). A previous study has shown that less active fish can grow better at the parr stage (Adriaenssens and Johnsson 2010). This indicates that, while being at a disadvantage during the fry stage, the more passive individuals may perform better than more active fish during the parr stage. Indeed, **Paper 6** indicate that more active parr seem to be dependent on finding good habitat (as inferred from home range - with smaller home range being assumed to signal better habitat quality) to achieve high growth rates, while more passive fish perform equally well in different habitats.

The combined findings of this thesis suggest that activity affect survival and growth rates differently in fry and parr. Furthermore, previous studies on salmonids suggest that the effects may change yet again if the fish migrates to an open environment (Biro et al. 2004, 2006, 2007). Thus, future studies following individuals across ontogenetic stages could be conducted to investigate changes in selection pressure on different behavioural types over ontogeny. Such studies could also give insights into the long-term stability of the behavioural types.

Main conclusions:

- Survival is positively influenced by activity in fry
- Survival appears not to be influenced by activity in parr
- Growth appears not to be influenced by activity in fry
- Growth is influenced by an interaction between activity and habitat quality in parr

The future for the pace-of-life syndrome hypothesis

"All models are wrong, but some are useful" is an aphorism commonly used in statistics, attributed to G. E. P. Box (e.g. Box 1976). It simply means that the real world is too complex to be described by a model, but also that a model can be good enough to describe the important phenomena in which one is interested to a satisfying degree. One criterion for usefulness should be the ability to make predictions from the model. We started off this thesis with a description of the *r*- and *K*-selection hypothesis. This "model" did fail in several predictions (see e.g. Stearns 1992; Reznick et al. 2002), partly because it did not contain a high enough level of complexity (e.g. it lacked a factor of extrinsic mortality). Based on the findings of this thesis, the POLS hypothesis fails to predict fitness trade-offs associated with behavioural variation in the brown trout model system. Particularly, for brown trout fry the activity appears to be positively associated with survival. Deviations from the general POLS hypothesis have been known for a time, both at the inter-specific level (e.g. Munshi-South and Wilkinson 2010; Grant and Grant 2011) and at the intra-specific level (e.g. Adriaenssens and Johnsson 2010, 2013; Farwell and McLaughlin 2009). It is also clearly stated in one of the major publications on the POLS that future research should avoid "[...] *the temptation to generalize and simplify a complex reality, in a way that has been detrimental to the r- and K-selection model in ecology*[...]" (Réale et al. 2010). However, this makes the POLS model useful only if we take the potential pitfalls into account. Consequently, while being useful, it is also dangerous in the sense that the wrong predictions for associations among traits may be made based on it. The most important conclusion from this thesis is that care must be taken not to assume trait correlations or ecological consequences of single traits only because the POLS hypothesis predicts such associations. The usefulness of the POLS is in formulation of hypotheses to be tested, not in drawing conclusions based on single findings.

References

Abbott JC, Dill LM. 1985. Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). Can J Fish Aquat Sci 42: 1702–6

Adriaenssens B, Johnsson JI. 2010. Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. Behav Ecol 22: 135–43

Adriaenssens B, Johnsson JI. 2013. Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. Ecol Lett 16: 47–55

Alexander GR. 1977. Consumption of small trout by large predatory brown trout in the north branch of the Au Sable River, Michigan. Michigan Department of Natural Resources Fisheries Division. Fisheries Research Report No. 1855

Ali M, Nicieza A, Wootton RJ. 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish 4: 147-90

Allan JD, Castillo MM. 2007. Stream Ecology: Structure and Function of Running Waters. Second edition. Dordrecht: Springer Science & Business Media

Allan IRH, Ritter JA. 1977. Salmonid terminology. J Cons Int Explor Mer 37: 293–9

Álvarez D, Nicieza AG. 2005a. Compensatory response "defends" energy levels but not growth trajectories in brown trout, *Salmo trutta* L. Proc R Soc B 272: 601–7

Álvarez D, Nicieza AG. 2005b. Is metabolic rate a reliable predictor of growth and survival of brown trout (*Salmo trutta*) in the wild? Can J Fish Aquat Sci 649: 643–9

Andersson MÅ, Laursen DC, Silva PIM, Höglund E. 2013. The relationship between emergence from spawning gravel and growth in farmed rainbow trout *Oncorhynchus mykiss*. J Fish Biol 83: 214–9

Angilletta MJ, Steury TD, Sears MW. 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integr Comp Biol 44: 498-509

Arendt JD. 1997. Adaptive intrinsic growth rates: an integration across taxa. Q Rev Biol 72: 149-77

Arendt J, Wilson DS, Stark E. 2001. Scale strength as a cost of rapid growth in sunfish. Oikos 93: 95-100

Arnold SJ. 1994. Multivariate inheritance and evolution: a review of concepts. In: Boake CRB, ed. Quantitative Genetic Studies of Behavioral Evolution. Chicago: University of Chicago Press. p. 17-48

ArtDatabanken. 2015. Artportalen - Rapportsystem för växter, djur och svampar. https:// www.artportalen.se/. Electronic version accessed 2015-07-03

Auperin B, Geslin M. 2008. Plasma cortisol response to stress in juvenile rainbow trout is influenced by their life history during early development and by egg cortisol content. Gen Comp Endocrinol 158: 234–9

Bachman RA. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. Trans Am Fish Soc 113: 1–32

Bar N. 2014. Physiological and hormonal changes during prolonged starvation in fish. Can J Fish Aquat Sci 71: 1447-58

Beacham TD, Withler FC, Morley RB. 1985. Effect of egg size on incubation time and alevin and fry size in chum salmon (*Oncorhynchus keta*) and coho salmon (*Oncorhynchus kisutch*). Can J Zool 63: 847–50

Bell AM. 2007. Future directions in behavioural syndromes research. Proc R Soc B 274: 755–61

Bell A. 2012. Randomized or fixed order for studies of behavioral syndromes? Behav Ecol 24: 16-20

Bell AM, Sih A. 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecol Lett 10: 828–34

Bell AM, Dingemanse NJ, Hankison SJ, Langenhof MBW, Rollins K. 2011. Early exposure to nonlethal predation risk by size-selective predators increases somatic growth and decreases size at adulthood in threespined sticklebacks. J Evol Biol 24: 943–53

Bilton HT. T, Robins GLL. 1973. The effects of starvation and subsequent feeding on survival and growth of Fulton Channel sockeye salmon fry (*Oncorhynchus nerka*). J Fish Res Board Canada 30: 1–5

Birkesten O. 1963. Ädelfisk. Stockholm: Åhlén & Åkerlunds Boktryckeri

Biro PA, Stamps JA. 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends Ecol Evol 25: 653–9

Biro PA, Abrahams M V., Post JR. 2007. Direct manipulation of behaviour reveals a mechanism for variation in growth and mortality among prey populations. Anim Behav 73: 891–6

Biro PA, Abrahams M V., Post JR, Parkinson EA. 2004. Predators select against high growth rates and risk–taking behaviour in domestic trout populations. Proc R Soc Lond B 271: 2233–7

Biro PA, Abrahams M V., Post JR, Parkinson EA. 2006. Behavioural trade-offs between growth and mortality explain evolution of submaximal growth rates. J Anim Ecol 75: 1165–71

Bisther M, Roos A. 2006. Uttern i Sverige 2006. Online publication: Världsnaturfonden WWF. Available at: http://www.wwf.se/source.php?id=1120430

Bjornn T, Reiser D. 1991. Habitat requirements of salmonids in streams. Am Fish Soc Spec Publ 19: 83–138

Blackburn EH, Gall JG. 1978. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. J Mol Biol 120: 33–53

Blake RW, Chan KHS. 2006. Cyclic feeding and subsequent compensatory growth do not significantly impact standard metabolic rate or critical swimming speed in rainbow trout. J Fish Biol 69: 818–27

Blanckenhorn WU. 2005. Behavioral causes and consequences of sexual size dimorphism. Ethology 111: 977-1016

Boersma B, Wit JM. 1997. Catch-up growth. Endocr Rev 18: 646-61

Bohlin T. 1977. Habitat selection and intercohort competition of juvenile sea-trout *Salmo trutta*. Oikos 29: 112–7

Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML. 2003. The ecology of individuals: incidence and implications of individual specialization. Am Nat 161: 1–28

Box GEP. 1976. Science and statistics. J Am Stat Ass 71: 791–9

Brett JR. 1979. Environmental factors and growth. In: Hoar WS, Randall DJ, Brett JR, eds. Fish Physiology, vol VIII - Bioenergetics and growth. Orlando: Academic Press Inc. p. 599–675

Briffa M, Sneddon LU. 2010. Contest behavior. In: Westneat DF, Fox CW, eds. Evolutionary Behavioral Ecology. New York: Oxford University Press, Inc. p. 246-65

Brown ME. 1957. Experimental studies on growth. In: Brown ME, ed. The Physiology of Fishes, Vol. I - Metabolism. New York: Academic Press Inc. p. 361-400

Brännäs E. First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. Evol Ecol 9: 411-20

Brännäs E, Jonsson S, Lundqvist H. 2003. Influence of food abundance on individual behaviour strategy and growth rate in juvenile brown trout (*Salmo trutta*). Can J Zool 81: 684–691

Brännäs E, Jonsson S, Brännäs K. 2004. Density-dependent effects of prior residence and behavioural strategy on growth of stocked brown trout (*Salmo trutta*). Can J Fish Aquat Sci 82: 1638–46

Budaev SV. 1998. How many dimensions are needed to describe temperament in animals: a factor reanalysis of two data sets. Int J Comp Psychol 11: 17-29

Bull CD, Metcalfe NB. 1997. Regulation of hyperphagia in response to varying energy deficits in overwintering juvenile Atlantic salmon. J Fish Biol 50: 498–510

Bull CD, Metcalfe NB, Mangel M. 1996. Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. Proc R Soc Lond B 263: 13–8

Burns JG. 2008. The validity of three tests of temperament in guppies (*Poecilia reticulata*). J Comp Psychol 122: 344–56

Burton T, Hoogenboom MO, Armstrong JD, Groothuis TGG, Metcalfe NB. 2011a. Egg hormones in a highly fecund vertebrate: do they influence offspring social structure in competitive conditions? Funct Ecol 25: 1379–88

Burton T, Killen SS, Armstrong JD, Metcalfe NB. 2011b. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proc R Soc B 282: 20111778

Burton T, Hoogenboom MO, Beevers ND, Armstrong JD, Metcalfe NB. 2013a. Among-sibling differences in the phenotypes of juvenile fish depend on their location within the egg mass and maternal dominance rank. Proc R Soc B 280: 20122441

Burton T, McKelvey S, Stewart DC, Armstrong JD, Metcalfe NB. 2013b. Early maternal experience shapes offspring performance in the wild. Ecology 94: 618–26

Careau V, Thomas D, Humphries MM, Réale D. 2008. Energy metabolism and animal personality. Oikos 117: 641-53

Carlson SM, Seamons TR. 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evol Appl 1: 222-38

Carney Almroth B, Johnsson JI, Devlin R, Sturve J. 2012. Oxidative stress in growth hormone transgenic coho salmon with compressed lifespan - a model for addressing aging. Free Radic Res 46: 1183–9

Carter AJ, Feeney WE, Marshall HH, Cowlishaw G, Heinsohn R. 2013. Animal personality: what are behavioural ecologists measuring? Biol Rev 88: 465–75

Castanheira MF, Conceição LEC, Millot S, Rey S, Bégout M-L, Damsgård B, Kristiansen T, Höglund E, Øverli Ø, Martins CIM. 2015. Coping styles in farmed fish: consequences for aquaculture. Rev Aquacult doi:10.1111/raq.12100

Churchfield S. 1985. The feeding ecology of the European water shrew. Mamm Rev 15: 13-21

Clapham PJ. 2000. The Humpback Whale - Seasonal Feeding and Breeding in a Baleen Whale. In: Mann J, Connor RC, Tyack PL, Whitehead H, eds. Cetacean Societies. Chicago: University of Chicago Press. p. 173-96

Class B, Brommer JE. 2015. A strong genetic correlation underlying a behavioural syndrome disappears during development because of genotype – age interactions. Proc R Soc B 282: 20142777

Colgan P. 1993. The motivational basis of fish behaviour. In: Pitcher TJ, ed. Behaviour of Teleost Fishes. Second edition. London: Chapman & Hall. P. 31-55

Congdon JD, Dunham AE, Tinkle DW. 1982. Energy budgets and life histories of reptiles. In: Gans C, Pough FH, eds. Biology of the Reptilia, vol 13, Physiology. London: Academic Press. p. 233-71

Conover DO, Present TM. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologia 83: 316-324

Conrad JL, Sih A. 2009. Behavioural type in newly emerged steelhead *Oncorhynchus mykiss* does not predict growth rate in a conventional hatchery rearing environment. J Fish Biol 75: 1410–26

Conrad JL, Weinersmith KL, Brodin T, Saltz JB, Sih A. 2011. Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. J Fish Biol 78: 395-435

Crisp DT. 1993. The environmental requirements of salmon and trout in fresh water. Freshw Forum 3: 176-202

Crossin GT, Sundström LF, Vandersteen WE, Devlin RH. 2015. Early life-history consequences of growth-hormone transgenesis in rainbow trout reared in stream ecosystem mesocosms. PLoS ONE 10: e0120173

Cuthbert JH. 1979. Food studies of feral mink *Mustela vison* in Scotland. Fish Manage 10: 17-26

Cutts CJ, Metcalfe NB, Taylor AC. 1998. Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. J Fish Biol 52: 1026–37

Cutts CJ, Brembs B, Metcalfe NB, Taylor AC. 1999. Prior residence, territory quality and life-history strategies in juvenile Atlantic salmon (*Salmo salar* L.). J Fish Biol 55: 784–94

Damsgård B, Dill LM. 1998. Risk-taking behavior in weight-compensating coho salmon, *Oncorhynchus kisutch*. Behav Ecol 9: 26–32

Davey A. 2011. Grey Wagtail with catch. Adrian Davey Wildlife Photography, May 20, 2011. http://adriandaveywildlifephotography.blogspot.se/2011/05/grey-wagtail-with-catch.html

Dawson A, Hinsley SA, Ferns PN, Bonser RC, Eccleston L. 2000. Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. Proc R Soc Lond B 267: 2093-8

Degerman E, Sers B. 2001. Elfiske. Fiskeriverket Informerar 1999:3 (Revised edition, 2010-08-24)

Degerman E, Östergren J, Moberg B, Svensson L, Olsson B, Ljunggren N, Söderman M, Engstedt O, Douglas G, Norlin J, Vallin L, Karlsson A. 2012. Havsöring i Sverige 2012 - status, fiske och förvaltning. Rapport 2012:2. Bromma: Sveriges sportfiske- och fiskevårdsförbund

Degerman E, Nyberg P, Sers B. 2001. Havsöringens ekologi. Fiskeriverket Informerar 2001:10

Degerman E, Nilsson PA, Nyström P, Nilsson E, Olsson K. 2007. Are fish populations in temperate str-

eams affected by crayfish? – A field survey and prospects. Environ Biol Fish 78: 231-9
Dellefors C, Faremo U. 1988. Early sexual maturation in males of wild sea trout, *Salmo trutta* L., inhibits smoltification. J Fish Biol 33: 741–9
Desjardins JK, Fernald RD. 2010. What do fish make of mirror images? Biol Lett 6: 744–7
Dieter LD, Atkinson AM, Yparraguirre DR. 2014. Mallard feeding on kokanee salmon eggs, Taylor Creek, California. Calif Fish Game 100: 573-5
Dill LM. 1983. Adaptive flexibility in the foraging behavior of fishes. Can J Fish Aquat Sci 40: 398-408
Dill LM, Ydenberg RC, Fraser AHG. 1981. Food abundance and territory size in juvenile coho salmon (*Oncorhynchus kisutch*). Can J Zool 59: 1801–9
Dingemanse NJ, Wright J, Kazem AJN, Thomas DK, Hickling R, Dawnay N. 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. J Anim Ecol 76: 1128–38
Dmitriew CM. 2011. The evolution of growth trajectories: what limits growth rate? Biol Rev 86: 97-116
Duckworth RA. 2006. Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. Behav Ecol 17: 1011–9
Edelsparre AH, McLaughlin RL, Rodríguez MA. 2013. Risk taking not foraging behavior predicts dispersal of recently emerged stream brook charr (*Salvelinus fontinalis*). Ecosphere 4: 73
Einum S, Fleming IA. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. Proc R Soc Lond B 266: 2095-100
Einum S, Fleming IA. 2000a. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution 54: 628-39
Einum S, Fleming IA. 2000b. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405: 565–7
Elliott JM. 1994. Quantitative Ecology and the Brown Trout. Oxford: Oxford University Press
Elliott JM. 2009. Validation and implications of a growth model for brown trout, *Salmo trutta*, using long-term data from a small stream in north-west England. Freshw Biol 54: 2263–75
Elliott JM, Hurley MA. 1998. A new functional model for estimating the maximum amount of invertebrate food consumed per day by brown trout, *Salmo trutta*. Freshw Biol 39: 339–49
Elliott JM, Hurley MA, Fryer RJ. 1995. A new, improved growth-model for brown trout, *Salmo trutta*. Funct Ecol 9: 290-8
Erlinge S. 1969. Food habits of the otter *Lutra lutra* L. and the mink *Mustela vison* Schreber in a trout water in southern Sweden. Oikos 1969: 1-7
Eschmeyer, WN (ed). 2015. Catalog of Fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Electronic version accessed 2015-07-02
Espmark ÅM, Eriksen MS, Salte R, Braastad BO, Bakken M. 2008. A note on pre-spawning maternal cortisol exposure in farmed Atlantic salmon and its impact on the behaviour of offspring in response to a novel environment. Appl Anim Behav Sci 110: 404–9
FAO (Food and Agriculture Organization of the United Nations). Aquaculture. http://www.fao.org/fishery/aquaculture/en. Electronic version accessed 2015-07-03
Farwell M, Fuzzen MLM, Bernier NJ, McLaughlin RL. 2014. Individual differences in foraging behavior and cortisol levels in recently emerged brook charr (*Salvelinus fontinalis*). Behav Ecol Sociobiol 68: 781–90
Farwell M, McLaughlin RL. 2009. Alternative foraging tactics and risk taking in brook charr (*Salvelinus fontinalis*). Behav Ecol 20: 913–21
Finkel T, Holbrook NJ. 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408: 239-47
Finstad AG, Forseth T, Ugedal O, Næsje TF. 2007. Metabolic rate, behaviour and winter performance in juvenile Atlantic salmon. Funct Ecol 21: 905–12
Fleming C. 2012. Dipper catches a salmon par. Wildlife in a suburban garden, March 10, 2012. http://parrotletsuk.typepad.com/wldlife_in_a_suburban_gar/2012/03/dipper-catches-a-salmon-par.html
Forseth T, Næsje TF, Jonsson B, Hårsaker K. 1999. Juvenile migration in brown trout: a consequence of energetic state. J Anim Ecol 68: 783–93
Froese, R. and D. Pauly (eds). 2015. FishBase. http://www.fishbase.org. Electronic version accessed 2015-07-03
Frost WE. 1946. Observations on the food of eels (*Anguilla anguilla*) from the Windermere catchment area. J Anim Ecol 15: 43-53
Frost WE, Brown ME. 1967. The Trout. London: Collins
Futuyma DJ. 2009. Evolution. Second edition. Sunderland: Sinauer Associates, Inc.
Gallup GG. 1968. Mirror-image stimulation. Psychol Bull 70: 782-93
Geiger C. 1984. Graureiher *Ardea cinerea* und Fischbestand in Fließgewässern. Ornithol Beobachter 81: 111-31
Gerell R. 1967. Food selection in relation to habitat in mink (*Mustela vison* Schreber) in Sweden. Oikos 18:233-46
Giger T, Excoffier L, Day PJR, Champigneulle A, Hansen MM, Powell R, Largiadèr CR. 2006. Life history shapes gene expression in salmonids. Curr Biol 16: R281–2
Godin J-GJ. Evading predators. In: Godin J-GJ, ed. Behavioural Ecology of Teleost Fishes. Oxford: Oxford University Press. p. 191-236
Gomes NM, Shay JW, Wright WE. 2010. Telomere biology in Metazoa. FEBS Lett 584: 3741-51
Gotthard K. 2000. Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. J Anim Ecol 69: 896-902
Gotthard K. 2001 Growth strategies of ectothermic animals in temperate environments. In: Atkinson D, Thorndyke M, eds. Environment and Animal Development. Oxford: BIOS Scientific Publishers. p. 287-304
Gray P, Charleston M. 2011. Swimming Against the Tide - Restoring Salmon to the Tyne. Ellesmere: Medlar Press
Grant JWA, Noakes DLG. 1987. Movers and stayers: foraging tactics of young-of-the-year brook charr, *Salvelinus fontinalis*. J Anim Ecol 56: 1001–13
Grant PR, Grant BR. 2011. Causes of lifetime fitness of Darwin's finches in a fluctuating environment. Proc Natl Acad Sci USA 108: 674-9
Greenwood A, Churchfield S, Hickey C. 2002. Geographical distribution and habitat occurrence of the Water Shrew (*Neomys fodiens*) in the Weald of South-East England. Mamm Rev 32: 40-50
Gregory PT, Isaac LA. 2004. Food habits of the grass snake in southeastern England: is *Natrix natrix* a generalist predator? J Herpetol 38: 88-95
Hallet C. 1982. Étude du comportement de prédation du Martin-Pêcheur *Alcedo atthis* (L.): taille préférentialle de capture du chabot *Cottus gobio* L. et de la truite *Salmo trutta* L. Rev Ecol (Terre Vie) 36: 211-22
Harman D. 1956. Aging: a theory based on free radical and radiation chemistry. J Gerontol 11: 298-300
He L, Pei Y, Jiang Y, Li Y, Liao L, Zhu Z, Wang Y. 2015. Global gene expression patterns of grass carp following compensatory growth. BMC Genomics 16: 184
Hector KL, Nakagawa S. 2012. Quantitative analysis of compensatory and catch-up growth in diverse taxa. J Anim Ecol 81: 583-93
Heggenes J, Krog OMW, Lindas OR, Dokk JG, Bremnes T. 1993. Homeostatic behavioral responses in a

changing environment - brown trout (*Salmo trutta*) become nocturnal during winter. J Anim Ecol 62: 295–308
Heiderose, Fischer-Nagel A. 1991. Öringen [Aus dem Leben der Forellen]. Stockholm: Bokförlag Carlsen [Swedish translation: Ulf Svedberg]
Héland M. 1999. Social organization and territoriality in brown trout juveniles during ontogeny. In: Baglinière J-L, Maisse G, eds. Biology and Ecology of Brown Trout and Sea Trout. Chichester: Praxis Publishing Ltd. p. 115–43
Hirshfield MF, Tinkle DW. 1975. Natural selection and the evolution of reproductive effort. Proc Natl Acad Sci USA 72: 2227-31
Hoogenboom MO, Armstrong JD, Groothuis TGG, Metcalfe NB. 2012. The growth benefits of aggressive behavior vary with individual metabolism and resource predictability. Behav Ecol 24: 253-61
Houston AI, McNamara JM. 1999. Models of adaptive behaviour: an approach based on state. Cambridge: Cambridge University Press
Huntingford FA. 1976. The relationship between antipredator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. Anim Behav 24: 245–60
Huntingford FA, Garcia de Leaniz C. 1997. Social dominance, prior residence and the acquisition of profitable feeding sites in juvenile Atlantic salmon. J Fish Biol 51: 1009-14
Huntingford F, Adams C. 2005. Behavioural syndromes in farmed fish: implications for production and welfare. Behaviour 142: 1207–21
Hurst TP. 2007. Causes and consequences of winter mortality in fishes. J Fish Biol 71: 315–45
Höjesjö J, Johnsson JI, Axelsson M. 1999. Behavioural and heart rate responses to food limitation and predation risk: an experimental study on rainbow trout. J Fish Biol 55: 1009–19
Höjesjö J, Johnsson JI, Bohlin T. 2004. Habitat complexity reduces the growth of aggressive and dominant brown trout (*Salmo trutta*) relative to subordinates. Behav Ecol Sociobiol 56: 286-9
Höjesjö J, Stradmeyer L, Griffith S, Armstrong J. 2010. Effects of competing brown trout on habitat use by Atlantic salmon parr: controlled investigations in a laboratory stream. In: Kemp P, editor. Salmonid Fisheries: Freshwater Habitat Management. Chichester: Blackwell Publishing Ltd. p. 194-208
Höjesjö J, Adriaenssens B, Bohlin T, Jönsson C, Hellström I, Johnsson JI. 2011. Behavioural syndromes in juvenile brown trout (*Salmo trutta*); life history, family variation and performance in the wild. Behav Ecol Sociobiol 65: 1801–10
Ivanova MN, Svirskaya AN. 2013. Size hierarchy in young pike *Esox lucius*. J Ichthyol 53: 333-46
Jenkins TM. 1969. Social structure, position choice and micro-distribution of two trout species (*Salmo trutta* and *Salmo gairdneri*) resident in mountain streams. Anim Behav Monogr 2: 55–123
Jensen AJ, Johnsen BO, Saksgård L. 1989. Temperature requirements in Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and Arctic char (*Salvelinus alpinus*) from hatching to initial feeding compared with geographic distribution. Can J Fish Aquat Sci 46: 786–9
Jensen LF, Hansen MM, Pertoldi C, Holdensgaard G, Mensberg K-LD, Loeschcke V. 2008. Local adaptation in brown trout early life-history traits: implications for climate change adaptability. Proc R Soc B 275: 2859–68
Jennings B J, Ozanne SE, Dorling MW, Hales CN. 1999. Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. FEBS Lett 448: 4-8
Jobling M. 1985. Growth. In: Tytler P, Calow P, eds. Fish energetics: new perspectives. London: Croom Helm. p. 213-30
Jobling M. 1985. Physiological and social constraints of fish with special reference to Arctic charr, *Salvelinus alpinus* L. Aquaculture 44: 83-90
Jobling M. 2010. Are compensatory growth and catch-up growth two sides of the same coin? Aquacult Int 18: 501-10
Jobling M, Johansen SJS. 1999. The lipostat, hyperphagia and catch-up growth. Aquacult Res 30: 473–8
Johansson M. 1971. Lokala och temporära variationer i ett öringbestånd i Jörlandaån. Trebetygsarbete, Department of Zoology, Göteborg university
Johnson JC, Sih A. 2005. Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. Behav Ecol Sociobiol 58: 390–6
Johnsson JI. 1993. Big and brave: size selection affects foraging under risk of predation in juvenile rainbow trout, *Oncorhynchus mykiss*. Anim Behav 45: 1219-25
Johnsson JI, Bohlin T. 2005. Compensatory growth for free? A field experiment on brown trout, *Salmo trutta*. Oikos 111: 31–8
Johnsson JI, Bohlin T. 2006. The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proc R Soc B 273: 1281–6
Johnsson JI, Jönsson E, Björnsson BT. 1996a. Dominance, nutritional state, and growth hormone levels in rainbow trout (*Oncorhynchus mykiss*). Horm Behav 30: 13–21
Johnsson JI, Petersson E, Jönsson E, Björnsson BT, Järvi T. 1996b. Domestication and growth hormone alter antipredator behaviour and growth patterns in juvenile brown trout, *Salmo trutta*. Can J Fish Aquat Sci 53: 1546–54
Johnsson JI, Nöbbelin F, Bohlin T. 1999a. Territorial competition among wild brown trout fry: effects of ownership and body size. J Fish Biol 54: 469–72
Johnsson JI, Petersson E, Jönsson E, Järvi T, Björnsson BT. 1999b. Growth hormone-induced effects on mortality, energy status and growth: a field study on brown trout (*Salmo trutta*). Funct Ecol 13: 514–22
Jones MH, Seeb JE, Warheit KI, Seamons TR, Quinn TP, Seeb LW. 2015. Consequences of emergence timing for the growth and relative survival of steelhead fry from naturally spawning wild and hatchery parents. Trans Am Fish Soc 144: 977-89
Jonsson B, Jonsson N. 2011. Ecology of Atlantic salmon and brown trout. Habitat as a template for life histories. Dordrecht: Springer Science+Business Media, Inc.
Jönsson E, Johnsson JI, Björnsson BT. 1998. Growth hormone increases aggressive behavior in juvenile rainbow trout. Horm Behav 33: 9–15
Kalleberg H. 1958. Observations in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. and *S. trutta* L.). Rep Inst Freshw Res Drottingholm 39: 55–98
Kamler E. 1992. Early life history of fish: an energetics approach. London: Chapman & Hall
Kamler E. 2008. Resource allocation in yolk-feeding fish. Rev Fish Biol Fish 18: 143–200
Kaspersson R. 2010. Age-Class Interactions in Atlantic Salmon and Brown Trout: Effects on habitat use and performance. PhD thesis, Department of Zoology, University of Gothenburg
Kelly CD. 2008. The interrelationships between resource-holding potential, resource-value and reproductive success in territorial males: how much variation can we explain? Behav Ecol Sociobiol 62: 855–71
Klemetsen A. 2013. The most variable vertebrate on Earth. J Ichthyol 53: 781-91
Kohane MJ, Parsons PA. 1988. Domestication. Evolutionary change under stress. Evol Biol. 23: 31-48
Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis

MAW, Blokhuis HJ. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev 23: 925–35

Korte SM, Koolhaas JM, Wingfield JC, McEwen BS. 2005. The Darwinian concept of stress: Benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. Neurosci Biobehav Rev 29: 3–38

Kortet R, Hedrick A. 2007. A behavioural syndrome in the field cricket *Gryllus integer*: intrasexual aggression is correlated with activity in a novel environment. Biol J Linn Soc 91: 475–82

Kortet R, Vainikka A, Janhunen M, Piironen J, Hyvärinen P. 2014. Behavioral variation shows heritability in juvenile brown trout *Salmo trutta*. Behav Ecol Sociobiol 68: 927-34

Kotrschal A, Rogell B, Bundsen A, Svensson B, Zajitschek S, Brännström I, Immler S, Maklakov AA, Kolm N. 2013. Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. Curr Biol 23: 168–71

Krause J, Loader SP, McDermott J, Ruxton GD. 1998. Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. Proc R Soc Lond B 265:2373–9

Krause J, Loader SP, Kirkman E, Ruxton GD. 1999. Refuge use by fish as a function of body weight changes. Acta Ethol 2:29–34

Kvingedal E, Einum S. 2011. Prior residency advantage for Atlantic salmon in the wild: effects of habitat quality. Behav Ecol Sociobiol 65: 1295–303

Kyne MJ, Smal CM, Fairley JS. 1989. Food of otters *Lutra lutra* in the Irish Midlands and a comparison with that of mink *Mustela vison* in the same region. Proc R Ir Acad B 89: 33-46

Lack D. 1947. The significance of clutch-size. Ibis 89: 302-52

Lahti K, Huuskonen H, Laurila A, Piironen J. 2002. Metabolic rate and aggressiveness between brown trout populations. Funct Ecol 16: 167–74

Lahti K, Laurila A, Enberg K, Piironen J. 2001. Variation in aggressive behaviour and growth rate between populations and migratory forms in the brown trout, *Salmo trutta*. Anim Behav 62: 935–44

Laine VN, Herczeg G, Shikano T, Vilkki J, Merilä J. 2014. QTL analysis of behavior in nine-spined sticklebacks (*Pungitius pungitius*). Behav Genet 44: 77–88

Lankford TE, Billerbeck JM, Conover DO. 2001. Evolution of intrinsic growth and energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia menidia*. Evolution 55: 1873-81

Law R. 1979. Optimal life histories under age-specific predation. Am Nat 114: 399-417

Le Cren ED. 1961. How many fish survive? Yb River Boards Ass 9: 57-64

Lee W-S, Metcalfe NB, Monaghan P, Mangel M. 2011. A comparison of dynamic-state-dependent models of the trade-off between growth, damage, and reproduction. Am Nat 178: 774-86

Lee W-S, Monaghan P, Metcalfe NB. 2013. Experimental demonstration of the growth rate–lifespan trade-off. Proc R Soc B 280: 20122370

Lelong A, Bolliet V, Bancel D, Gaudin P. 2008. Detection of elemental composition and caloric value variability at individual level in newly emerged brown trout *Salmo trutta* L. J Fish Biol 72: 2695–9

Lima SL. 1998. Nonlethal effects in the ecology of predator-prey interactions. BioScience 48: 25-34

Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68: 619-40

Lindström K. 1992. The effect of resource holding potential, nest size and information about resource quality on the outcome of intruder-owner conflicts in the sand goby. Behav Ecol Sociobiol 30: 53-8

Lochmiller RL, Deerenberg C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88: 87-98

Lucas MC. 1994. Heart rate as an indicator of metabolic rate and activity in adult Atlantic salmon, *Salmo salar*. J Fish Biol 44: 889–903

Luttbeg B, Sih A. 2010. Risk, resources and state-dependent adaptive behavioural syndromes. Phil Trans R Soc B 365: 3977-90

MacArthur RH, Wilson EO. 1967. The Theory of Island Biogeography. Princeton: Princeton University Press

MacCrimmon HR. 1972. World distribution of rainbow trout (*Salmo gairdneri*): further observations. J Fish Res Board Can 29: 1788-91

MacCrimmon HR, Marshall TL, Gots BL. 1970. World distribution of brown trout, *Salmo trutta*: further observations. J Fish Res Board Can 27: 811-8

MacCrimmon HR, Gots BL, Campbell JS. 1971. World distribution of brook trout, *Salvelinus fontinalis*: further observations. J Fish Res Board Can 28: 452-6

MacKay JRD, Haskell MJ. 2015. Consistent individual behavioral variation: the difference between temperament, personality and behavioral syndromes. Animals 5: 455-78

Magurran AE. 1993. Individual differences and alternative behaviours. In: Pitcher TJ (ed). Behaviour of Teleost Fishes, Second edition. London: Chapman & Hall. p. 441-78

Mangel M. 2008. Environment, damage and senescence: modelling the life-history consequences of variable stress and caloric intake. Funct Ecol 22: 422-30

Mangel M, Munch SB. 2005. A life-history perspective on short- and long-term consequences of compensatory growth. Am Nat 166: E155-76

Mangel M, Stamps J. 2001. Trade-offs between growth and mortality and the maintenance of individual variation in growth. Evol Ecol Res 3: 611-32

Marcil-Ferland D, Festa-Bianchet M, Martin AM, Pelletier F. 2013. Despite catch-up, prolonged growth has detrimental fitness consequences in a long-lived vertebrate. Am Nat 182: 775-85

Marquiss M, Leitch AF. 1990. The diet of Grey Herons *Ardea cinerea* breeding at Loch Leven, Scotland, and the importance of their predation on ducklings. Ibis 132: 535-49

Maynard Smith J. 1982. Evolution and the Theory of Games. Cambridge: Cambridge University Press

Maynard Smith J, Parker GA. 1976. The logic of asymmetric contests. Anim Behav 24: 159-75

Mayr E. 1961. Cause and effect in biology. Science 134: 1501-6

McCarthy ID. 2009. Temporal repeatability of relative standard metabolic rate in juvenile Atlantic salmon and its relation to life history variation. J Fish Biol 57: 224–38

McFarland WL. 1925. Salmon of the Atlantic. New York: Parke, Austin and Lipscomb

McLaughlin RL. 2001. Behavioural diversification in brook charr: adaptive responses to local conditions. J Anim Ecol 70: 325-37

McLaughlin RL, Grant JWA, Kramer DL. 1992. Individual variation and alternative patterns of foraging movements in recently-emerged brook charr (*Salvelinus fontinalis*). Behaviour 120: 286–301

McLaughlin RL, Grant JWA, Kramer DL. 1994. Foraging movements in relation to morphology, water-column use, and diet for recently emerged brook trout (*Salvelinus fontinalis*) in still-water pools. Can J Fish Aquat Sci 51: 268-79

McLaughlin RL, Ferguson MM, Noakes DLG. 1999. Adaptive peaks and alternative foraging tactics in brook charr: evidence of short-term divergent selection for sitting-and-waiting and actively searching. Behav Ecol Sociobiol 45: 386–95

McNamara JM, Houston AI. 1996. State-dependent life histories. Nature 380: 215-21

McNaughton SJ. 1983. Compensatory plant growth as a response to herbivory. Oikos 40: 329-36

Metcalfe NB. 1998. The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 55: 93–103

Metcalfe NB, Monaghan P. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16: 254-60

Metcalfe NB, Monaghan P. 2003. Growth versus lifespan: perspectives from evolutionary ecology. Exp Gerontol 38: 935-40

Metcalfe NB, Wright PJ, Thorpe JE. 1992. Relationships between social status, otolith size at first feeding and subsequent growth in Atlantic salmon (*Salmo salar*). J Anim Ecol 61: 585–9

Metcalfe NB, Taylor AC, Thorpe JE. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Anim Behav 49: 431–6

Millidine KJ, Armstrong JD, Metcalfe NB. 2009. Juvenile salmon with high standard metabolic rates have higher energy costs but can process meals faster. Proc R Soc B 276: 2103–8

Mittelbach GG, Ballew NG, Kjelvik MK. 2014. Fish behavioral types and their ecological consequences. Can J Fish Aquat Sci 71: 927–44

Mobley KB, Ruiz RC, Johansson F, Englund G. Bokma F. 2013. No evidence that stickleback spines directly increase risk of predation by an invertebrate predator. Evol Ecol Res 15: 189-98

Montiglio P-O, Garant D, Bergeron P, Dubuc Messier G, Réale D. 2014. Pulsed resources and the coupling between life-history strategies and exploration patterns in eastern chipmunks (*Tamias striatus*). J Anim Ecol 76: 720–8

Morgan IJ, Metcalfe NB. 2001. Deferred costs of compensatory growth after autumnal food shortage in juvenile salmon. Proc R Soc London B 268: 295–301

Morris MRJ, Fraser DJ, Eddington J, Hutchings JA. 2011. Hybridization effects on phenotypic plasticity: experimental compensatory growth in farmed-wild Atlantic salmon. Evol Appl 4: 444–58

Munch SB, Conover DO. 2003. Rapid growth results in increased susceptibility to predation in *Menidia menidia*. Evolution 57: 2119-27

Munshi-South J, Wilkinson GS. 2010. Bats and birds: exceptional longevity despite high metabolic rates. Ageing Res Rev 9: 12-9

NASCO (The North Atlantic Salmon Conservation Organization). 2008. Working Group Report on Social & Economic Values of Salmon. Online publication: NASCO. Available at: http://www.nasco.int/pdf/socioeconomics/se_rep2008.pdf

Nelson JA. 2011. Energetics — Energetics: An Introduction. In: Farell AP, ed. Encyclopedia of Fish Physiology. San Diego: Academic Press. p. 1563-5

Neregård L, Sundt-Hansen L, Björnsson BTh, Johnsson JI. 2008. Growth hormone affects behaviour of wild brown trout *Salmo trutta* in territorial owner-intruder conflicts. J Fish Biol 73: 2341-51

Newton C. 2013. The Trout's Tale: The Fish That Conquered an Empire. Ellesmere: Medlar Press

Nicieza AG, Metcalfe NB. 1997. Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. Ecology 78: 2385–400

Nicieza AG, Álvarez D. 2009. Statistical analysis of structural compensatory growth: how can we reduce the rate of false detection? Oecologia 159: 27-39

Norton WHJ, Stumpenhorst K, Faus-Kessler T, Folchert A, Rohner N, Harris MP, Callebert J, Bally-Cuif L. 2011. Modulation of fgfr1a signaling in zebrafish reveals a genetic basis for the aggression–boldness syndrome. J Neurosci 31: 13796-807

Näslund I, Degerman E, Nordwall F. 1998. Brown trout (*Salmo trutta*) habitat use and life history in Swedish streams: possible effects of biotic interactions. Can J Fish Aquat Sci 55: 1034-42

Näslund J, Aldvén D, Závorka L. 2015a. Eggs from anadromous adults provide marine-derived nutrients to Atlantic salmon and brown trout parr in late autumn - observations from a Swedish coastal stream. Environ Biol Fish doi:10.1007/s10641-015-0436-y

Näslund J, Bererhi B, Johnsson JI. 2015b. Design of emergence test arenas can affect the results of boldness assays. Ethology 121: 556-65

Obuch J. 2004. Typy potravy sovy obyčajnej (*Strix aluco*) v Národnom parku Muránska planina [Types of diet of Tawny Owl in the Muránska Planina National Park]. Reussia 1: 291-301

Obuch J, Karaska D. 2010. The Eurasian eagle-owl (*Bubo bubo*) diet in the Orava Region (N Slovakia). Slovak Raptor J 4: 83-9

O'Connor KI, Taylor AC, Metcalfe NB. 2000. The stability of standard metabolic rate during a period of food deprivation in juvenile Atlantic salmon. J Fish Biol 57: 41–51

Ojanguren AF, Reyes-Gavilán FG, Braña F. 1996. Effects of egg size on offspring development and fitness in brown trout, *Salmo trutta* L. Aquaculture 147: 9–20

Oliveira RF, Gonçalves DM. 2008. Hormones and social behaviour of teleost fish. In: Magnhagen C, Braithwaite VA, Forsgren E, Kapoor BG, eds. Fish Behaviour. Enfield: Science Publishers. p. 61-150

Olovnikov AM. 1973. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon? J Theor Biol 41: 181–90

Ostrovsky I. 1995. The parabolic pattern of animal growth – determination of equation parameters and their temperature dependencies. Freshw Biol 33: 357-71

Parrish DL, Behnke RJ, Gephard SR, McCormick SD, Reeves GH. 1998. Why aren't there more Atlantic salmon (*Salmo salar*)? Can J Fish Aquat Sci 55: 281-7

Pauliny A, Devlin RH, Johnsson JI, Blomqvist D. 2015. Rapid growth accelerates telomere attrition in a transgenic fish. BMC Evol Biol 15: 159

Pavlov DS, Savvaitova KA. 2008. On the problem of ratio of anadromy and residence in salmonids (Salmonidae). J Ichthyol 48: 778-91

Pavlov DS, Kostin VV, Ponomareva VYu. 2010. Behavioral differentiation of underyearlings of the Black Sea salmon *Salmo trutta labrax*: rheoreaction in the year preceding smoltification. J Ichthyol 50: 270-80

Pearl R. 1928. The Rate of Living. London: University of London Press Ltd

Peck MA, Herrmann J-P, Ewest B, Stäcker S, Temming A. 2014. Relationships between feeding, growth and swimming activity of European sprat (*Sprattus sprattus* L.) post-larvae in the laboratory. Environ Biol Fishes 98: 1117-27

Pedersen BH. 1997. The cost of growth in young fish larvae, a review of new hypotheses. Aquaculture 155: 259–69

Pettersson JCE. 2002. Migration in brown trout *Salmo trutta* - causes and consequences. PhD thesis, Department of Zoology, Göteborg University

Pianka ER. 1970. On r- and K-selection. Am Nat 104: 592-7

Pianka ER. 1972. r and K selection or b and d selection? Am Nat 106: 581-8

Pianka ER. 1974. Evolutionary Ecology. New York: Harper & Row

Pottinger TG, Carrick TR. 1999. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. Gen Comp Endocrinol 116: 122–32

Pottinger TG, Carrick TR. 2001. Stress responsiveness affects dominant-subordinate relationships in rainbow trout. Horm Behav 40: 419–27

Promislow DEL, Harvey PH. Living fast and dying young: a comparative analysis of life-history variation among mammals. J Zool (Lond) 220: 417-37

Prosek J. 2003. Trout of the World. New York:Abrams

Puckett KJ, Dill LM. 1985. The energetics of feeding territoriality in juvenile coho salmon (*Oncorhynchus kisutch*). Behaviour 92: 97–111

Pustovrh G, Snoj A, Bajec SS. 2014. Molecular phylogeny of *Salmo* of the western Balkans, based upon multiple nuclear loci. Genet Sel Evol 46: 7

Railsback SF, Harvey BC, Hayse JW, Lagory KE. 2005. Tests of theory for diel variation in salmonid feeding activity and habitat use. Ecology 86: 947–59

Raven P. 1986. The size of minnow prey in the diet of young Kingfishers *Alcedo atthis*. Bird Study 33: 6-11

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82: 291-318

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P-O. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Philos Trans R Soc B 365: 4051–63

Reed HS. 1921. The rate of growth following an initial period of suppression. Am Nat 55: 539–55

Régnier T, Bolliet V, Labonne J, Gaudin P. 2010. Assessing maternal effects on metabolic rate dynamics along early development in brown trout (*Salmo trutta*): an individual-based approach. J Comp Physiol B 180: 25–31

Régnier T, Bolliet V, Gaudin P, Labonne J. 2012a. Female effects on offspring energetic status and consequences on early development in yolk feeding brown trout (*Salmo trutta*). J Exp Zool A 317: 347–58

Régnier T, Labonne J, Gaudin P, Bolliet V. 2012b. Influence of energetic status on ontogenetic niche shifts: emergence from the redd is linked to metabolic rate in brown trout. Oecologia 168: 371–80

Reid D, Armstrong JD, Metcalfe NB. 2012. The performance advantage of a high resting metabolic rate in juvenile salmon is habitat dependent. J Anim Ecol 81: 868–75

Reist JD. 1980. Predation upon pelvic phenotypes of brook stickleback, *Culaea inconstans*, by selected invertebrates. Can J Zool 58: 1253-8

Reznick D, Bryant MJ, Bashey F. 2002. *r*- and *K*-selection revisited: the role of population regulation in life-history evolution. Ecology 83: 1509-20

Richardson JML. 2001. A comparative study of activity levels in larval anurans and response to the presence of different predators. Behav Ecol 12: 51-8

Ricklefs RE. 2000. Density dependence, evolutionary optimization, and the diversification of avian life histories. Condor 102: 9-22

Ricklefs RE, Wikelski M. 2002. The physiology/life-history nexus. Trends Ecol Evol 17: 462-8

Rikardsen AH, Thorpe JE, Dempson JP. 2004. Modeling the life history variation of Arctic charr. Ecol Freshw Fish 13: 305–11

Robinson BW, Wardrop SL. 2002. Experimentally manipulated growth rate in threespine sticklebacks: assessing trade offs with developmental stability. Environ Biol Fish 63: 67-78

Robinson WD, Hau M, Klasing KC, Wikelski M, Brawn JD, Austin SH, Tarwater C, Ricklefs RE. 2010. Diversification of life histories in New World birds. Auk 127: 253-62

Roff DA. 1986. Predicting body size with life history models. BioScience 36: 316-23

Roff DA. 1992. Evolution Of Life Histories: Theory and Analysis. New York: Chapman & Hall

Rollinson N, Hutchings JA. 2010. Why does egg size increase with maternal size? Effects of egg size and egg density on offspring phenotypes in Atlantic salmon (*Salmo salar*). Evol Ecol Res 12: 949–60

Rollinson N, Hutchings JA. 2011. Body size-specific maternal effects on the offspring environment shape juvenile phenotypes in Atlantic salmon. Oecologia 166: 889–98

Rollinson N, Hutchings JA. 2013. The relationship between offspring size and fitness: integrating theory and empiricism. Ecology 94: 315–24

Rombough P. 2011. The energetics of embryonic growth. Respir Physiol Neurobiol 178: 22–9

Rubner M. 1908. Das Problem der Lebensdauer und seine Beziehung zu Wachstum und Ernährung. Munich, Berlin: Verlag R. Oldenburg

Ruckelshaus MH, Levin P, Johnson JB, Kareiva PM. 2002. The Pacific Salmon Wars: what science brings to the challenge of recovering species. Annu Rev Ecol Syst 33: 665-706

Sacks A. 2004. Salmon Doubts. Cupertino: Alternative Comics

Saikkonen A, Kekäläinen J, Piironen J. 2011. Rapid growth of Atlantic salmon juveniles in captivity may indicate poor performance in nature. Biol Conserv 144: 2320–7

Schjolden J, Backström T, Pulman KGT, Pottinger TG, Winberg S. 2005. Divergence in behavioural responses to stress in two strains of rainbow trout (*Oncorhynchus mykiss*) with contrasting stress responsiveness. Horm Behav 48: 537–44

Schjolden J, Winberg S. 2007. Genetically determined variation in stress responsiveness in rainbow trout: behavior and neurobiology. Brain Behav Evol 70: 227–38

Schluter D, Rambaut A. 1996 Ecological speciation in postglacial fishes [and discussion]. Phil Trans R Soc B 351: 807-14

Seger J, Brockmann HJ. 1987. What is bet-hedging? Oxford Surv Evol Biol 4: 182-211

Senner NR, Conklin JR, Piersma T. 2015. An ontogenetic perspective on individual differences. Proc R Soc B 282: 20151050

Sih A, Kats LB, Maurer EF 2003. Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. Anim Behav 65: 29-44

Sih A, Bell A, Johnson JC. 2004a. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol 19: 372–8

Sih A, Bell A, Johnson JC, Ziemba RE. 2004b. Behavioral syndromes: an integrative overview. Q Rev Biol 79: 211-44

Sih A, Bell A, Johnson JC. 2010. Behavioral syndromes. In: Westneat DF, Fox CW, eds. Evolutionary Behavioral Ecology. New York: Oxford University Press, Inc. p. 516-30

Sih A, Cote J, Evans M, Fogarty S, Pruitt J. 2012. Ecological implications of behavioural syndromes. Ecol Lett 15: 278-89

Sih A, Mathot KJ, Moirón M, Montiglio P-O, Wolf M, Dingemanse NJ. 2015. Animal personality and state–behaviour feedbacks: a review and guide for empiricists. Trends Ecol Evol 30: 50–60

Sigourney DB, Letcher BH, Obedzinski M, RA Cunjak. 2008. Size-independent growth in fishes: patterns, models and metrics. J Fish Biol 72: 2435–55

Sigourney DB, Letcher BH, Obedzinski M, RA Cunjak. 2013. Interactive effects of life history and season on size-dependent growth in juvenile Atlantic salmon. Ecol Freshw Fish 22: 495-507

Sinha VRP, Jones JW. 1967. On the food of the freshwater eels and their feeding relationships with the salmonids. J Zool (Lond) 153: 119-37

Skoglund H, Barlaup BT. 2006. Feeding pattern and diet of first feeding brown trout fry under natural conditions. J Fish Biol 68: 507–21

Skoglund H, Einum S, Forseth T, Barlaup BT. 2012. The penalty for arriving late in emerging salmonid juveniles: differences between species correspond to their interspecific competitive ability. Funct Ecol 26: 104-11

Slaney PA, Northcote TG. 1974. Effects of prey abundance on density and territorial behavior of young rainbow trout (*Salmo gairdneri*) in laboratory stream channels. J Fish Res Board Can 31: 1201–9

Sloman KA. 2010. Exposure of ova to cortisol prefertilisation affects subsequent behaviour and physiology of brown trout. Horm Behav 58: 433–9

Smulders TV. 2015. Interpreting measurements of heritability: comment on Croston et al. Behav Ecol 10.1093/beheco/arv120

Sogard SM. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull Mar Sci 60: 1129–57

Speakman JR. 2005. Body size, energy metabolism and lifespan. J Exp Biol 208: 1717-30

Speakman JR, Selman C, McLaren JS, Harper EJ. 2002. Living fast, dying when? The link between aging and energetics. J Nutr 132: 1583-97

Stamps JA. 2007. Growth-mortality tradeoffs and 'personality traits' in animals. Ecol Lett 10: 355-63

Stearns SC. 1992. The Evolution of Life Histories. New York: Oxford University Press Inc.

Stoks R, De Block M, Van De Meutter F, Johansson F. 2005. Predation cost of rapid growth: behavioural coupling and physiological decoupling. J Anim Ecol 74: 708-15

Sundström LF, Devlin RH, Johnsson JI, Biagi CA. 2003. Vertical position reflects increased feeding motivation in growth hormone transgenic coho salmon (*Oncorhynchus kisutch*). Ethology 109: 701–12

Sundström LF, Lõhmus M, Devlin RH, Johnsson JI, Biagi CA, Bohlin T. 2004a. Feeding on profitable and unprofitable prey: Comparing behaviour of growth-enhanced transgenic and normal coho salmon (*Oncorhynchus kisutch*). Ethology 110: 381–96

Sundström LF, Lõhmus M, Johnsson JI, Devlin RH. 2004b. Growth hormone transgenic salmon pay for growth potential with increased predation mortality. Proc R Soc B 271: S350-2

Sundström LF, Petersson E, Höjesjö J, Johnsson JI, Järvi T. 2004c. Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. Behav Ecol 15: 192–8

Sundström LF, Kaspersson R, Näslund J, Johnsson JI. 2013. Density-dependent compensatory growth in brown trout (*Salmo trutta*) in nature. PLoS ONE 8: e63287

Sundt-Hansen L, Neregård L, Einum S, Höjesjö J, Björnsson BTh, Hindar K, Økland F, Johnsson JI. 2009. Growth enhanced brown trout show increased movement activity in the wild. Funct Ecol 23: 551-8

Sundt-Hansen L, Einum S, Neregård L, Björnsson BTh, Johnsson JI, Fleming IA, Devlin RH, Hindar K. 2012. Growth hormone reduces growth in free-living Atlantic salmon fry. Funct Ecol 26: 904–11

Svensson L, Mullarney K, Zetterström D. 2010. Fågelguiden: Europas och Medelhavsområdets fåglar i fält. Stockholm: Bonnier Fakta

Symons PEK. 1968. Increase in aggression and in strength of the social hierarchy among juvenile Atlantic salmon deprived of food. J Fish Res Board Can 25: 2387–401

Sæther B-E. 1988. Pattern of covariation between life-history traits of European birds. Nature 331: 616-7

Thorlacius M, Hellström G, Brodin T. 2015. Behavioral dependent dispersal in the invasive round goby *Neogobius melanostomus* depends on population age. Curr Zool 61: 529-542

Thorpe JE, Mangel M, Metcalfe NB, Huntingford FA. 1998. Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. Evol Ecol 12: 581–99

Tieleman IB, Williams JB, Ricklefs RE, Klasing KC. 2005. Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. Proc R Soc B 272: 1715-20

Tinoco AB, Näslund J, Delgado MJ, de Pedro N, Johnsson JI, Jönsson E. 2014. Ghrelin increases food intake, swimming activity and growth in juvenile brown trout (*Salmo trutta*). Physiol Behav 124: 15-22

Toms CN, Echevarria DJ, Jouandot DJ. 2010. A methodological review of personality-related studies in fish: focus on the shy-bold axis of behavior. Int J Comp Psychol 23: 1–25

Torday P. 2007. Salmon fishing in the Yemen. London: Weidenfield & Nicholson

Tunney TD, Steingrímsson SÓ. 2012. Foraging mode variation in three stream-dwelling salmonid fishes. Ecol Freshw Fish 21: 570-80

Vehanen T. 2003. Adaptive flexibility in the behaviour of juvenile Atlantic salmon: short-term responses to food availability and threat from predation. J Fish Biol 63: 1034–45

von Zglinicki T. 2002. Oxidative stress shortens telomeres. Trends Biochem Sci 27: 339–44

Vøllestad LA, Lillehammer T. 2000. Individual variation in early life-history traits in brown trout. Ecol Freshw Fish 9: 242–7

Wahle RA. 1992. Body-size dependent anti-predator mechanisms of the American lobster. Oikos 65: 52-60

Wang T, Hung CCY, Randall DJ. 2006. The comparative physiology of food deprivation: from feast to famine. Annu Rev Physiol 68: 223-51

Weatherley AH, Gill HS. 1981. Recovery growth following periods of restricted rations and starvation in rainbow trout, *Salmo gairdneri* Richardson. J Fish Biol 18: 195-208

Werner EE, Anholt BR. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am Nat 142: 242-72

West GB, Brown JH, Enquist BJ. 2001. A general model for ontogenetic growth. Nature 413: 628-31

Wiersma P, Muñoz-Garcia A, Walker A, Williams JB. 2007. Tropical birds have a slow pace of life. Proc Natl Acad Sci USA 104: 9340-5

Wiersma P, Nowak B, Williams JB. 2012. Small organ size contributes to the slow pace of life in tropical birds. J Exp Biol 215: 1662-9

Wikelski M, Spinney L, Schelsky W, Scheuerlein A, Gwinner E. 2003. Slow pace of life in tropical sedentary birds: a common-garden experiment on four stonechat populations from different latitudes. Proc R Soc Lon B 270: 2383-8

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am Nat 100: 687-90

Williams JPG. 1981. Catch-up growth. J Embryol Exp Morphol 65: 89-101

Willson MF. 1997. Variation in salmonid life histories: patterns and perspectives. Res. Pap. PNW-RP-498. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station.

Wilson ADM, McLaughlin RL. 2007. Behavioural syndromes in brook charr, *Salvelinus fontinalis*: prey-search in the field corresponds with space use in novel laboratory situations. Anim Behav 74: 689–98

Wilson ADM, McLaughlin RL. 2010. Foraging behaviour and brain morphology in recently emerged brook charr, *Salvelinus fontinalis*. Behav Ecol Sociobiol 64: 1905–14

Wilson PN, Osbourn DF. 1960. Compensatory growth after undernutrition in mammals and birds. Biol Rev 35: 324-61

Wolf M, McNamara JM. 2012. On the evolution of personalities via frequency-dependent selection. Am Nat 179: 679-92

Wolf M, van Doorn GS, Leimar O, Weissing FJ. 2013. The evolution of animal personalities. In: Carere C, Maestripieri D, eds. Animal Personalities: Behavior, Physiology, and Evolution. Chicago: University of Chicago Press. p. 252-275

Wootton RJ. 1984. A functional biology of sticklebacks. Berkeley and Los Angeles: University of California Press

Zera AJ, Harshman LG. 2001. The physiology of life history trade-offs in animals. Annu Rev Ecol Syst 32: 95-126

Zera AJ, Harshman LG, Williams TD. 2007. Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics. Annu Rev Ecol Evol Syst 38: 793-817

Öhlund G, Nordwall F, Degerman E, Eriksson T. 2008. Life history and large-scale habitat use of brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) - implications for species replacement patterns. Can J Fish Aquat Sci 65: 633-44

Økland F, Jonsson B, Jensen AJ, Hansen LP. 1993. Is there a threshold size regulating seaward migration of brown trout and Atlantic salmon? J Fish Biol 42: 541–50

Øverli Ø, Sørensen C, Pulman KGT, Pottinger TG, Korzan W, Summers CH, Nilsson GE. 2007. Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. Neurosci Biobehav Rev 31: 396-412

Acknowledgements

I would like to thank...

My supervisor Jörgen, for all the support, guidance, and advice. Thank you for being permissive and letting me do side-projects... many of them went quite well in the end, and I believe that there still are some more papers to write up from them. I really learned a lot during my time in the Salmonid Ecology Group.

My examiners during these years, First Lotta K and later Staffan A

The co-supervisors Johan H and Angela P. Thank you for guidance and discussions.

David A, Libor Z, Barbara K, Rasmus K, and Fredrik S for good (and sometimes quite tough) times in the field (and in the lab and in the office...).

Malte A and Bart A for very helpful discussions and advice during the thesis-writing period.

Niklas W and Markus L from Sportfiskarna for good and interesting collaborations.

Malin R and Eli K for good collaboration and good times at Ims and elsewhere.

Ana and Andrea, our Spanish guests, for great work and company during their time in Gothenburg.

All the rest of the PhD-students at the department - I would like to write all your names, but I am afraid of forgetting someone...

People from Smoltpro, BiFiNe, and SalmoInvade

All the friendly people I met at conferences and courses in Tanzania, St Andrews, Uppsala, Tjärnö, Bergen, New York City, Toulouse, Girona, Älvkarleby, Norwich, Hull, Kristineberg, Lund, and Gothenburg.

People in the "Thursday poker club".

Fellow students during my BSc and MSc studies.

The administrative staff, your support is great!

The faculty staff for interesting discussions on biology, science in general, politics, computer problems, university organization, publication strategies, teaching and all kinds of other topics during lunches, parties, fika, etc.

All students working and helping within my/our projects: Caroline B, Lin S, David H, Per S, David B, Britt S, Badreddine B, Erik L., Leo P, Joakim B, Filip E, Karl F, Fredrik W, and all the students in "my" project groups in the course *Organismers adaptationer*.

All people who voluntarily (or not, it doesn't matter...) helped out in the field, in hatcheries, and in the lab.

All the co-authors on the papers that did not make it into this thesis.

All teachers from preschool to university, with special thanks to the science teachers from 7th - 9th grade and 'high school'. I also need to thank Lt Svensson and Kn Mild from Ing2 who provided insights into certain aspects of work ethics which proved to be surprisingly useful during the PhD studies.
Marabou Apelsinkrokant for invaluable support during fieldwork.
All the fishes... (I'm sorry for what some of you had to experience. It was intentional, but still...)

Financial support was provided by Helge Ax:son Johnsons Stiftelse, Wilhelm och Martina Lundgrens Vetenskapsfond, Adlerbertska Forskningsstiftelsen, Berghaus donationsfond, University of Gothenburg travel fund, Formas (grants to J.I. Johnsson), and University of Gothenburg.

Jag vill tacka min familj för att ni stöttat mig genom alla år.

Till sist vill jag tacka Christina, för ditt stöd och sällskap. Utan dig hade det inte gått lika bra.

This thesis was typeset in LaTeX using ShareLaTeX (http://www.sharelatex.com/).


SEG
Salmonid Ecology Group

Paper 1

Telomere dynamics in wild brown trout: effects of compensatory growth and early growth investment

Joacim Näslund [1]
Angela Pauliny [1]
Donald Blomqvist [1]
Jörgen I. Johnsson [1]

This manuscript was originally published by Springer-Verlag Berlin Heidelberg
Oecologia 2015, 177(4), 1221-1230. doi: 10.1007/s00442-015-3263-0
(Received 1 April 2014; accepted 2 February 2015; published online 20 February 2015)

Corresponding author: J. Näslund (joacim.naslund@gmail.com)

[1] Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

Abstract

After a period of food deprivation, animals often respond with a period of faster than normal growth. Such responses have been suggested to result in decreased chromosomal maintenance, which in turn may affect the future fitness of an individual. Here, we present a field experiment in which a food deprivation period of 24 days was enforced on fish from a natural population of juvenile brown trout (*Salmo trutta*) at the start of the high-growth season in spring. The growth of the food-deprived fish and a non-deprived control group was then monitored in the wild during 1 year. Fin tissue samples were taken at the start of the experiment and 1 year after food deprivation to monitor the telomere dynamics, using reduced telomere length as an indicator of maintenance cost. The food deprived fish showed partial compensatory growth in both mass and length relative to the control group. However, we found no treatment effects on telomere dynamics, suggesting that growth-compensating brown trout juveniles are able to maintain their telomeres during their second year in the stream. However, body size at the start of the experiment, reflecting growth rate during their first year of life, was negatively correlated with change in telomere length over the following year. This result raises the possibility that rapid growth early in life induces delayed costs in cellular maintenance.

Keywords: chromosomal maintenance, field experiment, growth deprivation, growth rate, mortality

Introduction

After periods of food deprivation, many organisms regain control size, i.e. the size they would have had if not being growth restricted (e.g. Reed 1921; Dmitriew 2011). Such recovery growth can be attained either through faster than normal growth rate, a response called "compensatory growth", or by extending the growing period (Nicieza and Álvarez 2009; Jobling 2010; Hector and Nakagawa 2012). These two growth strategies can also be elicited together (Jobling 2010).

Compensatory growth may come with several costs, immediate or delayed, to the recovering individuals (reviewed by Mangel and Stamps 2001; Metcalfe and Monaghan 2001; Hector and Nakagawa 2012). Accordingly, a number of studies have shown increased mortality following periods of compensatory growth in animals (Metcalfe and Monaghan 2003; Johnsson and Bohlin 2006; Lee et al. 2013). In order to accelerate growth, animals often increase their food intake per time unit, which may force them to reduce vigilance and increase mortality risk due to predation (Munch and Conover 2003; Álvarez 2011). In addition, cellular maintenance has been suggested to be a key to understanding the energy allocation trade-offs during growth compensation (Arendt 1997; Mangel and Munch 2005). For instance, accelerated growth rate may result in higher production of, and susceptibility to, reactive oxygen species (Metcalfe and Alonso-Alvarez 2010), which can

lead to increased damage on, e.g., the chromosomes' telomeric ends (von Zglinicki 2002). The telomeres are hexanucleotide repeats [in salmonid fish $(T_2AG_3/C_3TA_2)_n$; Abuín et al. (1996)], with associated proteins, located distally on eukaryotic chromosomes (Blackburn and Gall 1978; Gomes et al. 2010). Their functions include prevention of chromosomal degradation and end-to-end fusion (Blackburn 2001). In addition to oxidative damage, telomeres are also shortened at every cell division due to the end-replication problem (Olovnikov 1973) and C-strand processing (Huffman et al. 2000), and consequently the telomere length has been used as an indicator of cellular aging (Aubert and Lansdorp 2008). Attrition of telomeres to a critically short length leads to chromosomal instability and causes cell malfunction and disease (Hemann et al. 2001; Wong and Collins 2003). Thus, with reduced maintenance, the rate of telomere shortening may increase, resulting in reduced survival and, hence, fitness.

Telomeres have been suggested as useful fitness indicators in, e.g., stress response and growth studies (Monaghan and Haussmann 2006; Horn et al. 2010). In birds, this seems to be valid, as observations on wild king penguins (*Aptenodytes patagonicus*), where chicks undergoing recovery growth stress showed both higher levels of oxidative damage and accelerated telomere loss (Geiger et al. 2012). Furthermore, chicks dying during the experimental period had the shortest telomeres, indicating that telomeres indeed were indicators of life-expectancy (Geiger et al. 2012), in accordance with studies on other birds and reptiles (Pauliny et al. 2006; Bize et al. 2009; Olsson et al. 2011). In fish, there are as yet very few studies on the links between growth stress and telomere dynamics. Recent laboratory studies have shown that rapidly growing growth-hormone transgenic coho salmon (*Oncorhynchus kisutsch*) have higher levels of oxidative damage (Carney Almroth et al. 2012), but information on telomere shortening in fish is currently lacking in this context.

By initially manipulating growth of wild brown trout (*Salmo trutta*), and subsequently recapturing fish at different times during 1 year, we monitored their individual growth and telomere dynamics in a natural stream following food deprivation. We predicted that food-deprived trout would recover body size when compared to non-deprived conspecifics, in accordance with previous studies from the same geographic area (Johnsson and Bohlin 2005, 2006; Sundström et al. 2013). Given a compensatory growth response, we also predicted that compensating individuals would have a higher degree of telomere deterioration as a result of reduced cellular maintenance. Ina ddition, we explored relationships between body size at 1 year of age, reflecting growth investment early in life, and telomere length.

Materials and methods

Study population

The subjects were juvenile brown trout, from River Norumsån, a small soft-water coastal stream in south-western Sweden (58°2.474'N, 11°50.410'E). Trout in this population are predominantly sea-run as adults, with the main seaward migration occurring during spring, 2 years after hatching (Bohlin et al. 1996).

Fish sampling and treatment

On 13 April 2010, 280 trout between 65 and 103 mm were collected by electrofishing (L-1000, 200–400 V, straight DC; LugAB, Luleå, Sweden). We chose to use this size span, which is likely to represent 1-year-old fish at the time of capture (Bohlin et al. 1996), to avoid age-dependent fitness effects (Horn et al. 2010). The sampling stretch was 500 m long and still contained trout after the sampling. The collected fish were transported to the University of Gothenburg were they were kept for acclimation in a 750-l flow-through tank with water temperature maintained at 12 °C, without access to food. On 15 April 2010, the fish were anaesthetised (2-phenoxyethanol, 0.5 ml l^{-1}), weighed to the nearest 0.1 g (wet mass), measured to the nearest mm (fork length) and implanted with unique passive integrated transponders (PIT-tags) into the abdominal cavity for individual identification. All experimental fish had the adipose fin clipped for recognition in the field when being recaptured. Half of the fish ($n = 140$) were randomly assigned to the food-deprived group (henceforth: DEPR) and kept in the laboratory without access to food for 22 additional days (total deprivation time: 24 days). Previous experiments have shown that brown trout of this size can be held in the laboratory without food, at similar temperature as used in this study, for at least 1 month without increasing mortality or impairing health (Johnsson and Bohlin 2006). The other half of the fish (the control group; henceforth: CTRL) were released into the wild the following day. No significant differences in initial fork length [mean ± SD, (min–max) mm; DEPR: 77.4 ± 8.6, (66–103); CTRL: 77.4 ± 10.0, (66–103)] or initial wet mass [mean ± SD, (min–max) g; DEPR: 4.6 ± 1.9, (2.6–11.6); CTRL: 4.6 ± 2.2, (2.6–13.4)] between the two groups were detected (see also Fig. 1; Table 1). The size distribution was positively skewed, possibly due to emerging size bimodality (see, e.g., Heggenes and Metcalfe 1991) or inclusion of some smaller sized 2-year-olds. Three fish died during the food deprivation period.

Release and recapture

A 250-m stretch of River Norumsån, situated within the original capture area, was divided into ten consecutive release sections. The CTRL fish were released on 16 April 2010, while the DEPR fish were released on 7 May. Each of the treatment groups was divided into five equally sized sub-groups and these were released into alternating sections to reduce effects of prior residency from the CTRL fish on the DEPR fish (Johnsson et al. 1999). After release, the fish were, however, free to move within the stream.

The first recapture was conducted on 10 May 2010 to get an estimate of size differences between DEPR and CTRL fish. Second and third recapture were conducted on 7 June and 27 September 2010, respectively. At these three recaptures, we fished the experimental stretch plus an additional distance of 125 m downstream and 200 m upstream, using one-pass electrofishing. All captured fish were released back into the stream close to the capture site after being anaesthetised, PIT-scanned, weighed and measured. The final recapture was conducted on 4 and 5 April 2011, using two-pass electrofishing; this time, we fished stretches 100 m upstream and downstream of the experimental stretch. The final recapture was conducted prior to the start of the main smolt migration to the sea in this stream, as estimated based on previous studies (Bohlin et al. 1993). Also, fishing earlier in the winter/spring is normally ineffective as low water temperatures reduce catchability.

Tissue sampling

For telomere analysis, we collected fin clips (approx. 2 mm^2) from the tips of the paired pelvic fins, which were assumed to have comparable telomere lengths. At the initial measurements on 15 April 2010, all fish had the tip of the right pelvic fin clipped, while the left pelvic fin was clipped from the recaptured fish at the final recapture. Fin clips were immediately put in 95 % ethanol and, after transport to the department, stored at -80 °C for up to 2 years.

Telomere assay

Relative telomere length (rTL) was measured using a quantitative real-time PCR (qPCR) protocol (Cawthon 2002), adapted for use in brown trout. Here, we follow applicable MIQE guidelines to ensure quality and transparency in our qPCR analyses (Bustin et al. 2009; Horn et al. 2010).

Pre-assay preparations

Genomic DNA was extracted from fin clips using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and the manufacturer's protocol. We assessed DNA quantity and quality with a spectrophotometer (NanoDrop 2000c; NanoDrop Products, Wilmington, USA) and prepared a working stock of 10 ng μl^{-1} in autoclaved and aliquoted purified water (Milli-Q; EMD Millipore, Billerica, USA). The concentration of these working stocks were re-analysed and diluted with purified water to a final sample concentration of 0.5 ng μl^{-1}.

Determination of telomere length by qPCR

Quantitative real-time PCR records the accumulating fluorescent signal as amplification of the target DNA proceeds (Higuchi et al. 1993). For each sample, the fractional cycle number (C_q) at which the signal reaches a set threshold above baseline fluorescence is determined. Thus, the C_q value of a sample is inversely proportional to the starting amount of template DNA, e.g. telomere repeats (see ESM Fig. S1). Telomeric content per cell (a proxy measurement for telomere length; e.g. Cawthon 2002) was determined as the number of telomere repeats (T) per number of reference gene copies (S). Building on Cawthon (2002), we obtained a relative measure of individual telomere length by comparing the T/S ratio (Eq. 2) of each focal sample to that of the calibrator samples (included on all plates).

We used β-actin as a reference gene. Forward and reverse β-actin primers were designed in Beacon Designer (PREMIER Biosoft, Palo Alto, USA) based on the published β-actin mRNA sequence of a related salmonid species (Oncorhynchus mykiss, GenBank accession No. AF157514). For amplification of telomeric repeats, universal primers were used. For all primer sequences, see ESM Table S2. Both PCR reactions were optimised using the machine's gradient function, and amplicon size as well as specificity was confirmed by agarose gel electrophoresis (data not shown). Each qPCR reaction contained 4 ng DNA in a total volume of 20 μl 1× KAPA SYBR Fast Mastermix (2.5 mM final $MgCl_2$) (KAPA Biosystems, Woburn, USA). Final concentrations of forward and reverse primers for the telomere amplification were 100 and 200 nM, respectively, whereas 350 nM was used for each of the β-actin primers. Reactions were set up manually and amplified on a qPCR machine (iCycler/MyiQ; Bio-Rad, Hercules, USA) using FrameStar PCR plates (4titude, Wotton, UK). Reaction conditions included an initial denaturation at 95 °C for 4 min, followed by 25 (telomere) or 40 (β-actin) cycles of 95 °C for 15 s and 56 °C for 1 min. After

each run was completed, a melt curve (55–95, 0.5 °C increase cycle^{-1}) was generated to assess PCR specificity. Corresponding telomere and β-actin amplifications were carried out on different plates (but the same well position) and right after each other on the same day, using aliquots from the same preparation of sample and dilutions.

All experimental samples were analysed in triplicate, and average values were used in the subsequent analyses. In 1 out of 550 (0.18 %) cases, one of the triplicates deviated more than one amplification cycle from the average of the other two measurements, and was therefore conservatively excluded in subsequent analyses. Individuals were randomly assigned to 1 of 12 plates, but repeat samples from the same individual were analysed on the same plate for optimal comparison. The intra-plate coefficient of variation (samples run in triplicate) ranged between 0.02 and 3.70 % (telomere) and between 0.03 and 1.64 % (β-actin). Eight samples (four per treatment) were loaded on all plates to assess inter-plate variability (calibrator samples). The coefficient of variation of these samples ranged between 0.77 and 4.57 % (telomere), and between 0.30 and 2.29 % (β-actin). To estimate the amplification efficiency of each plate, a standard curve consisting of five serial 1:10 dilutions of one sample was analysed in triplicate (10,000 fold range, 50–0.005 ng DNA per well, with the middle quantity roughly matching that of samples being analysed). Standard curves were generated by the iQ5 2.0 software (Bio-Rad), and PCR efficiencies (E) calculated as:

$$E = 10^{(m^{-1})} \tag{1}$$

where m is the slope. PCR efficiencies were generally high in the investigated range (50–0.005 ng), as was the linearity of the model (all $R^2 > 0.988$). Standard curve characteristics as well as E of all plates are presented in ESM Table S1, while one example is shown in ESM Fig. S1. On each plate, a negative control (no template control, NTC) was included in triplicate. The C_q of NTCs for the telomere and β-actin amplifications was at least 5.6 and 5.2 cycles higher, respectively, than with template present (mean difference, telomere: 11.1 cycles; β-actin: 9.0 cycles). Thus, fluorescence signals derived from samples were approximately 1,024 times stronger than background noise ($E^{cycles} = 210 = 1.024$), assuming a PCR efficiency of 100 % ($E = 2$, reflecting a perfect two-fold increase in number of copies per cycle). Relative telomere length (T/S ratio) was calculated using a mathematical model for relative quantification (Pfaffl 2001). In this model, the T/S ratio of the target amplicon (telomere repeats) is calculated based on E and the C_q difference (ΔC_q) between the calibrator sample and the focal sample, and expressed in comparison to the reference amplicon

(β-actin), following the formula:

$$T/S\ ratio = (E_{target})^{\Delta C_{q_{target}}} / (E_{reference})^{\Delta C_{q_{reference}}} \quad (2)$$

This model results in more reliable and exact estimates of relative telomere length (Pfaffl 2001), since it does not presume optimal and identical PCR efficiencies $E = 2$ for the target and reference amplifications (as is the case for the commonly used "$\Delta\Delta C_q$" or Livak method; Livak and Schmittgen 2001). Change in relative telomere length (ΔrTL) between the two sampling periods for each focal sample was calculated as average estimates based on all eight calibrator samples:

$$\Delta rTL = (\sum_{r=1}^{n=8}(b_r - a_r)) \cdot n^{-1} \quad (3)$$

where a_r and b_r is the T/S ratio based on calibrator sample r at the first and second sampling, respectively.

Data analysis

Growth over time was analysed as specific growth rate, calculated as:

$$SGR = 100 \cdot (log_e(S_{i+1}) - log_e(S_i)) \cdot t^{-1} \quad (4)$$

where S_i is size (i.e. mass or length) at the start of the period and S_{i+t} is size after time t. Condition factor of the fish was calculated as:

$$CF = 10^5 \cdot M \cdot L^{-3} \quad (5)$$

where M is wet mass and L is fork length. Data from each recapture were analysed separately since the same individuals were not captured every time. Size, growth, condition factor and ΔrTL were analysed using linear models. Factors used were: initial fork length (i.e. at the time of the first measurement) (L_I), initial body mass (M_I), and treatment (TR), with L_I and M_I used as continuous covariates and TR a fixed factor. Models used and results for each analysis are shown in Table 1. Approximate normality and equal variances of residuals for each value of the covariate, and homogeneity of slopes, was assumed. Graphical investigation of these traits suggested that the assumption was reasonable (for an example, see ESM Fig. S2). It can be noted that there was significant deviation from unimodality in initial length (Hartigans' dip test: $D = 0.0375$, $P = 0.0045$), but, as this pattern was similar in both treatment groups, analyses were carried out assuming normality. Sample size is noted in Fig. 1. As the results in the growth and size analyses are interconnected, and as the results

are following a general expected pattern over time, no corrections were made for multiple testing.

For data on change in telomere length of the fin tissue (ΔrTL), we assumed homogeneity of slopes, approximate normality and equal variances of data for each value of the covariate. Graphical investigation suggested that these assumptions were reasonable (ESM Fig. S3). Sample size is noted in Fig. 2. ΔrTL was also analysed by separate one-sample t tests for each treatment group, to detect whether average ΔrTL of each treatment group deviated from zero. In addition, we analysed ΔrTL in a linear model using specific growth rate in mass (SGR_M) over the whole experimental period as an independent variable. As ΔrTL appeared to be influenced by specific growth rate and since this variable is strongly dependent on initial size, we also used a size corrected specific growth rate measure ($CSGR_M$) as a continuous independent variable to analyse ΔrTL. This was done to investigate whether faster than expected SGR_M, over the whole experimental period, influenced ΔrTL. $CSGR_M$ was obtained by subtracting the expected SGR (given the initial mass) from the observed value. Expected values were obtained using parameter estimates from a linear regression including all data on initial mass and SGR over the whole experimental period:

$$Expected\ SGR = 0.063 - 0.13 \cdot M_I \tag{6}$$

Pearson correlation analyses were carried out to investigate the relationships between growth, size and ΔrTL.

For the survival analysis, we used recapture status (yes or no) at the final sampling as an estimate of survival. Data was analysed using a generalized linear model for binomial distribution with a logit link function. The model included TR, L_I, and their interaction. The dataset contained all fish which did not die prior to release (140 CTRL fish and 137 DEPR fish).

Statistical analyses were made in SPSS 20.0 (IBM, Armonk, USA).

Results

Compensatory growth

Statistics and illustrations of the growth patterns are presented in Table 1 and Fig. 1. After the laboratory treatment (i.e. at first recapture), the DEPR fish were significantly smaller than the CTRL fish in mass, length and also had a lower condition factor. After release, the DEPR trout caught up with the CTRL in condition factor within 28 days. This effect was driven

Table 1: Statistical models and results.

			Covariate		TR		
Model	Time	d.f.	F	P	F	P	Effect(TR)
Variable: $log_e(M)$							
$\sim TR$	I	1, 278	-	-	0.1219	0.73	n.s.
$\sim log_e(M_I) + TR$	R1	1, 121	2331	< 0.001	690.5	< 0.001	CTRL > DEPR
$\sim log_e(M_I) + TR$	R2	1, 114	366.1	< 0.001	78.42	< 0.001	CTRL > DEPR
$\sim log_e(M_I) + TR$	R3	1, 137	258.9	< 0.001	21.91	< 0.001	CTRL > DEPR
$\sim log_e(M_I) + TR$	R4	1, 93	79.19	< 0.001	7.158	0.009	CTRL > DEPR
Variable: SGR_M							
$\sim log_e(M_I) \times TR$*	I - R1	1, 121	1.667	0.20	86.01	< 0.001	CTRL > DEPR
$\sim log_e(M_I) + TR$	R1 - R2	1, 52	8.541	0.0051	16.10	< 0.001	CTRL < DEPR
$\sim log_e(M_I) + TR$	R2 - R3	1, 62	14.62	< 0.001	4.205	0.045	CTRL < DEPR
$\sim log_e(M_I) + TR$	R3 - R4	1, 72	16.08	< 0.001	< 0.1	0.85	n.s.
$\sim log_e(M_I) + TR$	I - R4	1, 91	57.97	< 0.001	7.312	0.008	CTRL > DEPR
Variable: $log_e(L)$							
$\sim TR$	I	1, 278	-	-	< 0.1	0.91	n.s.
$\sim log_e(L_I) + TR$	R1	1, 121	4874	< 0.001	529.1	< 0.001	CTRL > DEPR
$\sim log_e(L_I) + TR$	R2	1, 114	398.3	< 0.001	97.94	< 0.001	CTRL > DEPR
$\sim log_e(L_I) + TR$	R3	1, 138	243.6	< 0.001	22.33	< 0.001	CTRL > DEPR
$\sim log e(L_I) + TR$	R4	1, 93	76.61	< 0.001	6.467	0.013	CTRL > DEPR
Variable: SGR_L							
$\sim log_e(L_I) \times TR$**	I - R1	1, 121	< 0.1	0.79	10.32	0.0017	CTRL > DEPR
$\sim log_e(L_I) + TR$	R1 - R2	1, 52	5.241	0.026	9.064	0.0040	CTRL > DEPR
$\sim log_e(L_I) + TR$	R2 - R3	1, 63	8.038	0.0061	5.692	0.020	CTRL < DEPR
$\sim log_e(L_I) + TR$	R3 - R4	1, 72	12.84	< 0.001	1.271	0.26	n.s.
$\sim log_e(L_I) + TR$	I - R4	1, 91	44.95	< 0.001	5.544	0.021	CTRL > DEPR
Variable: CF							
$\sim TR$	I	1, 278	-	-	1.411	0.24	n.s.
$\sim TR$	R1	1, 122	-	-	162.5	< 0.001	CTRL > DEPR
$\sim TR$	R2	1, 115	-	-	0.8746	0.35	n.s.
$\sim TR$	R3	1, 138	-	-	0.5154	0.47	n.s.
$\sim TR$	R4	1, 94	-	-	0.2856	0.59	n.s.
Variable: ΔrTL							
$\sim log_e(M_I) + TR$	I - R4	1, 90	4.851	0.030	0.276	0.601	n.s.
$\sim SGR_M$	I - R4	1, 90	6.610	0.012	-	-	-
$\sim CSGR_M$	I - R4	1, 90	2.299	0.133	-	-	-

The Effect(TR)-column show the direction of the observed treatment effects, if significant, where DEPR = food deprived fish and CTRL = control fish. Significant results ($P < 0.05$) in bold.
Variables: M - wet mass (g); SGR_M - specific growth rate, wet mass; L - fork length; SGR_L - specific growth rate, fork length; CF - condition factor; ΔrTL - change in telomere length of fin tissue.
Model factors: M_I - initial wet mass; L_I - initial fork length; TR - treatment (fixed factor, two levels: food deprived and control); SGR_M - specific growth rate in wet mass; $CSGR_M$ - deviation from expected SGR given initial wet mass. All models include an intercept.
Time: I ("initial") = April 15, 2010; R1 = May 10, 2010; R2 = June 7, 2010; R3 = September 27, 2010; R4 = April 4-5, 2011.
* Interaction included: $F = 7.586$, $P = 0.007$
** Interaction included: $F = 6.700$, $P = 0.011$

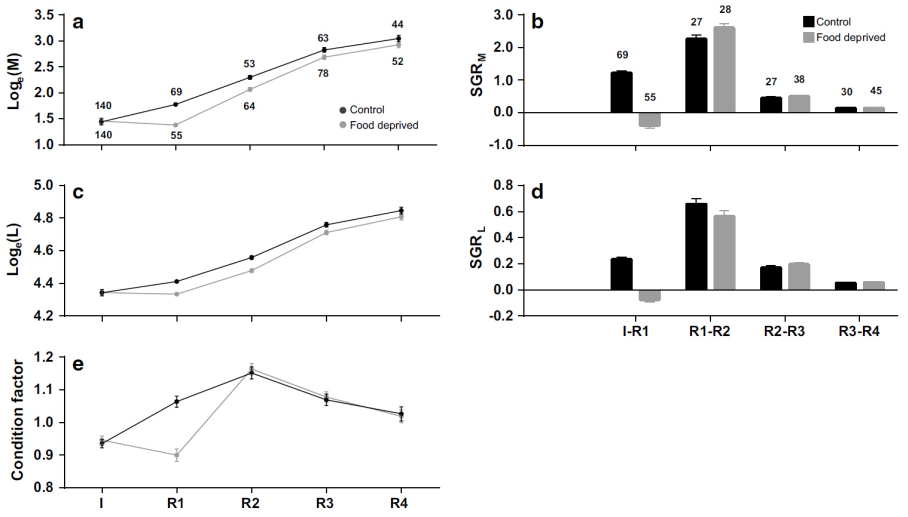


Figure 1: Size (a, c), growth (b, d) and condition factor (e) data for brown trout (*Salmo trutta*) over the experimental period. SGR: specific growth rate as % change per day in wet mass (SGR_M), or fork length (SGR_L). I: time for first measurements (15 April 2010); R1, R2 and R3: first (10 May 2010), second (7 June 2010) and third (27 September 2010) recapture, respectively; R4: final recapture (4–5 April 2011). Figures show the estimated marginal means based on the mean of the covariate in each model (see Table 1). Error bars estimated 95 % confidence intervals. Sample sizes for each analysis are noted next to the data in (a) and (b). Sample sizes in (c, e) are the same as in (a), and sample sizes in (d) are the same as in (b).

by compensatory growth in mass, which also continued over the summer. Length growth was slower in DEPR fish during the regaining of condition, but a compensatory response could be observed later in the summer. Over the winter, there was no difference in specific growth rate in either mass or length between the treatments. At the final recapture, there were still significant differences between treatments but the mean differences between treatments had decreased since the DEPR fish were released. Together, these data suggest that there was no extended growth period in the DEPR group in late autumn. Analysing the specific growth rates over the whole experimental period showed that CTRL fish did grow faster on average. This supports the conclusion that, while there was indeed compensatory growth going on, the result was only partial compensation. Specific growth rate for both wet mass and fork length was dependent on initial size in all periods except during the treatment period. Specific growth rate of smaller individuals was higher, which is normal in fish (Brett 1979).

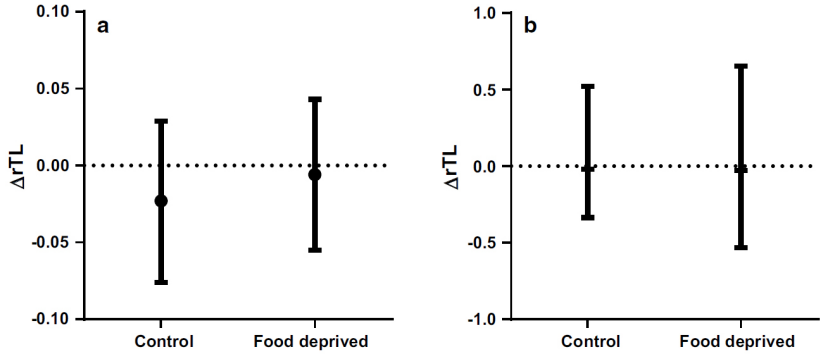


Figure 2: Change in relative telomere length (ΔrTL; negative values denote a reduction in rTL and vice versa) over the experimental period (15 April 2010–4–5 April 2011). (a) Estimated marginal means with error bars showing 95 % confidence intervals. Data are corrected for initial body length (L_I) and valuated at the mean $log_e(L_I) = 4.335$ mm (back-transformation: $e^{4.335} = 76.3$ mm). (b) Median and range of raw data. Note the different scales of the y-axes in (a) and (b). Sample sizes: control $n = 43$; food-deprived $n = 50$.

Telomere length

A linear model containing the natural logarithm of initial mass and treatment as independent variables showed a negative effect of initial mass ($P = 0.030$) but no effect of food deprivation ($P = 0.64$) on change in telomere length over the experimental period (Table 1; Fig. 2). No net change in telomere length over the experimental period was detected for either the DEPR group (mean ± SEM: -0.010 ± 0.028; $|t| = 0.37$, $df = 49$, $P = 0.71$), or the CTRL group (mean ± SEM: -0.018 ± 0.022; $|t| = 0.83$, $df = 42$, $P = 0.41$), and neither for the two groups pooled (mean ± SEM: -0.014 ± 0.018; $|t| = 0.78$, $df = 92$, $P = 0.44$). Analysing the data with specific growth rate in mass (SGR_M) over the whole experimental period (including the treatment period) as independent variable indicated a significant effect of specific growth rate ($P = 0.012$; Table 1), with a positive relationship between ΔrTL and SGR_M. Usage of the deviation from expected specific growth rate ($CSGR_M$) as independent variable led to worse, and non-significant, fit compared to when SGR_M was used as independent variable. Pearson correlation analyses illustrating relationships between growth, size and ΔrTL are presented in Fig. 3.

Survival

Compared to CTRL fish, a slightly higher proportion of the DEPR group was caught at the final recapture [DEPR: 52 individuals (38 %), CTRL:

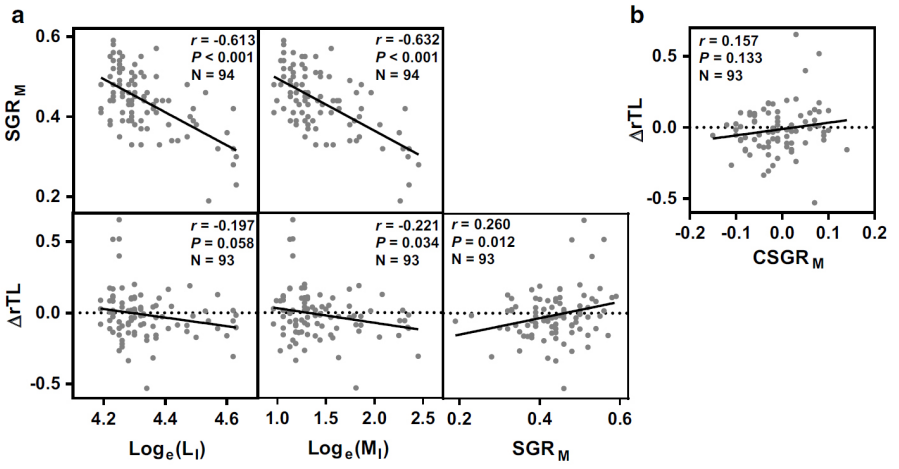


Figure 3: (a) Correlations between change in relative telomere length over the experimental period (ΔrTL negative values denote a reduction in rTL and vice versa), L_I initial length and (M_I) mass, and specific growth rate in mass (SGR_M) over the experimental period. (b) Correlation between the deviation from expected specific growth rate based on initial mass ($CSGR_M$) and ΔrTL. Regression lines are drawn to aid the interpretation of the correlations.

44 individuals (31 %)]. No interaction effect was detected between initial length and treatment (Wald $\chi^2 = 2.24$, $P = 0.135$). After removing the interaction term, we found no main effects of treatment (Wald $\chi^2 = 1.302$, $P = 0.254$) or initial length (Wald $\chi^2 = 0.548$, $P = 0.459$).

CTRL fish were overrepresented in non-recaptured fish from the upper tail of the initial size distribution (i.e. initial length over 90 mm; DEPR: 4 missing, 7 captured; CTRL: 15 missing, 3 captured; see ESM Fig. S4).

Discussion

The growth analyses indicated compensatory growth in wild brown trout following food deprivation, although only partially, i.e. without complete catch-up in growth over the experimental period. The condition factor was restored first, mediated by an increase in body mass at the expense of length increment. Later in the growth season, when normal body condition was recovered, the DEPT fish partly compensated both mass and length until late autumn when growth slowed down to similar rates in both DEPT and CTRL fish. However, the overall growth during the whole experimental period was faster on average for the CTRL group. The overall pattern of the compensatory growth closely resembles the response in a previous experiment in the same stream (Johnsson and Bohlin 2006).

Although the fish were compensating in size, we found no effects on change in telomere length in the tissue of the pelvic fins. Instead, the fish appeared to maintain their telomeres over the experimental year. The telomerase enzyme, which helps to maintain telomere length, has been shown to be ubiquitously active in several tissues in another salmonid fish, the rainbow trout (*Oncorhynchus mykiss*) (Klapper et al. 1998), and the results of this study indicate that telomeres can be maintained during compensatory growth, possibly due to retained activity of telomerase.

Even though we found no evidence of reduced telomere maintenance as a cost of compensatory growth, it is still possible that reduced maintenance activity in the cells is a consequence of accelerated growth rate, as suggested by Mangel and Munch (2005). For instance, there may be increased oxidative damage on other parts of the chromosomes, mitochondria or on proteins, which may also lead to reduced longevity (reviewed in Mangel and Munch 2005).

Our data suggest the possibility that telomere dynamics can be affected by previous growth investment prior to the experiment. Regardless of treatment, initial body size at the start of the experiment was negatively correlated to change in telomere length (ΔrTL) over the experimental period. Analysing the effect of deviation from expected growth rate ($CSGR_M$), i.e. a size corrected estimate of SGR, suggested that faster than normal growth (positive values of $CSGR_M$) had little effect on ΔrTL (lower correlation than absolute SGR). Instead, size at the start of the experiment may be the better variable for explaining ΔrTL. We can assume that initial size in the cohort of 1-year-old fish roughly reflects growth investment during their first year of life. The fish may differ slightly in age, but hatching in the wild is relatively synchronized, so size differences at 1 year of age are largely attributable to differential growth (Elliott 1994). However, it should be noted that some of the largest fish included in the experiment may have been smaller individuals from a previous year class, and some of the effects may be related to age. Still, a possible interpretation of these results is that rapid growth early in life results in delayed costs of cellular maintenance, which is consistent with previous work on delayed costs of rapid growth (Metcalfe and Monaghan 2001). Individuals vary in life-history strategies due to evolutionary trade-offs shaped by age-related mortality resulting from predation, parasites and variation in resource abundance and distribution (Williams 1966; Stearns 1989). In stream-living salmonids, like brown trout, the first period after emergence could be a selective bottleneck as the majority of the hatched fry die during their first summer (Elliott 1994). During this period, it is vital to grow

out of the small size ranges vulnerable to a range of predators which can remove a large proportion of the under-yearling trout (Alexander 1979). In addition, smaller individuals have lower energy stores and are therefore also more vulnerable to food deprivation (Elliott 1994). These early conditions likely select for rapid growth close to the physiological maximum at early life stages (Sibly et al. 1985), in which the fastest growing individuals may trade-off higher survival probability against delayed costs of cellular maintenance and repair (Arendt 1997). Further manipulative experiments on young-of-the-year trout will be necessary to test this hypothesis.

We found no effects on mortality due to compensatory growth, which is in contrast with the study by Johnsson and Bohlin (2006) but in accordance with two other studies (Johnsson and Bohlin 2005; Sundström et al. 2013). Among-study differences in magnitude of compensation and mortality in the wild may depend on the duration of food deprivation as well as year-to-year variations in environmental factors like winter temperature, ice condition, parasite population density, predation pressure, or intraspecific competition. Among initially large fish (> 90 mm at initial sampling) that were not recaptured at the final sampling, CTRL fish were overrepresented. Even though fish were recaptured prior to the estimated migration timing in spring, some larger fish may have started their downstream movement early, as larger smolt tend to migrate earlier in our study stream (Bohlin et al. 1993). This could be an effect of fewer early migrants in the DEPR group, but this inference is largely speculative and requires further investigations in future experiments. Adding the potential early migrants to the surviving fish did not change the result of similar survival between treatment groups, but it may have obscured signs of size-dependent mortality.

In conclusion, 1-year-old brown trout responded to food deprivation with partial compensatory growth, but there was no evidence for increased mortality from compensatory growth or size-dependent mortality. We found no evidence of reduced chromosomal maintenance in compensating fish, but a negative correlation between initial body size and subsequent change in telomere length which may reflect delayed costs of growth acting on telomere maintenance.

Acknowledgements

This study was performed in accordance with Swedish animal welfare laws and was approved by the Gothenburg ethical committee for animal research, license number 113-2008. Funding was provided to J.I.J. and D.B. by the Swedish Research Council Formas, and to A.P. by Stiftelsen Oscar och Lili Lamms Minne. We thank David Hammenstig, Caroline Blomgren and Lin Sandquist for assistance in the field and Johanna Borlid for assistance in the laboratory. We also thank Neil Metcalfe for valuable comments on the manuscript.

Author contribution statement

A.P., D.B. and J.I.J. conceived the experiments. J.N., A.P., D.B. and J.I.J. designed the experiments. J.N. and J.I.J. performed the experiments. J.N. and A.P. analysed the data. J.N. wrote the manuscript; other authors provided editorial advice.

References

Abuín M, Martínez P, Sánchez L. 1996. Localization of the repetitive telomeric sequence $(TTAGGG)_n$ in four salmonid species. Genome 39:1035–1038

Alexander GR. 1979. Predators of fish in coldwater streams. In: Clepper H (ed) Predator-prey systems in fisheries management. Sport Fishing Institute, Washington DC, pp 153–170

Álvarez D. 2011. Behavioral responses to the environment. Effects of compensatory growth on fish behavior. In: Farell AP, Stevens ED, Cech JJ, Richards JG (eds) Encyclopedia of fish physiology. Academic, San Diego, pp 752–757

Arendt JD. 1997. Adaptive intrinsic growth rates: an integration across taxa. Q Rev Biol 72:149–177

Aubert G, Lansdorp PM. 2008. Telomeres and aging. Physiol Rev 88:557–579

Bize P, Criscuolo F, Metcalfe NB, Nasir L, Monaghan P. 2009. Telomere dynamics rather than age predict life expectancy in the wild. Proc R Soc B 276:1679–1683

Blackburn EH. 2001. Switching and signaling at the telomere. Cell 106:661–673

Blackburn EH, Gall JG. 1978. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. J Mol Biol 120:33–53

Bohlin T, Dellefors C, Faremo U. 1993. Timing of sea-run brown trout (*Salmo trutta*) smolt migration: effects of climatic variation. Can J Fish Aquat Sci 50:1132–1136

Bohlin T, Dellefors C, Faremo U. 1996. Date of smolt migration depends on body-size but not age in wild sea-run brown trout. J Fish Biol 49:157–164

Brett JR. 1979. Environmental factors and growth. In: Hoar WS, Randall DJ, Brett JR (eds) Fish Physiology, vol VIII., Bioenergetics and growth. Academic Press, Orlando, pp 599–675

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55:611–622

Carney Almroth B, Johnsson JI, Devlin R, Sturve J. 2012. Oxidative stress in growth hormone transgenic coho salmon with compressed lifespan—a model for addressing aging. Free Radic Res 46:1183–1189

Cawthon RM. 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res 30:e47

Dmitriew CM. 2011. The evolution of growth trajectories: what limits growth rate? Biol Rev 86:97–116

Elliott JM. 1994. Quantitative ecology and the brown trout. Oxford University Press, Oxford

Geiger S, Le Vaillant M, Lebard T, Reichert S, Stier A, Le Maho Y, Criscuolo F. 2012. Catching-up but telomere loss: half-opening the black box of growth and ageing trade-off in wild king penguin chicks. Mol Ecol 21:1500–1510

Gomes NMV, Shay JW, Wright WE. 2010. Telomere biology in Metazoa. FEBS Lett 584: 3741–3751

Hector KL, Nakagawa S. 2012. Quantitative analysis of compensatory and catch-up growth in diverse taxa. J Anim Ecol 81:583–593

Heggenes J, Metcalfe NB. 1991. Bimodal size distributions in wild juvenile Atlantic salmon populations and their relationship with age at smolt migration. J Fish Biol 39:905–907

Hemann MT, Strong MA, Hao LY, Greider CW. 2001. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell 107:67–77

Higuchi R, Fockler C, Dollinger G, Watson R. 1993. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology 11:1026–1030

Horn T, Robertson BC, Gemmell NJ. 2010. The use of telomere length in ecology and evolutionary biology. Heredity 105:497–506

Huffman KE, Levene SD, Tesmer VM, Shay JW, Wright WE. 2000. Telomere shortening is proportional to the size of the G-rich telomeric 3'-overhang. J Biol Chem 275:19719–19722
Jobling M. 2010. Are compensatory growth and catch-up growth two sides of the same coin? Aquacult Int 18:501–510
Johnsson JI, Bohlin T. 2005. Compensatory growth for free? A field experiment on brown trout, *Salmo trutta*. Oikos 111:31–38
Johnsson JI, Bohlin T. 2006. The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proc R Soc B 273:1281–1286
Johnsson JI, Nöbbelin F, Bohlin T. 1999. Territorial competition among wild brown trout fry: effects of ownership and body size. J Fish Biol 54:469–472
Klapper W, Heidorn K, Kühne K, Parwaresch R, Krupp G. 1998. Telomerase activity in "immortal" fish. FEBS Lett 434:409–412
Lee W-S, Monaghan P, Metcalfe NB. 2013. Experimental demonstration of the growth rate—life-span trade-off. Proc R Soc B 280:20122370
Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_t}$ method. Methods 25:402–408
Mangel M, Munch SB. 2005. A life-history perspective on short- and long-term consequences of compensatory growth. Am Nat 166:E155–E176
Mangel M, Stamps J. 2001. Trade-offs between growth and mortality and the maintenance of individual variation in growth. Evol Ecol Res 3:583–593
Metcalfe NB, Alonso-Alvarez C. 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. Funct Ecol 24:984–996
Metcalfe NB, Monaghan P. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16:254–260
Metcalfe NB, Monaghan P. 2003. Growth versus lifespan: perspectives from evolutionary ecology. Exp Gerontol 38:935–940
Monaghan P, Haussmann MF. 2006. Do telomere dynamics link lifestyle and lifespan? Trends Ecol Evol 21:47–53
Munch SB, Conover DO. 2003. Rapid growth results in increased susceptibility to predation in *Menidia menidia*. Evolution 57:2119–2127
Nicieza AG, Álvarez D. 2009. Statistical analysis of structural compensatory growth: how can we reduce the rate of false detection? Oecologia 159:27–39
Olovnikov AM. 1973. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon? J Theor Biol 41:181–190
Olsson M, Pauliny A, Wapstra E, Uller T, Schwartz T, Blomqvist D. 2011. Sex differences in sand lizard telomere inheritance: paternal epigenetic effects increases telomere heritability and offspring survival. PLoS ONE 6:e17473
Pauliny A, Wagner RH, Augustin J, Szép T, Blomqvist D. 2006. Ageindependent telomere length predicts fitness in two bird species. Mol Ecol 15:1681–1687
Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45
Reed HS. 1921. The rate of growth following an initial period of suppression. Am Nat 55:539–555
Sibly R, Calow P, Nichols N. 1985. Are patterns of growth adaptive? J Theor Biol 112:553–574
Stearns SC. 1989. Trade-offs in life-history evolution. Funct Ecol 3:259–268
Sundström LF, Kaspersson R, Näslund J, Johnsson JI. 2013. Density-dependent compensatory growth in brown trout (*Salmo trutta*) in nature. PLoS ONE 8:e63287
von Zglinicki T. 2002. Oxidative stress shortens telomeres. Trends Biochem Sci 27:339–344
Williams GC. 1966. Adaptation and Natural Selection. Princeton University Press, Princeton
Wong JMY, Collins K. 2003. Telomere maintenance and disease. Lancet 362:983–988

This page intentionally left (almost) blank

Paper 2

Behaviour in a novel environment is associated with body size, but not affected by recent feeding history, in brown trout *Salmo trutta* fry

Joacim Näslund [1]
Lin Sandquist [1]
Jörgen I. Johnsson [1]

This manuscript has been submitted for publication in a peer-reviewed academic journal

Corresponding author: J. Näslund (joacim.naslund@gmail.com)

[1]Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

Abstract

Energetic status and body size are inconstant bodily states generally considered to have feedback effects on the behaviour of fish. Feedbacks can be either negative, like the starvation-threshold feedback (e.g. lowered state-values increase risk-taking because of the need to attain food, and vice versa), or positive the state-dependent safety feedback (higher state-values make individuals less vulnerable when expressing risky behaviours). Few studies have investigated such feedback effects during the early-life critical period, when rapid growth is important regardless of energetic status. In a laboratory experiment we investigated effects of body size and energetic status on the emergence latency from a start-box into an unknown environment in brown trout Salmo trutta fry. Energetic status was manipulated by feeding the fish high and low food rations in different combinations over two consecutive periods. Social status and acute hunger effects were controlled for experimentally by rearing in isolation, and ad libitum feeding the day before trials, respectively. No effects of energetic status on behaviour were detected, but initially larger fish had shorter emergence latency. Overall, these results indicate that behaviour, as expressed in the emergence test, was not modified based on recent feeding conditions. However, when dividing fish based on behavioural types, using an automated cluster analysis, continuously well-fed fish from the more active cluster appeared to have relatively shorter emergence latency than previously food restricted groups, in line with the state-dependent safety feedback.

Keywords: Behavioural types, Compensatory growth, Energetic status, Salmonidae, State-dependent behaviour

Introduction

Animals that are displaced into unfamiliar environments need to explore the surroundings to decide whether to stay or to leave, and if they stay, how to utilize the area. Individuals with longer latency to start such exploration are generally considered being more risk-averse, i.e. they respond more cautiously to environmental uncertainty (Brown and Braithwaite 2004; Kortet et al. 2007; Farewell and McLaughlin 2009). However, any time spent in one area is traded off against the opportunity to search for more profitable areas and staying in one place also restricts the collection of information about the novel environment (Dall 2010).

Laboratory studies on fish have found that individuals with higher energetic status (Krause et al. 1998, 1999; but see Dowling and Godin 2002) and larger body size (Krause et al. 1998; Dowling and Godin 2002; Brown and Braithwaite 2004; but see Harris et al. 2010) tend to emerge later from shelter than individuals with lower energy reserves and/or smaller body size. These size and energy dependent behavioural patterns are consistent with general energy intake vs. risk avoidance trade-offs and suggests state-dependency of behaviour (Houston and McNamara 1999; Luttbeg and Sih 2010).

There are several possible feedback mechanisms involved in state-dependent behavioural expression. The 'starvation-threshold' feedback suggests that individuals with small energy reserves should take more risks to avoid starving to death (i.e. 'starvation avoidance' behaviour), while individuals with larger reserves can afford to be more cautious. Starvation avoidance can also be related to body size as relatively smaller fish have generally higher basal metabolism relative to their body size (Clarke and Johnston 1999) and less storage capacity, and should therefore need to eat more often to avoid starvation. Relatively larger fish may also be more cautious to protect attained fitness assets ('asset-protection' behaviour) (Clark 1994). In addition to these negative feedback mechanisms, there can also be positive feedback mechanisms leading to state-dependent behaviours (Luttbeg and Sih 2010). For instance, larger individuals may be more able to obtain and defend good territories and can consequently attain even more assets. Larger individuals may also be relatively safer in any given situation due to their larger body protecting them from some gape-limited predators, or due to higher capacity to escape predator attempts (Rice et al. 1987; Godin 1997). Larger individuals may thus appear to be more risk-taking, while actually being at less risk despite their behavioural traits. These positive feedbacks are consistent with the principle of 'state-dependent safety'.

The early juvenile stage is a time of very rapid growth in fish (Pedersen 1997), and generally strong selection against small body size suggests that attaining a larger body is important (Perez and Munch 2010, but see Good et al 2001). After emergence the fry compete for territories close to the spawning redds (Titus 1990) and missed growth opportunities may increase the risk of being outcompeted. Slow early growth may also restrict later life-history choices by delaying the timing of ontogenetic niche shifts, such as maturation or smoltification (Metcalfe and Thorpe 1992; Cutts et al. 1999). Attaining larger size and higher bodily condition (i.e. higher asset values) may thus be especially beneficial in early competition, resulting in a general aim for maximising growth rates in fry.

Some studies suggest distinct behavioural groups with fry being either territorial, taking residence close to the nest, or passive, drifting downstream to find areas with less competition (brown trout *Salmo trutta*: Héland 1999; Atlantic salmon *S. salar*: Bujold et al. 2004). There are also observations of different behavioural types having differential foraging tactics within sections of a stream (brook char *Salvelinus fontinalis*: Grant and Noakes 1987b; McLaughlin et al. 1999; Atlantic salmon: Huntingford et al. 1988). At present, however, the exact mechanisms influencing behavioural characteristics in young salmonids are not fully investigated.

The aims of this study are (i) to investigate the relationship between bodily assets (energetic status and size) and behaviour in young salmonids during a major selective bottleneck in the early life-stage of brown trout, and (ii) to investigate whether distinct behavioural strategies could be detected in brown trout fry after a month of social isolation (the approach for this aim was exploratory, using automated cluster analysis). To address the first aim, we conducted a laboratory experiment on recently emerged brown trout fry from a natural stream. We split fish into four groups which were fed different combinations of high (H) and low (L) food rations over two consecutive periods (groups: HH, HL, LH; and LL; first and second letter denoting ration during first and second period respectively). The fish were subsequently trialled in standardized start-box emergence test (SBET) to assess risk-taking propensity. We hypothesised that behaviour would be state-dependent, and as a working hypothesis we used the starvation-threshold feedback. Thus, we predicted that food restricted fish (HL, LH, LL) would be more risk-prone than well-fed fish (HH) as a consequence of more active search for food. Particularly, we expected LH fish to be most risk-prone, as these fish could be in a state of compensatory growth (Bilton and Robins 1973; Damsgård and Dill 1998). Regarding size effects we again used the starvation-threshold feedback as a working hypothesis, predicting that larger fish should be more risk-averse than smaller ones, as smaller fish should have relatively higher demand for food, given their energy storage capacity and mass-specific metabolic rate. Alternative predictions were based on the state-dependent safety feedback, where larger and well-fed fish should appear more risk-prone.

Materials and methods

Capture

On June 3, 2011, we captured 136 recently emerged brown trout fry (swim-up occurs in mid-May to early June, personal observation), with a size range of 28.1–37.4 mm, on their hatching grounds in the stream Norumsån, Sweden (N58° 2.589', E11° 50.759'), using electrofishing (LUGAB L-600, Lug AB, Sweden; straight DC, 200-300 V). The hatching ground is located 2.4 km up-steam from the sea and consists of a shallow (typically down to 10-15 cm deep) 50 m section with gravel and a few deeper pools with finer bottom substrate (ca 30 cm deep). The population is predominantly anadromous, but a proportion of the fish (mainly males) stay as stream residents throughout their lives (Bohlin et al. 1984).

Housing

The fry were transported in 25-l insulated cool boxes by car to the university, where all fish were placed in one 70-l holding aquarium, equipped with sand and plastic fanwort plants, for seven days. During this time the fish were fed frozen chironomid larvae ("bloodworms"; Fina Fisken, Sweden; approximately 5 to 10 larvae $\cdot$ fish^{-1} $\cdot$ day^{-1}). On June 10, we put the fish individually into visually isolated 1.3-l plastic tanks (bottom area: 170 $\times$ 110 mm; water depth: 70 mm), and fed them five bloodworms each. Each tank was equipped with 5 mm of sand substrate and an airstone connected to a pressurised air system. Water (10-13°C) flowed through each tank, supplied by the in-house circulating system. Individual housing was chosen to avoid social effects on growth and behaviour.

Food manipulation

At the start of the experiment the fish were randomly split into two feeding groups ($n = 60$): high food ration (H) and restricted food ration (L); see Table 1. These rations were given over 12 days (Period 1). At the end of Period 1, five fish from H and six fish from L had died. Furthermore, one fish which had been on high ration but lost mass was removed from the experiment as it did not fulfil the criteria for the treatment (i.e. being well fed). The two feeding groups were split in half by random assignment of the remaining fish, creating two sub-groups from each initial feeding group. One sub-group from each initial feeding group was given high food ration, and the other sub-groups were given restricted rations, see Table 1. These latter rations were provided over 23 days (Period 2). This resulted in four treatment groups (n denote final sample size): (i) continuous high food ration (HH; $n = 18$); (ii) continuous restricted food ration (LL; $n = 20$); (iii) initially high food ration, switched to restricted food ration (HL; $n = 21$); and (iv) initially restricted food ration, switched to high food ration (LH; $n = 20$). The supplied food consisted of thawed bloodworms.

The initial low ration was calculated to approximately allow for maintenance of physiological functions, but not growth, following equations in Elliott et al. (1995, eqn. 4) and Elliott and Hurley (1998, eqn. 11), using the chironomid energy content measured by Wissing and Hasler (1971) (average energy content: 5630 Cal $\cdot$ g dry mass^{-1}). Food rations were the same for all fish within a treatment, specifically calculated for the largest of our fish at the start of the experiment (0.44 g wet mass). Thus, the smallest fish got slightly more food relative to their mass than the larger fish, but the maintenance ration still represent a very restricted food intake

Table 1: Food rations for the treatment groups during the experiment. Chironomids are commonly one of the major food items of brown trout fry (Skoglund and Barlaup 2006)

Day of experiment	Number of chironomids per fish per day			
	HH	HL	LH	LL
0	5	5	5	5
1-12	10	10	2	2
13-17	10	2	10	2
18-27	12	3	15	3
28-29	12	4	18	4
30-35	12	4	19	4
36-39 (Trial days: 37-40)	*	*	*	*
Total (1-35)	386	192	386	96
% of HH ration (1-35)	100%	50%	100%	25%

* A fish being trialled the coming day was given 19 chironomids; otherwise it was given the same ration as day 35

for all fish. During the course of the experiment the rations were adjusted for growth and bodily condition of the fish, based on daily visual inspection (see Table 1 for details).

Growth monitoring and analyses

We recorded wet mass (M [g]; precision: 0.01 g; Kern EW 3000-2M, Kern & Sohn GmbH, Germany) and took digital photographs (Canon EOS 40D with EF-S 17-85 IS USM [at 70 mm focal length]; Canon Inc., Japan) of all fish at three time points: (i) the day before the start of the food manipulation (June 10); (ii) the day we switched the food ration for the HL and LH groups (June 24); and (iii) the day after food manipulation ended (16 July). Mass measurements were taken before feeding, so that the fish had not been fed for 24 h prior to the weighing. From the digital photographs we measured fork length (L [mm]; from the tip of the snout to the end of the central caudal fin ray; precision: 0.1 mm) using ImageJ 1.45 software (Rasband 2011). To compare experimental growth with growth in nature, we also sampled fish from the same area as the experimental fish on July 12 for mass and length measurements ($N = 13$). During all measurements fish were anaesthetised with 2-phenoxyethanol ($0.5\ \mathrm{ml} \cdot \mathrm{l}^{-1}$).

Growth rate in wet mass was analysed as specific growth rate (SGR_M; % change per day):

$$SGR_M = 100 \cdot (ln(M_{t_1}) - ln(M_{t_0})) \cdot (t_1 - t_0)^{-1} \quad (1)$$

where t_0 and t_1 are the initial and final time-point in days, respectively.

Growth rate in fork length was analysed as absolute growth rate (AGR_L; mm per day):

$$AGR_L = L_{t_1} - L_{t_0} \cdot (t_1 - t_0)^{-1}. \quad (2)$$

Condition factor (K) was calculated as:

$$K = 10^5 \cdot M \cdot L^{-3}. \quad (3)$$

We analysed initial and final size (fork length and wet body mass) using generalised linear mixed models (GLMM; Gaussian distribution, identity link-function, residual method for degrees of freedom, model-based covariance) including treatment (TR) as a fixed factor and date ($DATE$) and their interaction ($TR \times DATE$). Growth was analysed separately for Period 1 and Period 2 using general linear models (GLM; Gaussian distribution, identity link-function), including TR as fixed factor and fork length (L) at the start of each period as covariates. We initially tested the interaction $TR \times L$ for significance in all growth analyses, but removed it from final analyses if there was low evidence for effects of this term (i.e. $p > 0.1$).

Start-box emergence test (SBET) trials

On July 17 we started the behavioural trials. We ran 20 trials each 24 hour period, and consequently the tests spanned over four days. Following the end of the feeding treatment period, all fish where kept on their final ration until one day prior being tested in the behavioural trial. To be able to investigate effects of bodily energetic state (condition), and not effects of immediate hunger levels, the fish were fed ad libitum (19 bloodworms) on the day before trials. On the trial day the subject fish were not fed.

The behavioural test consisted of a SBET where the animal is put in a start-box connected to a larger unfamiliar area, typically by a closable gate controlled by the experimenter (see e.g. Näslund et al. 2015a). After having acclimated to the start-box for a certain period of time, the gate-way to the adjacent area is opened and the animal is free to emerge from the start-box. Individuals emerging faster from the start-box are typically considered bolder (more prone to taking risks) than individuals with longer latency to emerge (Brown and Braithwaite 2004; Kortet et al. 2007; Farwell and McLaughlin 2009). However, there have been arguments that these tests actually measure a combination of boldness and exploratory tendency (Burns 2008). Generally, there are uncertainties in what behaviours appropriately reflect "boldness", and different measures can often give different results (e.g. Réale et al. 2007; Toms et al. 2010; Carter et al. 2012). Therefore, we avoid further reference to the term and simply refer to the

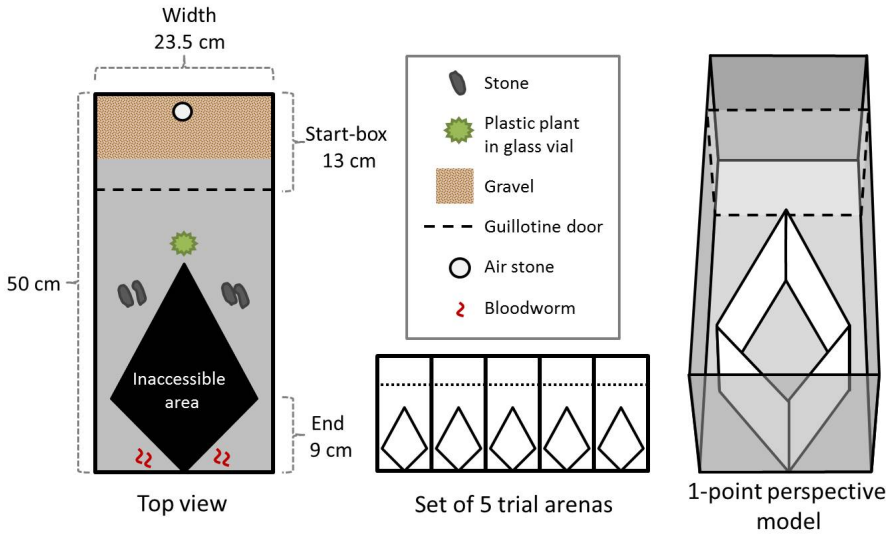


Figure 1: Design of the emergence test arenas, which were built in sets of five. The start-box had no cover and there was no flow through the tank (initial water temperature ≈13°C; room temperature ≈18°C).

behaviour as "emergence latency". The main rationale for the test is that emergence into an unfamiliar area constitutes a potential risk.

In the SBET trials, we used four sets of five adjoined trial arenas (arena design depicted in Fig. 1). Behaviour was recorded with a HD-cam recorder (Sony Handycam HDR-XR155, Sony Corp., Japan) in a standardized fashion; starting with the leftmost arena in set 1, continuing with the leftmost arena in set 2, and so on in set 3 and set 4, followed by second leftmost arena in set 1, set 2, set 3, set 4, etc. in a total of five filming rounds. Each round, one fish from each treatment was filmed. Fish from all treatments were trialled in each arena. Arenas were screened off from the rest of the room and the experimenter by black plastic bags with 30x20 cm openings cut out for filming.

SBET protocol

Fish were released into the start-box of the trial arena (Fig. 1) and left to acclimatise and familiarise with the start-box for a minimum of 30 min (depending on filming order). After acclimatisation the guillotine-door dividing the start-box (known area) and the rest of the arena (unknown area) was lifted by the experimenter using a remote pulley system. The fish was filmed until it reached one of the end-compartments, where food was pro-

vided, or for a maximum of 10 minutes.

Behavioural analyses

Recorded films from the trials were analysed manually using Adobe Premiere CS3 (Adobe Systems, Inc., USA). We measured emergence latency (time from the guillotine-door was lifted until the head was out of the start-box), and the time to reach one of the end-compartments after first emergence from the start-box. Fish that did not leave the start-box within ten minutes was recorded at a ceiling value of 600s. One LH fish was excluded from behavioural analysis as it was obviously diseased, having difficulties maintaining equilibrium during the trial.

Emergence latency data were ln-transformed due to positive skew, and analysed using GLMM (Gaussian distribution, identity link-function, Satterthwaite approximation of degrees of freedom, and robust covariance). The model included the explanatory variables food treatment (TR; fixed) and initial fork length (L_I). An initial model including trial day (DAY) as a random factor and the $TR \times L_I$ interaction was run to investigate effects of these terms. As we found no strongly supported effects of these terms (DAY : $\mathrm{Var}(DAY) = 0.26 \pm 0.35$ SE, $p = 0.40$; $TR \times L_I$: $F_{3,68} = 0.015$, $p = 0.99$), they were not included in the final model. We also ran three alternative GLMs using initial fork length (L_I), final fork length (L_F), or final condition factor (K_F) as single explanatory variables. These three models were compared using the finite sample Akaike Information Criterion (AIC_C), with lower values indicating better model fit.

Behaviour outside of the start-box was scored as time to reach an end-section. However, there were no treatment or size differences detected, so we refrain from expanding on the analysis and the results in this paper.

To investigate whether distinct behavioural groups could be discerned, we used the SPSS TwoStep Cluster procedure (distance measure: log-likelihood), set to automatically categorize a number of clusters (maximally five) (SPSS Inc. 2001). Cluster quality was based on the average silhouette measure of cohesion and separation from the SPSS output. The scale for this measure ranges from -1.0 to 1.0, with values > 0.5 considered good quality (SPSS Inc. 2001). The cluster analysis was based only on the emergence latency data. Time form first emergence to entering an end-section was not included since it contained a relatively large number of censored data (i.e. fish that never emerged). As we did find two clusters (see Results), supplementary analyses were carried out to explore whether there were treatment differences in emergence latency (ln-transformed) within

each cluster. The data was split based on cluster assignment, and separate GLMs (Gaussian distribution, identity link-function) were run for each cluster, using TR and LI as explanatory variables. L_I was removed from the model if $p > 0.1$ to create a reduced model. We also compared initial size between the clusters, using a t-test.

To compare differences between treatment groups we used pairwise contrasts (Fisher's least significant difference; LSD).

General information

Statistical analyses were run using IBM SPSS Statistics 22 (IBM Corp., USA). The experiment was conducted in accordance with Swedish law and regulations and was approved by the Ethical Committee on Animal Experiments in Gothenburg, Sweden (ethical licence number 8-2011).

Results

Size

Wet mass and fork length showed the same qualitative responses to experimental treatments, with statistically significant main effects (DAY and TR) and interaction effects ($DAY \times TR$) (all $p < 0.001$; for estimated marginal means, see Fig. 2). There were no significant differences in initial size among treatment groups (LSD: all $p > 0.3$). The final size of LH was on average slightly smaller than that of HH but without significant difference between the groups (LSD: $p > 0.30$), indicating that the LH fish had almost compensated size as compared to the HH. Size did not differ significantly between LL and HL either (LSD: $p > 0.15$). All other pairwise contrasts were statistically significant with $p < 0.001$.

The fish from the field sample (July 12) had a mean fork length of 39.8 mm (95% confidence interval: 36.5-43.1 mm; range: 30.1-48.7 mm). In comparison, the HH fish from the lab (July 15) had a mean fork length of 35.7 mm (95% confidence interval: 34.7-36.7 mm; range: 32.4-39.7 mm). The fish with longest final length in the experiment was 39.9 mm (from the LH treatment). Means and 95% confidence intervals for ln-transformed wet mass and fork length of the field sample are presented in Fig. 2a and 2d for comparison with experimental fish.

Table 2: Summary of statistical results of growth analyses. Statistically significant effects marked in bold. Illustrations of the effect sizes for treatment groups are presented in Fig. 2.

Response variable		Wald χ^2	p	Effect*
SGR_M (P1)	TR	167.160	< 0.0005	HH $\approx$ **HL** $>$ **LH** $\approx$ LL
	L1	42.643	< 0.0005	B: **-0.174** (-0.227 – -0.122)
SGR_M (P2)	TR	198.571	< 0.0005	**LH** $>$ **HH** $>$ **HL** $\approx$ LL
	L2	11.152	0.001	B: **-0.096** (-0.152 – -0.039)
AGR_L (P1)	TR	146.369	< 0.0005	HH $\approx$ **HL** $>$ **LH** $\approx$ LL
	L1	19.620	< 0.0005	B: **-0.008** (-0.012 – -0.005)
AGR_L (P2)	TR	123.291	< 0.0005	**LH** $>$ **HH** $>$ **HL** $\approx$ LL
	L2	8.246	0.004	B: **-0.009** (-0.015 – -0.003)
Total mass growth (P1+P2)	TR	102.392	< 0.0005	HH $\approx$ **LH** $>$ **HL** $>$ **LL**
	L1	1.780	0.182	B: -0.004 (-0.010 – 0.002)
Total length growth (P1+P2)	TR	93.929	< 0.0005	HH $\approx$ **LH** $>$ **HL** $>$ **LL**
	L1	20.939	< 0.0005	B: **-0.336** (-0.480 – -0.192)

SGR_M: specific growth rate in mass (%$\cdot$ day^{-1});
AGR_L: absolute growth rate in fork length (mm $\cdot$ day^{-1});
P1: Period 1; P2: Period 2; TR: Treatment (HH, HL, LH, LL; see Table 1);
L1: Fork length at the start of Period 1; L2: Fork length at the start of Period 2.
* TR effects assessed with pairwise comparisons (least significant difference) among estimated marginal means at the mean of the covariate;
"$>$" denote significant difference ($p \leq 0.05$), "$\approx$" denote non-significant difference ($p > 0.05$).
Covariate effects (L1, L2) given as parameter estimates B (95% confidence interval).

Growth

During Period 1 the HH and HL treatments grew faster than LH and LL (Table 2, Fig. 2). During Period 2 LH grew faster than HH, which in turn grew faster than HL and LL (Table 2, Fig. 2). There were general negative relationships between length at the start of each period and growth (Table 2). No significant difference was seen in total growth between HH and LH, both growing more than HL, which in turn grew more than LL (Table 2, Fig. 2).

Emergence latency

There were no significant treatment effects (TR) on emergence latency ($F_{3,73} = 0.82$, $p = 0.49$). The HH treatment had the shortest average emergence latencies of all treatment groups, but the data for all treatments were largely overlapping (Fig. 3a). The covariate L_I (initial size) had a small effect ($F_{3,73} = 5.53$, $p = 0.021$), with initially larger fish having shorter emergence latency (coefficient for slope: -0.17 ± 0.071 SE; Fig. 3b). Comparing single factor GLMs (L_I vs. L_F vs. K_F), we could conclude that L_I (AIC$_C$: 306.1) was a better explanatory factor than both L_F (AIC$_C$: 307.1) and K_F (AIC$_C$: 309.5), albeit the difference between L_I and L_F being only slight. The slope for L_F resembled that of L_I but was less steep (coefficient for slope: -0.12 ± 0.073 SE).

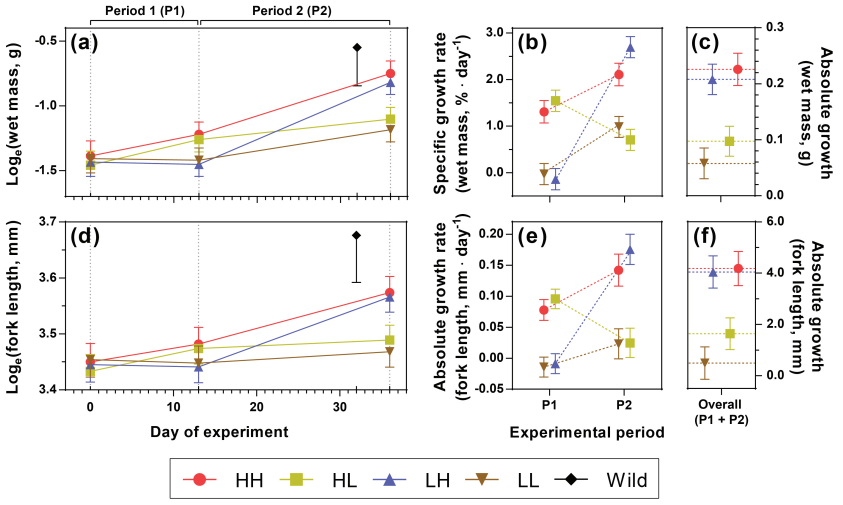


Figure 2: Growth patterns for the experimental fish: (a) mean wet mass; (b) specific growth rate in mass, adjusted for initial size (marginal means); (c) absolute growth in mass over the experiment, adjusted for initial size (marginal means); (d) mean fork length; (e) absolute growth rate in fork length, adjusted for initial size (marginal means); (f) absolute growth in fork length over the experiment, adjusted for initial size (marginal means). Error bars show 95% confidence intervals [in (a) and (d) either upper or lower interval (symmetrical) is drawn for readability of the figure]. HH: high ration P1, high ration P2; HL: high ration P1, low ration P2; LH: low ration P1, high ration P2; LL: low ration P1, low ration P2; Wild: fish sampled directly from the stream Norumsån; exact rations are shown in Table 1.

Behavioural clusters

The TwoStep cluster analysis resulted in two clusters being discerned. The cluster quality was good with an average silhouette measure of cohesion and separation of 0.7. One cluster (Cluster A) was composed of individuals with longer emergence latency (62.8% of the individuals) and the second cluster (Cluster B) was composed of individuals with short emergence latency (37.2% of the individuals). The clusters were separated at an emergence latency of approximately 34 seconds. The number of fish assigned to each cluster was relatively similar among treatments (see Fig. 4). Levene´s test for unequal variances indicated that the variation in size was higher in Cluster B, then in Cluster A ($p = 0.031$). Welch's t-tests (assuming unequal variances) showed that there was no significant differences between the clusters in average initial length ($|t| = 1.58$, $p = 0.12$; Fig. 4).

The separate analyses of emergence latency for each cluster indicated that treatment had no effect on long-latency fish (Cluster A: Wald $Chi^2 = 2.23$, $p = 0.53$), but may have affected the short-latency fish (Cluster B:

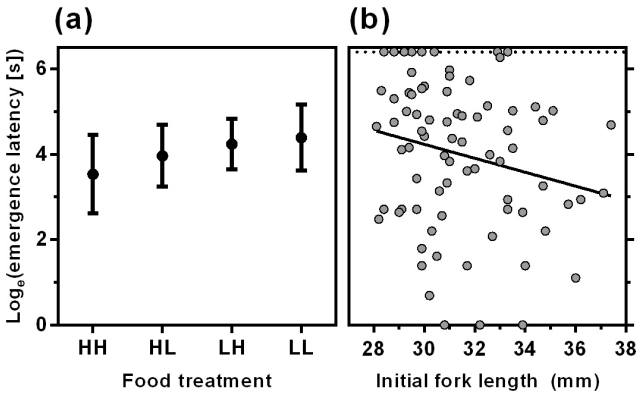


Figure 3: Effects of (a) food ration treatment (estimated marginal means, with 95% confidence intervals), and (b) initial size. Dotted line in (b) marks maximal trial time.

Wald $Chi^2 = 14.2$, $p = 0.003$); no effects of initial length were detected in either analysis ($p > 0.1$) (Fig. 4). In Cluster B, HH differed from all other treatments, having shorter emergence latency (LSD: all $p < 0.015$) (Fig. 4).

Discussion

Our results show that (1) behaviour in SBET was not significantly affected by energetic status as manipulated by feeding history during the previous month, (2) initial size was a significant explanatory variable for emergence latency in the SBET, and (3) individuals cluster into two distinct behavioural groups based on emergence latency in the SBET. We also note that the growth patterns in the different feeding groups followed the patterns predicted by the food rations given. Notably, the LH group seemed to elicit a compensatory growth response, as we had assumed at the start of the experiment.

Growth patterns in the treatment groups

The growth patterns of the treatment groups followed expectations, with fish on high rations growing faster than fish on low rations. The fastest average growth was seen in the LH group during refeeding after initial food restriction, and these fish ended up at approximately the same average size as the HH group. This high growth rate may be a sign of a compensatory

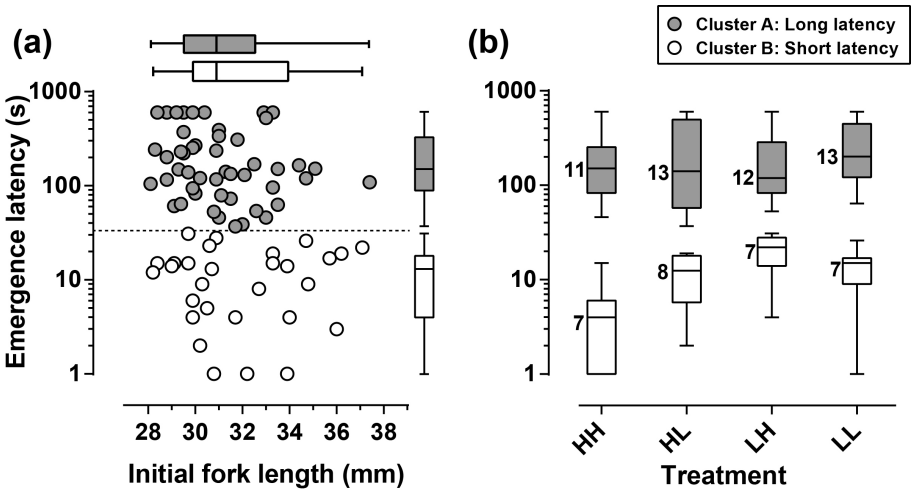


Figure 4: Behavioural clusters: (a) cluster assignment in relation to initial fork length with dotted line marking the approximate separation at emergence latency of 34 seconds (note that maximal trial time is 600 s; thus, the boxplots for Cluster A underestimate the maximal emergence latencies); (b) emergence latency of the different treatments, separated based on cluster assignment with sample size noted beside each boxplot. Box hinges show the first and third quartile, the line inside the box shows the second quartile (median), and the whiskers show minimum and maximum values.

growth response, as observed in older fish (age 1+) from the same population (Johnsson and Bohlin 2006, Sundström et al. 2013; Näslund et al. 2015b). Results from another study, on hatchery reared sockeye salmon, also suggest compensatory growth responses at the fry stage (Bilton and Robins 1973). It should be noted that the LH group was fed a higher ration than HH during the refeeding period, and thus could eat more food each day. However, throughout the experiment we commonly observed that food was left over in all H groups, suggesting that all H rations were indeed ad libitum rations. Still, fish in the wild appeared to be able to grow at substantially faster rates than our experimental fish, assuming that our experimental fish were representative of the population when they were initially caught. There are some potential explanations for this difference in growth between experimental and wild fish. Firstly, the growth may have been low during acclimation, which is often observed in older trout juveniles (age $>$ 1 year) brought to laboratory (authors' personal observations). However, the fry in this study fed without apparent restraints from the first day in captivity, so this explanation appears unlikely to us. Secondly, the fry were reared in isolation from other individuals, which may have reduced their willingness to feed due to isolation stress (suggested by

Jonsson and Jonsson 2011). Thirdly, lack of competition may decrease the drive to feed at maximal capacity (Johnsson 2003). Fourthly, we only fed the fish once a day which may limit the overall daily intake compared to fish in the wild which potentially can feed continuously, keeping the stomach full at all times. During visual observations we observed high stomach fullness in experimental fish on high food rations, but as wild fish were substantially larger when measured, it appears that these fish must have had even higher general food intake than what was possible to achieve in the laboratory, using our feeding regime. A fifth possibility is that wild fish forage on more energetically profitable food. However, chironomids are commonly observed as the main food source for brown trout fry (e.g. Nilsson 1956; Skoglund and Barlaup 2006).

SBET behaviour in relation to feeding history (energetic state)

Energetic state, as manipulated by food rations, did not significantly affect the behaviour as expressed in the SBET. With respect to the starvation-threshold feedback, the pattern of the average values for the treatment groups is opposite to what would be expected. Thus, the evidence is pointing against this particular hypothesis. Previous studies on salmonids have shown that starved individuals alter their behaviour, presumably to increase their food intake. For instance, restricted food rations have been shown to increase aggression, activity and risk-taking in Atlantic salmon (Symons 1968; Gotceitas and Godin 1991; Vehanen 2003), coho salmon Oncorhynchus kisutch (Damsgård and Dill 1998), and rainbow trout O. mykiss (Höjesjö et al. 1999). Importantly, as our aim was to study effects of bodily energetic state rather than acute hunger effects due to empty stomachs, we fed all fish in excess the day before trial to level out such acute hunger effects. Given that compensatory growth responses in general can be maintained over long time periods of feeding (e.g. over months in age 1+ brown trout; Näslund et al. 2015b), alterations of behaviour due to low energetic bodily state are expected to be more than direct effects of empty stomachs. This kind of pre-assay feeding has not been part of the above cited studies where effects were discovered which may explain some of the differences between our results and previous findings. Fish used in these studies were also all substantially larger than the fry used in our study, and may have a more developed behavioural flexibility, which could also explain the difference in results. A larger body can stand energy deprivation for a longer time compared to a smaller body, which could allow for more risk-averse responses to food shortages. Larger predatory fish, including salmonids,

appear to be adapted to variable food supply, with a high capacity to capitalise on food when it is abundant (Armstrong and Schindler 2011). Thus, while larger fish may adapt their behaviour in relation to food abundance, fry could have too small energy stores for such flexibility. An alternative explanation could be that the cognitive capacity allowing for plasticity in behaviour is not yet fully developed (Blaxter 1986).

In hatchery environments, salmonid fry can stand starvation for several weeks, but mortality starts to increase above baseline levels after one to three weeks (Atkins 1906; Bilton and Robins 1973). In contrast, larger juveniles (fork length > 65 mm) can be starved for at least a month without apparent effect on their mortality (e.g. Näslund et al. 2015b). Speculatively, the lack of behavioural adjustment to body state indicated in our study could be due to a limited ability to increase appetite-adjusting hormone levels, possibly as an adaptive strategy keeping the fry at optimal energy expenditure when foraging. While salmonid fry respond to artificially elevated levels of e.g. growth-hormone by increased growth in hatchery environments, the effect in natural environments is the opposite, supposedly due to individuals spending more than the optimal amount of energy on foraging (Sundt-Hansen et al. 2012). This suggests that too high levels of this hormone can be maladaptive for fry in nature. Slight elevations of appetite still appear possible, given that the growth response of the LH group in our experiment reflects compensatory growth.

SBET behaviour in relation to body size

Among the variables investigated (L_I, L_F and K_F), L_I (initial fork length) was the variable that best explained individual variation in behaviour, albeit marginally. This suggests that the observed differences in behaviour could have been present already before treatments began. In general, larger fish had shorter emergence latency, which is opposite to the pattern predicted by the starvation-threshold feedback. Instead, trout fry behaviour seems to follow the state-dependent safety feedback. This is in contrast to studies on other fish, with similar body size, which tend to support state-dependent behaviour based on the starvation-threshold feedback, with larger fish being more risk-averse (Krause et al. 1998, 1999; Brown and Braithwaite 2004). We hypothesise that the difference in behavioural ecology among species may explain the different results among studies. In contrast to many small bodied species which are commonly schooling, trout fry are highly territorial and obtaining a good territory early has many advantages, e.g. prior-residency effects (Titus 1990; Johnsson et al 1999; Jonsson

and Jonsson 2011). In artificial streams without predators, salmonids tend to occupy feeding stations based on social rank, with the most dominant fish choosing the most profitable spot (Fausch 1984; Huntingford et al. 1988). However, according to foraging theory, an individual should find a territory which minimises risk of death per unit energy demanded (Gilliam and Fraser 1987). Thus, in natural environments the best territories may, in addition to providing rich food abundance, also have access to good sheltering opportunities. An active (and likely more aggressive, see Näslund and Johnsson 2015) behavioural strategy could be indicative of a general interference competition strategy, where fish obtain and defend a good and relatively safe territory. However, this will be at the expense of defending the territory. Trout not being able to find or hold territories of sufficient quality may be better off using alternative behavioural strategies, based on exploitation competition (Gotceitas and Godin 1991; Nielsen 1992; Elliott 2002; Skoglund and Barlaup 2006). These alternative strategies may be either conditional 'best-of-a-bad-situation' strategies or evolutionary stable strategies with equal fitness evolved by frequency dependent selection (Magurran 1993).

In a previous study, also using trout fry in a SBET, some smaller individuals took longer to emerge than what was generally seen in larger individuals (Näslund et al. 2015a). This pattern strongly resembles the size-relationship in the present study. Näslund et al. (2015a) suggested several potential explanations: (1) ontogenetic effects, with smaller fish being recently emerged and retaining the geopositive and photonegative behaviour of the yolk-sac fry; (2) subordination with smaller fish being behaviourally suppressed by larger dominant individuals; and (3) general personality differences where smaller fish tend to be more risk-averse. From the present study we can reject the two first hypotheses, because all fry used were long past the yolk-sac fry stage, and subordination was impossible since fish were individually reared. Thus, we suggest that personalities, or behavioural types (see discussion on behavioural clustering below), may explain the differences. The size effects could originate from different behavioural types having different growth rates, or experiencing different selection pressures (see "Clustering of behavioural types" below).

The test situation is likely stressful for the individuals as it involves many novel stimuli and unnatural situations. We know that the fish in general were not too stressed to eat in the test situation, as we observed consumption of the provided bloodworms in all cases where fish entered the end-section. Thus, feeding motivation may have been a driving force for the fish to leave the start-box, and if so, larger fish may have a stronger drive

to feed. However, it may be that the faster emergence of larger sized fish in our study is also a consequence of a higher drive to search for territories in a novel environment. During the fry stage, when there are many intraspecific competitors, there may be time-constraints in finding a territory (Cutts et al. 1999).

Some studies on salmonid fish at similar life-stages have found that larger individuals are less risk-prone, in contrast to our study (Grant and Noakes 1987a; Mikheev et al. 1994). In these studies, the experiments included direct predator stimuli, which may have induced differences among fish of different size. Thus, the opposite results found in our study may only be applicable to situations where predator presence is uncertain.

Clustering of behavioural types

We found that the clustering analysis separated our fish into two separate groups based on their emergence latency. Importantly, these clusters were detected despite one month of social isolation, which suggests that this behaviour is not a consequence of social hierarchies – unless effects of such hierarchies linger from the period spent in the wild prior to capture. The number of fish in each cluster was relatively similar among treatments, suggesting that treatment did not affect clustering. When exploring emergence latency separately for each cluster, it appeared that Cluster B-fish from the HH treatment emerged faster than the other groups, while no differences were apparent in Cluster A-fish. As the results originate from ad hoc analyses, thy need to be interpreted with caution, but the result from the Cluster B-analysis are in favour of the state-dependent safety feedback, and goes against the starvation-threshold feedback.

In general, the clustering favours the idea that different strategies exist in recently emerged brown trout fry (Héland 1999). It is possible that the different behavioural types are adaptations to different environmental conditions (Závorka et al. 2015). For instance, Cluster A may have higher fitness in more variable environments where territoriality has low pay-off due to changing conditions, while Cluster B has better fitness in more stable and predictable conditions where establishing a territory is beneficial (see e.g. Magurran 1993; Huntingford and Garcia de Leaniz 1997; Skoglund and Barlaup 2006; Závorka et al. 2015). Alternatively, or complementarily, the clusters may reflect different foraging strategies as in brook char (McLaughlin et al. 1994, 1999). Brook char fry with different behavioural feeding strategies (active or sedentary) also behave differently when emerging from cover, with active fish emerging sooner than sedentary fish (Farwell

and McLaughlin 2009). The behavioural strategies in this species seem to be associated to stress coping style, with passive fish having higher stress response (cortisol expression) (Farwell et al. 2014). It is possible that variation in behaviour in our experiment also depend on stress responsiveness, given the relatedness of the species (both being salmonids) and their ecology being similar.

The different behavioural types detected in this study may reflect individual personality, which have previously been described for brown trout juveniles (see. e.g. Höjesjö et al. 2012; Adriaenssens and Johnsson 2013; Kortet et al. 2014).

Conclusions

Our results suggest that brown trout fry are not particularly responsive to previous food levels, with respect to their SBET behaviour. Potentially, fish belonging to the more active of the two detected behavioural clusters (i.e. the fish with relatively short emergence latencies), were modifying their behaviour, with previously food restricted fish taking longer time to enter into a novel area. Body size, however, influenced behaviour with larger individuals having faster emergence latency. Overall, our results do not support state-dependent behaviour as predicted by the starvation-threshold feedback hypothesis. Instead, we find evidence for a state-dependent safety feedback, where high state individuals are able to perform more risky behaviours (i.e. having shorter latency to enter an unknown environment).

Acknowledgements

Rasmus Kaspersson is thanked for his assistance in the field. J.N. received financial support from Helge Ax:son Johnsons stiftelse, and Wilhelm och Martina Lundgrens Vetenskapsfond. J.I.J. was financed by a grant from the Swedish Research Council Formas. J.N and J.I.J. conceived and designed the experiment. J.N. and L.S. performed the experiment. J.N. analysed the data and wrote the paper. J.I.J. and L.S. reviewed and approved drafts of the paper.

References

Adriaenssens, B. and Johnsson, J.I. 2013. Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. Ecol Lett 16: 47–55

Armstrong, J.B. and Schindler, D.E. 2011. Excess digestive capacity in predators reflects a life of feast and famine. Nature 476: 84–87

Atkins, C.G. 1906. Experiments in fasting of fry. Trans Am Fish Soc 35: 123–142

Bilton, H.T. and Robins, G.L. 1973. The effects of starvation and subsequent feeding on survival and growth of Fulton Channel sockeye salmon fry (*Oncorhynchus nerka*). J Fish Res Board Can 30: 1–5

Blaxter, J.H.S. 1986. Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. Trans Am Fish Soc 115: 98–114

Bohlin, T., Dellefors, C. and Faremo, U. 1984. Early sexual maturation of male sea trout and salmon - an evolutionary model and some practical implications. Rep Inst Freshw Res Drottningholm 63: 17–25

Brown, C. and Braithwaite, V.A. 2004. Size matters: a test of boldness in eight populations o the poeciliid *Brachyraphis episcopi*. Anim Behav 68: 1325–1329

Bujold, V., Cunjak, R.A., Dietrich, J.P. and Courtemanche, D.A. 2004. Drifters versus residents: assissing size and age differences in Atlantic salmon (*Salmo salar*) fry. Can J Fish Aquat Sci 61: 273–282

Burns, J.G. 2008. The validity of three tests of temperament in guppies (*Poecilia reticulata*). J Comp Psychol 122: 344–356

Carter, A.J., Marshall, H.H., Heinsohn, R. and Cowlishaw, G. 2012. How not to measure boldness: novel object and antipredator responses are not the same in wild baboons. Anim Behav 84: 603–609

Clark, C.W. 1994. Antipredator behaviour and the asset-protection principle. Behav Ecol 5: 159–170

Clarke, A. and Johnston, N.M. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J Anim Ecol 68: 893–905

Cutts, C.J., Brembs, B., Metcalfe, N.B. and Taylor, A.C. 1999. Prior residence, territory quality and life-history strategies in juvenile Atlantic salmon (*Salmo salar* L). J Fish Biol 55: 784–794

Dall, S.R.X. 2010. Managing risks: the perils of uncertainty. In: Westneat, D. and Fox, C., eds. Evolutionary Behavioral Ecology. New York, NY: Oxford University Press, Inc. pp. 194–206

Damsgård, B. and Dill, L.M. 1998. Risk-taking behavior in weight-compensating coho salmon, *Oncorhynchus kisutch*. Behav Ecol9: 26–32

Dowling, L.M. and Godin, J.-G.J. 2002. Refuge use in a killifish: influence of body size and nutritional state. Can J Zool 80: 782-788

Elliott, J.M. 1989. The critical-period concept for juvenile survival and its relevance for population regulation in young sea trout, *Salmo trutta*. J Fish Biol 35: 91–98

Elliott, J.M. 2002. Shadow competition in wild juvenile sea-trout. J Fish Biol 61: 1268–1281

Elliott, J.M. and Hurley, M.A. 1998. A new functional model for estimating the maximum amount of invertebrate food consumed per day by brown trout, *Salmo trutta*. Freshw Biol 39: 339–349.

Elliott, J.M., Hurley, M.A. and Fryer, R.J. 1995. A new, improved growth model for brown trout, *Salmo trutta*. Funct Ecol 9: 290–298.

Farwell, M. and McLaughlin, R.L. 2009. Alternative foraging tactics and risk taking in brook charr (*Salvelinus fontinalis*). Behav Ecol20: 913–921

Farwell, M., Fuzzen, M.L.M., Bernier, N.J. and McLaughlin, R.L. 2014. Individual differences in foraging behavior and cortisol levels in recently emerged brook charr (*Salvelinus fontinalis*). Behav Ecol Sociobiol 68: 781–790

Fausch, K.D. 1984. Profitable stream positions for salmonids: relating specific growth rate to net energy gain. Can J Zool 62: 441–451

Gilliam, J.F. and Fraser, D.F. 1987. Habitat selection under predation hazard: test of a model with foraging minnows. Ecology 68: 1856–1862

Godin, J.-G.J. 1997. Evading predators. In: Godin, J.-G.J., ed. Behavioural Ecology of Teleost Fishes. Oxford: Uxford University Press. pp. 191–136

Good, S.P., Dodson, J.J., Meekan, M.G. and Ryan, D.A.J. 2001. Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. Can J Fish Aquat Sci 58: 1187–1195

Gotceitas, V. and Godin, J.-G.J. 1991. Foraging under the risk of predation in juvenile Atlantic salmon (*Salmo salar* L.): effects of social status and hunger. Behav Ecol Sociobiol 29: 255–261

Grant, J.W.A. and Noakes, D.L.G. 1987a. Escape behavior and use of cover by young-of-the-year brook trout, *Salvelinus fontinalis*. Can J Fish Aquat Sci 44: 1390–1396

Grant, J.W.A. and Noakes, D.L.G. 1987b. Movers and stayers: foraging tactics of young-of-the-year brook charr, *Salvelinus fontinalis*. J Anim Ecol 56: 1001–1013

Harris, S., Ramnarine, I.W., Smith, H.G. and Pettersson, L.B. 2010. Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy Poecilia reticulata. Oikos 119: 1711–1718

Héland, M. 1999. Social organization and territoriality in brown trout juveniles during ontogeny. In: Baglinière, J.-L. and Maisse, G., eds. Biology and Ecology of the Brown Trout and Sea Trout. Chichester, UK: Praxis Publishing Ltd. pp. 115–143

Houston, A.I. and McNamara, J.M. 1999. Models of adaptive behaviour: an approach based on state. Cambridge, UK: Cambridge University Press.

Huntingford, F.A. and Garcia de Leaniz, C. 1997. Social dominance, prior residence and the acquisition of profitable feeding sites in juvenile Atlantic salmon. J Fish Biol 51: 1009–1014

Huntingford, F.A., Metcalfe, N.B. and Thorpe, J.E. 1988. Choice of feeding station in Atlantic salmon, *Salmo salar*, parr: effects of predation risk, season and life history strategy. J Fish Biol 33: 917–924

Höjesjö, J., Johnsson, J.I. and Axelsson, M. 1999. Behavioural and heart rate responses to food limitation and predation risk: an experimental study on rainbow trout. J Fish Biol 55: 1009–1019

Höjesjö, J., Adriaenssens, B., Bohlin, T., Jönsson, C., Hellström, I. and Johnsson, J.I. 2011. Behavioural syndromes in juvenile brown trout (*Salmo trutta*); life history, family variation and performance in the wild. Behav Ecol Sociobiol 65: 1801–1810

Johnsson, J.I. 2003. Group size influences foraging effort independent of predation risk: an experimental study on rainbow trout. J Fish Biol 63: 863–870

Johnsson, J.I. and Bohlin, T. 2006. The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proc R Soc B 273: 1281–1286

Johnsson, J.I., Nöbbelin, F. and Bohlin, T. 1999. Territorial competition among wild brown trout fry: effects of ownership and body size. J Fish Biol 54: 469–472

Kortet, R., Rantala, M.J. and Hedrick, A. 2007. Boldness in anti-predator behaviour and immune defence in field crickets. Evol Ecol Res 9: 185–197

Kortet, R., Vainikka, A., Janhunen, M., Piironen, J., and Hyvärinen, P. 2014. Behavioral variation shows heritability in juvenile brown trout Salmo trutta. Behav Ecol Sociobiol 68: 927–934

Krause, J., Loader, S.P., McDermott, J. and Ruxton, G.D. 1998. Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. Proc R Soc Lond B 265: 2373–2379

, J., Loader, S.P., Kirkman, E. and Ruxton, G.D. 1999. Refuge use by fish as a function of body weight changes. Acta Ethol 2: 29–34

Luttbeg, B. and Sih, A. 2010. Risk, resources and state-dependent adaptive behavioural syndromes. Phil Trans R Soc B 365: 3977–90

Magurran, A.E. 1993. Individual differences and alternative behaviours. In: Pitcher TJ, ed. Behaviour of Teleost Fishes, Second edition. London, UK: Chapman and Hall. p. 441–477

McLaughlin, R.L., Grant, J.W.A, and Kramer, D.L. 1994. Foraging movements in relation to morphology, water-column use, and diet for recently emerged brook trout (*Salvelinus fontinalis*) in still-water pools. Can J Fish Aquat Sci 51: 268-279

McLaughlin, R.L., Ferguson, M.M. and Noakes, D.L.G. 1999. Adaptive peaks and alternative foraging tactics in brook charr: evidence of short-term divergent selection for sitting-and-waiting and actively searching. Behav Ecol Sociobiol 45: 386–395

Metcalfe, N.B. and Thorpe, J.E. 1992. Early predictors of life-history events: the link between first feeding date, dominance and seaward migration in Atlantic salmon, *Salmo salar* L. J Fish Biol 41: 93–99

Mikheev, V.N., Metcalfe, N.B., Huntingford, F.A. and Thorpe, J.E. 1994. Size-related differences in behaviour and spatial distribution of juvenile Atlantic salmon in a novel environment. J Fish Biol 45: 379–386

Nielsen, J.L. 1992. Microhabitat-specific foraging behaviour, diet, and growth of juvenile coho salmon. Trans Am Fish Soc 121: 617–634

Nilsson, N.-A. 1956. On the feeding habits of trout in a stream of northern Sweden. Rep Inst Freshw Res Drottningholm 37: 154-166

Näslund, J. and Johnsson, J.I. 2015. State-dependent behavior and alternative behavioral strategies in brown trout (*Salmo trutta* L.) fry. PeerJ PrePrints 3:e1524 doi:10.7287/peerj.preprints.1244v1

Näslund, J., Bererhi, B. and Johnsson, J.I. 2015a. Design of emergence test arenas can affect the results of boldness assays. Ethology 121: 556–565

Näslund, J., Pauliny, A., Blomqvist, D. and Johnsson, J.I. 2015b. Telomere dynamics in wild brown trout: effects of compensatory growth and early growth investment. Oecologia 177: 1221–1230

Pedersen, B.H. 1997. The cost of growth in young fish larvae, a review of new hypotheses. Aquaculture 155: 259–269

Perez, K.O. and Munch, S.B. 2010. Extreme selection on size in the early lives of fish. Evolution 64: 2450–2457

Rasband, W.S. 2011 ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/

Réale, D., Reader, S.M., Sol, D., McDougall, P.T. and Dingemanse, N.J. 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82: 291–318

Rice, J.A., Crowder, L.B. and Binkowski, F.P. 1987. Evaluating potential sources of mortality for larval bloater (*Coregonus hoyi*): starvation and vulnerability to predation. Can J Fish Aquat Sci 44: 467–472

Skoglund, H. and Barlaup, B.T. 2006. Feeding pattern and diet of first feeding brown trout fry under natural conditions. J Fish Biol 68: 507-521

SPSS Inc. 2001. The SPSS TwoStep Cluster Component. A scalable component enabling more efficient customer segmentation. SPSS White paper - technical report. SPSS Inc., USA. 9 pp.

Sundström, L.F., Kaspersson, R., Näslund, J. and Johnsson, J.I. 2013. Density-dependent compensatory growth in brown trout (*Salmo trutta*) in nature. PLoS ONE 8: e63287

Sundt-Hansen, L., Einum, S., Neregård, L., Björnsson, B.Th., Johnsson, J.I., Fleming, I.A., Devlin, R.H. and Hindar, K. 2012. Growth hormone reduces growth in free-living Atlantic salmon fry. Funct Ecol 26: 904–911

Symons, P.E.K. 1968. Increase in aggression and in strength of the social hierarchy among juvenile Atlantic salmon deprived of food. J Fish Res Board Can 25: 2387–2401

Titus, R.G. 1990. Territorial behavior and its role in population regulation of young brown trout (*Salmo trutta*): new perspectives. Ann Zool Fenn 27: 119–130

Toms, C.N., Echevarria, D.J. and Jouandot, D.J. 2010. A methodological review of personality-related studies in fish: focus on the shy-bold axis of behaviour. Int J Comp Psychol 23: 1–25

Vehanen, T. 2003. Adaptive flexibility in the behaviour of juvenile Atlantic salmon: short-term responses to food availability and threat from predation. J Fish Biol 63: 1034–1045

Wissing, T.E. and Hasler, A.D. 1971. Intraseasonal change in caloric content of some freshwater invertebrates. Ecology 52: 371–373

Závorka, L., Aldvén, D., Näslund, J., Höjesjö, J. and Johnsson, J.I. 2015. Linking lab activity with growth and movement in the wild: explaining pace-of-life in a trout stream. Behav Ecol 26: 877–884

Paper 3

State-dependent behavior and alternative behavioral strategies in brown trout (*Salmo trutta* L.) fry

Joacim Näslund [1]
Jörgen I. Johnsson [1]

This manuscript has been submitted for publication in a peer-reviewed academic journal, and a previous version of the manuscript is available as a non-peer-reviewed pre-print:
PeerJ PrePrints 2015, 3, e1524. doi: 10.7287/peerj.preprints.1244v1

Corresponding author: J. Näslund (joacim.naslund@gmail.com)

[1]Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

Abstract

Animals generally adjust their behavior in response to bodily state (e.g. size and energy reserves) to optimize energy intake in relation to mortality risk, weighing predation probability against starvation. Here we investigated whether brown trout adjust their behavior in relation to feeding history (energetic status) and body size during a major early-life selection bottleneck, when fast growth also appear to be important. We manipulated growth using different food ration schemes over two consecutive time periods (P1 = 12 days, P2 = 23 days), excluding social effects through individual isolation. During these experimental periods the fish were fed either high or low food rations in a crossed design. In behavioral trials following the treatment, where acute hunger levels were standardized among all treatments, fish that were initially fed high rations (P1) and thereafter low rations (P2) had on average 15-21% higher swimming activity than the other groups, but large within-treatment variation rendered only weak statistical support for the effect. Furthermore, fish on low ration during P2 tended to be more aggressive than fish on high ration. Size was related to behavioral expression, with larger fish being more active and aggressive. Swimming activity and active aggression were positively correlated, forming a behavioral syndrome in the studied population. Based on these behavioral traits we could also distinguish two behavioral clusters, one consisting of more active and aggressive individuals, and the other consisting of less active and aggressive individuals. This indicates that two behavioral strategies may exist in young brown trout.

Keywords: animal personality, behavioral syndrome, compensatory growth, food restriction, mirror aggression, open-field activity, repeatability

Introduction

Food restriction reduces body condition in animals, which in severe cases may lead to energy depletion and death from starvation. Thus, it is likely that food restriction alters the animal's behavior to reduce the risk of starvation, a pattern which has been observed in many different taxa. For instance, green sea turtles *Chelonia mydas* in poor body condition select more profitable, but also more risky, foraging areas than turtles in good body condition (Heithaus et al. 2007). Similarly, Atlantic salmon *Salmo salar* juveniles subjected to restricted feeding become less risk averse than well fed conspecifics, as shown by their increased diurnal activity out of shelter (Orpwood et al. 2006).

In growing animals, food restriction commonly leads to higher than normal foraging rates (hyperphagia) when food becomes available again, which aids in compensatory growth of the body (Ali et al. 2003; Dmitriew 2011). The occurrence of hyperphagia and compensatory growth following starvation suggests that growth rates are generally below their maximal levels under normal energetic conditions (Arendt 1997; Ali et al. 2003). The effects of food restriction on behavior are generally thought to be

linked to the production-mortality trade-off hypothesis where behavior adjusts the foraging intensity optimally to minimize mortality risk (Gilliam and Fraser 1987; Fiksen and Jørgensen 2011). This trade-off incorporates two main feedback systems (Luttbeg and Sih 2010; Sih et al. 2015). On the one hand there is the negative 'starvation-threshold' feedback system consisting of starvation avoidance (SA) at the one end, and asset protection (AP) at the other (Sih 1980; Lima 1986; Pettersson and Brönmark 1993; Clark 1994; Heithaus et al. 2007; Luttbeg and Sih 2010). These feedback mechanisms (SA-AP) mean that lower-asset individuals (i.e. with relatively low predicted fitness, e.g. small body size or low energy reserves) will accept higher risks, because they need to increase their assets, while higher-asset individuals can afford to avoid risk at the expense of some of their assets (e.g. energy reserves). On the other hand there is a positive feedback mechanism based on state-dependent safety (SDS) (Clark 1994; Luttbeg and Sih 2010). Here, the high asset values (e.g. large energy reserves or body size) lead to higher competitive ability, and reduce risks due to predator gape-limits or increased vigor (Mittelbach 1981; Peterson and Wroblewski 1984; Werner and Gilliam 1984; Travis et al. 1985; but see Lima 1986). The influence of these feedback systems could differ in strength in different environmental contexts (e.g. depending on population density, predator abundance, predator guild composition, or ontogenetic time constraints) (e.g. Ludwig and Rowe 1990). SDS and SA-AP may be elicited together, e.g. with lager individuals being more safe than smaller (SDS), but with SA-AP acting within each size class. If SA-AP is strong, then studies on individual behavioral consistency (a component of animal personality; see e.g. Bell 2007) need to take bodily state into account. Here, we investigate the relationships between bodily state (energy state as manipulated by recent feeding history, as well as body size) and behavior in young juvenile brown trout *Salmo trutta*. We also investigated the occurrence of personality in our experimental individuals.

Our primary aim was to investigate state-dependent behavior in young individuals. Like in many other animals with high fecundity, the early juvenile stage of brown trout is a major selective bottleneck (e.g. Degerman et al. 2001). Thus, the early life of trout is typically described as a critical period where individuals need to grow rapidly regardless of bodily state, due to selection against small sized individuals through predation or competition (Elliott 1990; Perez and Munch 2010). To explore whether or not these fish adjust their growth and behavior in relation to their bodily state, we manipulated food rations of individual trout and subsequently scored their behavior in standardized laboratory tests. Specifically, we tested effects

of food ration on swimming activity, boldness and aggression. Activity and boldness were assumed to be related to risk taking, and aggression have been found to be important to obtain a territory, which is beneficial for foraging efficiency (Elliott 1990, 2002; Johnsson and Björnsson 1994; Johnsson et al. 1999). In line with studies on older stages of salmonid fish (e.g. Johnsson et al. 1996; Nicieza and Metcalfe 1997; Höjesjö et al. 1999; Vehanen 2003; Orpwood et al. 2006), expression of these behaviors was predicted to be relatively higher in low-asset fish (i.e. fish being starved), as foraging would be important to regain lost body growth for these individuals. Particularly, we predicted that the group being initially starved and subsequently re-fed for a short period of time would have the highest activity, boldness and aggression, as these fish were assumed to be in the midst of a compensatory growth phase. Compensatory growth has been observed repeatedly in older juveniles of brown trout from the same population as used in this study (Johnsson and Bohlin 2006; Sundström et al. 2013; Näslund et al. 2015a). Alternatively, the trout fry may mainly follow SDS, with larger fish being more active, aggressive and bold. A strong SDS effect could possibly drive all fish to maximize their individual capacity to express these behavioral traits, since the larger they get the safer and more competitive they are. Indeed, some studies indicate that young fish are maximizing growth with little capability to increase their foraging efforts (Pedersen 1997; Conceição et al. 1998; Peck et al. 2014). In contrast to many previous studies, we standardize acute hunger levels, to measure effects of energetic state only.

Our second aim of this study was to investigate whether our fish showed personality traits. Specifically we investigated whether they were consistent in their behavioral expression, whether different behavioral characters were correlated (indicative of behavioral syndromes, see Sih et al. 2004), and whether these characteristics of personality were related to bodily state (energetic state or body size). Distinct personalities are generally assumed to be the behavioral expressions of general life-history strategies (Stamps 2007; Réale et al. 2010). We predicted that behaviors would be correlated and repeatable, in line with previous studies of yearling brown trout (Höjesjö et al. 2011; Hoogenboom et al. 2012; Adriaenssens and Johnsson 2013; Kortet et al. 2014), with an explorative approach regarding the relationship with bodily state.

Our third aim of this study was to investigate whether brown tout fry group into separate behavioral strategies, or into a single continuum of behavioral expression. It has been suggested that there are two, more or less distinct, behavioral strategies adopted by emerging salmonid fry

(Héland 1999; Skoglund and Barlaup 2006). One behavioral strategy is to quickly establish and actively defend a territory (active and aggressive strategy), while the other is to hide and nocturnally disperse downstream from the nest and away from the main area of competition (passive and shy strategy). These strategies have been suggested to be independent of social environment, as the passive strategy has been observed also in isolated fish, i.e. in absence of a social hierarchy (Héland 1999). Generally, personality traits are often discussed as if they in a dichotomous way (e.g. fast vs. slow pace-of-life [Réale et al. 2010]; proactive vs. reactive coping style [Koolhaas et al. 1999]), but few studies investigate the actual distribution of behavioral traits in a population. Given the previous descriptions of brown trout fry behavior, being either active or passive, we predicted that behavioral clusters exist.

Materials and methods

Study population characteristics

We used fish from a natural population of sea trout, the anadromous form of the brown trout, from the coastal stream Norumsån in Sweden (N58° 2.589', E11° 50.759'). The adult sea trout spawns in rivers in late autumn, the eggs hatch in early spring, and fry emerges from the gravel in late spring (May-June) (Degerman et al. 2001). At this point the fry emerge to start to feed and establish territories (Elliott 1994; Héland 1999). In Norumsån, juveniles normally stay in the stream for one or two summers before migrating to the sea the following spring, typically at a size of 70 - 160 mm (Bohlin et al. 1993, 1996). However, depending on body condition in the previous year, up to half of the one-year old males, and a lower proportion of females, mature precociously and stay as stream residents (Dellefors and Faremo 1988; Bohlin et al. 1994). Thus, restricted growth at early stages may have extensive effects on life-history decisions.

Capture and housing

We captured 144 recently emerged fry on one of the stream's main spawning grounds on June 5, 2012, using electrofishing (L-600; Lug AB, Sweden; straight DC, 200-300 V) and brought them to the laboratory. All fish were initially put in one 70 l holding aquarium, equipped with sand and plastic fanwort plants, for seven days. During this time we supplied the fish with thawed chironomid larvae, approximately 5-10 larvae per fish and day.

Table 1: Food rations for the treatment groups during the experiment.

Day of experiment		Number of chironomids per fish per day			
		HH	HL	LH	LL
0		5	5	5	5
1-12		10	10	2	2
13-17		10	2	10	2
18-27		12	3	15	3
28-35		12	4	18	4
36*		Satiation	Satiation	Satiation	Satiation
37*	Trial 1	12	4	18	4
38*		Satiation	Satiation	Satiation	Satiation
39*	Trial 2	-	-	-	-
Total (1-35)		386	192	368	96
% of HH ration (1-35)		100%	50%	95%	25%

* Behavioral trial period

During treatments fish were housed individually in ten 55 l polypropylene storage boxes with removable transparent lids (Nordiska Plast, Sweden), each modified to contain 12 equally sized compartments (bottom area: 100 × 150 mm; water depth: 100 mm; see drawing in electronic supplement, Fig. S1). Water (average temperature: 11.5°C; range: 10.3 - 11.9°C) flowed through all compartments, supplied by the in-house circulating system. All compartments had 5 mm of sand as bottom substrate. During the experiment the boxes were covered with lids to prevent escape by jumping. Rearing boxes were lit by fluorescent tubes with the armature covered by black garbage bags to reduce light intensity (illuminance above the boxes was ca. 100 lux).

Food manipulation (treatment)

At the start of the experiment the fish were randomly split into two feeding groups ($n = 60$): high food ration (H) and restricted food ration (L); see Table 1. These rations were given over 12 days (Period 1). At the end of Period 1, twelve fish had died (H: 4; L: 8). Furthermore, eight fish which had been on high ration but lost in mass were removed from the experiment as they did not fulfil the criteria for the treatment (i.e. being well fed). The two feeding groups were split in half by random assignment of the remaining fish, creating two sub-groups from each initial feeding group. One sub-group from each initial feeding group was given high food rations, and the other sub-groups were given restricted rations, see Table 1. These latter rations were provided over 23 days (Period 2). This resulted in four treatment groups (n denote final sample size): (i) continuous high food ration (HH; $n = 23$); (ii) continuous restricted food ration (LL; $n = 21$); (iii) initially high food ration, switched to restricted food ration (HL;

$n = 23$); and (iv) initially restricted food ration, switched to high food ration (HL; $n = 22$). The supplied food consisted of thawed chironomid larvae (Akvarieteknik, Sweden). Chironomids constitute a major part of the natural food eaten by brown trout at the early fry stage (Nilsson 1956; Skoglund and Barlaup 2006). Food rations were the same for all fish within a treatment. Thus, the smallest fish received slightly more food relative to their mass than the larger fish, but the maintenance rations should regardless represent a very restricted food intake for all fish. Food rations were based on a previous experiment (Näslund et al. 2015b) and during the course of the experiment the rations were adjusted for growth and bodily condition of the fish, based on daily visual inspection (Table 1). Leftover bloodworms were removed using a disposable pipette the day after each feeding before the provision of new food; the pipette was also dipped in compartments without leftovers to standardize disturbance. The food manipulation lasted for 35 days.

Growth monitoring

We recorded wet mass (precision: 0.01 g; Kern EW 3000-2M, Kern & Sohn GmbH, Germany) and took digital photographs (Canon EOS 40D; lens: EF-S 17-85 IS USM [at 70 mm focal length]; Canon Inc., Japan) of all fish at three time points: (i) the day before the start of the food manipulation (day 0; June 9); (ii) the day we switched the food ration for the HL and LH groups (day 12); and (iii) the day prior to the last day of food manipulation (day 34). Mass was recorded before feeding, leaving fish unfed for 24 h prior to the weighing. From the digital photographs we measured fork length (from the tip of the snout to the end of the central caudal fin ray; precision: 0.1 mm) using ImageJ 1.45 (http://imagej.nih.gov/ij/). During handling the fish were anaesthetized with 2-phenoxyethanol ($0.5 \text{ ml} \cdot \text{l}^{-1}$).

Growth rate in wet mass (M) was analyzed as specific growth rate (SGR_M; % change per day):

$$SGR_M = 100 \cdot (ln(M_{t_1}) - ln(M_{t_0})) \cdot (t_1 - t_0)^{-1} \quad (1)$$

where t_0 and t_1 are the initial and final time-point in days, respectively.

Growth rate in fork length (L) was analyzed as absolute growth rate (AGR_L; mm per day):

$$AGR_L = 100 \cdot (L_{t_1} - L_{t_0}) \cdot (t_1 - t_0)^{-1}. \quad (2)$$

Growth analyses

Abbreviations for models, dependent variables and factors are found in Table 2.

Initial and final size (fork length and wet body mass) was analyzed using a GLMM (Gaussian distribution, identity link function) with the factors TR and DATE and their interaction TR × DATE. Growth was analyzed separately for Period 1 and Period 2 using GLM (Gaussian distribution, identity link function), including TR and FL at the start of each period. The interaction TR × FL was tested for significance in all growth analyses, but sequentially removed if there was low evidence for effects of this term (i.e. $p > 0.1$).

One LL fish grew substantially faster than all other LL ($SGR_M = 1.9\%$; cf. Fig. 1b) fish during the second experimental period, and was removed from all analyses investigating treatment effects, as it was likely erroneously fed.

Behavioral trials

Behavioral trials were conducted on the second (trial 1; day 36) and fourth (trial 2; day 38) day after the end of the feeding treatment. To measure

Table 2: Descriptions of abbreviations used to describe statistical analyses.

Statistical methods	
LM	Linear model
GLM	Generalized linear model
GLMM	Generalized linear mixed model
ICC	Intraclass correlation
PCA	Principal component analysis
Dependent variables	
SGR_M	Specific growth rate in wet mass (% per day)*
AGR_L	Absolute growth rate in fork length (mm per day)*
$Act1/Act2$	Swimming activity score, trial 1/trial 2**
$Boldn1/Boldn2$	Boldness score, trial 1/trial 2**
$AAggr1/AAggr2$	Active aggression score, trial 1/trial 2**
$PAggr1/PAggr2$	Passive confrontation score, trial 1/trial 2**
Independent factors	
TR	Food treatment***
	Categorical between-subject factor (fixed; four levels)
FL	Fork length (mm) at the time of the trials
	Continuous factor
DAY	Trial day****
	Categorical within-subject factor (fixed; two levels)
DATE	Date of size-measurement*****
	Categorical within-subject factor (fixed; three levels)

* see Materials and methods: Growth monitoring
** see Materials and methods: Behavioral analyses
*** see section "Food manipulation" in Materials and methods
**** see section "Behavioral trials" in Materials and methods
***** see section "Growth monitoring and analyses" in Materials and methods

effects of bodily state rather than immediate hunger effects, all fish were fed once to satiation the day before trials. On trial days fish were given rations corresponding to the final feeding treatment rations after the trial. On each trial day, single fish were put into opaque white trial arenas (area: 28 × 19 cm, water level: 5 cm; Slugis, Ikea, The Netherlands), where behavior was recorded from above, using web-cameras (Creative VF0520; Creative Labs, Singapore) mounted on the ceiling. Nineteen fish could be recorded simultaneously. Over one trial, water temperature in the trial arena generally rose from 12.0 – 12.3°C to 13.7 – 14°C.

Trial protocol

Three consecutive behavioral tests (modified versions of the tests used in Adriaenssens and Johnsson 2013) were conducted on each trial day. First the fish were left to swim around in the barren white environment for 15 minutes (*forced open-field test*). Secondly, we lowered down a novel object (trial 1: M6 hardware nut glued to a red 10 × 10 mm plastic bead; trial 2: stainless steel screw 3 × 10 mm) into one corner of the arena, using a clear nylon line attached to the object, and left the fish for another 15 min (*novel-object test*). Lastly, we put a mirror into the container (hiding the novel object behind the mirror) into one of the short sides of the container and let the fish interact with the mirror image for 10 min (*mirror-aggression test*), after which the trial ended and the fish was put back into its home tank.

Behavioral analyses

Behavior was scored manually from recorded videos using Adobe Premier CS3 (Adobe Systems Inc., USA). Abbreviations for statistical models, dependent variables and independent factors are found in Table 2.

In the open-field test we scored swimming activity (*Act1/Act2*). The trial arena was divided by lines into a grid of 12 equal-sized rectangles (70 × 63.3 mm; Fig. 2a). The number of line-crossings, between the 10th and the 15th minute after release into the arena, was recorded as a measure of activity. The whole body had to cross the line to count as a crossing.

In the novel-object test we scored boldness (*Boldn1/Boldn2*). Four zones were delimited (Fig 2b), based on the distance to the novel object: zone 1 (0 – 84 mm distance), zone 2 (85 – 169 mm distance), zone 3 (170 – 254 mm distance), and zone 4 (> 255 mm distance). The location of the head of the fish was scored every tenth second between the 10th and the

15th minute after the novel object was put into the arena. The mean score was used as a measure of boldness.

In the mirror-aggression test we scored aggression toward a mirror image. A "confrontation-zone" was delimited at 3 cm distance from the mirror (Fig. 2c). If the fish was inside this zone with its head, it was scored as confronting the mirror reflection, except for when the body was facing away from the mirror at an angle of $> 45°$. If the fish was swimming actively against the mirror, or swimming towards the mirror at an angle of $> 45°$ inside the zone, the behavior was scored as being actively aggressive (*AAggr1/AAggr2*). If the fish was inside the zone but not moving, or being faced toward the mirror at an angle $\leq 45°$, or $\leq 45°$ away from the mirror, the behavior was scored as passive confrontation (*PAggr1/PAggr2*). For a graphical illustration of the definitions, see Fig. $2c_2$. These behaviors were scored every tenth second between the 5th and the 10th minute after the mirror was inserted into the arena.

In all cases, lines and zones used to score behavior in the trial arenas were drawn on plastic film which was put on the computer LCD-monitor when analyzing the recorded films. Statistical analyses were conducted in SPSS 22 (IMB Corp., USA), if not stated otherwise.

Behavioral scores from each test were analyzed using GLMMs (covariance type: compound symmetry; robust covariance estimates; residual method for degrees of freedom estimation). Factors included in the models were TR, DAY and FL. Initially we also included the interactions TR $\times$ DAY and TR $\times$ FL, but these interactions were not significant in any of the analyses (all $p > 0.2$) and therefore removed from the final models. Pairwise contrasts for fixed factors were checked if $p \leq 0.1$. From the results of the *AAggr*-GLMM, a pattern occurred where fish ending on low ration seemed to be more aggressive. As an ad-hoc analysis, we pooled the TR-levels HH and LH, and HL and LL, and ran the model again. In addition, as there was substantial variation in growth rate within treatment groups, we conducted complimentary analyses where we modelled behavioral scores as linear functions of specific growth rate during Period 2, without including treatment group as a factor.

Repeatability of the scored behaviors was analyzed by ICC, using the PSYCH package in R 3.0.3 (R Core Team, 2014). Correlations among different behaviors in the different tests (Table 3) were used to combine data into principal components in a PCA. However, as the novel-object test did not appear to result in any informative behaviors with respect to boldness (see electronic supplement, Section 2), *Boldn1* and *Boldn2* were not included in the PCA. It can be noted that, if included, these variables would

load in a separate component, *Boldn1* positively and *Boldn2* negatively (data not shown). Out of the confrontation scores, we chose to include only *AAggr1/AAggr2* in the PCA (see Results: Aggression for details). The component obtained from the PCA, including *Act1*, *Act2*, *AAggr1* and *AAggr2*, was analyzed using a GLM (Gaussian distribution, identity link-function), including TR and FL; the interaction was initially included, but removed in the final analysis as it was non-significant ($p = 0.3$).

To investigate whether distinct behavioral groups could be discerned, we used two-step cluster-analysis (distance measure: log-likelihood), set to automatically categorize a number of clusters (maximally five). The cluster analysis was based on the variables *Act1*, *Act2*, *AAggr1* and *AAggr2*. Detected clusters were analyzed using binomial GLM (logit link-function), including TR and FL. Furthermore, to investigate whether detected clusters were set already prior to the experiment, we analyzed the cluster assignment using a binomial GLM with only initial body size (i.e. fork length prior to the onset of the feeding treatments) as a factor.

Ethical note

The experimental procedures were approved by the Ethical Committee on Animal Experiments in Gothenburg, Sweden (ethical license number 8-2011). Food rations where continuously assessed for adequacy with respect to fish survival, based on visual inspection of fish condition, behavior and mortality. Although most fish fed on the provided food from the first day in the lab, some fish never started to feed. Such failure of feeding in some young salmonid fry (so called "pin-heads") is commonly noted in lab and hatchery environments.

Results

Growth

The initial mean sizes of HL and LL groups were slightly, but significantly, larger than the HH and LH groups and as a consequence there was no significant differences among groups in size at the end of the treatment (wet mass: Fig. 1a, Table S2, S3; fork length: Fig. 1d, Table S6, S7). During Period 1 the growth rates were faster for fish on high ration; in general high ration fish showed positive growth, while low ration fish showed negative growth (SGR_M, Fig. 1b, Table S4; AGR_L: Fig. 1e; Table S8). During Period 2, all treatment groups differed in SGR_M, with the LH group

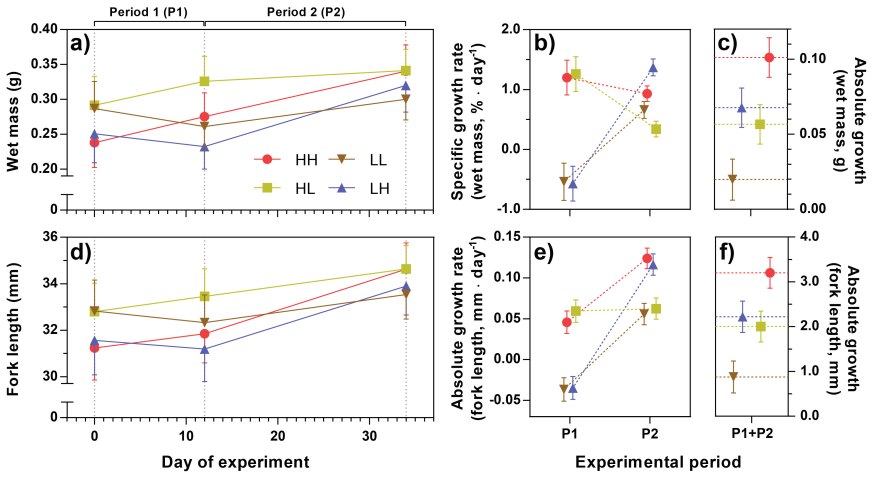


Figure 1: Growth patterns for the experimental fish: (a) mean wet mass; (b) specific growth rate in mass, adjusted for initial size; (c) absolute growth in mass over the experiment, adjusted for initial size; (d) mean fork length; (e) absolute growth rate in fork length, adjusted for initial size for P2); (f) absolute growth in fork length over the experiment, adjusted for initial size. Error bars show 95% confidence intervals. Detailed statistics are found in the electronic supplement (Section 3). For details on treatment groups (HH, HL, LH and LL) see Table 1.

growing at the fastest rate: LH > HH > LL > HL (Fig. 1b, Table S4). For AGR_L in Period 2, the high ration groups grew faster than low ration fish: HH ≈ LH > HL ≈ LL (Fig. 1e, Table S8). Looking at the absolute growth over the whole experiment (Period 1 + Period 2), HH grew most rapidly, followed by LH and HL, and LL grew slowest (wet mass: Fig. 1c, Table S5; fork length: Fig. 1f, Table S9).

Confidence intervals are presented for evaluation of treatment effects (Fig. 1); for detailed results of GLMs and GLMMs, along with contrast estimates and their p-values, see electronic supplement (Section 3, Table S2-S9).

Open-field activity

Body size had a significant effect on swimming activity, where larger fish were more active (FL: $F_{1,172} = 28.879$; $p < 0.001$) (Fig. 2g). In the GLMM, treatment was not a significant factor (TR: $F_{3,172} = 2.115$; $p = 0.100$), but pairwise contrasts suggested that the HL group tended to be more active (HL vs. LH [21% higher]: $p = 0.036$; HL vs. HH [17% higher]: $p = 0.079$; HL vs LL [15% higher]: $p = 0.076$) (Fig. 2d). Trial day had no significant

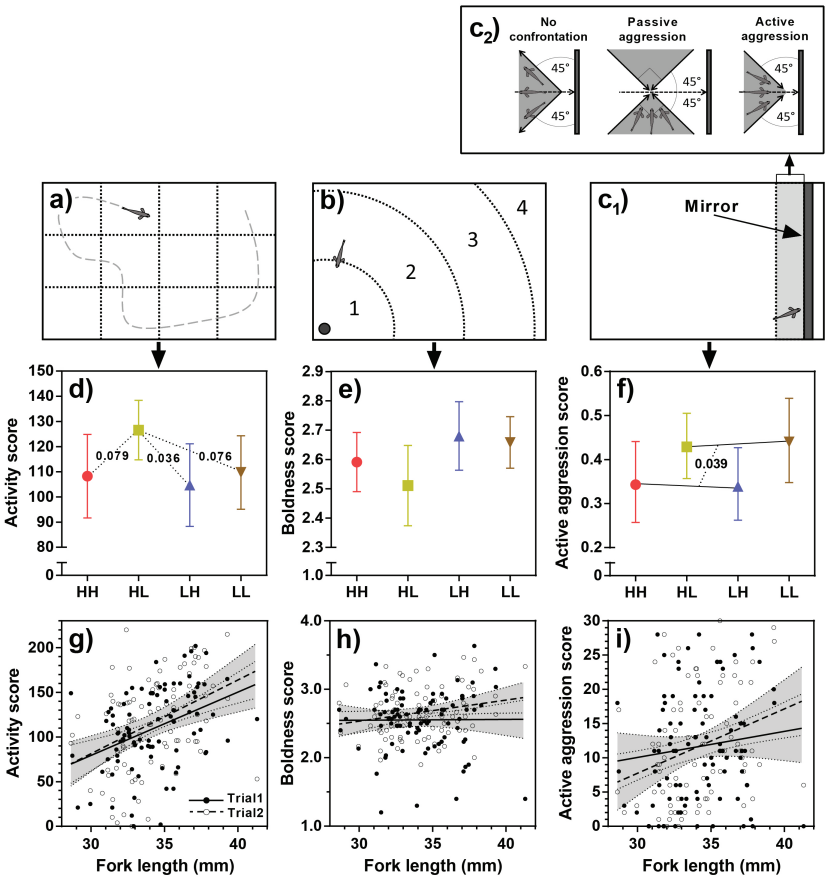


Figure 2: Results from the behavioral trials. First panel-row (a-c): top-view schematic illustrations of the behavioral arenas for (a) forced open-field test, (b) novel object test (numbers indicate distance-zones, as described in Materials and Methods), and (c1) mirror aggression test (dark grey zone: mirror; light grey zone: "confrontation zone"). Definitions of aggression based on fish position relative to the mirror within the confrontation zone are shown in c_2. Second panel-row (d-f): estimated means, with 95% confidence intervals, based on the GLMMs (i.e. combining both behavioral trials) for (d) activity score (significant and trend contrasts connected with dotted lines and p-values), (e) boldness score, and (f) active aggression score (dotted line indicate significant difference in ad hoc analysis combining HH and LH, and LH and LL, along with p-value). Third panel-row (e-i): body size effects on (g) activity score, (h) boldness score, and (i) active aggression score. Gray areas show 95% confidence limits. For details on treatment groups (HH, HL, LH and LL) see Table 1.

effect (DAY: $F_{1,175} = 1.205$; $p = 0.274$). Regression analyses indicated that there were negative effects of specific growth rate on activity during Period 2 (see electronic supplement, Fig. S2).

Swimming activity was generally repeatable (Table 4). However, re-

peatability seemed to be higher for HL and LH fish than for HH and LL, albeit with overlapping confidence intervals for ICC.

Novel-object boldness

No significant treatment effect was detected (TR: $F_{3,172} = 1.446$; $p = 0.231$) (Fig. 2e), neither was there any effect of body size (FL: $F_{1,172} = 2.236$; $p = 0.137$) (Fig. 2h). Fish tended to be slightly further away from the novel object on the second trial day compared to the first trial day (DAY: $F_{1,172} = 3.092$; $p = 0.080$). Regression analyses did not indicate any effects of specific growth rate during Period 2 of the feeding period ($R^2 \leq 0.02$, $p > 0.18$).

Individual boldness scoring was not found to be repeatable between the two trial days (Table 4).

In general, boldness scoring was found to be largely reflecting a random swimming pattern for most individuals; i.e. for the majority of the individuals, the number of times a fish was found in each zone did not deviate from what was expected based on the size of each zone (for analyses and further discussion see electronic supplement, Section 2).

Mirror aggression

Total confrontation levels towards the mirror (i.e. *AAggr + PAggr*) were generally very high and close to the maximum score (electronic supplement Fig. S3), leading to the *PAggr1* and *PAggr2* being largely complementarily, negatively correlated, to *AAggr1* and *AAggr2* respectively (this is the reason why we only included *AAggr* in the PCA and why we only report results on *AAggr*; for illustration of *PAggr* scores see electronic supplement, Fig S3,). For active aggression scores, no significant effects were detected for treatment (TR: $F_{3,172} = 1.464$; $p = 0.226$) or trial day (DAY: $F_{1,172} = 0.001$; $p = 0.974$) (Fig. 2f). Larger fish were more aggressive (FL: $F_{1,175} = 5.913$; $p = 0.016$) (Fig. 2i). Pooling fish with respect to the ration given during the second feeding period (i.e. HH+LH, and HL+LL) revealed that fish reared on low ration during the second experimental period were more aggressive (TR_{Pooled}: $F_{1,174} = 4.345$; $p = 0.039$) (Fig. 2f). Regression analyses indicated that there negative effects of specific growth rate on aggression during Period 2 of the feeding period (see electronic supplement, Fig. S2).

Active aggression was repeatable overall (Table 4). However, repeatability seemed to be higher for HH and LL fish than for HL and LH, albeit with overlapping confidence intervals for ICC.

Table 3: Relationships among behavioral variables. Pearson correlation coefficient r (left table, below diagonal); significance p (left table, above diagonal); principle component analysis summary (right table). *Act*: swimming activity; *AAggr*: active aggression; *PAggr*: passive confrontation; *Boldn*: boldness; 1: first trial; 2: second trial. Significant correlations are marked bold.

Correlation matrix									Principal component analysis	
N=90	*Act1*	*Act2*	*AAggr1*	*AAggr2*	*PAggr1*	*PAggr2*	*Boldn1*	*Boldn2*	Communalities	Factor loadings
Act1	—	***	**	NS	¤	NS	NS	¤	0.499	0.706
Act2	**0.439**	—	**	**	*	NS	NS	NS	0.594	0.771
AAggr1	**0.335**	**0.290**	—	**	***	¤	NS	NS	0.462	0.680
AAggr2	0.172	**0.363**	**0.300**	—	¤	***	NS	NS	0.401	0.633
PAggr1	-0.180	**-0.224**	**-0.507**	-0.187	—	*	NS	NS	—	—
PAggr2	-0.077	-0.131	-0.187	**-0.464**	**0.233**	—	NS	NS	—	—
Boldn1	-0.043	0.173	0.038	-0.030	-0.074	0.072	—	NS	—	—
Boldn2	0.192	-0.003	0.151	-0.003	-0.023	0.140	-0.058	—	—	—

¤ = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = not significant, $p > 0.1$.

Table 4: Repeatability of behaviors as indicated by the intraclass correlation coefficient (ICC). N: sample size; F: F-statistic. Numbers within brackets denote 95% confidence interval of ICC. Significant ICC are bold. For details on treatment groups (HH, HL, LH and LL) see Table 1.

		Activity		Boldness		Active aggression	
	N	ICC	F	ICC	F	ICC	F
Overall	90	**0.43*****	2.5	-0.066	0.88	**0.30****	1.9
		(0.25 – 0.58)		(-0.27 – 0.14)		(0.11 – 0.48)	
HH	23	0.25	1.7	-0.31	0.53	**0.48****	2.9
		(-0.16 – 0.60)		(-0.63 – 0.11)		(0.11 – 0.74)	
HL	23	**0.59*****	3.9	0.062	1.1	0.048	1.1
		(0.25 – 0.80)		(-0.35 – 0.45)		(-0.36 – 0.44)	
LH	22	**0.68*****	5.3	0.033	1.1	0.23	1.6
		(0.38 – 0.85)		(-0.38 – 0.44)		(-0.20 – 0.59)	
LL	21	0.22	1.5	-0.078	0.86	**0.47***	2.8
		(-0.22 – 0.59)		(-0.48 – 0.35)		(0.072 – 0.75)	

* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

Principal component analysis

In the PCA we extracted one single component (PC1), as judged from both Cattell's scree test and the Kaiser–Guttman criterion (eigenvalue > 1). All included variables loaded positively on PC1 (see correlation matrix, communalities and factor loadings in Table 3). Thus, higher values of swimming activity and active aggression were represented by higher values of PC1. PC1 explained 48.9% of the variation in the included data and the eigenvalue was 1.96. Sampling adequacy as indicated by the KMO-test (0.649) and Bartlett's sphericity-test ($p < 0.001$) was regarded as acceptable, but results should be treated with some caution due to the KMO-value being < 0.7 (following Budaev 2010).

Given the factor loadings from the PCA (Table 3), PC1 is indicating the presence of a behavioral syndrome between swimming activity and active aggression in the subject fish. The PC1 scores were not significantly different among treatments (TR: Wald $\chi^2 = 5.9$; $df = 3$; $p = 0.117$), but higher scores were associated with longer bodies (FL: Wald $\chi^2 = 20.235$; $df = 1$; $p < 0.001$) (Fig 3b), indicating that larger fish were more active and aggressive.

Cluster analysis

Two behavioral groups were detected in the cluster analysis. In general, lower activity and lower aggression were associated with one cluster (Cluster A, 44.9% of individuals, Fig. 3a), and higher activity and higher aggression were associated with the other cluster (Cluster B, 55.1% of individuals, Fig. 3a). In concordance with the other results on activity and aggression, larger body size increased the probability of being assigned to Cluster B

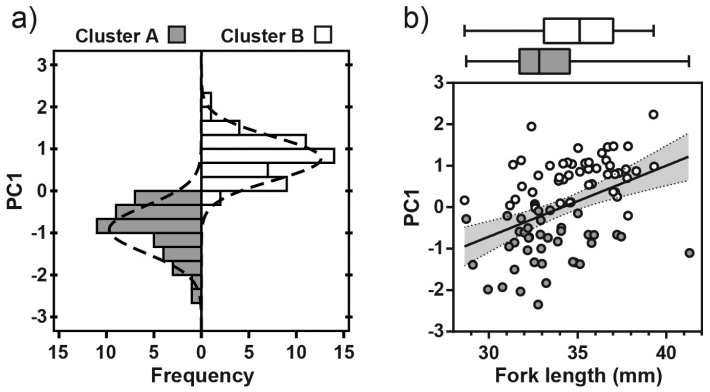


Figure 3: Clustering of behavioral types: (a) distribution of individuals into the two clusters in relation to their score of the extracted principal component, PC1 (Cluster A = less active and less aggressive; Cluster B = more active and more aggressive); (b) relationship between PC1 and body size (fork length). Box-plots on top of the graph show the fork length of the two clusters; box hinges show the first and third quartile, the line inside the box shows the second quartile (median), and the whiskers show minimum and maximum values. Regression line with 95% confidence interval is shown for both clusters combined. Grey = Cluster A; White = Cluster B.

(Fig. 3b) (FL: Wald $\chi^2 = 10.685$; $df = 1$; $p = 0.001$). Treatment group did not affect the probability of being assigned to a particular cluster (TR: Wald $\chi^2 = 3.552$; $df = 3$; $p = 0.314$). Behavioral clusters were defined already prior to the onset of the experiment, as initial fork length alone was a significant predictor of cluster assignment (Wald $\chi^2 = 11.520$; $df = 1$; $p = 0.001$, see Fig. S4).

Discussion

The results presented here provide some evidence for state-dependent behavior in brown trout fry, but not following the pattern we predicted. We predicted that the LH group (initially starved and subsequently re-fed at high rations and assumed to have entered a compensatory growth phase) would be more active due to being in a hyperphagic state, but this effect could not be confirmed. Instead we found that the treatment group with a negative change in food ration in Period 2 (HL) tended to be more active in the open-field test than the other groups. We also found that food restricted fish in Period 2 (i.e. HL + LL pooled) showed higher levels of active aggression than fish fed high rations. This is in contradiction to Hoogenboom et al. (2012), who detected no effects among trout of similar age. However, the fish in their study were scored in groups which may

have affected aggression levels of subordinate fish. Nicieza and Metcalfe (1997) showed that older juveniles of Atlantic salmon increased aggression after being food restricted, in line with our findings. The prediction that initially starved and subsequently re-fed fish should be more aggressive than all other groups was not realized. Both activity and aggression were negatively correlated with growth rate during Period 2, albeit with relatively low R^2 values, indicating large inter-individual variation (see Fig S2, electronic supplement). Smaller trout in general have faster growth rate (Jonsson and Jonsson 2011), as long as they are not being suppressed by dominant individuals (e.g. Brown 1957). Here, smaller fish were indeed growing faster, as expected by the fact that the fish were reared without competition for food. The finding that larger individuals were generally more active and more aggressive indicates that larger fish are more likely to belong to a more territorial behavioral type (i.e. Cluster 2 in this study, see further discussion below).

No effects were detected for the behavior in the novel-object test. In fact, this test seemed to be largely uninformative in the way it was carried out here (see electronic supplement, section 2). It should be noted here, that other designs of novel-object tests for recently emerged brow trout fry have proved to be useful (e.g. Sundström et al. 2004). Overall the effects of treatment appeared to be relatively small, compared to the general behavioral expression, in agreement with another recent study on the same life-stage of brown trout from the same population (Näslund et al. 2015b).

The overall pattern of our results suggest that the scope for adjustments of behavior is limited in brown trout fry, which further suggests that fry are under general pressure to attain a larger size, to avoid predation and increase competitive ability. Similar results have been obtained for juvenile stages of other fish species (e.g. Peck et al. 2014), as well as for larval insects (Brodin and Drotz 2011). Early survival of brown trout is largely dependent on whether the fish can attain a territory or not during a critical period, which corresponds to the experimental period for this study, and is negatively influenced by increased population density (Elliott 1990). It should be noted, that the fish were not stimulated by any predator models during trials, and thus the conclusion that state-dependent safety is of large importance for the trout fry behavior may be less valid when individuals perceive direct predation risk. Other studies have shown that salmonid juveniles (slightly larger than our trout) rely on asset protection, i.e. larger fish take fewer risks, when directly attacked by model predators (Reinhardt and Healey 1999).

The brown trout fry showed individual consistencies in swimming ac-

tivity and aggression at similar levels as previously reported for this species (Hoogenboom et al. 2012; Adriaenssens and Johnsson 2013; Kortet et al. 2014). Interestingly, the treatment groups tended to differ in repeatability of these traits. Regarding activity, the groups which experienced a switch in their food ration (HL and LH) showed relatively stronger repeatability than the stable ration groups (HH and LL). Repeatability in the latter two groups was not statistically significant, although showing similar patterns as the former two groups. Previous studies have shown that environmental factors can affect the strength of personality traits (e.g. behavioural syndromes being stronger in the presence of a predator; Bell and Sih 2007) and cognitive abilities (e.g. higher ability when food rations have changed during the juvenile stage; Kotrschal and Taborsky 2010). Possibly, stability of food ration may affect the consistency of behavioural traits. Further investigation into the strength of repeatability in different environments is warranted.

Activity and aggression were generally positively correlated in the brown trout fry, forming a behavioral syndrome. This behavioral syndrome has also been observed in juveniles of European eel (Geffroy et al. 2015), and in adults of several fish species (reviewed in Conrad et al. 2011). When adding the same behavioral variables into a cluster analysis, two general clusters could be discerned – one with lower activity and aggression (Cluster A), and one with higher activity and aggression (Cluster B). The clustering of two general behavioral types is in line with much of the previous literature describing the biology of early brown trout stages. In general, two behavioral groups are discerned when fry emerges from the spawning gravel. One group takes station close to the nest, and the other, having delayed formation of static swimming behavior, drift downstream away from the nest (Cuinat and Héland 1979; Héland 1999). The downstream drifters have been suggested to constitute a group of individuals with the strategy of forming territories in areas where there are less competition (Héland 1999; Skoglund and Barlaup 2006). Trout fry show these different behaviors even if reared in isolation (Héland 1999), a finding which is supported by our results. In general for salmonids, the early emerging fry are the ones taking station close to the nests and become dominant over later emerging fry (Mason and Chapman 1965; Chandler and Bjornn 1988; Metcalfe and Thorpe 1992). This dominance could potentially lead to a size advantage during the rest of the juvenile stage and thereby earlier smoltification (preadaptation for seaward migration), as shown in hatchery studies (Metcalfe and Thorpe 1992). Dominant fish can chose the best foraging grounds, and also have precedence in choosing when to forage, and can thereby optimize

food intake in relation to risk (Alanärä et al. 2001). Some evidence suggest that early emergers have basal higher metabolic rate, which could lead to higher activity levels (Metcalfe et al. 1995). This, in turn, would further support the inference that the active group is constituted of early emergers. Similar strategies are also found in wild brook char *Salvelinus fontinalis* fry, but in this species the strategies appear to be associated with stress reactivity (i.e. cortisol expression) (Farwell and McLaughlin 2009; Farwell et al. 2014).

In some cases, a passive strategy may not be viable during the early critical period, as indicated by high mortalities in non-territorial fry in their first months of life in the Black Brows Beck, Britain (Elliott 1990). In other cases, like in the tributaries to the Norwegian river Daleelva, non-territorial drifting fry do not seem to starve and may thus not be outcompeted; instead this appears to be a specific dispersal strategy (Skoglund and Barlaup 2006). The possibility of coexistence of different behavioral types is likely positively influenced by territory availability and environmental complexity (Höjesjö et al. 2004; Hoogenboom et al. 2012; Reid et al. 2012), which likely differ among study sites and over time. The different clusters of behavioral types could be a result of frequency dependent selection based on underlying physiological mechanisms (e.g. metabolic rate or stress reactivity) as modelled by Wolf and McNamara (2012). However, studies on young hatchery reared salmonids have indicated that agonistic behavior, which is part of the behavioral syndrome in our study, show virtually no heritability (Vøllestad and Quinn 2003; Kortet et al. 2014). Still, salmonid selection programs seem to be able to create genetic strains with altered aggression levels compared to wild populations, indicating that there actually is a genetic component for the behavioural expression (Huntingford and Adams 2005). Substantial among-sibling variation in behavior has previously been found in brown trout, attributed to the location of the eggs in the egg sac and possibly pre-natal hormone exposure (Burton et al. 2011, 2013). Thus, behavioral strategies of individual fry may be depending on embryonal environment, which can vary within females (Jonsson and Jonsson 2014). For instance, within-egg size variation in female fish (southern pygmy perch Nannoperca australis) can be influenced by e.g. environmental predictability, with more variation in unpredictable environments (Morrongiello et al. 2012). If within-female differences in investment into eggs affect behavior, e.g. through effects on metabolic rate (Régnier et al. 2012), this may be an indication of bet-hedging with different behavioral types performing well in different situations, utilizing different niches, or different competitive strategies (e.g. Grant and Noakes 1987; Skoglund and Barlaup 2006; Závorka

et al. 2015). In this way the offspring from a single female may have a wider total niche breadth. Given the many non-genetic factors which can affect offspring behavior, the frequency of behavioral types in a population may be an effect of selection for intra-female variation in offspring phenotypes and fine-tuned each generation through environmental effects, rather than being an effect of direct genetic inheritance of specific behavioral traits.

It is not yet known whether brown trout retain their behavioral strategy, or personality, over longer time-periods (for similar issues see e.g. Groothuis and Trillmich 2011). Possibly, if the low-activity fish would retain their passive behavior over time, they may in fact perform equally well, or even better, than active individuals at later life-stages (e.g. Adriaenssens and Johnsson 2010; Závorka et al. 2015).

In summary, we argue that behavior in brown trout fry is influenced by recent food availability after experimentally controlling for acute hunger effects, albeit with effects being relatively weak due to inter-individual variation. Size was associated with behavior, with larger fish being more active and more actively aggressive on average. We found evidence for both consistent individual differences in activity and active aggression, and a behavioral syndrome where activity and active aggression were positively correlated. Finally, two distinct behavioral groups could be discerned, after removing effects of social environment for a month prior to behavioral trials, suggesting two behavioral strategies in brown trout fry.

Acknowledgements

We thank Lin Sandquist and Christina Claesson for their assistance during field collection and in the lab. J.N. received financial support from Helge Ax:son Johnsons stiftelse, and Wilhelm och Martina Lundgrens Vetenskapsfond. J.I.J. was financed by a grant from the Swedish Research Council Formas.

References

Adriaenssens B, Johnsson JI (2010) Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. Behav Ecol 22:135–143

Adriaenssens B, Johnsson JI (2013) Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. Ecol Lett 16:47–55

Alanärä A, Burns MD, Metcalfe NB (2001) Intraspecific resource partitioning in brown trout: the temporal distribution of foraging is determined by social rank. J Anim Ecol 70:980–986

Ali M, Nicieza A, Wootton RJ (2003) Compensatory growth in fishes: a response to growth depression. Fish Fish 4:147–190

Arendt JD (1997) Adaptive intrinsic growth rates: an integration across taxa. Q Rev Biol 72:149–177

Bell AM (2007) Future directions in behavioural syndromes research. Proc R Soc B 274:755-761

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

Bohlin T, Dellefors C, Faremo U (1996) Date of smolt migration depends on body-size but not age in wild sea-run brown trout. J Fish Biol 49:157–164

Bohlin T, Dellefors C, Faremo U (1993) Optimal time and size for smolt migration in wild sea trout (*Salmo trutta*). Can J Fish Aquat Sci 50:224–232

Bohlin T, Dellefors C, Faremo U (1994) Probability of first sexual maturation of male parr in wild sea-run brown trout (*Salmo trutta*) depends on condition factor 1 yr in advance. Can J Fish Aquat Sci 51:1920–1926

Brodin T, Drotz MK (2011) Larval behavioral syndrome does not affect emergence behavior in a damselfly (*Lestes congener*). J Ethol 29:107-113

Brown ME (1957) Experimental studies on growth. In: Brown ME (ed) The Physiology of Fishes, Vol. I - Metabolism. Academic Press Inc., New York, pp 361–400

Budaev SV (2010) Using principal components and factor analysis in animal behaviour research: caveats and guidelines. Ethology 116:472–480

Burton T, Hoogenboom MO, Armstrong JD, Groothuis TGG, Metcalfe NB (2011) Egg hormones in a highly fecund vertebrate: Do they influence offspring social structure in competitive conditions? Funct Ecol 25:1379–1388

Burton T, Hoogenboom MO, Beevers ND, Armstrong JD, Metcalfe NB (2013) Among-sibling differences in the phenotypes of juvenile fish depend on their location within the egg mass and maternal dominance rank. Proc R Soc B 280:20122441

Chandler GL, Bjornn TC (1988) Abundance, growth, and interactions of juvenile steelhead relative to time of emergence. Trans Am Fish Soc 117:432–443

Clark CW (1994) Antipredator behavior and the asset-protection principle. Behav Ecol 5:159–170

Conceição LEC, Dersjant-Li Y, Verreth JAJ (1998) Cost of growth in larval and juvenile African catfish (*Clarias gariepinus*) in relation to growth rate, food intake and oxygen consumption. Aquaculture 161:95–106

Conrad JL, Weinersmith KL, Brodin T, Saltz JB, Sih A (2011) Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. J Fish Biol 78:395–435.

Cuinat R, Héland M (1979) Observations sur la devalaison d'alevins de truite commune (*Salmo trutta* L.) dans le Lissuraga. Bull Fr Piscic 274:1–17 [In French]

Degerman E, Nyberg P, Sers B (2001) Havsöringens ekologi. Fiskeriverket Informerar 2001:10. Fiskeriverkets Sötvattens-laboratorium, Örebro. Available at: http://www.havochvatten.se/ [In Swedish]

Dellefors C, Faremo U (1988) Early sexual maturation in males of wild sea trout, *Salmo trutta* L., inhibits smoltification. J Fish Biol 33:741–749

Dmitriew CM (2011) The evolution of growth trajectories: what limits growth rate? Biol Rev 86:97–116

Elliott JM (1990) Mechanisms responsible for population regulation in young migratory trout, *Salmo trutta*. III. The role of territorial behaviour. J Anim Ecol 59:803–818

Elliott JM (1994) Quantitative ecology and the brown trout. Oxford University Press, Oxford

Farwell M, McLaughlin RL (2009) Alternative foraging tactics and risk taking in brook charr (*Salvelinus fontinalis*). Behav Ecol 20:913–921

Farwell M, Fuzzen MLM, Bernier NJ, McLaughlin RL (2014) Individual differences in foraging behavior and cortisol levels in recently emerged brook charr (*Salvelinus fontinalis*). Behav Ecol Sociobiol 68:781–790

Fiksen Ø, Jørgensen C (2011) Model of optimal behaviour in fish larvae predicts that food availability determines survival, but not growth. Mar Ecol Prog Ser 432:207–219

Geffroy B, Sadoul B, Bardonnet A (2015) Behavioural syndrome in juvenile eels and its ecological implications. Behaviour 152:147–166

Gilliam JF, Fraser DF (1987) Habitat selection under predation hazard: test of a model with foraging minnows. Ecology 68:1856–1862

Grant JWA, Noakes DLG (1987) Movers and stayers: foraging tactics of young-of-the-year brook charr, *Salvelinus fontinalis*. J Anim Ecol 56:1001–1013

Groothuis TGG, Trillmich F (2011) Unfolding personalities: the importance of studying ontogeny. Dev Psychobiol 53:641–655

Heithaus MR, Frid A, Wirsing AJ, Dill LM, Fourqurean JW, Burkholder D, Thomson J, Bejder L (2007) State-dependent risk-taking by green sea turtles mediates top-down effects of tiger shark intimidation in a marine ecosystem. J Anim Ecol 76:837–844

Héland M (1999) Social organization and territoriality in brown trout juveniles during ontogeny. In: Baglinière J-L, Maisse G (eds) Biol. Ecol. Brown Trout Sea Trout. Praxis Publishing Ltd., Chichester, pp 115–143

Hoogenboom MO, Armstrong JD, Groothuis TGG, Metcalfe NB (2012) The growth benefits of aggressive behavior vary with individual metabolism and resource predictability. Behav Ecol 24:253–261

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

Höjesjö J, Johnsson JI, Axelsson M (1999) Behavioural and heart rate responses to food limitation and predation risk: an experimental study on rainbow trout. J Fish Biol 55:1009–1019

Höjesjö J, Johnsson JI, Bohlin T (2004) Habitat complexity reduces the growth of aggressive and dominant brown trout (*Salmo trutta*) relative to subordinates. Behav Ecol Sociobiol 56:286–289

Höjesjö J, Adriaenssens B, Bohlin T, Jönsson C, Hellström I, Johnsson JI (2011) Behavioural syndromes in juvenile brown trout (*Salmo trutta*); life history, family variation and performance in the wild. Behav Ecol Sociobiol 65:1801–1810

Johnsson JI, Björnsson BT (1994) Growth hormone increases growth rate, appetite and dominance in juvenile rainbow trout, *Oncorhynchus mykiss*. Anim Behav 48:177-186

Johnsson JI, Bohlin T (2006) The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proc R Soc B 273:1281–1286

Johnsson JI, Jönsson E, Björnsson BT (1996) Dominance, nutritional state, and growth hormone levels in rainbow trout (*Oncorhynchus mykiss*). Horm Behav 30:13–21

Johnsson JI, Nöbbelin F, Bohlin T (1999) Territorial competition among wild brown trout fry: effects of ownership and body size. J Fish Biol 54:469–472

Jonsson B, Jonsson N (2011) Ecology of Atlantic Salmon and Brown Trout: Habitat as a template for Life Histories. Springer Science + Business Media B.V., Heidelberg

Jonsson B, Jonsson N (2014) Early environment influences later performance in fishes. J Fish Biol 85:151–188

Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MAW, Blokhuis HJ (1999) Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev 23:925–935

Kortet R, Vainikka A, Janhunen M, Piironen J, Hyvärinen P (2014) Behavioral variation shows heritability in juvenile brown trout *Salmo trutta*. Behav Ecol Sociobiol 68:927–934

Kotrschal A, Taborsky B (2010) Environmental change enhances cognitive abilities in fish. PLoS Biol 8:e1000351

Lima SL (1986) Predation risk and unpredictable feeding conditions: determinants of body mass in birds. Ecology 67:377–385

Ludwig D, Rowe L (1990) Life-history strategies for energy gain and predator avoidance under time constraints. Am Nat 135:686–707

Luttbeg B, Sih A (2010) Risk, resources and state-dependent adaptive behavioural syndromes. Phil Trans R Soc B 365:3977–3990

Mason JC, Chapman DW (1965) Significance of early emergence, environmental rearing capacity, and behavioral ecology of juvenile coho salmon in stream channels. J Fish Res Board Can 22:173–190

Metcalfe NB, Taylor AC, Thorpe JE (1995) Metabolic rate, social status and life-history strategies in Atlantic salmon. Anim Behav 49:431–436

Mittelbach GG. (1981) Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology 62:1370–1386

Morrongiello JR, Bond NR, Crook DA, Wong BB (2012) Spatial variation in egg size and number reflects trade-offs and bet-hedging in a freshwater fish. J Anim Ecol 81:806–817

Nicieza AG, Metcalfe NB (1997) growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. Ecology 78:2385–2400

Nilsson N-A. (1956) On the feeding habits of trout in a stream of northern Sweden. Rep - Inst Freshw Res, Drottningholm 38:154–166

Näslund J, Pauliny A, Blomqvist D, Johnsson JI (2015a) Telomere dynamics in wild brown trout: effects of compensatory growth and early growth investment. Oecologia 177:1221-1230

Näslund J, Sandquist L, Johnsson JI (2015b) Behaviour in a novel environment is associated with body size, but not affected by recent feeding history in brown trout *Salmo trutta* fry. Submitted manuscript

Orpwood JE, Griffiths SW, Armstrong JD (2006) Effects of food availability on temporal activity patterns and growth of Atlantic salmon. J Anim Ecol 75:677–685

Peck MA, Herrmann J-P, Ewest B, Stäcker S, Temming A (2014) Relationships between feeding, growth and swimming activity of European sprat (*Sprattus sprattus* L.) post-larvae in the laboratory. Environ Biol Fish 98:1117-1127

Pedersen BH (1997) The cost of growth in young fish larvae, a review of new hypotheses. Aquaculture 155:259–269

Perez KO, Munch SB (2010) Extreme selection on size in the early lives of fish. Evolution 64:2450–2457

Peterson I, Wroblewski JS (1984) Mortality rate of fishes in the pelagic ecosystem. Can J Fish Aquat Sci 41:1117–1120

Pettersson LB, Brönmark C (1993) Trading off safety against food: state dependent habitat choice and foraging in crucian carp. Oecologia 95:353–357

R Core Team (2014) R: A language and environment for statistical computing, version 3.0.3. R Foundation for Statistical Computing, Vienna, Austria

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P-O (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. Phil Trans R Soc B 365:4051–4063

Régnier T, Boillet V, Gaudin P, Labonne J (2012) Female effects on offspring energetic status and consequences on early development in yolk feeding brown trout (*Salmo trutta*). J Exp Zool 317A:347–358

Reid D, Armstrong JD, Metcalfe NB (2012) The performance advantage of a high resting metabolic rate in juvenile salmon is habitat dependent. J Anim Ecol 81:868–875

Reinhardt UG, Healey MC (1999) Season- and size-dependent risk taking in juvenile coho salmon: experimental evaluation of asset protection. Anim Behav 57:923–933

Sih A (1980) Optimal behavior: can foragers balance two conflicting demands? Science 210:1041–1043

Sih A, Bell AM, Johnson JC, Ziemba RE (2004) Behavioural syndromes: an integrative overview. Q Rev Biol 79:211–244

Sih A, Mathot KJ, Moirón M, Montiglio P-O, Wolf M, Dingemanse NJ (2015) Animal personality and state-behaviour feedbacks: a review and guide for empiricists. Trends Ecol Evol 30:50–60

Skoglund H, Barlaup BT (2006) Feeding pattern and diet of first feeding brown trout fry under natural conditions. J Fish Biol 68:507–521

Stamps JA (2007) Growth-mortality tradeoffs and "personality traits" in animals. Ecol Lett 10:355–363

Sundström LF, Petersson E, Höjesjö J, Johnsson JI, Järvi T (2004) Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance, Behav Ecol 15:192–198

Sundström LF, Kaspersson R, Näslund J, Johnsson JI (2013) Density-dependent compensatory growth in brown trout (*Salmo trutta*) in nature. PLoS ONE 8:e63287

Travis J, Keen WH, Juilianna J (1985) The role of relative body size in a predator-prey relationship and larval anurans between naliads dragonfly. Oikos 45:59–65

Vehanen T (2003) Adaptive flexibility in the behaviour of juvenile Atlantic salmon: short-term responses to food availability and threat from predation. J Fish Biol 63:1034–1045

Vøllestad LA, Quinn TP (2003) Trade-off between growth rate and aggression in juvenile coho salmon, *Oncorhynchus kisutch*. Anim Behav 66.561–568

Werner EE, Gilliam JF (1984) Then ontogenetic niche and species interactions in size-structured populations. Ann Rev Ecol Syst 15: 393–425

Wolf M, McNamara JM (2012) On the evolution of personalities via frequency-dependent selection. Am Nat 179: 679–692

Závorka L, Aldvén D, Näslund J, Höjesjö J, Johnsson JI (2015) Linking lab activity with growth and movement in the wild: explaining pace-of-life in a trout stream. Behav Ecol 26: 877–884

Paper 4

Can a faster pace-of-life be safer? Investigating the growth-mortality trade-off in brown trout during the early-life selective bottleneck

Joacim Näslund [13]
Per Saarinen Claesson [23]
Jörgen I. Johnsson [1]

This manuscript has been submitted for publication in a peer-reviewed academic journal

Corresponding author: J. Näslund (joacim.naslund@gmail.com)

[1] Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden
[2] Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden
[3] Equal contribution

Abstract

The early life stage is one of the main survival bottlenecks for many fish species, making performance during this period important for individuals. Body size, growth rate and activity have all been found to affect fitness in fish, the effects have rarely been investigated for early life stages in natural conditions. In two separate experiments, we attempted to induce compensatory growth in wild-caught brown trout fry (Salmo trutta) under laboratory conditions by exposing the fry to a period of restricted food followed by a period of re-feeding. During the laboratory period, we scored activity in open-field tests before food restriction and during re-feeding to investigate changes in activity. In the midst of the presumed compensatory state the fish were released back into their native habitat. After a month we recaptured the fish for evaluation of growth and mortality in the natural environment. In the laboratory, we detected full compensation in body condition, and partial compensation in mass, but no compensation in length. No signs of growth compensation could be detected in the field. No difference in recapture rates between compensating and control fish was detected, suggesting that compensating individuals did not just die. Together, these results indicate that the environment limited the capacity for compensatory growth rates in the fry. We found that open-field activity was a repeatable trait, despite manipulations of energetic state, and that a high activity level had positive effects on survival under natural conditions. Body size was positively related to survival in the first experiment (early summer), but not in the second experiment (late summer). The results are in conflict with the general expectation that high activity should be more risky in natural environments. Based on our results and previous studies, we hypothesise that activity may be associated with territoriality, and that successful territorial behaviour can be a relatively safe life-style given that a good territory provide both growth-opportunities, improving competitive ability, and shelter against predators.

Keywords: Compensatory growth, Open-field activity, Behavioural consistency, State-dependency, Personality, Survival

Introduction

In recent years, the connections between individual differences in behaviour and life-history traits have gained increased attention (e.g. Stamps 2007; Biro and Stamps 2008; Réale et al. 2010), and a holistic model connecting physiology, behaviour, and life-history, have been proposed in the pace-of-life syndrome hypothesis (POLS; Réale et al. 2010). This hypothesis predicts relationships among traits largely based on the growth-mortality trade off where certain physiological and behavioural traits promote faster growth and fitness benefits through size, but at the expense of higher mortality probability associated with e.g. reduced investment in maintenance and defence, and higher risk taking propensity (Arendt 1997; Stamps 2007). However, most studies have been made in artificial environments and the links between performance in these environments and links to performance in the wild are not always clear (Adriaenssens and Johnsson 2009; Niemelä

and Dingemanse 2014; Fisher et al. 2015). Given the large differences between artificial and natural environments, studies performed in more natural environments are of high relevance to resolve what the ecological consequences of individual differences in behaviour are (Dingemanse and Réale 2005; Adriaenssens and Johnsson 2009; Archard and Braithwaite 2010; Mittelbach et al. 2014; Niemelä and Dingemanse 2014).

In animals with high fecundity the early life stages are typically major bottlenecks for survival (Pianka 1974). For salmonid fish, like brown trout Salmo trutta (the model species in this paper) most of the mortality of a year-class occurs at the fry stage following the alevin (yolk-sac fry) stage, which is spent safely buried in the stream substrate (Le Cren 1961; Mortensen 1977; Elliott 1989; Milner et al. 2003). Identifying individual traits affecting survival during this early life stage is therefore important to understand the selective forces shaping the population.

Small coastal streams on the Swedish west coast mainly contain juvenile brown trout (commonly $> 90\%$ of fish individuals), making these streams well suited as model systems for investigating intra-specific ecological dynamics. Associations between individual differences in behaviour, growth, and mortality in juvenile brown trout have been studied in these coastal streams in several previous experiments, but the majority has been conducted after the critical summer period (Adriaenssens and Johnsson 2010, 2013; Závorka et al. 2015). The one study conducted during the critical period suggests that alternative behavioural types (based on boldness measures) may be equally successful in natural environments (Höjesjö et al. 2011). In older trout, a negative relationship between explorative tendency and growth has been shown in age 1+ fish (Adriaenssens and Johnsson 2010), and a positive association between activity and survival (as inferred from recapture rates) has been shown in age 0+ fish (after the critical period) (Adriaenssens and Johnsson 2013). A subsequent study suggests that the performance of more active fish depends on the habitat to a higher extent than it does for more passive fish (Závorka et al. 2015).

The growth-mortality trade-off underlying the POLS hypothesis, can also be applied to the reasoning about adaptive growth rates on the individual level (Arendt 1997; Stamps 2007; Álvarez 2011). Even within individuals we would expect faster growth to be associated with relatively higher risk taking and mortality probability. Like many other animals (Wilson and Osbourn 1960; Ali et al. 2003; Dmitriew 2011), larger individuals of brown trout (age 1+) are able to go through compensatory growth in both mass and length after periods of food deprivation (Johnsson and Bohlin 2005, 2006; Sundström et al. 2013; Näslund et al. 2015b). However, it

is not known whether compensatory growth also occurs in wild salmonid fry. Laboratory studies suggest that compensatory growth could be possible (Bilton and Robins 1973; Näslund and Johnsson 2015; Näslund et al. 2015c), but field studies are needed to confirm these results in natural environments. A study on Atlantic salmon S. salar, a species closely related to the brown trout, have indicated that fry with increased drive to forage (through implantation of growth-hormone, GH) grow faster than control fry in a hatchery environment, but slower in natural environments (Sundt-Hansen et al. 2012). This suggests that endogenous plasma GH levels may be adapted to maximize growth under limiting environmental conditions (Sundt-Hansen et al. 2012).

In two separate experiments, replicating the methodology, we investigate four main questions regarding the performance of brown trout fry: Does (i) activity, as scored in an open field test, and (ii) bodily state (size and energetic status) affect survival in the wild?, (iii) Is compensatory growth elicited after food restriction?, and (iv) Does open-field activity change when changing the bodily state?

Materials and methods

The study was conducted in 2013 and included two separate experiments in two small coastal streams on the Swedish west coast. In both streams the vast majority of all fish are brown trout (only small populations of European eel *Anguilla anguilla* co-occur with the trout), with the populations being predominantly anadromous. In both experiments, young-of-the year (YOY) brown trout were subjected to two different food schedules, either constant high-ration feeding (henceforth called "HR-HR"), or low-ration feeding followed by high-ration re-feeding (henceforth called "LR-HR").

Experiment 1 - Stream Norumsån

The first experiment was conducted on fish from stream Norumsån, between June 11 and Aug 16. On June 11 we captured approximately 300 recently emerged fry, using electro-fishing (200-300V straight DC, keeping electric current at ca. 1 A; L-1000, LugAB, Sweden). All fish were caught on a 50 m long brown trout spawning ground (58°2.593'N, 11°50.758'E; mean width 3.4 m, mean depth 0.12 m), located 2.4 km upstream from the sea. The fish were transported in 25-l cool boxes for 46 km by car to the zoology facility of University of Gothenburg, where they were put temporarily in an 80 l glass tank for acclimatization to laboratory conditions. The tank was supplied

with flow-through water from the in-house circulating system (temp) and air stones connected to a pressurized air system to keep the water well oxygenated. The tank was furnished with gravel, bundles of plastic plants and shelter structures (PVC pipes and plates). Fish were treated with salt water (5 ‰sea salt) for one hour before the freshwater flow was connected to the holding tank, as a treatment for potential exoparasitic organisms.

Individual marking and data collection in the laboratory

The day after being captured we took fish haphazardly from the acclimatization tank for individual marking and distribution to experimental tanks. Each fish was anaesthetized (2-phenoxyethanol, 0.5 $ml \cdot L^{-1}$) and tagged subcutaneously with visible implant elastomers (VIE; Northwest Marine Technology, USA). VIE were injected at the base of the dorsal fin (Leblanc and Noakes 2009; two colours representing each of the two treatment groups) and at the base of the anal fin (Olsen and Vøllestad 2001). In total, five colours of VIE (yellow, red, orange, pink and blue) were used. All individuals in each compartment uniquely tagged, but colour combinations were the same for several fish among compartments. When needed (e.g. at recapture after stream-release), identification among individuals with the same colour combination was based on the unique individual body colouration pattern [parr marks and melanic spots; used successfully for the same purpose in other salmonids (Garcia de Leaniz et al. 1994; Yagyu et al. 2007)] and the shape of the VIE tags, which was visible on the photos. Previous studies on brown trout have suggested no effects of VIE on growth or survival (Olsen and Vøllestad 2001).

Directly after tagging the wet mass of the fish was weighed to the nearest 0.1 g (Kern EW 3000-2M, Kern & Sohn GmbH, Germany) after water was blotted off its body. Thereafter it was placed in a Petri dish filled with water, standing on a light pad and photographed dorsally and laterally (right side) along with a millimetre scale (photography setup described in Näslund 2014). Body length (standard length; i.e. excluding the caudal fin) was measured digitally to the nearest 0.1 mm from the lateral photographs in ImageJ 1.47t (http://imagej.nih.gov/ij/). Two length measurements of each fish were made and their mean was used in analyses.

Fish remaining in the acclimatization tank after we tagged the experimental individuals were kept to replace fish dying during the initial acclimatization period in the treatment tanks.

Experimental tanks and fish distribution

The experimental tanks consisted of four replicate flow-through PVC tanks (120 cm wide, 50 cm long and 20 cm high), each divided into five separate compartments (each 50 cm long and 24 cm wide); in total 20 compartments. Each compartment was supplied with a 1 cm thick layer of sand (diameter $<$ 0.5 mm) and ten granite rocks (diameter: 5 to 10 cm) evenly spread across the bottom area. Water flow was ca. 1.1 l $\cdot$ min^{-1} in each compartment and the outflow was located at the opposite short side from the inflow at a level maintaining the water depth at ca. 10 cm; keeping the water volume at ca. 12 l in each compartment. Water temperature varied between 10.5 and 12.3°C during the entire experimental period. Distribution of fish into experimental tanks was randomized prior to tagging, so that groups of 20 fish (based on capture order from the acclimatization tank) were each randomized into each of the 20 experimental tank compartments. In total we kept ten fish in each tank compartment.

Laboratory feeding treatments

The laboratory period was split into two periods P1 (15 days) and P2 (11 days), starting the day after capture. The first four days of P1, we supplied all fish with equal amounts of food (10% of body mass). During this period we replaced dead individuals in the experimental tanks with fish being left over after the tagging. During the following two days behaviour of the individuals were scored (see below). Feeding treatments started on the seventh day of P1. HR-HR groups were fed a high ration each day of the experiment, while the LR-HR groups were fed low ration during the last 9 days of P1, followed by high rations during P2. Food consisted of a mixture of pre-frozen invertebrates (commercial food for aquarium fish; Akvarieteknik, Sweden), and was supplied once a day during P1 and generally twice a day during P2. Food was thawed prior to feeding. Details on rations, feeding schedule, and food composition, as well as dates of measurements, are given in Appendix A.

Behavioural scoring

To score activity we performed open field tests, which is a well-functioning assay in terms of testing the activity of an individual and has been shown to be repeatable (Burns 2008; Adriaenssens and Johnsson 2013; Näslund and Johnsson 2015). All individuals were tested twice; the first trial was performed during the two days preceding the initial treatment period and

the second trial five or six days into the second treatment period. The ten open field tanks (scoring tanks) consisted of white plastic boxes (30cm high $\times$ 25.5 cm wide $\times$ 17 cm long; Slugis, IKEA, The Netherlands) filled with 5 cm of water (ca. 11-12.7 °C). All tanks were video recorded from above using web cameras (Creative VF0520, Creative Labs, Singapore). We scored all fish from each tank compartment at the same time. Fish were caught by a hand net and immediately put individually in 1 l plastic containers filled with 2 dl water. After catching all fish from one compartment the containers with the fish were moved to the scoring tanks, into which the fish were released individually within a few seconds. Fish from five randomly selected compartments from each treatment were scored on the first day and the rest on the second day. The same order was used at both scoring occasions. Fish were scored for 30 minutes, whereupon each individual was identified with a UV-flashlight and returned to its original compartment.

All video recordings were analysed in Adobe Premier Pro CS3 (Adobe Systems, Inc., USA) by adding a grid net (4 $\times$ 3 rectangles, 64 mm $\times$ 57 mm) over each open field tank on the computer screen, and then counting number of whole-body line crossings for ten minutes after first movement in the scoring tank.

Stream-release and recapture

To investigate performance in the wild we released the fish back into stream Norumsån at the same area where they were captured after the laboratory period (release date: 12 July). Prior to release, we electrofished the stream section and removed the same number of fish as was released. The disturbance from electrofishing and the removal of individuals was supposed to facilitate re-establishment of experimental individuals in the area.

After 34 days we recaptured the experimental fish during two days using electrofishing with three removals (Bohlin et al. 1989) in the 50 m stream section where the fish were released. All YOY (tagged and untagged) and older fish were counted each pass (identification of tagged fish was not possible due to time limitation). The next day we fished buffer zones consecutive to the release section (50 m upstream and 100 m downstream) until $\leq$ 1 tagged fish was caught in a pass (in effect one or two removals per 50 m stream length). All untagged fish caught were kept in corves until all electrofishing was finished, and were weighed and measured in the field to estimate the natural size distribution. We also measured all the older brown trout in the area. Experimental fish were transported back to the laboratory for final measurements (following the procedure de-

scribed above). After measurements the fish were killed by an overdose of 2-phenoxyethanol (1 ml $\cdot$ l^{-1} until death) and preserved for subsequent analyses (not presented here).

Mortality

A total of 69 fish died between experimental start and release. This corresponds to a mortality rate of 1.3% per day. No difference in mortality between treatments was found during the pre-treatment and feeding period P1, where 17 LR-HR and 16 HR-HR fish died. Mortality differences were higher in the second period where 25 LR-HR and 11 HR-HR fish died. Mortality rates were similar to those found in natural populations of brown trout fry during the same period of the year (Mortensen 1977), as well as to other laboratory studies on young juvenile brown trout (Westley et al. 2013).

Experiment 2 – Stream Stenunge å

The second experiment was conducted on fish from stream Stenunge å, between July 17 and Sept 19. On July 17 we captured approximately 200 fry, using electrofishing (same method as above). All fish were caught in a 175 m long section of the stream (58°4.829'N, 11°52.307'E; mean width 1.8 m, mean depth 0.10 m), located 4.9 km upstream from the sea. In contrast to Experiment 1, the fish were not captured on a spawning ground, and consequently the density of fish was much lower. The fish were transported in 25-l cool boxes for 50 km by car to the university, where they were handled as in Experiment 1.

Individual marking and data collection in the laboratory

Procedures were the same as in Experiment 1, except for individual marking which was applied on three placed of each fish: at the base of the anal fin (treatment identification), at the base of the dorsal fin (individual identification), and at the base of the adipose fin (individual identification). VIE colours used were red, blue, yellow and orange.

Experimental tanks and fish distribution

Due to smaller catch of fish from the stream and larger size of the fish, we used groups of eight fish instead of ten (as in Experiment 1). Otherwise, methods were the same as in Experiment 1.

Laboratory feeding treatments

Same procedure as in Experiment 1.

Behavioural scoring

Same procedure as in Experiment 1.

Stream-release and recapture

On August 14, all fish were released in three batches, separated by 40 m in the middle of the section where the fish were caught. Due to the substantially lower density of 0+ fish in Stenunge å (Experiment 2), as compared to the spawning area in stream Norumsån (Experiment 1), we did not remove any fish from the section prior to releasing the experimental fish.

After 37 days in the stream, fish were recaptured using electrofishing over two consecutive days. The first day we fished the original capture section, using three pass-removal; the second day we fished buffer zones (50 m upstream and 100 m downstream), using two-pass removal in the downstream buffer and one-pass removal in the upstream buffer (no tagged fish were caught there). Numbers of tagged and untagged YOY, as well as older fish were counted for each pass.

Mortality

A total of 21 fish died between experimental start and stream-release, corresponding to a mortality rate of $0.5\% \cdot \text{day}^{-1}$. During pre-treatment period and feeding period P1 nine LR-HR and seven HR-HR fish died. In contrast to Experiment 1 mortality decreased over the second feeding period with four LR-HR and one HR-HR fish dying. Unexpectedly, one food restricted fish (initial size: 46.4 mm, 1.40 g) appeared to have consumed another fish in the same compartment (initial size 27.4 mm, 0.24 g), as it was found with extreme stomach fullness and a mass of 1.79 g at the end of the restriction period. This fish was not included in analyses.

General calculations and statistical analyses

Standardized mass-specific growth rate (Ω; $\% \cdot \text{day}_{-1}$), was calculated following Ostrovsky (1995):

$$\Omega = 100 \cdot (m_{t_2}^b - m_{t_1}^b)/(b \cdot (t_2 - t_1)) \quad (1)$$

where t_1 and t_2 is the start and end time (days of experiment) for which growth rate is investigated, m is blotted wet mass, and b is the allometric mass exponent for the relationship between growth rate and body size (taken to be 0.31 for brown trout, following Elliott et al. 1995). The standardized growth rate was used due to the fact that the fish were held in arenas where dominance hierarchies could easily be influencing the growth rate. Normally, smaller trout have a higher mass-specific growth rate (Brett 1970), but in dominance hierarchies the opposite pattern often emerges (Brown 1957).

It should be noted that the first period (P1) included a period of feeding at 10% of body mass, as well as a period of restriction (2% of body mass) for LR-HR. Thus, general growth rates for this group are overestimated with respect to the restriction period (Fig. 1).

Absolute growth in standard length (L) per day (LGR; mm), and condition factor (K) were calculated using Eqn. 2, and 3 respectively.

$$LGR = (L_{t_2} - L_{t_1})/(t_2 - t_1) \tag{2}$$

$$K = 10^5 \cdot m \cdot L^{-3} \tag{3}$$

Catchability of YOY and older fish was estimated based on the captures from the experimental sections, using the three-pass removal formula in Bohlin et al (1989).

Size (L and $\ln(m)$) and condition factor (K) were analysed in SPSS 22.0 (IMB Corp., USA) using Generalized Linear Mixed Models (GLMM) based on Gaussian distribution and identity link-function, including the following independent variables: TR (treatment: fixed factor; two levels: HR-HR and LR-HR), TP (time point for measurement: fixed within-subject factor; four levels: TP1, TP2, TP3, TP4), $TR{\times}TP$ (interaction between treatment and time-point), and $CP(TR)$ (rearing compartment within treatment: random effect block, 20 levels (compartment 1 to 20).

Growth (Ω and LGR) was analysed in SPSS 22.0 using GLMM (Gaussian distribution, identity link-function) including independent variables: TR (see above), PD (experimental period: fixed within-subject factor; three levels: P1, P2, and P3), $TR \times PD$ (interaction between treatment and experimental period), and $CP(TR)$ (see above).

Stream survival (recapture probability) was analysed in R Studio (http://www.rstudio.org/) using Generalized Linear Models (GLM) within the package nlme (Pinheiro et al. 2012). Independent variables were: TR (See above), SL (initial standard length: continuous variable), ACT (activity at the first behavioural trial: continuous variable). We also ran a GLMM also including $CP(TR)$ (See above), but as there were no quantitative or

qualitative effects on the results, as compared with the GLM. Furthermore, we also checked potential interactions between the independent variables included in the GLM, but none of these indicated any significant effects ($p > 0.05$) and were consequently dropped. In the results, we report only the GLM results without interactions.

To analyse whether activity changed in the LR-HR fish as compared to the HR-HR fish, we compared mean change in activity over the two trials, using t-tests. General changes (without taking treatment into account) in activity between the first and the second trial were analysed using paired t-tests. Repeatability of activity between the two trials were analysed using the intraclass correlation coefficient (ICC), using the psych package for R (Revelle 2015).

Effects of activity (first trial) on growth rate in the streams were analysed using linear regressions.

Results

Growth patterns

For the analyses of growth, size, and condition we focus on the interaction terms (growth: $TR \times PD$; size and condition: $TR \times TP$; Table 1) and the pairwise comparisons between treatment groups for each period (Fig. 1), as our hypotheses concern treatment differences in growth rate depending on experimental period (PD) and differences in size and condition depending on time-point (TP). Main effects were generally significant, but this is expected by the feeding treatments and not associated to any hypotheses (see Table 1 and Fig. 1 for statistics and effects).

In both experiments, the HR-HR fish were growing faster in both length and mass during P1. During re-feeding in the laboratory the LR-HR fish showed a significantly higher growth rate in body mass, but lower growth rate in body length, as compared to HR-HR. The condition factor was higher for LR-HR at the end of P2 (but only significantly so in Experiment 2), indicating that the fish compensated body mass at the expense of growth in length. After release into their natural environment, we could not detect any differences in growth rate among treatment groups, suggesting that the compensatory growth detected in the laboratory had ceased.

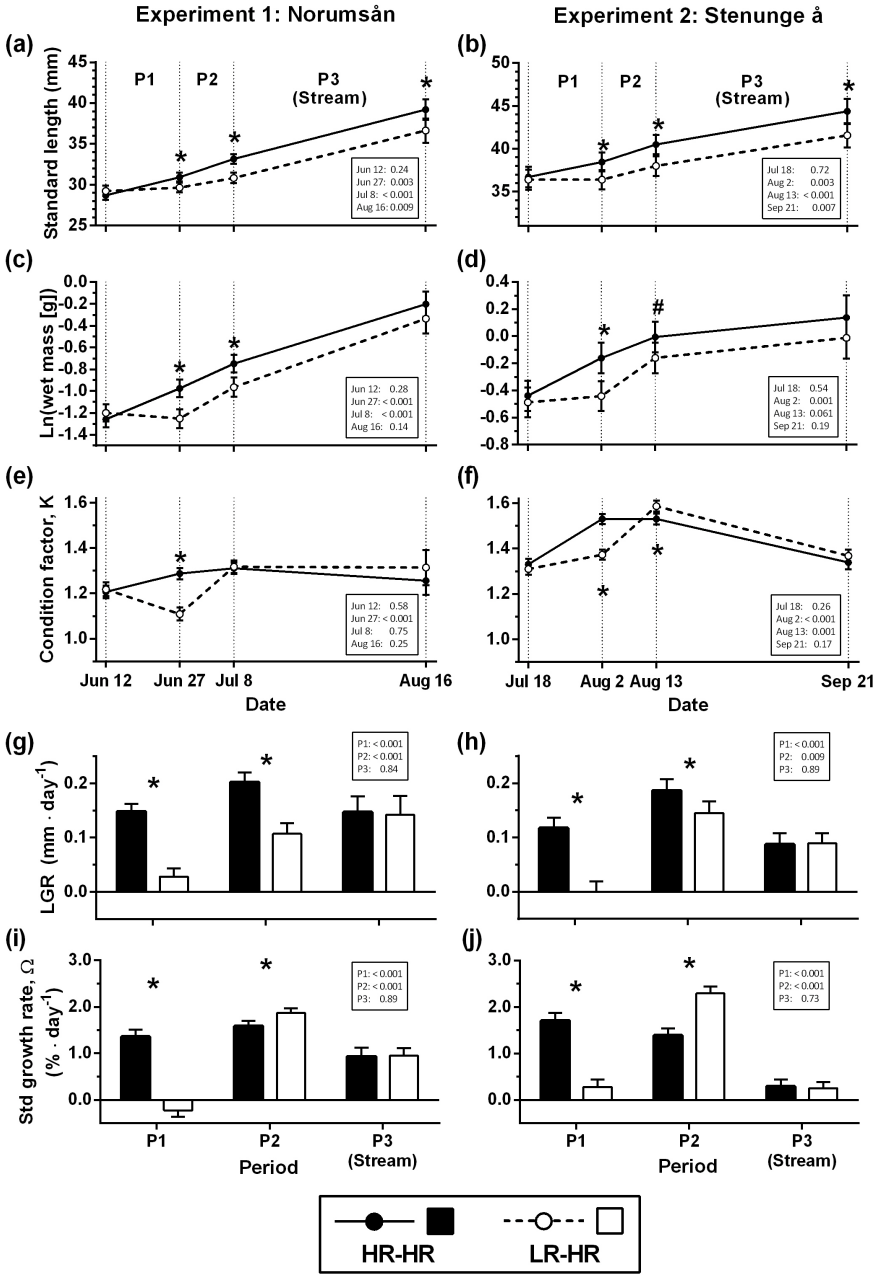


Figure 1: Size (a-d), condition factor (e-f), and growth (g-j) over the experiments. Significant pairwise comparisons indicated with asterisks (*), near-significant ($0.1 > p > 0.05$) pairwise comparisons indicated with hashes (#). Actual p-values for pairwise comparisons are given within boxes in each figure.

Table 1: Summary statistics for generalised linear mixed models of size (standard length, L; wet mass, Ln(m)), condition factor (K), and growth (standardised mass-specific growth rate in wet mass, Ω; absolute growth rate in standard length, LGR). Independent variables: TR – food treatment, TP – time-point for measurement, PD – experimental period, CP(TR) – compartment within treatment (for details see Materials and Methods).

Model	TR	TP or PD	Interaction	CP(TR)
Experiment 1: Norumsån				
$L \sim$	$F_{1,425} = 8.77$	$F_{3,425} = 378$	$F_{3,425=63.6}$	Estimate: 5.64
TR+TP+TR×TP+CP(TR)	$p = 0.003$	$p < 0.001$	$p < 0.001$	95% CI: 4.38-7.25
$Ln(m) \sim$	$F_{1,425=9.90}$	$F_{3,425} = 158$	$F_{3,425} = 10.6$	Estimate: 0.006
TR+TP+TR×TP+CP(TR)	$p = 0.002$	$p < 0.001$	$p < 0.001$	95% CI: 0.002-0.017
$K \sim$	$F_{1,425=1.74}$	$F_{3,425} = 60.5$	$F_{3,425} = 41.8$	Estimate: 0.006
TR+TP+TR×TP+CP(TR)	$p = 0.19$	$p < 0.001$	$p < 0.001$	95% CI: 0.004-0.009
$\Omega \sim$	$F_{1,296=41.0}$	$F_{2,296} = 219$	$F_2, 296 = 132$	Estimate: 0.040
TR+PD+TR×PD+CP(TR)	$p < 0.001$	$p < 0.001$	$p < 0.001$	95% CI: 0.016-0.100
$LGR \sim$	$F_{1,296} = 55.3$	$F_{2,296} = 35.1$	$F_{2,296} = 10.7$	Estimate: 0.000
TR+PD+TR×PD+CP(TR)	$p < 0.001$	$p < 0.001$	$p < 0.001$	95% CI: 1.95E-5-0.004
Experiment 2: Stenunge å				
$L \sim$	$F_{1,485} = 5.49$	$F_{3,485} = 205$	$F_{3,485} = 12.9$	Estimate: 22.6
TR+TP+TR×TP+CP(TR)	$p = 0.020$	$p < 0.001$	$p < 0.001$	95% CI: 17.8-28.7
$Ln(m) \sim$	$F_{1,484} = 6.22$	$F_{3,484} = 39.4$	$F_{3,484} = 2.42$	Estimate: 0.013
TR+TP+TR×TP+CP(TR)	$p = 0.013$	$p < 0.001$	$p = 0.066$	95% CI: 0.005-0.034
$K \sim$	$F_{1,484} = 3.45$	$F_{3,484} = 207$	$F_{3,484} = 49.9$	Estimate: 0.003
TR+TP+TR×TP+CP(TR)	$p = 0.064$	$p < 0.001$	$p < 0.001$	95% CI: 0.002-0.005
$\Omega \sim$	$F_{1,345} = 7.37$	$F_{2,345} = 258$	$F_{2,345} = 118$	Estimate: 0.043
TR+PD+TR×PD+CP(TR)	$p = 0.007$	$p < 0.001$	$p < 0.001$	95% CI: 0.013-0.143
$LGR \sim$	$F_{1,345} = 31.6$	$F_{2,345} = 61.4$	$F_{2,345} = 20.6$	Estimate: 0.001
TR+PD+TR×PD+CP(TR)	$p < 0.001$	$p < 0.001$	$p < 0.001$	95% CI: 0.000-5-0.003

Open-field activity

Open-field activity was repeatable between first and second trial in both experiments (Experiment 1: ICC = 0.44 (95% CI: 0.29-0.57), $F_{130,131} = 2.6$; Experiment 2: ICC = 0.55 (95% CI: 0.42-0.66), $F_{139,140} = 2.6$. ICC coefficients for each treatment group are noted in Fig. 2.

There were no significant differences in change in activity in either experiment (Exp. 1: $|t| = 0.73$, $p = 0.47$; Exp. 2: $|t| = 1.5$, $p = 0.13$), but a general change in activity was seen in Experiment 2, where fish were more active in the second scoring ($|t| = 4.3$, $p < 0.0001$). No such general pattern was seen in Experiment 1 ($|t| = 0.065$, $p = 0.95$).

Stream performance

In Experiment 1, 40 out of 131 released fish (30%) were recaptured after the 36 day period in the stream. 28% of the LR-HR fish and 33% of the HR-HR fish were recaptured. In Experiment 2, 69 out of 139 released fish (49.5%) were recaptured after the 36 day period in the stream. 55% of the LR-HR fish and 43% of the HR-HR fish were recaptured. Generally, no effects of feeding treatment were detected, but both experiments showed that more active individuals had higher recapture rate, suggesting higher survival of these fish (Table 2; Fig. 3). Initial standard length had a positive effect on

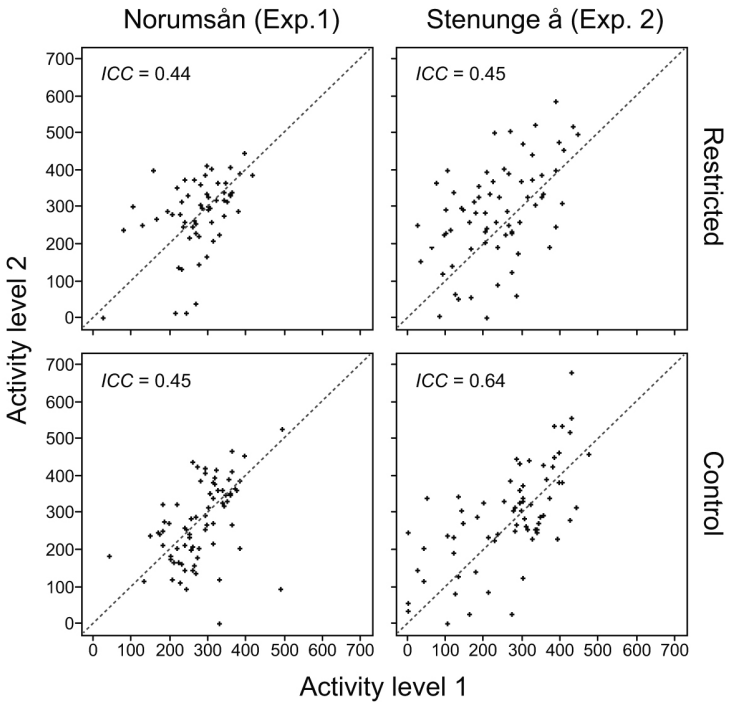


Figure 2: Activity levels as scored in forced open-field trials (activity level 1 = pre-manipulation trial; activity level 2 = trial during experimental period 2). Intraclass correlation coefficients (ICC) noted for each experimental group. Hashed diagonal line mark the 1:1 relationship.

recapture probability in Experiment 1, but not in Experiment 2 (Table 2; Fig. 4).

No association between open-field activity and growth rate (Ω) in the stream was detected (Exp. 1: $R^2 = 0.013$, $p = 0.48$; Exp. 2: $R^2 = 0.005$, $p = 0.58$).

Catchability

In Experiment 1, the catchability in the 50 m experimental section was estimated to be 0.51 ± 0.030 SE for fry and 0.74 ± 0.045 SE) for older trout. Based on the estimated population size of 0+ fish in the experimental area ($\hat{N} = 560.5$), we captured an estimated 88.3% (tagged + untagged individuals: $N_{capt} = 495$) of the 0+ population over three electrofishing passes. For older fish we estimated the capture to be 98.3% ($\hat{N} = 109.9$; $N_{capt} = 108$). Notably, there were quite high densities of both YOY (3.3 ind $\cdot$ m^2) and older fish (0.65 ind $\cdot$ m^2) in the experimental section.

Table 2: Summary of binomial GLMs presenting the effects of treatment, activity level and length on survival of brown trout fry during a period in natural conditions. Bold numbers are statistically significant ($p \leq 0.05$). Independent variables: TR – food treatment, ACT – activity, SL – initial standard length (for details see Materials and Methods).

Stream	N	Response variable	Independent variable	Estimate	z-value	p
Norumsån	131	Survival	TR	0.411	0.950	0.330
			ACT	0.003	2.268	0.023
			SL	0.116	2.722	0.006
Stenunge å	137	Survival	TR	0.361	1.533	0.097
			ACT	0.002	2.631	0.015
			SL	0.005	0.135	0.900

In Experiment 2, the catchability in the 175 m experimental section was estimated to be 0.60 ± 0.035 SE for fry and 0.83 ± 0.032 SE) for older trout. Based on the estimated population size of 0+ fish in the experimental area ($\hat{N} = 297.7$), we captured an estimated 93.7% (tagged + untagged individuals: $N_{capt} = 279$) of the 0+ population over three electrofishing passes. For older fish we estimated the capture to be 99.5% ($\hat{N} = 137.7$; $N_{capt} = 137$).

Discussion

Based on two separate experiments, with replicated methodology, we found several general results regarding the relationships among behaviour, energetic state and stream performance. First, in direct contrast to predictions based on the POLS hypothesis, higher open-field activity was positively related to recapture probability (used as a proxy for survival) in both experiments, suggesting that more active individuals were at lower risk of mortality than less active individuals. Second, we found that energetic state, as manipulated by previous feeding history did not affect an individual's chance of being recaptured in any experiment. Size, however, had a positive effect in the first experiment on younger fry, but not in the second where the fry were older. Third, both experiments showed that while compensatory growth was observed during the re-feeding period in the laboratory, this increased growth rate was not detected in the stream environment, suggesting that environmental conditions in the wild restricted growth rate in trout fry. Fourth, no apparent change in open-field activity was detected due to compensatory growth response in the laboratory.

If we relate the present and previous studies on brown trout juveniles in natural environments (Adriaenssens and Johnsson 2010, 2013; Höjesjö et al. 2011; Závorka et al. 2015) to the predictions derived from general hypotheses on the relationship between among-individual behavioural variation and

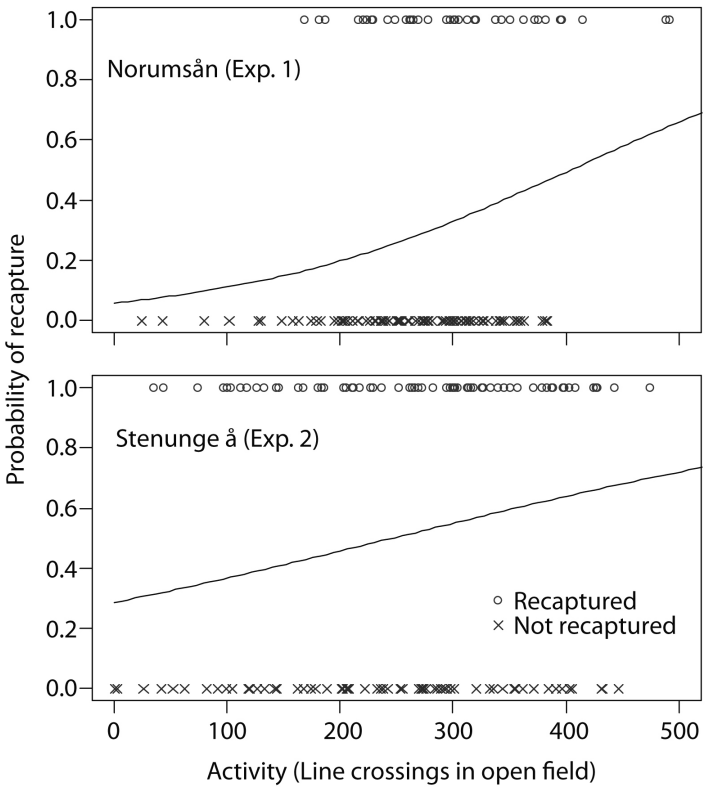


Figure 3: Predicted probability of recapture (used as a proxy for survival) for the brown trout fry (by GLM) depending on activity level. For details on statistics see Table 2.

performance in the wild (e.g. Biro and Stamps 2008; Réale et al. 2010), we can see some general deviations from the expectations. More active individuals are expected to be at higher risk due to higher predator exposure (Stamps 2007), but the opposite tend to be the situation in juvenile trout (Adriaenssens and Johnsson 2010, 2013; Závorka et al. 2015). The same pattern was found in both of our experiments which supports the claim that more active brown trout have a higher survivability. Outmigration from the experimental stream section is judged to be minor, as we found few VIE-tagged fishes in the buffer zones. Differential recapture probability of active and passive fish is another potential factor which could lead to the higher recapture rate of active individuals. However, our recapture rates appeared to be good, and electrofishing has previously been shown to not discriminate against personality types in one of our experimental streams (Stenunge å; see Adriaenssens and Johnsson 2013). Instead, we

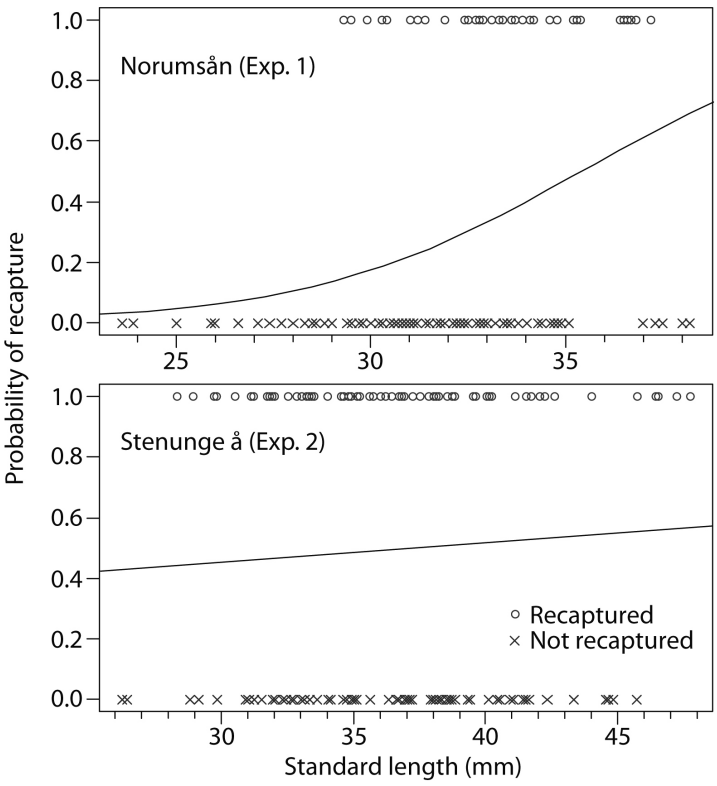


Figure 4: Predicted probability of recapture (used as a proxy for survival) for the brown trout fry (by GLM) depending on initial size (standard length). For details on statistics see Table 2.

argue that the higher apparent survival of active fish might be related to the territoriality of trout juveniles. More active trout are also more actively aggressive (Näslund and Johnsson 2015), so they may gain survival benefits when they can establish a good territory which optimises the energy intake with respect to mortality risk (Gilliam and Fraser 1987). Indeed, Réale et al. (2010) notes that the general pattern of the POLS hypothesis may be invalid in highly territorial species, where high levels of boldness, aggression and activity (but not dispersal tendency) can be beneficial for performance. Previous studies on salmonids which support the negative relationship between activity and survival probability have notably been made in lakes, where the fish likely swim more freely without defined territories (Biro et al. 2006, 2007). It is important to stress that our measure of activity is based on an artificial standardized test. Values from this test are not necessarily related to large-scale spatial activity in the wild. For

instance, open-field activity is not related with home-range size in age 1+ trout (Závorka et al. 2015), but may instead be related to activity within the home-range.

We found no effects of activity on growth rates in the streams (in line with results from Adriaenssens and Johnsson 2013), which could suggest that both more active and more passive fish were able to forage with similar efficiency. However, one should keep in mind that low activity appeared to be selected against. Thus, we may only see the well-growing fish in our recaptures.

Several studies in salmonid fish suggest that dominance could be a consequence of size rather than the opposite (Thorpe et al. 1992). This is further supported for brown trout fry, where clear individual differences in open-field activity and mirror aggression can be seen after a month of social isolation (Näslund and Johnsson 2015). In brown trout fry, size tend to be positively related to these behaviours, as well as negatively related to the time it takes for an individual to enter into a novel environment (Näslund and Johnsson 2015; Näslund et al. 2015a). However, if dominance was only associated to size, a month of isolation should probably make all fry more or less equally aggressive to an identically sized mirror image since they have no recent experience of any dominance hierarchy, and this was not observed in Näslund and Johnsson (2015). Instead, two distinct behavioural clusters were detected in that study. This observation fits with previous descriptions of salmonid fry behaviour, where some individuals are stationary belong to dominance hierarchies, while others are floaters without territories (e.g. Nielsen 1992; Héland 1999).

During the re-feeding period in the laboratory the growth rates in the food restricted groups showed that growth compensation occurred. Condition factor was completely compensated and body mass was partially compensated, showing that compensatory mass growth is physiologically possible in brown trout fry. This was particularly apparent in the second experiment, where a significantly higher condition factor was seen in the re-feeding fish, as compared to the controls. This supports results obtained in two previous studies where growth compensation was suggested to occur in individually housed brown trout fry (Näslund and Johnsson 2015; Näslund et al. 2015c). In the present study, food was given in excess in both treatment groups, so that all fish could eat ad libitum during the re-feeding period.

No compensation was detected for growth in body length. Instead the HR-HR fish grew faster than the mass compensating fish. However, this is the normal pattern for brown trout, as the fish first compensate in mass at

the expense of structural growth, before starting to compensate in structural growth (Näslund et al. 2015b). When released to the natural streams, the compensatory growth ceased in both experiments. This suggests that the environmental conditions in the streams limit the compensatory growth response in the fry. If fry are adapted at growing at the maximal rate allowed by the environment in the critical summer period, then there may not be an adaptive behavioural response to increase food intake. Open-field activity was not affected by growth compensation, in contrast to what was expected based on previous studies on older fish (Johnsson et al. 1996; Nicieza and Metcalfe 1997; Höjesjö et al. 1999; Vehanen 2003; Orpwood et al. 2006). The capacity to store energy in the body increases with body size, and the size of the energy stores likely affect the plasticity of the behaviour. Consequently, the flexibility of the endocrine systems affecting foraging behaviour may be more developed in larger individuals than in fry. Several studies suggest that salmonid fry can alter their behaviour when their endocrine systems are artificially enhanced above the normal levels, but this make them more susceptible to mortality in semi-natural environments, both with and without predators (e.g. Sundström et al. 2004; Crossin et al. 2015). Furthermore, while Atlantic salmon fry with exogenously increased GH-levels increased their growth rates above the normal in hatchery environments, their increased feeding motivation had negative consequences in the wild leading to slower growth rates than in sham-injected fry (Sundt-Hansen et al. 2012). Given that fry have a very high growth rate during the early summer (Mortensen 1977), it may not be adaptive to try to increase growth rate above the normal rate in nature. The graph of growth (Fig. 1) suggests that the fish from Norumsån (the younger of the two cohorts tested) continued to grow at a similar pace in nature as the control group showed in the laboratory. The fish from Stenunge å instead decreased their growth rate under natural conditions, but this may just be reflecting a normal season-dependent decrease in growth rate (Mortensen 1977).

Size had a positive effect on survival in the first experiment in Norumsån, but no effects in the second experiment in Stenunge å. This could indicate that size effects on survival disappear over summer. However, it may also be a consequence of smaller, less viable, fish being selected out from this population before we collected the experimental fish, or a sign of differential predation pressure in the two streams.

One possible reason for lack of signs of compensatory growth in the stream could be that compensating fish were more exposed to predation and thus removed from the population. However, we discard this hypothesis, as

no increased mortality was detected in fish that were released while being in a compensatory growth state. In fact, in Stenunge å, we caught a larger number of fish from the LH-HR group than from HR-HR growth.

General repeatability of open-field activity scores lie between 0.44 and 0.64, which is comparable with a previous study where trout fry were tested twice with a two day interval (Näslund and Johnsson 2015). The repeatability is within the typical range for animal behaviour, and even higher than what is typical for fish, suggesting that this behavioural trait is a relatively good measure of inter-individual behavioural variation (Bell et al. 2009). We should note that activity as tested in a, presumably, stressed state open-field test is not consistent with what is considered a 'pure' activity test to assess the activity-axis of personality, as such tests should be performed in a familiar area and in a non-stressed state (Réale et al. 2007; Conrad et al. 2011). Our measure is instead likely a measure of a combination of activity, boldness, and exploration. Still, this particular test appears to predict important ecological aspects of trout behaviour in natural environments (this study; Závorka et al. 2015). Adriaenssens and Johnsson (2013) used a very similar test, but with longer acclimation to reduce effects of exploratory behaviour and boldness.

From a wider perspective, our findings suggest that the prediction of a link between higher swimming activity, as scored in standardized laboratory tests, and higher mortality risk in the wild, is not supported. As suggested by Réale et al. (2010) there may be situations when the general POLS hypothesis is not valid, e.g. in highly territorial species. We therefore reiterate their advice that behavioural ecologists should avoid the temptation to generalise and simplify a complex reality based on the POLS without investigating the assumed relationships directly. We specifically note that ecological inference from laboratory-scored behaviour needs to be tested in nature for validity.

Acknowledgements

We thank Anders Hargeby for useful comments on a previous version of the manuscript. The work was funded by the Swedish Research Council Formas (J.I.J), Helge Ax:son Johnsons stiftelse (J.N.), and Wilhelm och Martina Lundgrens Vetenskapsfond (J.N).

Author contribution

J.N. and J.I.J. conceived the experiments. J.N., P.S.C. and J.I.J. designed the experiments. J.N. and P.S.C. performed the experiments. J.N. and P.S.C. analysed the data. J.N. and P.S.C. wrote the manuscript, with editorial advice from J.I.J.

References

Adriaenssens B, Johnsson JI. 2009. Personality and life-history productivity:consistent or variable association? Trends Ecol Evol 24: 179–180

Adriaenssens B, Johnsson JI. 2010. Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. Behav Ecol 22: 135–143

Adriaenssens B, Johnsson JI. 2013. Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. Ecol Lett 16: 47–55

Ali M. Nicieza A. Wootton R.J. 2003 Compensatory growth in fishes: a response to growth depression. Fish Fish 4: 147–190

Álvarez D. 2011. Behavioral responses to the environment — Effects of compensatory growth on fish behavior. Encyclopedia of Fish Physiology (eds A.P. Farrell, E.D. Stevens, J.J. Cech and J G Richards), pp. 752–757. Elsevier Inc., Electronic publication

Archard GA, Braithwaite VA. 2010. The importance of wild populations in studies of animal temperament. J Zool 281: 149–160

Arendt JD. 1997. Adaptive intrinsic growth rates: an integration across taxa. Q Rev Biol 72: 149–177

Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behaviour: a meta-analysis. Anim Behav 77: 771–783

Bilton HT, Robins GL. 1973. The effects of starvation and subsequent feeding on survival and growth of Fulton Channel sockeye salmon fry (*Oncorhynchus nerka*). J Fish Res Board Can 30: 1–5

Biro PA, Abrahams MV, Post JR. 2007. Direct manipulation of behaviour reveals a mechanism for variation in growth and mortality among prey populations. Anim Behav 73: 891–896

Biro PA, Abrahams MV, Post JR, Parkinson EA. 2006. Behavioural trade-offs between growth and mortality explain evolution of submaximal growth rates. J Anim Ecol 75: 1165–1171

Biro PA, Stamps JA. 2008. Are animal personality traits linked to life-history productivity? Trends Ecol Evol 23: 361–368

Bohlin T, Hamrin S, Heggberget TG, Rasmussen G, Saltveit SJ, Nielsen J. 1989. Electrofishing - Theory and practice with special emphasis on salmonids. Hydrobiologia 173: 9–43

Brett JR. 1970. Environmental factors and growth. Fish Physiology, Vol. VIII – Bioenergetics and growth (eds Hoar WS, Randall DJ, Brett JR), pp 599-675. Academic Press Inc., Orlando.

Brown ME. 1957. Experimental studies on growth. The Physiology of Fishes, Vol. I - Metabolism (ed Brown ME), pp 361-400. Academic Press Inc., New York.

Burns JG. 2008. The validity of three tests of temperament in guppies (*Poecilia reticulata*). J Comp Psychol 122: 344–356

Conrad JL, Weinersmith KL, Brodin T, Saltz JB, Sih A. 2011. Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. J Fish Biol 78: 395–435

Crossin GT, Sundström LF, Vandersteen WE, Devlin RH. 2015. Early life-history consequences of growth-hormone transgenesis in rainbow trout reared in stream ecosystem mesocosms. PLoS ONE 10: e0120173

Dingemanse N, Réale D. 2005. Natural selection and animal personality. Behaviour 142: 1159–1184

Dmitriew CM. 2011. The evolution of growth trajectories: what limits growth rate? Biol Rev 86: 97–116

Elliott JM. 1989. The critical-period concept for juvenile survival and its relevance for population regulation in young sea trout, *Salmo trutta*. J Fish Biol 35: 91–98

Elliott JM, Hurley MA, Fryer RJ. 1995. A new, improved growth-model for brown trout, *Salmo trutta*. Funct Ecol 9: 290–298

Fisher DN, Adèle J, Rodríguez-Muñoz R, Tregenza T. 2015. Behaviour in captivity predicts some aspects of natural behaviour, but not others, in a wild cricket population. Proc R Soc B 282: 20150708

Garcia de Leaniz C, Fraser N, Mikheev V, Huntingford F. 1994. Individual recognition of juvenile salmonids using melanophore patterns. J Fish Biol 45: 417–422

Gilliam JF, Fraser DF. 1987. Habitat selection under predation hazard: test of a model with foraging minnows. Ecology, 68: 1856–1862

Héland M. 1999. Social organization and territoriality in brown trout juveniles during ontogeny. Biology and Ecology of the Brown Trout and Sea Trout (eds J-L Baglinière, G Maisse), pp. 115–143. Praxis Publishing Ltd., Chichester.

Höjesjö J, Johnsson JI, Axelsson M. 1999. Behavioural and heart rate responses to food limitation and predation risk: an experimental study on rainbow trout. J Fish Biol 55: 1009–1019

Höjesjö J, Adriaenssens B, Bohlin T, Jönsson C, Hellström I, Johnsson JI. 2011. Behavioural syndromes in juvenile brown trout (*Salmo trutta*); life history, family variation and performance in the wild. Behav Ecol Sociobiol 65: 1801–1810

Johnsson JI, Bohlin T. 2005. Compensatory growth for free? A field experiment on brown trout, *Salmo trutta*. Oikos 111: 31–38

Johnsson JI, Bohlin T. 2006. The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proc R Soc B 273: 1281–6

Johnsson JI, Jönsson E, Björnsson BTh. (1996) Dominance, nutritional state, and growth hormone levels in rainbow trout (*Oncorhynchus mykiss*). Horm Behav 30: 13–21

Le Cren ED. 1961. How many fish survive? Yb River Boards Ass 9: 57–64

Milner NJ, Elliott JM, Armstrong JD, Gardiner R, Welton JS, Ladle M. 2003. The natural control of salmon and trout populations in streams. Fish Res 62: 111–125

Mittelbach GG, Ballew NG, Kjelvik MK. 2014. Fish behavioral types and their ecological consequences. Can J Fish Aquat Sci 71: 927–944

Mortensen E. 1977. Population, survival, growth and production of trout *Salmo trutta* in a small Danish stream. Oikos 28: 9–15

Nicieza AG, Metcalfe NB. 1997. Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. Ecology 78: 2385–2400

Nielsen JL. 1992. Microhabitat-specific foraging behavior, diet, and growth of juvenile coho salmon. Trans Am Fish Soc 121: 617-634

Niemelä PT, Dingemanse NJ. 2014. Artificial environments and the study of "adaptive" personalities. Trends Ecol Evol 29: 245–247

Näslund J. 2014. A simple non-invasive method for measuring gross brain size in small live fish with semi-transparent heads. PeerJ 2: e586

Näslund J, Johnsson JI. 2015. State-dependent behavior and alternative behavioral strategies in brown trout (*Salmo trutta* L.) fry. PeerJ PrePrints 3: e1524

Näslund J, Bererhi B, Johnsson JI. 2015a. Design of emergence test arenas can affect the results of boldness assays. Ethology 121: 556–565

Näslund J, Pauliny A, Blomqvist D, Johnsson JI. 2015b. Telomere dynamics in wild brown trout: effects of compensatory growth and early growth investment. Oecologia 177: 1221–1230

Näslund J, Sandquist L, Johnsson JI. 2015c. Behaviour in a novel environment is associated with body size, but not affected by recent feeding history in brown trout *Salmo trutta* fry. Submitted manuscript.

Olsen EM, Vøllestad LA. 2001. An evaluation of visible implant elastomer for marking age-0 brown trout. N Am J Fish Manage 21: 967–970

Orpwood JE, Griffiths SW, Armstrong JD. 2006. Effects of food availability on temporal activity patterns and growth of Atlantic salmon. J Anim Ecol 75: 677–685

Ostrovsky I. 1995. The parabolic pattern of animal growth – determination of equation parameters and their temperature dependencies. Freshw Biol 33: 357-371

Pianka ER. 1974. Evolutionary Ecology. Harper and Row, New York, NY.

Pinheiro J, Bates D, DebRoy S, Sarkar D. 2012. nlme: linear and nonlinear mixed effects models. R package version 3.1-103. R Foundation for Statistical Computing, Vienna.

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P-O. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Phil Trans R Soc B 365: 4051–4063

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82: 291–318.

Revelle W. 2015. Package 'psych'. R package version 1.5.6. R Foundation for Statistical Computing, Vienna.

Stamps JA. 2007. Growth-mortality tradeoffs and "personality traits" in animals. Ecol Lett 10: 355–363

Sundström LF, Lõhmus M, Johnsson JI, Devlin RH. 2004. Growth hormone transgenic salmon pay for growth potential with increased predation mortality. Proc R Soc B 271: S350–S352

Sundström LF, Kaspersson R, Näslund J, Johnsson JI. 2013. Density-dependent compensatory growth in brown trout (*Salmo trutta*) in nature. PLoS ONE 8: e63287

Sundt-Hansen L, Einum S, Neregård L, Björnsson BTh, Johnsson JI, Fleming IA, Devlin RH, Hindar K. 2012. Growth hormone reduces growth in free-living Atlantic salmon fry. Funct Ecol 26: 904–911

Thorpe JE, Metcalfe NB, Huntingford FA. 1992. Behavioural influences on life-history variation in juvenile Atlantic salmon, *Salmo salar*. Environ Biol Fish 33: 331–340

Vehanen T. 2003. Adaptive flexibility in the behaviour of juvenile Atlantic salmon: short-term responses to food availability and threat from predation. J Fish Biol 63: 1034–1045

Westley PAH, Stanley R, Fleming IA. 2013. Experimental tests for heritable morphological color plasticity in non-native brown trout (*Salmo trutta*) populations. PLoS ONE 8: e80401

Wilson PN, Osbourn DF. 1960. Compensatory growth after undernutrition in mammals and birds. Biol Rev 35: 324–363

Yagyu M, Nakamura H, Miyazaki T. 2007. A method for identifying individual Japanese charr, *Salvelinus leucomaenis*, using parr marks. Jpn J Ichthyol 54: 187–196

Závorka L, Aldvén D, Näslund J, Höjesjö J, Johnsson JI. 2015. Linking lab activity with growth and movement in the wild: explaining pace-of-life in a trout stream. Behav Ecol 26: 877–884

Appendix

Table 3: Details on treatment periods, food rations, feeding schedule, and food composition.

				Food composition* (wet mass)					
					RM (50%) TU (20%) WM (15%) BM (15%)	Total daily rations (% of body mass)		Feeding time	
	Treatment period	Date(s)	Experimental procedure	RM 100%	CY (10%)	HR-HR	LR-HR	Morning	Afternoon
Exp. 1		Jun 11	Capture	X		10	10	X	
	P1	Jun 12	Tagging, measurments	X		10	10		X
	P1	Jun 13-15	Acclimatisation	X		10	10	X	
	P1	Jun 16-17	Behavioural trials	X		10	10		X[a]
	P1	Jun 18-26	Feeding treatment		X	20	2	X	X[b]
	P2	Jun 27	Measurements		X	15	15		X
	P2	Jun 28-Jul 2	Feeding treatment		X	30	30	X	X
	P2	Jul 3-4	Behavioural trials		X	15	15		X[a]
	P2	Jul 5-7	Feeding treatment		X	30	30	X	X
	P2	Jul 8	Measurements		X	15	15		X
Exp. 2		Jul 17	Capture	X		10	10	X	
	P1	Jul 18	Tagging, measurments	X		10	10		X
	P1	Jul 19-21	Acclimatisation	X		10	10	X	
	P1	Jul 22-23	Behavioural trials	X		10	10		X[a]
	P1	Jul 24-Aug 1	Feeding treatment		X	20	2	X	X[b]
	P2	Aug 2	Measurements		X	15	15		X
	P2	Aug 3-7	Feeding treatment		X	30	30	X	X
	P2	Aug 8-9	Behavioural trials		X	15	15		X[a]
	P2	Aug 10-11	Feeding treatment		X	30	30	X	X
	P2	Aug 13	Measurements		X	15	15		X

* RM: "Red mosquito larvae" or "bloodworms" (Hexapoda, Chironomidae); TU: "Tubifex" (Clitellata, Naididae); WM: "White mosquito larvae" (Hexapoda, Chaoboridae); BM: "Black mosquito larvae" (Hexapoda, Culicidae); CY: "Cyclops" (Crustacea, Cyclopidae)

[a] Fish were fed after the behavioural trials

[b] Only HR-HR groups

Paper 5

Autumn food-restriction affects smoltification, but not over-winter performance in wild juvenile brown trout *Salmo trutta*

Joacim Näslund [1]
L. Fredrik Sundström [2]
Jörgen I. Johnsson [1]

This manuscript has been submitted for publication in a peer-reviewed academic journal

Corresponding author: J. Näslund (joacim.naslund@gmail.com)

[1]Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden
[2]Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden

Abstract

The winter is often considered as a survival bottleneck for stream-living fish. Juvenile salmonids generally become less active during this period, and while food intake continues to some extent, growth rates are typically low. Here we present the results of an over-winter field experiment where energy levels were manipulated in late autumn. Three groups of juvenile (age 1+) brown trout, from an anadromous population, were monitored with respect to over-winter growth rate and survival (as indicated by recapture rates). Two of the groups were brought in to the laboratory to be fed either high (HR), or low (LR) food rations; the third group remained in the stream (STR). Over-winter growth rates were relatively low in all groups, and no growth compensation could be detected. STR fish had better recapture rates, indicating that laboratory housing affected the performance in the stream. Comparing the two laboratory-housed groups, the LR group reached similar condition as the HR group in early spring, without indications of differences in survival. However, the onset of body silvering (indicating initiation of smoltification) was lower in the LR group. Thus, it appears that food-restriction during late autumn affect the onset of smoltification in juvenile brown trout. The results support previous findings from laboratory studies indicating that salmonids modify their over-winter foraging behaviour to end up at a targeted energy level at the end of winter. Such a modification appears to delay smoltification, but may not be costly in terms of over-winter mortality in the investigated stream.

Keywords: Growth rate, Salmonidae, Smoltification, Survival, Winter ecology

Introduction

Winter conditions in boreal and temperate regions can constitute challenges for many organisms. For stream-dwelling fish like salmonids, low water temperatures decrease the metabolism and reduce the scope for activity (Beamish 1978; Cunjak 1996; Huusko et al. 2007; 2013; Brown et al. 2011). Furthermore, the input of allochthonous food is low during this period due to low activity of terrestrial invertebrates (French et al. 2014), and the rate of food digestion and capacity of capturing drifting food are reduced by the low temperatures (Elliott 1972; Watz et al. 2013b, 2014). Salmonid fish also become predominantly nocturnal during this period (Heggenes et al. 1993; Fraser et al. 1993). Consequently, while salmonids often continue to feed though the winter (e.g. Elliott 1967; Cunjak and Power 1987; Bremset 2000; Finstad et al. 2004), the winter growth rates are low compared to other seasons (e.g. Swift 1961; Gardiner and Geddes 1980; Cunjak and Power 1986; Johnsson and Björnsson 2001; Bacon et al. 2004).

The winter is commonly considered a survival bottleneck for salmonids, but the evidence is conflicting (reviewed in Huusko et al. 2007; Carlson et al. 2008). Mortality likely depend on several interacting factors, like ice-formation, water temperature, discharge, shelter availability, predators, competition, and food availability (Cunjak 1996; Hurst 2007; Huusko et

al. 2007, 2013). Furthermore, the individual state (e.g. body size and energy stores) likely influences the ability to survive the winter in salmonid fish (Finstad et al. 2004; Biro et al. 2004; Hurst 2007). Finstad et al. (2004) suggested that the stored energy, rather than size, is the factor that determines an individual´s chance of survival. Thus, food-limitations prior to winter could potentially increase the over-winter mortality.

If food is restricted or withheld in spring, salmonid fish tend to respond with compensatory growth, i.e. faster than normal increase in body size, to catch up in size when food becomes available (Johnsson and Bohlin 2005, 2006; Sundström et al. 2013; Näslund et al. 2015b). However, the compensatory response often declines in autumn, and in winter the growth rate converges with that of individuals not being food-restricted, regardless of whether they have caught up in size or not (Johnsson and Bohlin 2005; Näslund et al. 2015b). Previous laboratory and hatchery studies indicate that food-restricted salmonids compensate lipid stores during winter time, but not length or mass, despite excess of food (Pirhonen and Forsman 1998, 1999; Morgan and Metcalfe 2001; Metcalfe et al. 2002). The difference in growth compensation patterns among seasons indicates that the growth response following periods of starvation is facultative (Metcalfe et al. 2002). While there are several laboratory and hatchery studies investigating the consequences of food-restriction out of the main growing season (i.e. in autumn and winter) in salmonids (e.g. Bull et al. 1996; Simpson et al 1996; Pirhonen and Forsman 1999; Morgan and Metcalfe 2001; Kiiskinen et al. 2003; Huusko et al. 2011), fewer studies have investigated effects on wild fish (e.g. Cunjak and Power 1986; Cunjak et al. 1987). In the laboratory, individuals can respond to depletion of their energy stores with increased activity and hyperphagia, but mainly to catch up in body condition (Bull et al. 1996; Simpson et al. 1996; Bull and Metcalfe 1997). However, laboratory studies lack the ecological complexity of real natural systems, and it is important to investigate experimental manipulations also in natural environments. In natural populations, some evidence suggest that depleted energy levels early in winter may not to be replenished later in winter, which could have dire consequences for survivability at the end of the winter season (Cunjak and Power 1986; Cunjak et al. 1987).

Brown trout juveniles living in coastal streams tend to migrate to sea a couple of years after hatching (commonly 1-3 years). This migration is preceded by a spectrum of morphological, behavioural, and physiological changes, collectively called 'smoltification' (Folmar and Dickhoff 1980). The decision to smoltify is thought to be taken as early as one year in advance based on growth pattern and condition, when fish that are not

going for smoltification can adopt an alternative life-history strategy of parr maturation (Bohlin et al. 1994). Timing in spring for smoltification is also dependent on size, with larger and faster growing fish smolting and migrating earlier in the season (Bohlin et al. 1993, 1996; Sundström et al. 2010). In general, the smolt migration appears to be an adaptive response to an inability to sustain high growth rates in the stream environment (Økland et al. 1993; Olsson et al. 2006). This occurs at a younger age, and at a smaller size, for faster growing fish than for fish with lower intrinsic growth rate (Økland et al. 1993). In hatchery reared fish the smoltification process has been shown to be affected by the fish's energetic status. For instance, in Lake Saimaa salmon (landlocked *Salmo salar*) the smoltification process seems to be temporarily switched off by poor nutritional conditions in winter (Kiiskinen et al. 2003). Another study indicated that food-restriction in early spring increased smolting in brown trout (*S. trutta*), with food-restriction in autumn and winter having no effect (Jones et al. 2015). However, hatchery salmonids and the hatchery environment often differ substantially from wild salmonids, particularly with respect to the growth rate and size achieved prior to smoltification. Thus, to get a picture of how lowered autumn energy levels affect smoltification in wild fish, manipulative studies using wild populations are necessary.

The aim of this study was to investigate the effect of food-restriction in late autumn on brown trout (age 1+) over-winter growth rate and mortality, and on condition and smoltification status in early spring. The novelty aspect of this study is to apply controlled food-restriction and investigate the effects of this manipulation in nature, using wild fish. With respect to growth and energy status, we hypothesised that compensatory growth in winter would be restricted, and that energetic levels at the end of winter would be similar among different autumn food treatment groups (i.e. fish would have a minimal acceptable condition, which they have to defend by modifying their winter foraging activity and/or energy expenditure). Accordingly we predicted that fish being food-restricted in late autumn would show little or no compensation in mass and length, but that the condition factor would be similar after winter. Regarding over-winter mortality, we hypothesised that food-restricted fish would need to forage more actively to keep their energy stores at the same levels as in non-restricted fish at the end of winter, and consequently be at higher risk of predation. Thus, the prediction was that food-restricted fish would have a higher mortality (as indicated by lower recapture rates in spring).

To test these predictions we conducted an over-winter field experiment, in which juvenile brown trout were fed at different rations in the laboratory

for a month in late autumn and thereafter released back into their native stream. In early spring we recaptured the fish and estimated over-winter growth rates and survival, and the level of morphological smoltification (body silvering). Data on external smoltification status was collected for descriptive purposes in relation to the treatments.

Material and Methods

Experimental area and population

The experiment was carried out in Jörlandaån, a coastal stream in south-western Sweden. The catchment area of the stream is 35 km2 and the mean river discharge is 0.4 $m^3 \cdot s^{-1}$ at the river mouth (maximal discharge is 10 $m^3 \cdot s^{-1}$ during snow melting in spring; minimal discharge is 0.0 $m^3 \cdot s^{-1}$ in summer) (Ramkvist 1975). The experimental section of the stream started 10.7 km upstream the outlet to the sea (57°58.534'N 11°55.594'E) and continued 700 m further upstream. This section of the stream is naturally meandering and runs through a forested area (broadleaf and mixed forest). The wetted width in the experimental section is 1.5-4 m, with varying depth (range 0 - 100 cm) and current speed. The stream bed mainly consists of sand and gravel, with large amounts of cobble and boulders. During cold winters, pool areas and the stream margins can freeze over, but riffle areas are typically ice-free; during mild winters the stream remains largely ice-free. Within the experimental section of the stream, brown trout is the dominant species (> 95% of individuals); other species present are European minnow *Phoxinus phoxinus* and European eel *Anguilla anguilla*. The brown trout population is predominantly anadromous, migrating to sea at a typical age of two years, but also contain stream-resident individuals (Pettersson 2002).

Stream water temperature was monitored from October 12, 2010, to March 6, 2011 using a temperature logger (StowAway Tidbit, Onset Computer Corp., USA). Comparative data on winter temperatures were obtained from the Swedish Meteorological and Hydrological Institute (SMHI 2015).

Capture and housing

On October 6, 2010, we captured 452 brown trout juveniles using electrofishing (LUGAB L-1000 [200-400 V, straight DC], LugAB, Sweden). The fish were brought to the Department of Zoology in Gothenburg where they

were anaesthetised (2-phenoxyethanol, 0.5 ml $\cdot$ l^{-1}), tagged with 12 mm passive integrated transponders (PIT-tags; ID 100, Trovan Ltd., United Kingdom), weighed (precision 0.1 g), and measured (fork length; precision 1 mm). The fish were randomly split into three groups: one group ($n = 152$) was released back into the stream the following day (henceforth called "STR"), and the other two groups (each $n = 150$) were kept in the laboratory until November 3 (28 days). One of the laboratory groups was assigned a low food ration (henceforth "LR") and the other was assigned a high food ration (henceforth "HR"). The LR and HR groups were split into five sub-groups (30 individuals in each), kept in separate 70 litre aquaria (bottom area: 35×65 cm) in the laboratory. Each tank had continuous water supply (10-12°C) from the in-house semi-closed circulation system and was furnished with gravel, stones, plastic plants and overhead covers made of grey PVC. Light was supplied by fluorescent tubes covered by thin black plastic bags to reduce the light levels. The fish were kept at high density in the laboratory to reduce aggression in the tanks, based on previous experience (authors' personal observations). Six individuals in the HR treatment, but none in the LR treatment, died during the laboratory period. Initial measurements for one HR individual were missing.

Laboratory food treatments

The HR fish were fed continuously through the laboratory treatment, in general four times per day, but less often during the initial 5 days when the fish were acclimating to laboratory conditions (see Fig. 1 for feeding schedule). To allow for a high food intake (i.e. four feedings per day, with time to digest the food in between) we extended the light period for the HR fish to 18:6 hours light:dark cycle (Fig. 1). The LR fish were fed approximately half of the days in the laboratory, and only at two times each feeding day (Fig. 1). The LR fish were kept at a natural light cycle (Fig. 1). At each feeding occasion we aimed at giving the fish food rations close to the ad libitum levels of the HR fish. Food consisted of frozen bloodworms (Chironomidae), chopped beef liver, or chopped compost worms (*Dendrobaena veneta*). Rations of approximately 3-5 g per tank were found to be close to ad libitum rations; all tanks were given the same food type, with the same wet mass, at each feeding occasion. For the initial laboratory acclimation period (5 days) the rations were smaller (1.5-3 g per tank), as the fish typically do not eat much during this phase.

During the laboratory period, each HR tank was supplied with 366 g food (73 g bloodworms, 186 g beef liver, and 107 g compost worms), while

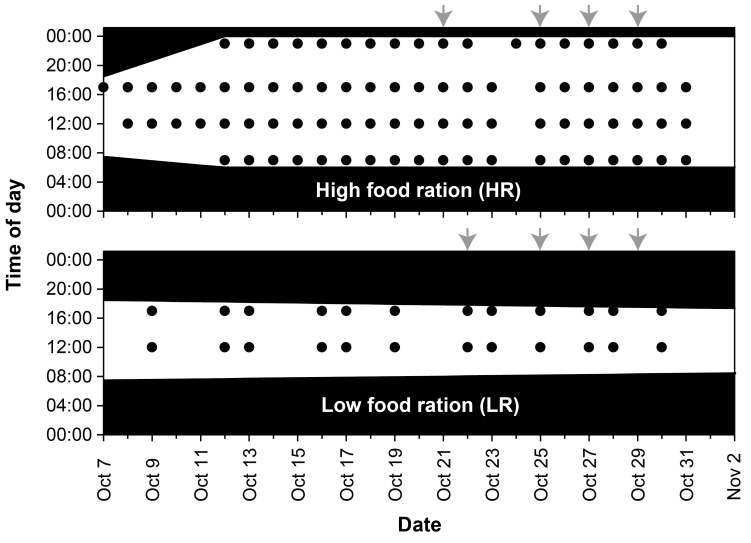


Figure 1: Change in light period (black = light off, white = light on) in the tanks, and feeding times (black dots) of the two laboratory treatment groups transferred to the laboratory on Oct 7 and released to back the stream on Nov 2. Grey arrows indicate cleaning of tank for removal of food remnants. Y-axis: 00:00 = midnight; 12:00 = midday.

each LR tank was supplied with 109 g food (10 g bloodworms, 60 g beef liver, and 39 grams compost worms).

All laboratory fish were weighed and measured (as described above) on November 2, after being starved for at least one day to empty the intestines of food.

Stream release and recaptures

On November 2 the experimental stream section was electrofished (one-pass fishing) for STR fish. All STR fish caught ($n = 26$) were weighed and measured and released back into the stream. On November 3 we released all laboratory fish into the experimental section in three equally sized, randomly divided, batches (approximately 50 m, 400 m, and 600 m upstream the start of the experimental section).

Early spring recapture electrofishing (two-pass fishing) was carried out on April 5-6 in the experimental stretch and in additional sections of 100 m immediately downstream and upstream of this area. The time was chosen to be able to catch also early migrating smolts. Water temperature during electrofishing was ranging between 2.1-3.1°C. Fish were anaesthetised, weighed, measured and scored for smoltification stage in the field. All fish were released back into the stream after recovering from anaesthesia.

Smoltification stage scoring

In April, smoltification stage was scored visually in the field using an ordinal scale with three classes (class 0: parr = no apparent silvering; parr marks clearly visible; class 1: pre-smolt = partial silvering, parr marks diffuse but visible; class 2: smolt = fully silvered, parr marks not/barely visible; scale modified after Johnston and Eales 1967 and Baglinière et al. 2000). Individuals scored as class 1 or class 2 were pooled to create a binomial smoltification stage variable (i.e. *silvering not initiated* = class 0; *silvering initiated* = class 1 + class 2).

Calculation of variables

Condition factor (K), i.e. mass (M) in g in relation to fork length (L) in mm, was calculated as

$$K = 10^5 \cdot M \cdot L^{-3}. \tag{1}$$

Specific growth rate in mass ($\% \cdot \text{day}^{-1}$; SGR_M) was calculated as

$$SGR_M = 100 \cdot (ln(M_{t_1}) - ln(M_{t_0})) \cdot (t_1 - t_0)^{-1}. \tag{2}$$

where t_0 and t_1 are the initial and final time-point in days (for a given investigated period), respectively.

Absolute growth in fork length (ΔL) was calculated as

$$\Delta L = L_{t_1} - L_{t_0}. \tag{3}$$

Evaluation of relative survival among groups was based on the number of recaptured fish in comparison to the initial number in each treatment group (excluding fish dead in the laboratory). As not all fish are captured during electrofishing, this measure is a relative measure of survival which underestimates the real survival. Here, we assume that recapture rate reflects the proportion of fish from each treatment group being present in the river at the time of recapture in spring.

Statistical analyses

To investigate changes in energy levels (condition factor) we analysed condition factor using a linear mixed model (LMM) with treatment (TR) as a fixed factor, time-point of measurement (TP) as a within-subject factor, and the interaction between these factors ($TR \times TP$). The same model was used to provide information on fork length and wet mass (ln-transformed) of the samples at each time-point.

Growth compensation was investigated by comparing mass growth rate (SGR_M) and growth in fork length (ΔL) using linear models (LM). Independent factors included in these LMs were: TR (fixed), initial fork length at the start of the experiment (L_I; covariate), and $TR \times L_I$. The two experimental periods ("treatment period" and "winter period") were analysed in separate models to allow for different factors to be included for each period-analysis (see selection criteria for model reduction below). The sample size of STR fish in the winter period analysis is lower than the total number of recaptured fish in spring in this group (see Table 1), due to the fact that some of the spring-recaptured fish were not recaptured in November (i.e. no growth rates could be calculated for these fish).

To explore whether the maintenance of the condition factor depended on the autumn post-treatment condition, we regressed the change in K between November and April on K in November.

The binary smolt stage variable and the spring recapture probability were analysed using generalised linear models (GLM) (binomial distribution, logit link-function), including independent factors: TR (fixed), L_I (covariate), and $TR \times L_I$.

In all analyses, covariates and the interactions including a covariate were included as control factors, but were subsequently removed from the model if they had p-values higher than 0.1 (i.e. we assumed no effect of the term in these cases). Final models are presented in Table 1.

Reported p-values are Holm-Bonferroni (H-B) corrected pairwise contrasts based on estimated marginal means from statistical models, unless otherwise stated. Main effect and interaction p-values are found in Table 1. Results were considered significant when $p \leq 0.05$. When $0.1 > p > 0.05$ the effects were treated as statistical trends of potential biological relevance.

All analyses were made in IBM SPSS Statistics 22.0 (IBM Corp., USA).

Stream temperature

Stream temperature over the experimental period is shown in Fig. 2. Low, stable temperatures around 0°C recorded between November 24, 2010, and March 28, 2011, could be associated to ice-formation. The logger was found close to the surface during recapture in April, probably being relocated by high flow. The winter was colder than normal for the geographic area, particularly in November and December (SMHI 2015; Fig. 2).

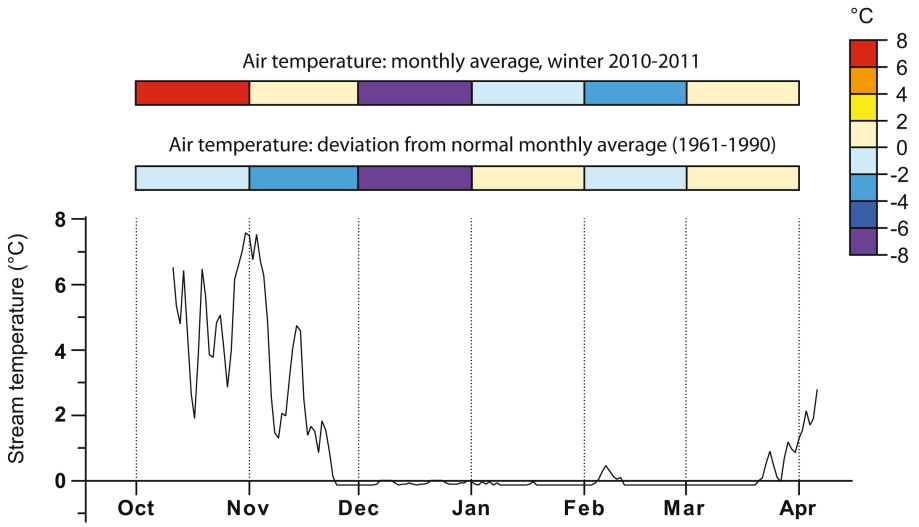


Figure 2: Temperature conditions during the study period. The line graph show stream water temperature as recorded by a temperature logger placed in the stream. Average monthly air temperature and deviation from normal monthly average (based on data from 1961 to 1990) is shown above the graph [data source: Swedish Meteorological and Hydrological Institute (SMHI 2015)].

Results

Growth

There were no significant differences in fork length (L; mean: 107 ± 13.1 SD), wet mass (M; mean: 13.3 ± 5.18 SD) or condition factor (K; mean: 1.03 ± 0.0494 SD) among the groups at the start of the experiment (all $p > 0.1$; Fig. 3).

The laboratory food treatments resulted in significant differences in both absolute length growth and specific mass growth rate, with LR growing less than both HR and STR (all $p < 0.001$; Fig. 4). HR and STR did not differ significantly, but STR tended to have a higher SGR_M ($p = 0.069$; Fig. 4), and a higher K in early November ($p < 0.001$; Fig. 3). During sampling of STR fish in November, we observed high stomach fullness in a large number of individuals, which seemed to depend on consumption of brown trout eggs (see electronic supplement, Fig. S1). The size of the fish (fork length) tended to influence the growth differently in the laboratory, as compared to the stream (overall effect, $TR \times L_I$: $p = 0.055$; Table 1); with the parameter estimates (B) of the slopes of LR and HR being significantly different (negative slopes; see Table 1) from STR (positive slope, but non-significant). Thus, in the laboratory the smaller fish grew relatively better

than larger ones, but this effect was not detected in the stream. During the treatment period LR had generally negative growth in both mass and length, while the other groups generally grew positively (see confidence intervals in Fig. 4).

Over winter there were no significant differences in either SGR_M or ΔL among recaptured fish from the different treatment groups, but SGR_M tended to be higher in LR than in STR ($p = 0.068$; Fig. 4). Condition factor generally decreased over winter (Fig. 3c).

A strong relationship was seen between change in condition factor over winter and the post-treatment condition factor, with fish having poorer condition after the treatment period having less change in the condition factor ($R^2 = 0.64$; $p < 0.001$; Fig. 5).

Recapture rates in spring

In April, 20 (14%) HR, 23 (15%) LR and 37(24%) STR fish were recaptured. The initial GLM indicated no $TR \times L_I$ interaction (Wald $\chi^2 = 1.9$, $p = 0.395$), so this term was removed from the model. The reduced model indicated a significant main effect of treatment (Wald $\chi^2 = 6.9$, $p = 0.032$), but no effect of initial fork length (Wald $\chi^2 = 0.17$, $p = 0.68$). H-B pairwise contrasts indicated that HR differed from STR ($p = 0.040$). Although LR had similar recapture probability as HR ($p = 0.59$), the former group did not differ significantly from STR ($p = 0.11$ [uncorrected $p = 0.056$]). However, a follow-up χ^2-test comparing laboratory-housed fish (LR + HR) against STR indicated that there was a general difference in recapture frequency caused by the laboratory environment ($\chi^2 = 6.4$, $p = 0.011$), with laboratory fish having lower recapture rate (which is assumed to indicate a relatively lower over-winter survival).

Smoltification status

The GLM modelling smoltification status (Fig. 6) showed a main effect of treatment (Wald $\chi^2 = 6.7$, $p = 0.035$) where more of the recaptured fish from both HR and STR had initiated silvering of the body, as compared to LR ($p = 0.001$ for both pairwise comparisons). HR and STR did not differ significantly from each other ($p = 0.35$). There was an effect of initial fork length (Wald $\chi^2 = 9.2$, $p = 0.002$), with larger fish being more likely to be silvered. However, there was also a significant interaction between initial fork length and treatment (Wald $\chi^2 = 6.3$, $p = 0.044$), caused by the STR group not showing size dependent onset of smoltification (as indicated by silvering).

Table 1: Summary of the statistical models of size, condition and growth (SGR_M – specific growth rate in mass; ΔL – absolute fork length growth). Model types: LM – linear model; LMM – linear mixed model. Explanatory variables: TP – time point; TR – treatment; L_I – initial length. Treatment groups: HR – high ration; LR – low ration; STR – stream. Estimated parameter values (B) for continuous variables, and interaction terms where these are included, are presented as footnotes.

Dep. variable	Model type	Final model	n	Test of effects (F-value; p-value)				
			(HR;LR;STR)	TR	TP	TP×TR	LI	LI×TR
Fork length	LMM	∼TP + TR + TP × TR	(a)	14.6; ***	8.82; ***	3.20; 0.014		
Ln(wet mass)	LMM	∼TP + TR + TP × TR	(a)	20.8; ***	8.71; ***	5.16; ***		
Condition factor	LMM	∼TP + TR + TP × R	(a)	25.9; ***	61.0; ***	11.7; ***		
$\Delta L_{Treatment}$	LM	∼LI + TR	144;150;26	71.9; ***			10.5; ***(b)	excluded
ΔL_{Winter}	LM	∼LI + TR	19;23;11	0.48; 0.62			13.7; ***(c)	excluded
$SGR_{M_{Treatment}}$	LM	∼LI + TR + LI × TR	144;150;26	3.08; 0.047			0.48; 0.49 (d)	2.92;0.055(d)
$SGR_{M_{Winter}}$	LM	∼TR	20;23;11	2.54; 0.089			excluded	excluded

*** $p \leq 0.001$
(a) Oct: (144;150;152), Nov: (145;150;26), Apr: (20;23;37)
(b) Negative effect (L_I: $B = -0.046$)
(c) Positive effect (L_I: $B = 0.057$)
(d) L_I: $B = 0.011$; Interaction: TR(STR) × L_I: $B = 0$ (reference); TR(HR) × L_I: $B = -0.022$ ($p = 0.016$); TR(LR) × L_I: $B = -0.018$ ($p = 0.045$)

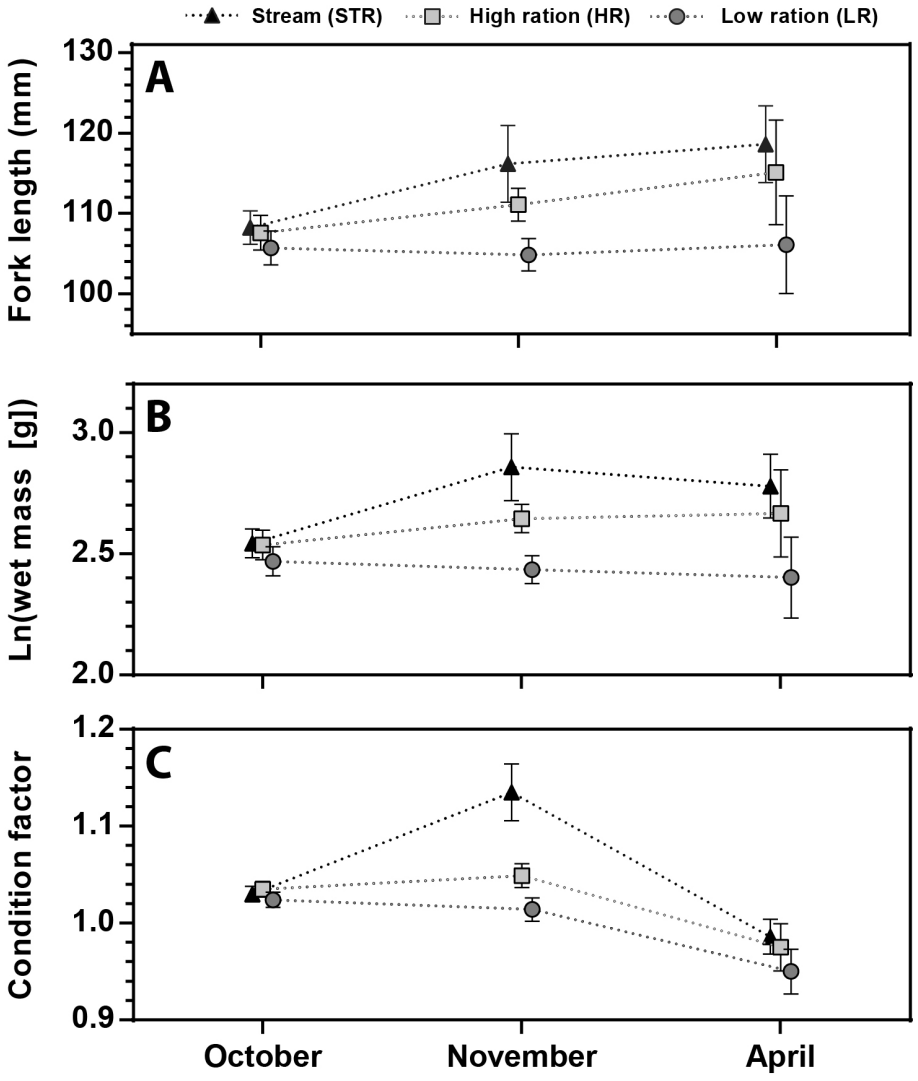


Figure 3: Estimated marginal means from linear mixed models (error bars show 95% confidence intervals) for fork length, wet mass (ln-transformed), and condition factor (K) at the different sampling times. Sample sizes are summarised in Table 1. Treatment groups and models are offset to increase the readability.

Discussion

The main discussion of observed effects concern differences between well-fed (HR) and food-restricted (LR) trout, as these two groups were treated similarly with respect to housing and handling in the laboratory. Com-

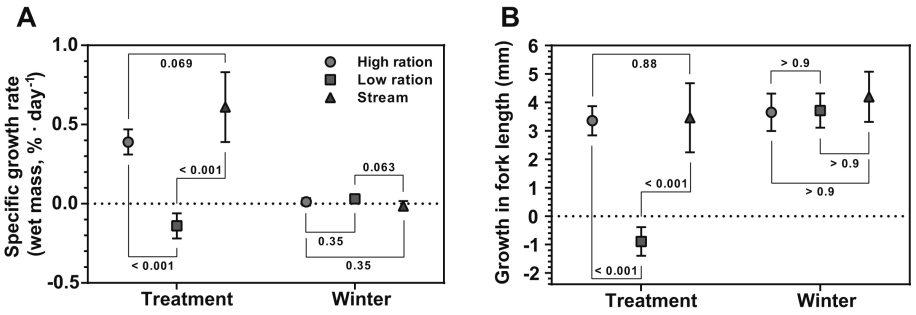


Figure 4: Estimated marginal means and 95% confidence intervals (error bars) for (A) specific growth rate in wet mass (treatment period corrected for body size; no effect of body mass detected for the winter period), and (B) absolute growth in fork length (both periods corrected for body mass). Numbers next to connecting lines between markers represent the p-values from pairwise contrasts.

parisons with the stream (STR) group are used mainly to discuss how the laboratory treatment could have affected the fish.

Treatment effects on over-winter performance

The results of this study show that, despite having lower condition factor at the onset of winter, the LR fish ended up with similar condition as the HR fish without any indications of lower survival. Furthermore, fish with poor condition following the autumn treatment period changed their condition factor less than fish with higher condition. Firstly, this implies that brown trout are able to adjust their winter foraging to avoid reaching a too low condition at the end of winter, which is in line with several previous laboratory studies (e.g. Simpson et al. 1996; Bull et al. 1996; Bull and Metcalfe 1997). Secondly, the lack of any indication for reduced survival in LR fish, as compared to HR fish, suggests that mortality was not depending on food-restriction in autumn. Recapture probability was likely relatively low, depending on low water temperatures when electrofishing, but we assume that the relative recapture frequency reflects the mortality. It is possible that the cold winter could have reduced mortality, as predation by endothermic predators could be limited in their choice of foraging grounds when parts of the stream are ice-covered (Hurst 2007; Huusko et al. 2007, 2013), which in turn may allow the trout to be more actively foraging. The ice-protection effect is supported by studies showing that ice-cover increases the willingness to forage, the swimming activity and the aggression, and reduces stress and ventilation rate in brown trout (Watz et al. 2013a, 2015). Studies on Atlantic salmon have also found that ice-cover may lead

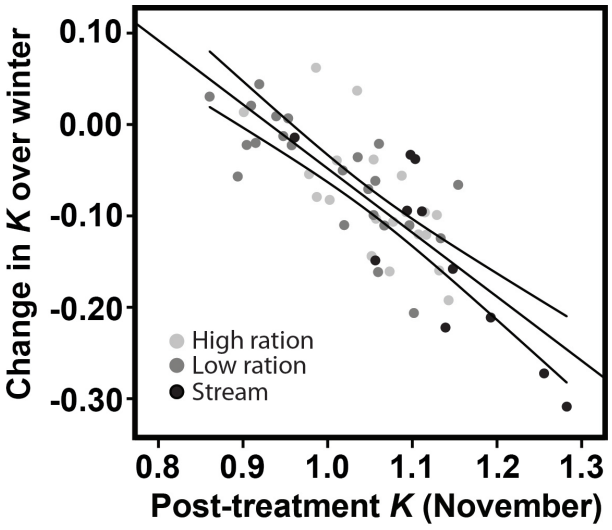


Figure 5: Regression of change in condition factor K over winter as dependent on post-treatment period condition factor (K in November).

to relatively low mortality, presumably due to the more stable conditions as compared to ice-free conditions (Linnansaari and Cunjak 2010).

Condition factor decreased over winter in all groups, suggesting a general reduction of stored energy reserves. This effect is considered to be normal for salmonid fish, and has been shown repeatedly (Gardiner and Geddes 1980; Cunjak 1988; Berg and Bremset 1998; Biro et al. 2004). Growth of LR fish was on average negative during the laboratory treatment, both in wet mass and fork length. Loss in mass is normal for food-restricted fish, and a slight decline in body length has been observed under harsh conditions in another study (Huusko et al. 2011). There was a trend for STR fish to grow more than HR fish in mass, but not in length during the treatment period (when HR fish were located in the laboratory, and the STR fish were in the stream). This effect was also evident in the estimate of condition factor, which consequently was higher for the STR fish. The high STR condition factor in November was likely an effect of high stomach fullness (which, consequently, is also reflected in their mass), as we observed that parr in the stream had started to eat eggs from spawning brown trout adults (Fig. S1). Laboratory-housed fish had empty stomachs when weighed in November, and may also have lost some days of growth due to the initial acclimation to laboratory environment. Overall, no tendency for compensatory growth in LR, as compared to HR, could be seen over winter, probably as sheltering and maintenance of energy levels

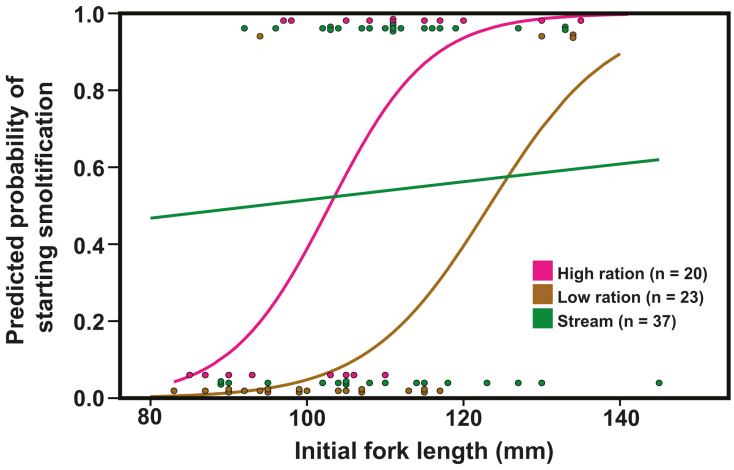


Figure 6: Predicted probabilities of having started smoltification, as indicated by silvering index, at the final recapture in April (from logistic regression).

is prioritized before regaining body growth (Heggenes et al. 1993; Bull et al. 1996; Bull and Metcalfe 1997). Growth rates over winter were similar among treatment groups. In general fish only grew very little between November and March (range of added length: 1 – 7 mm; range of change in mass: -3 – 3 g), in concordance with previous studies in the same general area (Johnsson and Björnsson 2001). A trend for STR fish to lose slightly more in mass than LR fish over winter is likely explained by their relatively greater stomach fullness in November (personal observation, indicated by the condition factor), rather than by differences in energy expenditure.

Treatment effects on timing of smoltification in spring

Food-restriction in late autumn affected the smoltification process, as fewer of the LR fish showed signs of silvering at the recapture in April, as compared to the HR and STR groups. Silvering is associated with parr-smolt transformation, when salmonids pre-adapt to a marine environment (Johnston and Eales 1967). In general, the silvering is reaching maximum levels several weeks prior to the full development of sea-water tolerance (Staley and Ewing 1992; Staurnes et al. 1993). Environmental light conditions may affect body silvering irrespective of smoltification status, but this could be an effect of being reared in artificial environments and is probably not seen in natural environments (Ewing and Birks 1982; Kazakov and Kozlov 1985). Thus, even if silvering has been criticised as an indicator of when the smoltification is finished (e.g. Ewing and Birks 1982; Kiiskinen et al. 2003),

it is still likely a suitable indicator that smoltification has been initiated in wild fish.

General food ration reductions over winter and spring have been shown to increase the numbers of smolts in hatchery populations of brown trout (e.g. Olsson et al. 2006; Wysujack et al. 2009; Jones et al. 2015), while food reductions in summer and autumn appear to have little effect (Pirhonen and Forsman 1998, 1999; Jones et al. 2015). However, it should be noted that even food-restricted trout in these hatchery studies are substantially larger with mean wet mass being two to three times higher than even the largest individuals in our study ($\approx$ 35 g). The association between energy levels, growth rate and life-history strategies could therefore be different in artificial and natural environments. It could also be that hatchery reared trout need to experience food-restriction in spring to initiate smoltification, as the conventional hatchery environment would provide enough energy to sustain high growth rates, taking away the need to migrate (Serrano et al. 2009). The energy depletion in our LR fish may instead have been relatively severe, and available energy could have been spent on maintaining life, rather than pre-adaptations for migration. In hatchery reared Lake Saimaa salmon, winter-fasted fish from the upper part of the size distribution showed lower levels of Na^+,K^+-ATPase, a physiological indicator for smoltification, before feeding was recommenced in spring (Kiiskinen et al. 2003). This indicates that the smoltification process may have been temporarily switched off in these fish. However, when the fish were fed again, they compensated both in size and expression of Na^+,K^+-ATPase quickly, and they achieved similar smolt status as continuously fed fish. In wild fish the potential for growth in the following winter period may be more limited than in hatcheries, where food is continuously provided. Whether or not the decision to smoltify at all, or just the timing of silvering was affected in our fish cannot be determined from our data, as infrastructure for monitoring of outmigration (e.g. PIT antennas or smolt traps) was not present in the stream. Like in the case of Saimaa salmon (Kiiskinen et al. 2003), it may be that well-fed fish with good condition prior to winter have started to smoltify, while the food-restricted ones still need to feed more to initiate smoltification. Given the water temperature at final sampling had risen to just about 3°C it seems unlikely that the spring growth season had started (Elliott 1976).

Restricted juvenile growth may increase the number of mature males (Pirhonen and Forsman 1999), which do not smoltify in brown trout (Bohlin et al. 1986). However, our study manipulated growth just before the spawning period, and the decision to mature should already have been

taken at that time. We did not investigate maturation status, but given random allocation to the treatment groups, the number of maturing parr should have been equal in all treatments.

Differences between fish kept in the laboratory and stream fish

Fish being kept in the laboratory for one month ('lab-treated', i.e. LR and HR) had generally lower recapture rates, indicating lower survival, as compared to STR fish. It is possible that the lab-treatment was stressful for the fish, and a recent study have indicated that high stress levels in autumn may reduce over-winter survival (Midwood et al. 2015). Lab-treated fish may also have had difficulties in acclimating back to the stream environment, leading to poorer survival. Stream temperatures at release (7-8 °C) were not much lower than laboratory temperatures (10-11°C), but the fluctuating nature of the stream water may affect the mortality of the lab-treated fish which had experienced a very stable environment over the previous month. It is possible that the late autumn is a more narrow survival bottleneck than the winter in stream salmonids (e.g. Linnansaari and Cunjak 2010), and problems in adjusting to the environment at this time may have carryover effects on winter survival. Recaptured lab-treated fish also showed size dependent silvering, in contrast to recaptured STR fish, indicating that they were affected by the time in the laboratory. Generally, larger fish not smoltifying seem to be missing from the lab-treated fish, but present in the STR group. It is possible that these fish were maturing parr, and that this particular group of fish from the lab-treatments were either particularly vulnerable, or dispersed more (looking for spawning opportunities), after release. Prior-residency effects, where STR fish had occupied the best over-winter territories before lab-treated fish were returned to the stream, may also have affected the lab-treated fishes' general performance (see e.g. Bohlin 1977; Bohlin et al. 2002). Speculatively, it may also be that lab-treated fish could have missed the late-autumn egg-boon, which was ongoing when they were released. Eggs from adult trout may be an important high-quality food resource important for over-winter performance (Näslund et al. 2015a). Thus, it is possible that mainly large-sized lab-treated fish had enough resources for early smoltification. LR and HR fish were all subjected to similar lab-treatment, so while the differences between these two groups and the STR group may be due to laboratory effects, the comparisons between LR and HR groups are still interpreted as valid, given that the effects of laboratory housing were additive.

Conclusions

Brown trout being food-restricted for a period in late autumn had similar growth over winter as conspecifics fed high food rations during the same period. Furthermore, both food-restricted and well-fed fish ended up decreasing their condition factor to similar levels until April, without costs of increased risk of mortality in the food-restricted group. However, food-restricted fish were showing a lower frequency of silvering (initiation of smoltification) at recapture in early spring, which indicate that they might delay seaward migration as a consequence of the autumn food-restriction.

Acknowledgements

We thank Rasmus Kaspersson, Lars-Erik Sundström, David Hammenstig, Johannes Björk and Bart Adriaenssens for assistance during electrofishing, tagging and/or for feeding the fish. J.I.J was supported by the Swedish Research Council Formas. Furthermore, three anonymous reviewers are thanked for their constructive comments. This study was performed in accordance with Swedish animal welfare laws and was approved by the Gothenburg ethical committee for animal research, license number 113-2008 and 8-2011.

References

Bacon PJ, Gurney WSC, Jones W, McLaren IS, Youngson AF. 2004. Seasonal growth patterns of wild juvenile fish: partitioning variation among explanatory variables, based on individual growth trajectories of Atlantic salmon (*Salmo salar*) parr. J Anim Ecol 74: 1–11

Baglinière JL, Ombredane D, Marchand F. 2000. Critères morphologiques pour l'identification des deux formes (rivière et mer) de truite (*Salmo trutta*) présentes sur un même basin. Bull Fr Pêche Piscicult 357/358: 375–383

Beamish FWH. 1978. Swimming capacity. In: Hoar WS, Randall DJ, eds. Fish Physiology, vol. VII. Locomotion. London: Academic Press, pp. 101–187.

Bell RJ. 2012. Winter feeding as an overwintering survival strategy in young-of-the-year winter flounder. Trans Am Fish Soc 141: 855–871

Berg OK, Bremset G. 1998. Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. J Fish Biol 52: 1272–1288

Biro PA, Morton AE, Post JR, Parkinson EA. 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). Can J Fish Aquat Sci 61: 1513–1519

Bohlin T. 1977. Habitat selection and intercohort competition of juvenile sea-trout *Salmo trutta*. Oikos 29: 112–117

Bohlin T, Dellefors C, Faremo U. 1993. Optimal time and size for smolt migration in wild sea trout (*Salmo trutta*). Can J Fish Aquat Sci 50: 224–232

Bohlin T, Dellefors C, Faremo U. 1994. Probability of first sexual maturation of male parr in wild sea-run brown trout (*Salmo trutta*) depends on condition factor 1 yr in advance. Can J Fish Aquat Sci 50: 224–232

Bohlin T, Dellefors C, Faremo U. 1996. Date of smolt migration depends on body-size but not age in wild sea-run brown trout. J Fish Biol 49: 157–164

Bohlin T, Sundström LF, Johnsson JI, Höjesjö J, Pettersson J. 2002. Density-dependent growth in brown trout: effects of introducing wild and hatchery fish. J Anim Ecol 71: 983–692

Bremset G. 2000. Seasonal and diel changes in behaviour, microhabitat use and preferences by young pool-dwelling Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*. Environ Biol Fish 59: 163–179

Brown RS, Hubert WA, Daly SF. 2011. A primer on winter, ice, and fish: what fisheries biologists should know about winter ice processes and stream-dwelling fish. Fisheries 36: 8–26

Bull CD, Metcalfe NB. 1997. Regulation of hyperphagia in response to varying energy deficits in overwintering juvenile Atlantic salmon. J Fish Biol 50: 498–510

Bull CD, Metcalfe NB, Mangel M. 1996. Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. Proc R Soc Lond B 263: 13–18

Carlson SM, Olsen EM, Vøllestad LA. 2008. Seasonal mortality and the effect of body size: a review and an empirical test using individual data on brown trout. Funct Ecol 22: 663–673

Cunjak RA. 1988. Physiological consequences of overwintering in streams: the cost of acclimitization? Can J Fish Aquat Sci 45: 443–452

Cunjak RA. 1996. Winter habitat of selected stream fishes and potential impacts from land-use activity. Can J Fish Aquat Sci 53: 267–282

Cunjak RA, Curry A, Power G. 1987. Seasonal energy budget of brook trout in streams: implications of a possible deficit in early winter. Trans Am Fish Soc 116: 817–828

Cunjak RA, Power G. 1986. Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). Can J Fish Aquat Sci 43: 1970–1981

Cunjak RA, Power G. 1987. The feeding and energetics of stream-resident trout in winter. J Fish Biol 31: 493–511

Elliott JM. 1972. Rates of gastric evacuation in brown trout, *Salmo trutta* L. J Anim Ecol 2: 1–18

Elliott JM. 1976. The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. J Anim Ecol 45: 923–948.

Ewing RD, Birks EK. 1982. Criteria for parr-smolt transformation in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 28: 185–194

Finstad AG, Ugedal O, Forseth T, Næsje TF. 2004. Energy-related juvenile winter mortality in a northern population of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 61: 2358–2368

Folmar LC, Dickhoff WW. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. Aquaculture 21: 1-37

Fraser NHC, Metcalfe NB, Thorpe JE. 1993. Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proc R Soc Lond B 252: 135–139

French WE, Vondracek B, Ferrington LC, Finlay JC, Dieterman DJ. 2014. Winter-feeding, growth and condition of brown trout *Salmo trutta* in a groundwater-dominated stream. J Freshw Ecol 29: 187–200

Gardiner WR, Geddes P. 1980. The influence of body composition on the survival of juvenile salmon. Hydrobiologia 69: 67–72

Heggenes J, Krog OMW, Lindås OR, Dokk JG. 1993. Homeostatic behavioural responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. J Anim Ecol 62: 295–308

Hurst TP. 2007. Causes and consequences of winter mortality in fishes. J Fish Biol 71: 315-345

Huusko A, Greenberg L, Stickler M, Linnansaari T, Nykänen M, Vehanen T, Koljonen S, Louhi P, Alfredsen K. 2007. Life in the ice lane: the winter ecology of stream salmonids. River Res Appl 23: 469–491

Huusko A, Mäki-Petäys A, Stickler M, Mykrä H. 2011. Fish can shrink under harsh living conditions. Funct Ecol 25: 628–633

Huusko A, Vehanen T, Stickler M. 2013. Salmonid habitats in riverine winter conditions with ice. In: Maddock I, Harby A, Kemp P, Wood P, eds. Ecohydraulics: An Integrated Approach. Chichester: John Wiley & Sons, pp. 177–192

Johnsson JI, Björnsson BTh. 2001. Growth-enhanced fish can be competitive in the wild. Funct Ecol 15: 654–659

Johnsson JI, Bohlin T. 2005. Compensatory growth for free? A field experiment on brown trout, *Salmo trutta*. Oikos 111: 31–38

Johnsson JI, Bohlin T. 2006. The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proc R Soc B 273: 1281–1286

Johnston CE, Eales JG. 1967. Purines in the integument of the Atlantic salmon (*Salmo salar*) during parr-smolt transformation. J Fish Res Board Can 24: 955–964

Jones DA, Bergman E, Greenberg LA. 2015. Food availability in spring affects smolting in brown trout (*Salmo trutta*). Can J Fish Aquat Sci doi: 10.1139/cjfas-2015-0106

Kazakov RV, Kozlov VV. 1985. Quantitative estimation of degree of silvering displayed by Atlantic salmon (*Salmo salar* L.) juveniles originating from natural populations and from fish-rearing farms. Aquaculture 44: 213–220

Kiiskinen P, Huskonen H, Hyvärinen H, Piironen J. 2003. Effects of daylength and winter fasting on growth and smolting of one-year-old Saimaa landlocked salmon (*Salmo salar m. sebago* Girard) under fish farm conditions. Ann Zool Fenn 40: 441–458

Linnansaari T, Cunjak RA. 2010. Patterns in apparent survival of Atlantic salmon (*Salmo salar*) parr in relation to variable ice conditions throughout winter. Can J Fish Aquat Sci 67: 1744-1754

Metcalfe NB, Bull CD, Mangel M. 2002. Seasonal variation in catch-up growth reveals state-dependent somatic allocations in salmon. Evol Ecol Res 4: 871–881

Midwood JD, Larsen MH, Boel M, Aarestrup K, Cooke SJ. 2015. An experimental field evaluation of winter carryover effects in semi-anadromous brown trout (*Salmo trutta*). J Exp Zool A doi: 10.1002/jez.1955

Morgan IJ, Metcalfe NB. 2001. Deferred costs of compensatory growth after autumnal food shortage in juvenile salmon. Proc R Soc Lond B 268: 295–301

Näslund J, Aldvén D, Závorka L. 2015a. Eggs from anadromous adults provide marine-derived nutrients to Atlantic salmon and brown trout parr prior to the onset of winter: observations from a Swedish coastal stream. Environ Biol Fish doi: 10.1007/s10641-015-0436-y

Näslund J, Pauliny A, Blomqvist D, Johnsson JI. 2015b. Telomere dynamics in wild brown trout: effects of compensatory growth and early growth investment. Oecologia 177: 1221–1230

Olsson IC, Greenberg LA, Bergman E, Wysujack K. 2006. Environmentally induced migration: the importance of food. Ecol Lett 9: 645-651

Pettersson JCE. 2002. Migration in brown trout *Salmo trutta* – causes and consequences. PhD thesis, Department of Zoology, Göteborg university.

Pirhonen J, Forsman L. 1998. Effect of prolonged feed restriction on size variation, feed consumption, body composition, growth and smolting of brown trout, *Salmo trutta*. Aquaculture 162: 203–217

Pirhonen J, Forsman L. 1999. Can smolting and maturation of hatchery-reared brown trout *Salmo trutta* L. be affected by food deprivation during the first and second years of rearing? Aquacult Res 30: 611–620

Ramkvist K. 1975. Naturvårdsinventering av Jörlanda- och Anråseån i Stenungsunds kommun. Länsstyrelsen i Göteborgs och Bohus län, Gothenburg, Sweden. (In Swedish)

Serrano I, Larsson S, Eriksson L-O. 2009. Migration performance of wild and hatchery sea trout (*Salmo trutta* L.) smolts – implications for compensatory hatchery programs. Fish Res 99: 210-215

Simpson AL, Metcalfe NB, Huntingford FA, Thorpe JE. 1996. Pronounced seasonal differences in appetite of Atlantic salmon parr, *Salmo salar*: effects of nutritional state and life-history strategy. Funct Ecol 10: 760–767

SMHI (Swedish Meteorological and Hydrological Institute). 2015. Klimatdata: Meteorologi: Temperatur. http://www.smhi.se/klimatdata/meteorologi/temperatur/ (Accessed: 2015-04-21; In Swedish)

Staley KB, Ewing RD. 1992. Purine levels in the skin of juvenile coho salmon (*Oncorhynchus kisutch*) during parr-smolt transformation and adaptation to seawater. Comp Biochem Physiol B 101: 447–452

Staurnes M, Lysfjord G, Hansen LP, Heggberget TG. 1993. Recapture rates of hatchery-reared Atlantic salmon (*Salmo salar*) related to smolt development and time of release. Aquaculture 118: 327–337

Sundström LF, Lõhmus M, Devlin RH. 2010. Migration and growth potential of coho salmon smolts: implications for ecological impacts from growth-enhanced fish. Ecol Appl 20: 1372-1383

Sundström LF, Kaspersson R, Näslund J, Johnsson JI. 2013. Density-dependent compensatory growth in brown trout (*Salmo trutta*) in nature. PLoS ONE 8: e63287

Swift DR. 1961. The annual growth-rate cycle in brown trout (*Salmo trutta* Linn.) and its cause. J Exp Biol 38: 595–604.

Watz J, Bergman E, Piccolo JJ, Greenberg L. 2013a. Effects of ice cover on the diel behavior and ventilation rate of juvenile brown trout. Freshw Biol 58: 2325-2332

Watz J, Piccolo J, Bergman E, Greenberg L. 2013b. Day and night drift-feeding by juvenile salmonids at low water temperatures. Environ Biol Fish 97: 505–513

Watz J, Bergman E, Piccolo J, Greenberg L. 2014. Prey capture rates of two species of salmonids (*Salmo trutta* and *Thymallus thymallus*) in an artificial stream: effects of temperature on their functional response. Mar Freshw Behav Physiol 47: 93–99

Watz J, Bergman E, Calles O, Enefalk Å, Gustafsson S, Hagelin A, Nilsson PA, Norrgård JR, Nyqvist D, Österling EM, Piccolo JJ, Schneider LD, Greenberg L, Jonsson B. 2015. Ice cover alters the behavior and stress level of brown trout *Salmo trutta*. Behav Ecol 26: 820-827

Wysujack K, Greenberg LA, Bergman E, Olsson IC. 2009. The role of the environment in partial migration: food availability affects the adoption of a migratory tactic in brown trout *Salmo trutta*. Ecol Freshw Fish 18: 52-59

Økland F, Jonsson B, Jensen AJ, Hansen LP. 1993. Is there a threshold size regulating seaward migration of brown trout and Atlantic salmon? J Fish Biol 42: 541–550

Paper 6

Linking lab activity with growth and movement in the wild: explaining pace-of-life in a trout stream

Libor Závorka [123]
David Aldvén [3]
Joacim Näslund [3]
Johan Höjesjö [3]
Jörgen I. Johnsson [3]

This manuscript was originally published by Oxford University Press on behalf of the International Society for Behavioral Ecology
Behavioral Ecology 2015, 26(3), 877-884. doi: 10.1093/beheco/arv029
(Received 18 August 2014; revised 21 February 2015; accepted 24 February 2015; published online 9 April 2015)

Corresponding author: L. Závorka (liborzavorka@email.cz)

[1]Institute for Environmental Studies, Charles University in Prague, Prague, Czech Republic
[2]Department of Zoology and Fisheries, Czech University of Life Sciences, Prague, Czech Republic
[3]Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

Abstract

Theory suggests that consistent individual differences in activity are linked to life history where high activity is associated with rapid growth, high dispersal tendency, and low survival (the pace-of-life syndrome hypothesis). We addressed this influential hypothesis by combining behavioral studies with fine-scale positional scoring in nature, estimating how individual movement strategies in brown trout (*Salmo trutta*) associate with fitness correlates (growth and survival) in the wild. Initial dispersal in the wild was positively related to the laboratory activity. Moreover, the growth of individuals with high laboratory activity decreased with increasing home range size, whereas the growth of individuals with lower laboratory activity increased slightly with increasing home range size. Survival in the wild was not associated with laboratory activity. Our results do not support the original pace-of-life syndrome hypothesis. As an alternative explanation, we suggest that the growth of individuals adopting a high-activity strategy is more sensitive to variation in resource abundance (indicated by home range size) than the fitness individuals adopting a more passive strategy.

Keywords: behavior repeatability, dispersal, exploration, growth rate, natural selection, survival

Introduction

Movements, fine-scale or long distance, allow an individual to switch habitats and to avoid unfavorable conditions (Bowler and Benton 2005; Brönmark et al. 2008). The decision to move should depend on individual-specific trade-offs between the fitness benefits and costs associated with movement (Dingemanse and de Goede 2004; Matthysen 2005; Hanski et al. 2006; Dingle and Drake 2007; Cote et al. 2010). Accordingly, within-population variability in movements has been described for a wide range of animal taxa (Jonsson and Jonsson 1993; Ball et al. 2001; Attisano et al. 2013). This behavior often differ consistently across individuals within a population from fine-scale movements often measured as behavioral traits in animal personality research (i.e., activity and exploration; Réale et al. 2007) to large-scale movement strategies in the wild linked to home range (John-Alder et al. 2009) and seasonal migration (Ball et al. 2001).

The ecological significance of consistent movement strategies is especially striking in species where such strategies are tightly linked to life history (Dixon et al. 1993; Musiani et al. 2007). Brown trout (*Salmo trutta* L.) is a highly plastic species showing a range of ecological adaptations including 3 basic life histories associated with movement: stream dwelling, lake migratory, and sea migratory (Klemetsen et al. 2003), which result from different trade-offs between reproduction, survival, and growth (Roff 1992; Hendry et al. 2004). Reproduction and survival in brown trout, as in

other organisms with indeterminate growth, are positively correlated with body size (Elliott 1994; Kingsolver and Huey 2008, but see Carlson et al. 2008). Thus, growth is an important fitness correlate that can be used to compare the success of alternative strategies at the juvenile stage (Stearns 1976).

Population density has been shown to affect growth rate, survival, and movement in salmonids and in other territorial species (Matthysen 2005; Einum et al. 2006). In high-density areas, individuals are facing a choice where they can either stay at the cost of reduced growth rate or move to another area with potentially better growth conditions (Einum et al. 2006). However, movement can also induce substantial costs, for example, energetic expenditure and increased risk of predation (Yoder et al. 2004).

The pace-of-life syndrome (POLS) concept (Réale et al. 2010) suggests that consistent differences in behavior and life-history traits can coevolve forming stable associations. According to this influential hypothesis, high activity and boldness or exploration is consistently associated with rapid growth and high mortality, whereas low activity and shyness or neophobia is associated with slow growth and low mortality (Biro et al. 2004, 2006; Stamps 2007; Cole and Quinn 2014). The POLS hypothesis has been supported by a number of studies conducted under captive conditions and/or with domesticated species (Biro and Stamps 2008; Adriaenssens and Johnsson 2009; Mittelbach et al. 2014). However, studies conducted under natural conditions have repeatedly failed to support the hypothesis (Dingemanse et al. 2004; Adriaenssens and Johnsson 2011, 2013; but see Smith and Blumstein 2008), suggesting that links between behavior and life-history traits are more variable in natural environments characterized by fluctuating resource abundance and competition levels (Adriaenssens and Johnsson 2009; Réale et al. 2010).

As indicated by the discussion above, the ecological and evolutionary significance of interindividual variation in activity and associated behavioral traits (boldness, exploration) is still poorly understood (Réale et al. 2007). Although laboratory scores of behavior often show some repeatability over time (Bell et al. 2009), it remains unclear to what extent behavioral variation carries over between different contexts. Indeed, the critical need for cross-context validation of behavior in both laboratory and natural conditions has recently been highlighted (Niemelä and Dingemanse 2014). Previous studies addressing associations between activity and life-history traits have used indirect measurements of activity and boldness like catchability to a net or a trap (Réale and Festa-Bianchet 2003; Biro et al. 2004, 2006) or open-field test (Murphy et al. 1994; Adriaenssens and Johnsson

2011, 2013), whereas detailed measurements of individual activity and its association with movement in the wild have been scarcer (Niemelä and Dingemanse 2014). This is likely due to limitations in methods available for animal positioning in the wild. Traditional technologies available for animal positioning (i.e., telemetry) are limited, as the size of the transmitter dictates the minimal body size of the focal animal (Jepsen et al. 2005). However, telemetry using passive integrated transponder (PIT) tags is a novel, effective, and low-invasive method for animal identification and tracking.

Here, we combine laboratory behavioral scoring with PIT-tag tele-metry in the wild using juvenile brown trout as a model to address predictions from the pace-of-life-syndrome hypothesis via the following questions: 1) To which extent are interindividual differences in activity, measured in a laboratory open-field test, associated with a) initial dispersal in an unfamiliar stream section and b) home range size in the stream? 2) Are differences in laboratory activity associated with growth and survival in the wild in concordance with the POLS hypothesis? 3) How are these associations influenced by population density?

Methods

Fish collection

In May 2013, 200 one-year-old brown trout (mean ± standard error [SE]: 78.10 ± 0.64 mm; range: 56–107 mm) were caught by electrofishing (Bohlin et al. 1989) in a 250-m-long section of Jörlandaån, a small coastal stream in western Sweden (57°58'N, 11°56'E) (Figure 1). In the laboratory, fish were housed in 3 holding tanks and starved for 1 day before tagging to get standardized initial measurements of the body size. The holding tank provided shelter (rocks and plastic plants) and fresh water from a flow-through filtration system (flow rate: 2 L min^{-1}). Photoperiod followed natural light cycles and temperature was kept at 11–13 °C throughout the experiment. After the acclimatization, trout were anesthetized (2-phenoxyethanol; 0.5 mL L^{-1}), and measurements of fork length (distance from the tip of the snout to the end of the central caudal fin ray) and body weight were taken, followed by tagging with 12-mm PIT tags (HDX ISO 11784/11785, Oregon RFID, Portland, OR) into the body cavity. Tagged fish were subsequently divided among 18 holding tanks (30 L, 30 × 32 × 34 cm, 11–12 individuals per tank) and left to recover for 5 days before behavioral scoring. Individuals were fed ad lib. with Chironomidae larvae during this period.

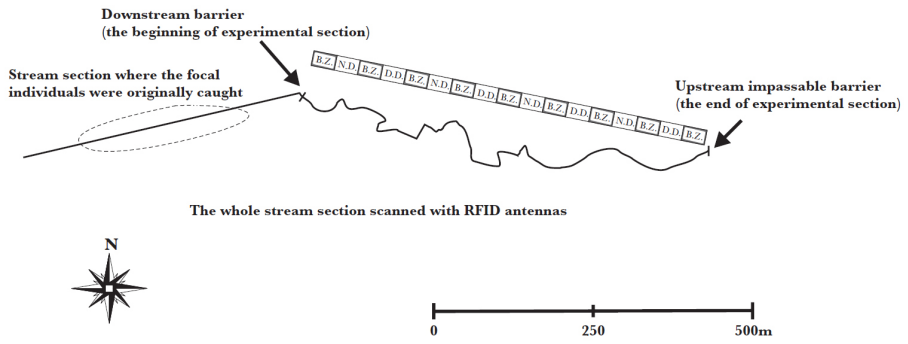


Figure 1: The experimental section of stream Jörlandaån. The rectangle illustrates the distribution of experimental subsections with manipulated population density. B.Z.: buffering zone; N.D.: subsection with normal population density; D.D. subsection with doubled.

Behavioral scoring

Each fish was individually scored for swimming activity in an open-field test. The open-field trials were conducted in barren white rectangular plastic tanks (61 × 45 cm, water level 10 cm), positioned underneath a dim fluorescent light tube and a camera (Logitech webcam C120, Romanel-sur-Morges, Switzerland).

The fish were not fed for 24 h prior to the trials in order to standardize hunger levels of individuals in trial. When subjected to the trial, fish were gently netted from the holding tank and placed individually into trial tanks. Individual activity was scored for 30 min, following the first swimming movement. The trial tanks were divided by a grid of 20 equal-sized squares.

Laboratory activity scores were derived from the number of crossings between squares, where each crossing represents a complete passage by an individual over the borderline into an adjacent square. After the scoring, all fish were scanned for individual PIT number and placed back into their holding tank; trial tanks were cleaned and filled with fresh water before a new batch of fish was entered. Trials were performed during 3 consecutive days from 8:30 AM until 7:00 PM under similar light and temperature conditions in order to standardize measured activity scores. There was no effect of day time on measured activity scores (Spearman's $\rho = -0.09$; $P = 0.193$). No mortality and no tag losses were observed during the laboratory period of the experiment. Fish activity was only scored once due to time constraints, as previous studies have found swimming activity to be repeatable over time in brown trout (repeatability in Adriaenssens and Johnsson 2013 was $R = 0.449$; $P = 0.006$).

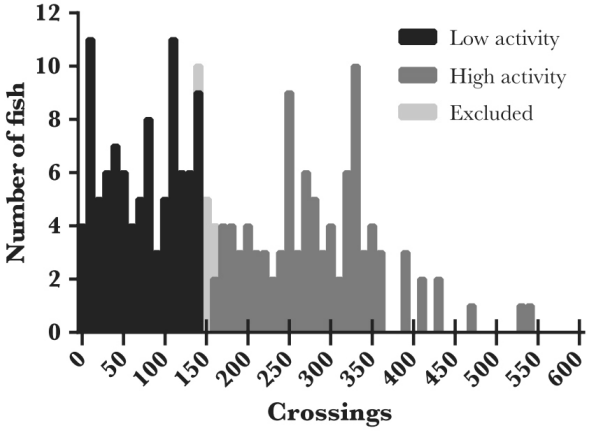


Figure 2: Distribution of activity scores of juvenile brown trout (activity scores were equal to the number of crossings between squares) in laboratory open-field test.

Density manipulation

Following behavioral scoring, individuals were divided into a low activity group and a high-activity group according to their laboratory activity score (Figure 2) and sorted in 8 release groups each containing 12 active and 12 passive individuals. The distribution of individuals was pseudo-randomized within the low- and high-activity groups, consequently each release group contained individuals with relatively high and low scores. Eight individuals with intermediate scores remained after the sorting and were removed from the experiment. The removal of surplus individuals resulted in a final number of 192 focal individuals (96 active and 96 passive individuals) that were released into the experimental stream section.

Each release group of focal individuals was put (on May 31) into one of eight 30-m-long experimental stream subsections in river Jörlandaån with manipulated trout density, either normal or doubled density. In subsections with normal density treatment, 24 resident age-1 trout were removed and replaced by 24 focal individuals. In the double density treatment, 24 resident age-1 trout were removed and replaced by 24 focal individuals and 24 introduced individuals, caught at least 200 m downstream from the used subsection (Figure 1). Removal of wild residents in experimental subsections was made to reduce prior residency effects in high-density subsections (Johnsson et al. 1999). Surplus individuals (8 fish) were released downstream the experimental section approximately 250 m below the barrier (Figure 1) in order to limit their homing migration back to the manipulated experimental stream section. All experimental subsections were separated

from each other by 50-m-long buffering zones, which were supposed to be beyond the distance of regular movement range for juvenile brown trout (Bridcut and Giller 1993; Höjesjö et al. 2014). The experimental section was surrounded and shaded by deciduous (mainly alder) and spruce trees. The stream holds a population of sea migratory brown trout, which is the most abundant fish species (> 95% by biomass); other species are European minnow *Phoxinus phoxinus* and European eel *Anguilla anguilla*. In addition to adult resident trout and eel, grey heron *Ardea cinerea* and American mink *Neovison vison* were predators observed to occur in the experimental area. The downstream limit of the experimental section was defined by a 0.25-m-high artificial weir. Movements upstream were hindered by a 1-m-high waterfall, an impassable obstacle for juvenile trout during the low water flow of the summer season. All fish appeared to be in good condition at the release.

Tracking and recapture

A longitudinal positioning of focal individuals was performed during the 3 summer months (June-August), using portable PIT-tag antennas (Oregon RFID). The first scanning took place 4 days after release, followed by biweekly scans, and the last scanning was performed 1 day before fish recapture on 3 September 2013. As individuals were able to move downstream from the experimental section; a stream section of 450 m below the lower weir was included in the scanning (Figure 1). Tracking was then conducted from this downstream point and upstream to the waterfall at the end of the experimental section, a total length 1400 m. Each observer was equipped with a GPS (eTrex Garmin, Olathe, KA), which automatically saved the position of the observer. Geographical positioning data of individual fish were obtained by synchronizing time stamps from the GPS and the RFID data logs. The repeatability and precision of positioning during the experiment was controlled against 3 fixed reference positions situated at the start, in the middle, and at the end of the scanned stream section (the standard deviation among scannings against these reference points was 2.4 m suggesting a good precision of positioning). During recapture, on September 4, focal fish were positioned with the PIT-tag antenna and subsequently caught by electric fishing. When the focal fish were not attracted to the electrofishing wand, we tried to displace the individual out from the shelter by moving the bottom substrate. Individuals that did not respond to the electrofishing and kept the position even after the disturbance of the substrate were considered being dead (i.e., PIT tag was lying on the bottom).

All recaptured fish (70 individuals) were anesthetized (2-phenoxyethanol), PIT scanned, and measured for mass and body length. After the recovery, all individuals were released back to the place where they were caught.

Data handling and statistics

Fish movement in the field was measured as a longitudinal distance between 2 successive fish positions following the middle of the stream channel (i.e., meandering of the stream was taken in to account while diagonal movements within the channel were ignored). The distance from the point of release to the position of first scanning was defined as initial dispersal. Home range size was estimated as the distance between the 2 most distant positions where the individual was detected during the study, excluding the initial dispersal. This is a common method used for estimating home range along the longitudinal gradient of the stream (Hodder et al. 2007). These 2 variables represented movement strategies of individuals in the wild. One of the recaptured individuals was detected only once after the initial dispersal (i.e., home range size could not be estimated) and was therefore excluded from analysis. In contrast to our expectations, initial dispersal of the majority of individuals exceeded the distance between the experimental sections that eroded the original experimental setup of manipulated population density. Consequently, we tested for the effect of population density only in relation to initial dispersal of individuals.

Initial dispersal was analyzed using gamma regression (log-link function), which is suitable for strictly positive continuous data with positive skew (Hardin and Hilbe 2007). The model contained laboratory activity, initial density (2 levels: high and low density), their interaction, and initial weight as independent variables. Non-significant interaction between laboratory activity and initial density was removed from the model, and we reported results for the model without the interaction. The model contained all individuals detected during the initial scanning, 4 days after the release. To graphically investigate relationships between activity in the lab and initial distance moved in the stream, we plotted a Loess line (Epanechnikov kernel with 50% of points to fit) through a scatter plot of all data points.

Home range size was analyzed using gamma regression (log-link function), with the model containing laboratory activity and initial weight as independent variables. The model contained all individuals recaptured at the end of the experiment.

Survival was analyzed based on the recapture rates at the end of the

experiment (on September 4). Individuals that were detected by PIT-tag antenna and caught by electrofishing gear were referred to as being alive, individuals detected by PIT-tag antenna but not caught referred to as dead, and undetected individuals were referred to as non-detected. There were 3 individuals referred as alive, which were not caught by the electrofishing gear, but they were displaced several meters upstream by the substrate disturbance. To investigate differences in laboratory activity among alive, dead, and non-detected individuals, we used linear model with status of individual at the final scanning (alive, dead, or non-detected) as an independent variable. Variance of data among the groups was homogenous (Levene's test: $P = 0.640$), and data were not normally distributed (Figure 2); however, model was robust against this assumption as the distribution of dependent variable did not differ among the groups (Schmider et al. 2010). Density treatment was not included in the analysis because many fish left experimental subsection shortly after release. The relationship between laboratory activity and the frequency of detection during the study was tested by nonparametric Spearman's rank correlation tests.

Individual growth was evaluated by specific growth rate (SGR) calculated as:

$$Observed\ SGR = \frac{\ln(final\ wet\ body\ weight - \ln(initial\ wet\ body\ weight)}{time\ between\ measurements\ (100\ days) * 100} \quad (1)$$

The influences of home range and laboratory activity on specific growth rate were analyzed using all recaptured fish. To analyze growth rate, we first calculated deviation from expected specific growth rate based on initial size. Specific growth rate was negatively related to initial length, as expected (Brett 1979). Therefore, we used the parameters from a linear regression (slope and intercept) on the pooled data of all recaptured fish to calculate expected growth rate:

$$Expected\ SGR = 1.54 - 0.0126 \cdot initial\ fork\ length \quad (2)$$

Deviation from expected growth rate was calculated as:

$$Deviation = Observed\ SGR - Expected\ SGR \quad (3)$$

The deviation from expected growth rate was analyzed using a linear model. The linear model included laboratory activity, home range size, and their interaction as continuous independent variables. Diagnosis of the initial model indicated presence of one outlying value (Cook's $D > 1$ for 1 individual [home range: 655 m; laboratory activity: 7]). This individual

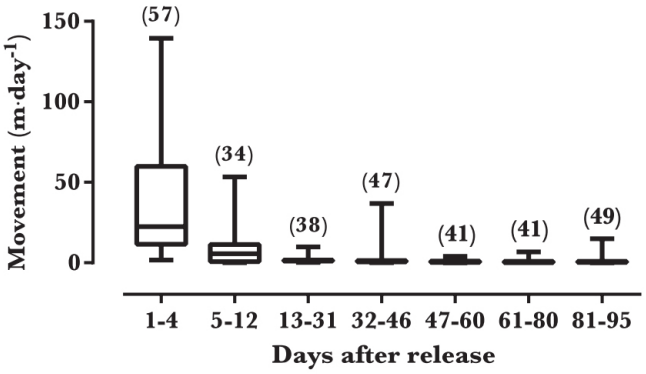


Figure 3: Mean movement per day of focal juvenile brown trout, which survived until the end of the experiment, between consecutive scannings. Boxplots (median with the box showing inter-quartile range) present the range of recorded movements, the number above each boxplot indicate sample size. Numbers on the x axis indicate a time span between the 2 consecutive scannings when the movements were recorded.

was excluded, and the deviation from expected growth rate was reanalyzed using the same linear model (data normally distributed [Shapiro-Wilk's test: $P = 0.637$]).

Results

General movements and frequency of detections

We recorded a decrease in the number of detected tags across the time, from 77% in the beginning to 58% at the end of the study. We detected 191 out of 192 individuals at least once during the study. The total number of recorded individual positions was 894. There was no relationship between laboratory activity and frequency of detections in the field (Spearman's $\rho = 0.093$; $P = 0.200$).

PIT-tag scanning in the stream revealed that most of the movements were occurring shortly after release. Thereafter movements large-ly ceased and positions were generally stable for individual fish (Figure 3). Homing behavior of focal individuals was generally weak, and the majority (165 out of 192 focal individuals) did not attempt to migrate downstream the artificial step to the stream section where they were originally caught.

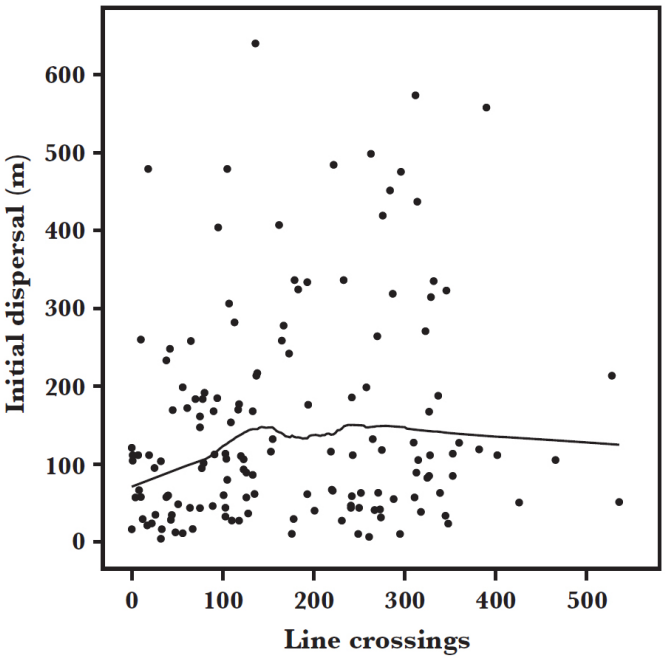


Figure 4: Correlation between laboratory activity (number of line crossings) and initial dispersal (4 days after release) of juvenile brown trout fitted by Loess line.

Lab activity and field movement strategy

Laboratory activity was positively related to initial dispersal in the wild ($F_{1,142} = 4.11$; $P = 0.044$; Figure 4). There was no significant correlation between initial fish length and laboratory activity (Spearman´s $\rho = 0.040$, $P = 0.578$). However, initially larger fish moved longer distances in the wild ($F_{1,142} = 5.48$; $P = 0.021$). Initial movement was not influenced by density treatment ($F_{1,142} = 0.29$; $P = 0.591$). Neither was there any effect of the interaction between density and laboratory activity ($F_{1,141} = 0.64$; $P = 0.426$). Home range size was not associated with laboratory activity ($F_{1,70} = 0.65$; $P = 0.422$) or initial size ($F_{1,70} = 0.51$; $P = 0.477$).

Survival

There was no significant difference in laboratory activity among individuals determined as alive, dead, and non-detected at the last scanning of the experiment ($F_{2,189} = 1.48$; $P = 0.229$): laboratory activity of alive (mean: 185; SE: 15.4; $N = 73$), dead (mean: 193; SE: 19.4; $N = 32$), and non-detected (mean: 158; SE: 13.1; $N = 87$) individuals.

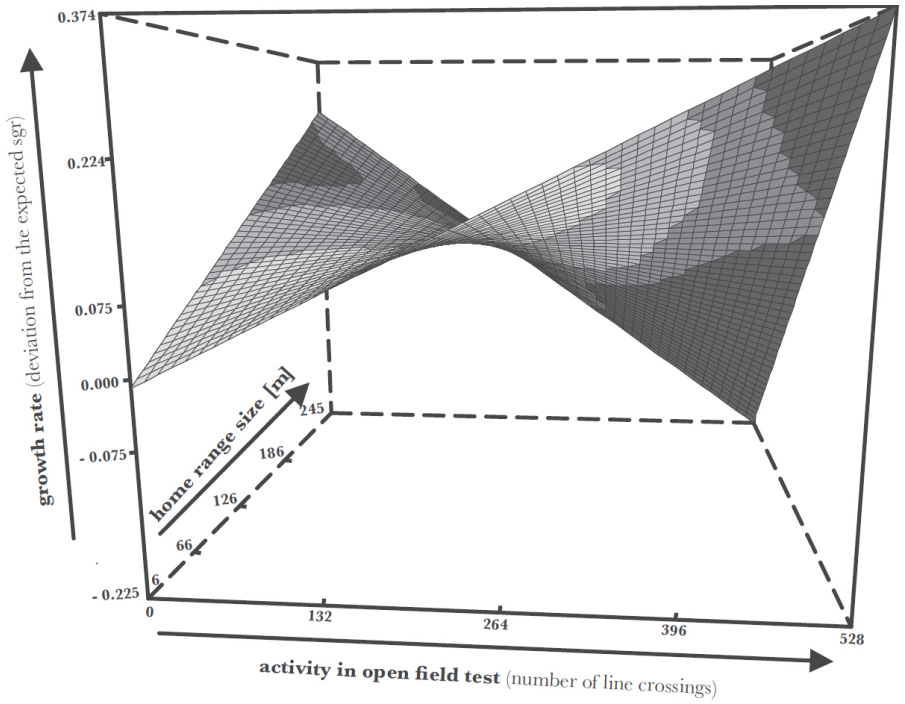


Figure 5: Surface plot displaying effect of interaction between laboratory activity and home range size on growth rate (i.e., deviation from the expected growth rate) in juvenile brown trout. The highest 75%, 50%, and 25% of the bivariate density estimate of laboratory activity and home range size are colored in with colors of increasing gray scale.

Growth

There was no overall association between growth (i.e., deviation from expected specific growth rate) and laboratory activity ($F_{1,64} = 1.787$; $P = 0.186$). However, growth was significantly influenced by an interaction between laboratory activity and home range size ($F_{1,64} = 4.471$; $P = 0.038$; Figure 5), where the growth of individuals with high laboratory activity decreased with increasing home range size, whereas the growth of individuals with lower laboratory activity increased slightly with increasing home range size.

Discussion

In this study, we investigated 1) to which degree laboratory activity is linked to movement strategies in the natural environment and 2) if individual differences in activity, growth, and survival in the wild are associated in

concordance with the POLS hypothesis. We also aimed to investigate 3) how these associations were influenced by population density. However, because the initial dispersal of the fish was higher than expected and there were no significant effects of density, we will focus on the first 2 questions in the discussion below.

Association between activity in the lab and movement strategies in the stream

We found that laboratory activity was positively related to the initial dispersal of individuals. In contrast, home range size was not correlated with laboratory activity and all individuals were predominantly stationary after the initial dispersal. The positive relation between laboratory activity and initial dispersal provides evidence that open-field tests reflect ecologically significant behavioral traits of animal personality (i.e., activity and exploration, see Réale et al. 2007), which are linked to dispersal tendency (Dingemanse and de Goede 2004). Visual exploration of the Loess line plotted between the laboratory activity and distance of initial dispersal shows that the activity was positively related to initial dispersal only in individuals with low activity (up to a lab activity score of approximately 150). This can be an indication that a highly active fish, as scored in the laboratory, can be constrained in their movements under natural conditions, for example, by the stream environment (complexity, obstacles, open stream sections without refuges, high predation sites, etc.) An alternative explanation might be that the increased activity is associated with low predictability in a behavioral syndrome (Biro and Adriaenssens 2013).

Link between activity and life-history traits

Our second main question was whether laboratory activity in trout was associated with growth and survival in the wild and whether this association was consistent with the POLS hypothesis. The key assumption of this hypothesis, a positive correlation between activity, growth rate, and mortality (Biro et al. 2004, 2006; Stamps 2007; Cole and Quinn 2014), was not confirmed by our data, as there was no significant correlation between laboratory activity and growth rate or survival. Growth rate in more active individuals decreased with increasing home range size (i.e., decrease of food abundance), whereas the growth of individuals with lower activity increased slightly with increasing home range size, indicating an interaction between life-history traits, environmental conditions, and behavioral traits linked to animal personality. Assuming a complete concordance (i.e., negative

correlation) between food abundance and home range size is an oversimplification of the natural system because home range size in salmonids is determined by multiple interacting factors including predation risk (Kim et al. 2011), competition (Keeley 2000), and habitat complexity (Kalleberg 1958). However, the energetic trade-off selects for the smallest home range size, which satisfies the energetic demands of the individual. Thus, an increase in food abundance is generally assumed to reduce the home range size (Hixon 1981; Grant 1997; Slavík et al. 2014).

We propose that more active individuals adopt a high gain/high cost strategy, as described in Metcalfe (1986), and are therefore able to yield a high net energy income and growth rate when conditions are optimal (e.g., high food availability). However, they suffer by low net energy income and growth rate under suboptimal conditions (low food availability) due to high activity–related maintenance costs. In contrast, less active individuals adopt a low gain/low cost strategy, allowing them to maintain a more constant growth rate under a wider range of environmental conditions (Figure 6). Thus, the link between these alternative strategies and fitness (i.e., growth) is dependent on environmental conditions. Although activity certainly increases metabolism, other characteristics of individual affecting energetic costs, like resting metabolic rate, are often (Biro and Stamps 2010; Réale et al. 2010), but not always (Houston 2010) positively correlated with activity.

Implications for POLS hypothesis: a new conceptual model

We suggest along with previous studies (Dingemanse et al. 2004; Dingemanse and de Goede 2004; Adriaenssens and Johnsson 2009, 2011, 2013; Höjesjö et al. 2011; Le Galliard et al. 2013; Montiglio et al. 2014) that the POLS hypothesis in its original form is too simplistic to explain the adaptive value of consistent behavioral traits under natural conditions, as exemplified by our stream model system, a complex and unpredictable environment with fluctuating selective pressures (Höjesjö et al. 2004). Based on the results of our field experiment and previous studies in similar experimental systems, we suggest an alternative hypothesis, complementary to the POLS concept (Réale et al. 2010), linking consistent interindividual variation in activity to reaction norms associating food abundance and growth rate (Figure 6). The general validity of our conceptual model is tentatively supported by other empirical studies conducted in similar experimental systems (Biro et al. 2004, 2006; Adriaenssens and Johnsson 2011, 2013; Höjesjö et al. 2011). When summarized, the results of these studies

are consistent with the hypothesis that different reaction norms link food abundance and growth rate for alternative behavioral strategies (Figure 6): in environments with stable and rich food habitats, more active individuals grow faster than passive because they are able to utilize abundant and predictable food resources (Biro et al. 2004, 2006), whereas environments with less predictable food abundance do not always meet costs of high activity and therefore passive or shy individuals can grow as fast as, or even faster than, active or bold individuals (Adriaenssens and Johnsson 2011, 2013; Höjesjö et al. 2011). The ecological relevance of these reaction norms may be even more general, also including other environmental variables where growth performance is optimal at intermediate conditions. For example, Armstrong et al. (2011) hypothesized similar curves for describing the relationship between growth and water velocity in salmon (*Salmo salar*) with different standard metabolic rate (gray lines in Figure 6). Even more generally, these norms could be viewed as an analogy to the performance of generalist and specialist strategies over a range of environmental conditions (Gilchrist 1995).

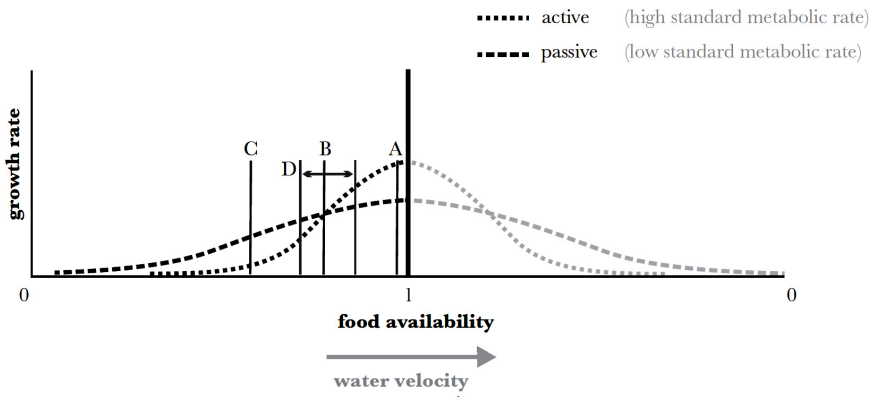


Figure 6: The hypothetical reaction norm associating environmental variation to growth for individuals adopting an extreme active or passive strategy, respectively. The units are arbitrary. Food availability is increasing from 0 to 1 (1 is equal to optimal conditions when individuals can feed *ad lib.*). Letters denote studies evaluating the growth of freshwater salmonid fish with different levels of activity in environments assumed to differ in food abundance — A: Biro et al. (2004, 2006) (conducted on wild and domesticated rainbow trout *Oncorhynchus mykiss*); B: Adriaenssens and Johnsson (2013) (conducted on wild brown trout) and Höjesjö et al. (2011) (hatchery reared wild brown trout); C: Adriaenssens and Johnsson (2011) (conducted on wild brown trout); D: present study (conducted on wild brown trout) — interval of values between dark gray vertical lines. Note that in the studies Biro et al. (2004, 2006), boldness was used as an explanatory behavioral trait; however, bold individuals in these studies were also more active. Shaded parts represent extended hypothetical reaction norms for variables where performance is assumed to be optimal at intermediate conditions (i.e., water velocity with a high level of food availability — Armstrong et al. 2011). Water velocity increases from the left to right.

Frequency-dependent selection acting in a fluctuating environment may allow coexistence of different activity in a natural population (Maynard Smith 1982). For example, according to our hypothesis (Figure 6), a population of mostly passive individuals can be invaded by a high-activity mutant at optimal or near optimal environmental conditions, but the fitness of the mutant strategy will vary more in response to environmental changes, allowing the passive strategy to increase in response to unpredictable fluctuating environmental conditions. Maternal effect has been shown to drive behavior and growth in juvenile brown trout (Höjesjö et al. 2011). Therefore, a parental bet-hedging strategy producing offspring with fixed activity can maintain within-family variation of behavior in unpredictable natural environments (Hamer et al. 2002).

Conclusions

In summary, we showed that laboratory activity often measured as behavioral traits in animal personality research (i.e., activity and exploration; Réale et al. 2007) associated with initial dispersal and growth of juvenile brown trout in the wild. However, our results do not fully support the original version of the pace-of-life-syndrome hypothesis as we found no constant trade-off between laboratory activity, growth rate, and survival. Taken together with previous studies (Biro et al. 2004, 2006; Adriaenssens and Johnsson 2011, 2013; Armstrong et al. 2011; Höjesjö et al. 2011; Figure 6), our results are more consistent with the hypothesis that the growth of individuals adopting active behavioral strategies is more sensitive to deviations from optimal environmental conditions than the growth of individuals adopting passive strategies.

Funding

This work was supported by the Swedish Research Council Formas (dnr 229-2009-1495) and Helge Ax:son Johnsons Stiftelse to D.A.

Acknowledgements

We thank P. Saarinen for assistance during behavioral scoring, J. Blomgren and F. Erkenborn for their help in field work and A. Höjesjö for assistance during fish tagging. We are also grateful to B. Adriaenssens for useful comments on the manuscript. These experiments were approved by the Ethical Committee for Animal Research in Göteborg (License dnr 8-211) and comply with current laws in Sweden. All authors designed the experiment. L.Z., D.A., and J.N. carried out the behavioral experiments, field tracking, and analyzed the data. L.Z. wrote a first draft and all authors were closely involved in the revision process of the manuscript.

References

Adriaenssens B, Johnsson JI. 2009. Personality and life-history productivity: consistent or variable association? Trends Ecol Evol 24:179–180.

Adriaenssens B, Johnsson JI. 2011. Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. Behav Ecol 22:135–143.

Adriaenssens B, Johnsson JI. 2013. Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. Ecol Lett 16:47–55.

Armstrong JD, Millidine KJ, Metcalfe NB. 2011. Ecological consequences of variation in standard metabolism and dominance among salmon parr. Ecol Freshw Fish 20:371–376.

Attisano A, Tregenza T, Moore AJ, Moore PJ. 2013. Oosorption and migratory strategy of the milkweed bug, *Oncopeltus fasciatus*. Anim Behav 86:651–657.

Ball J, Nordengren C, Wallin K. 2001. Partial migration by large ungulates: characteristics of seasonal moose *Alces alces* ranges in northern Sweden. Wildl Biol 7:39–47.

Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behaviour: a meta-analysis. Anim Behav 77:771–783.

Biro PA, Abrahams MV, Post JR, Parkinson EA. 2004. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. Proc R Soc B 271:2233–2237.

Biro PA, Abrahams MV, Post JR, Parkinson EA. 2006. Behavioural tradeoffs between growth and mortality explain evolution of submaximal growth rates. J Anim Ecol 75:1165–1171.

Biro PA, Adriaenssens B. 2013. Predictability as a personality trait: consistent differences in intraindividual behavioral variation. Am Nat 182:621–629.

Biro PA, Stamps JA. 2008. Are animal personality traits linked to life-history productivity? Trends Ecol Evol 23:361–368.

Biro PA, Stamps JA. 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends Ecol Evol 25:653–659.

Bohlin T, Hamrin S, Heggberget TG, Rasmussen G, Saltveit SJ. 1989. Electrofishing—theory and practice with special emphasis on salmonids. Hydrobiologia 173: 9–43.

Bowler DE, Benton TG. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biol Rev 80:205–225.

Brett JR. 1979. Environmental factors and growth. In: Hoar WS, Randall DJ, Brett JR, editors. Fish physiology, vol. VIII. Bioenergetics and growth. Orlando (FL): Academic Press, Inc. p. 599–675.

Bridcut E, Giller P. 1993. Movement and site fidelity in young brown trout *Salmo trutta* populations in a southern Irish stream. J Fish Biol 43:889–899.

Brönmark C, Skov C, Brodersen J, Nilsson PA, Hansson LA. 2008. Seasonal migration determined by a trade-off between predator avoidance and growth. PLoS ONE 3:e1957.

Carlson SM, Olsen EM, Vøllestad LA. 2008. Seasonal mortality and the effect of body size: a review and an empirical test using individual data on brown trout. Funct Ecol 22:663–673.

Cole EF, Quinn JL. 2014. Shy birds play it safe: personality in captivity predicts risk responsiveness during reproduction in the wild. Biol Lett 10:20140178.

Cote J, Clobert J, Brodin T, Fogarty S, Sih A. 2010. Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. Phil Trans R Soc B 365:4065–4076.

Dingemanse NJ, Both C, Drent PJ, Tinbergen JM. 2004. Fitness consequences of avian personalities in a fluctuating environment. Proc R Soc B 271:847–852.

Dingemanse NJ, de Goede P. 2004. The relation between dominance and exploratory behavior is context-dependent in wild great tits. Behav Ecol 15:1023–1030.

Dingle H, Drake V. 2007. What is migration? BioScience 57:113–121.

Dixon A, Horth S, Kindlmann, P. 1993. Migration in insects: cost and strategies. J Anim Ecol 62:182–190.

Einum S, Sundt-Hansen L, Nislow K. 2006. The partitioning of density dependent dispersal, growth and survival throughout ontogeny in a highly fecund organism. Oikos 3:489–496.

Elliott JM. 1994. Quantitative ecology and the brown trout. Oxford: Oxford University Press.

Gilchrist WG. 1995. Specialist and generalist in changing environments. I. Fitness landscape of thermal sensitivity. Am Nat 146:252–270.

Grant J. 1997. Territoriality. In: Godin JG, editor. Behavioural ecology of teleost fishes. New York: Oxford University Press. p. 81–103.

Hamer AJ, Lane SJ, Mahony MJ. 2002. The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. Oecologia 132:445–452.

Hanski I, Saastamoinen M, Ovaskainen O. 2006. Dispersal-related lifehistory trade-offs in a butterfly metapopulation. J Anim Ecol 75:91–100.

Hardin JW, Hilbe JM. 2007. Generalized linear models and extensions. 2nd ed. College Station (TX): Stata Press.

Hendry AP, Bohlin T, Jonsson B, Berg OK. 2004. To sea or not to sea? Anadromy versus non-anadromy in salmonids. In: Hendry AP, Stearns SC, editors. Evolution illuminated. New York: Oxford University Press. p. 92–125.

Hixon MA. 1981. An experimental analysis of territoriality in the California reef fish *Embiotoca jacksoni* Embiotocidae). Copeia 3:653–665.

Hodder KH, Masters JEG, Beaumont WRC, Gozlan RE, Pinder AC, Knight CM, Kenward RE. 2007. Techniques for evaluating the spatial behaviour of river fish. Hydrobiologia 582:257–269.

Höjesjö J, Adriaenssens B, Bohlin T, Jönsson C, Hellström I, Johnsson JI. 2011 Behavioural syndromes in juvenile brown trout (*Salmo trutta*); life history, family variation and performance in the wild. Behav Ecol Sociobiol 65:1801–1810.

Höjesjö J, Johnsson JI, Bohlin T. 2004. Habitat complexity reduces the growth of aggressive and dominant brown trout (*Salmo trutta*) relative to subordinates. Behav Ecol Sociobiol 56:286–289.

Höjesjö J, Johnsson JI, Bohlin T. 2014. Behavior and growth of juvenile brown trout (*Salmo trutta*) following upstream and downstream displacement. J Freshw Ecol 30:455-461.

Houston AI. 2010. Evolutionary models of metabolism, behaviour and personality. Phil Trans R Soc B 365:3969–3975

Jepsen N, Schreck C, Clements S, Thorstad EB. 2005. A brief discussion on the 2% tag/body mass rule of thumb. 5th Conference on Fish Telemetry held in Europe; 2003 Jun 09–13; Ustica, Italy. FAO/COISPA. p. 255–259.

John-Alder HB, Cox RM, Haenel GJ, Smith LC. 2009. Hormones, performance and fitness: natural history and endocrine experiments on a lizard (*Sceloporus undulatus*). Integr Comp Biol 49:393–407.

Johnsson JI, Nöbbelin F, Bohlin T. 1999. Territorial competition among wild brown trout fry: effects of ownership and body size. J Fish Biol 54:469–472.

Jonsson B, Jonsson N. 1993. Partial migration: niche shift versus sexual maturation in fishes. Rev Fish Biol Fish 3:348–365.

Kalleberg H. 1958. Observation in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. and *S.trutta*). Rep - Inst Freshw Res Drottningholm 39:55–98.

Keeley ER. 2000. An experimental analysis of territory size in juvenile steelhead trout. Anim Behav 59:477–490.

Kim JW, Wood JLA, Grant JWA, Brown GE. 2011. Acute and chronic increases in predation risk affect the territorial behaviour of juvenile Atlantic salmon in the wild. Anim Behav. 81:93–99.

Kingsolver JG, Huey, RB. 2008. Size, temperature, and fitness: three rules. Evol Ecol Res 10:251–268.

Klemetsen A, Amundsen P-A, Dempson JB, Jonsson B, Jonsson N, O'Connell MF, Mortensen E. 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecol Freshw Fish 12:1–59.

Le Galliard J-F, Paquet M, Cisel M, Montes-Poloni L. 2013. Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. Funct Ecol 27:136–144.

Matthysen E. 2005. Density-dependent dispersal in birds and mammals. Ecography 28:403–416.

Maynard Smith J. 1982. Evolution and the theory of games. Cambridge (UK): Cambridge University Press.

Metcalfe N. 1986. Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. J Fish Biol 28: 525–531.

Mittelbach GG, Ballew NG, Kjelvik MK. 2014. Fish behavioral types and their ecological consequences. Can J Fish Aquat Sci 71:927–944.

Montiglio PO, Garant D, Bergeron P, Dubuc Messier G, Réale D. 2014. Pulsed resources and the coupling between life-history strategies and exploration patterns in eastern chipmunks (*Tamias striatus*). J Anim Ecol 83:720–728.

Murphy PM, Purvis IW, Lindsay DR, Le Neindre P, Orgeur P, Poindron P. 1994. Measures of temperament are highly repeatable in merino sheep and some are related to maternal behaviour. Proc Aust Soc Anim Prod 20:247–250.

Musiani M, Leonard JA, Cluff HD, Gates CC, Mariani S, Paquet PC, Vilà C, Wayne RK. 2007. Differentiation of tundra/taiga and boreal coniferous forest wolves: genetics, coat colour and association with migratory caribou. Mol Ecol 16:4149–4170.

Niemelä PT, Dingemanse NJ. 2014. Artificial environments and the study of 'adaptive' personalities. Trends Ecol Evol 29:245–247.

Réale D, Festa-Bianchet M. 2003. Predator-induced natural selection on temperament in bighorn ewes. Anim Behav 65:463–470.

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Phil Trans R Soc B 365:4051–4063.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82:291–318.

Roff DA. 1992. Evolution of life histories: theory and analysis. New York: Chapman and Hall.

Schmider E, Ziegler M, Danay E, Beyer L, Bühner M. 2010. Is it really robust? Reinvestigating the robustness of ANOVA against violations of the normal distribution assumption. Methodol Eur J Res Methods Behav Soc Sci 6:147–151.

Slavík O, Horký P, Závorka L. 2014. Energy costs of catfish space use as determined by biotelemetry. PLoS ONE 9:e98997.

Smith BR, Blumstein DT. 2008. Fitness consequences of personality: a meta-analysis. Behav Ecol 19:448–455.

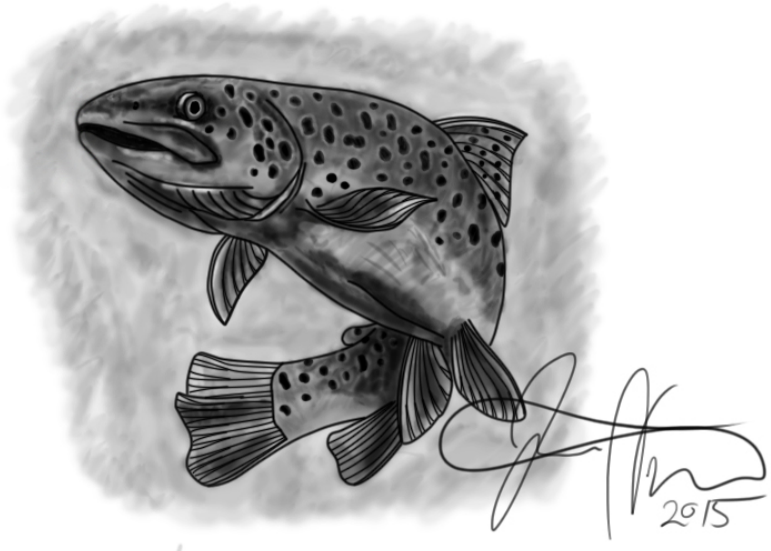
Stamps JA. 2007. Growth-mortality tradeoffs and 'personality traits' in animals. Ecol Lett 10:355–363.

Stearns SC. 1976. Life-history tactics: a review of the ideas. Q Rev Biol 51:3–47.

Yoder JM, Marschall EA, Swanson DA. 2004. The cost of dispersal: predation as a function of movement and site familiarity in ruffed grouse. Behav Ecol 15:469–476.

This page intentionally left (almost) blank

Supplementary material



Paper 1

qPCR standard curve

Table S 1: Summary of standard curve characteristics for all telomere and β-actin qPCR assays. On each of 12 telomere and 12 β-actin qPCR amplification plates, a standard curve was included in triplicate (for details see Methods). For the telomere and β-actin assays separately, the mean $\pm$ standard error, as well as the range is presented for the slope, the y-intercept, the coefficient of determination R^2 and PCR efficiency.

		Telomere	β-actin
Slope	Mean $\pm$ SE	$-3,181 \pm 0.020$	-3.237 ± 0.030
	Range	-3.357 to -3.072	-3.511 to -3.106
y-intercept	Mean $\pm$ SE	11.843 ± 0.030	25.528 ± 0.033
	Range	11.689 to 12.090	25.415 to 25.835
R^2	Mean $\pm$ SE	$0.999 \pm < 0.001$	0.994 ± 0.001
	Range	0.996 to 1.000	0.988 to 0.999
PCR efficiency	Mean $\pm$ SE	2.064 ± 0.009	2.039 ± 0.013
	Range	1.986 to 2.116	1.927 to 2.099

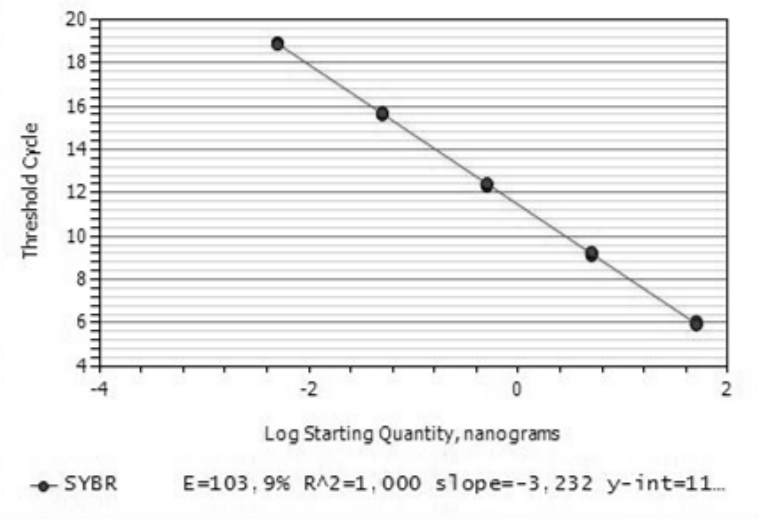


Figure S 1: The relationship between DNA quantity and number of PCR amplification cycles required to detect a fluorescent signal above background noise (threshold cycle, C_q) The threshold cycle numbers of five serial dilutions of a sample of known concentration (run in triplicate) are plotted against the log-transformed starting DNA quantity of each dilution (to yield a linear relationship). The resulting standard curve, included on each assay plate, is used to calculate assay characteristics such as PCR amplification efficiency E (based on the slope of the relationship) and assess methodological consistency (e.g. preparation of dilutions and repeatability of analysed triplicates) based on R^2.

Primer sequences

Table S 2: Summary of primer sequences used in telomere (Tel) and β-actin (actin) qPCR assays. The primer name, its nucleotide sequence as well as the source that provided the primer sequence is presented.

Primer	Sequence ($5' \rightarrow 3'$)
Tel 1b (forward)[12]	CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT
Tel 2b (reverse)[1]	GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT
Actin (forward)[13]	TGG CAT CAC ACC TTC TAC
Actin (reverse)[2]	AAT CTG GGT CAT CTT CTC C

Tests of assumptions for statistical analyses

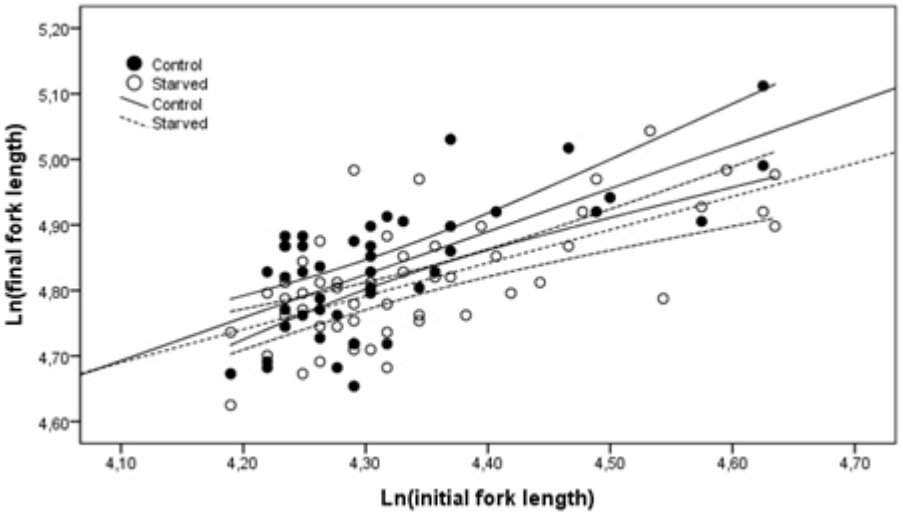


Figure S 2: Scatterplot of the natural logarithm of initial and final fork length. The graph was used to investigate assumptions of the linear model of final length. Approximate normality of residuals and homogeneity for each value of ln(initial wet weight) in each treatment group were both deemed to be reasonable assumptions. Homogeneity of slopes (parallel regression lines) was additionally analysed by adding the interaction of treatment (starved or control) and the covariate (initial length), and this term was found to be non-significant ($P > 0.100$). Similar analyses were made for all variables analysed in the analyses of size and growth variables.

[12] Designed by RM Cawthon. Eccles Institute of Human Genetics, University of Utah, USA

[13] Designed by B Wassmur. Dept. of Biol. Env. Sci., University of Gothenburg, Sweden

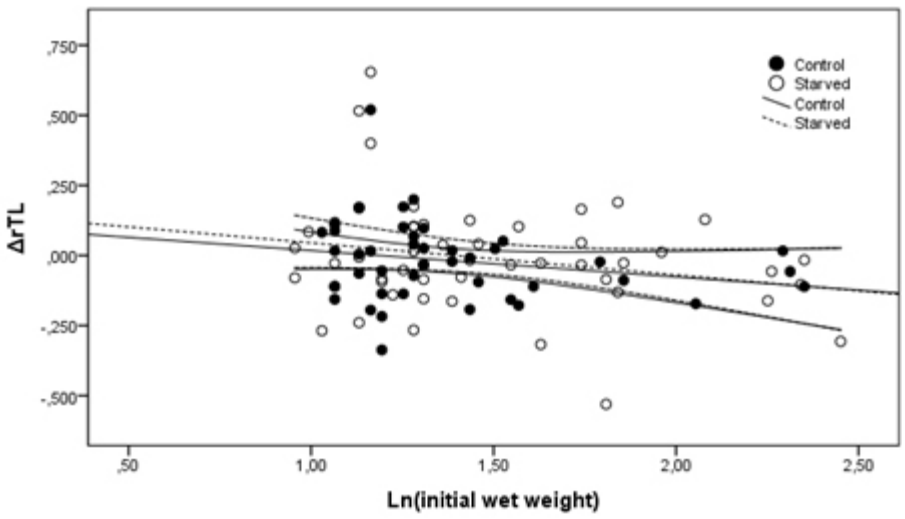


Figure S 3: Scatterplot of change in telomeric content over the experimental period (ΔrTL) and the natural logarithm of initial wet weight. The graph was used to evaluate the assumptions of the linear model of ΔrTL. Homogeneity of slopes (parallel regression lines), and approximate normality of residuals and homogeneity for each value of ln(initial wet weight) in each treatment group were all deemed to be reasonable assumptions. The apparent smaller variation in ΔrTL for the control group at higher values of initial weight is thought to be due to lower number of data points

Recapture data based on initial size modality

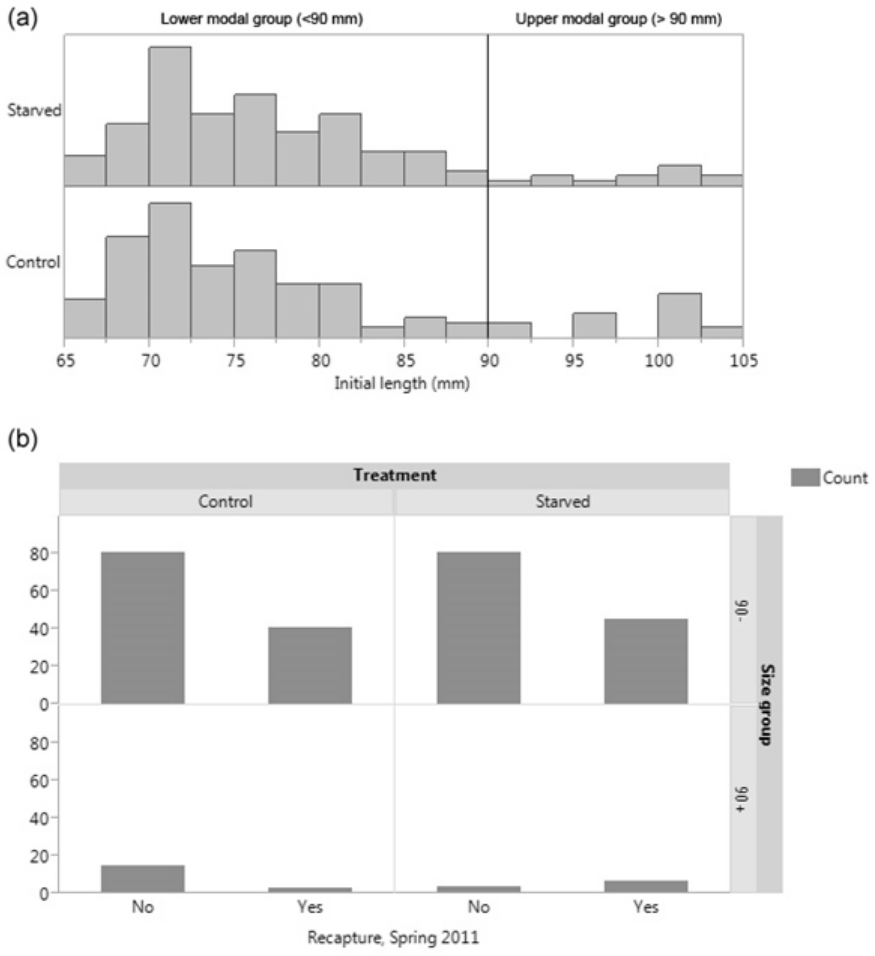


Figure S 4: (a) Histogram showing size distribution of brown trout at the start of the experiment, split into groups of smaller (less than 90 mm) and larger (more than 90 mm) sized of fish. (b) Summary of the frequency of recaptured fish in the different size groups, illustrating that more control fish from the upper modal group were not recaptured as compared to the starved fish. This could be a sign of early migration rather than mortality.

Paper 3

Rearing boxes

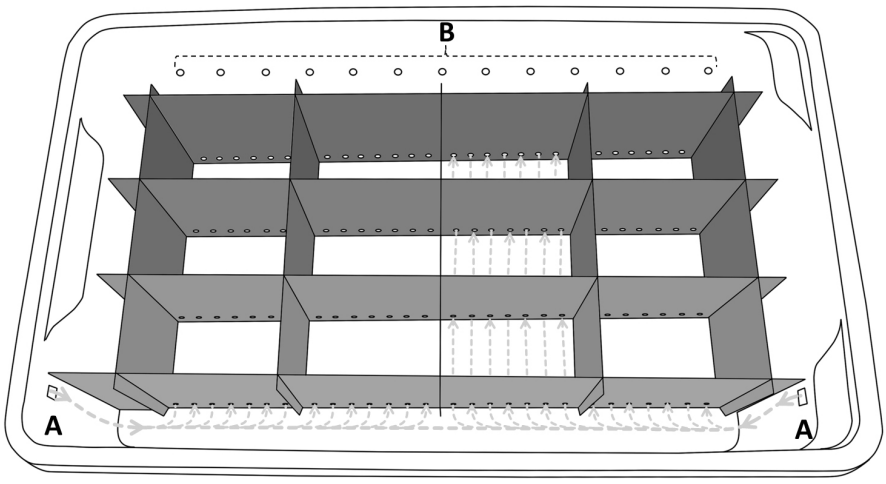


Figure S 1: Illustration of a rearing box used for individual rearing of brown trout fry (as viewed from above). Grey arrows show the flow through the fish compartments, from the inlets (A) to the outlets (B). The box was covered by a lid.

Boldness test: validation

The boldness test requires the fish to show reactions toward the object presented into the test arena. This may or may not be true.

To test whether the fish in our experiment reacted toward the novel objects used in this study with altered space utilization, we analyzed space usage for each fish using χ^2-tests. Higher than predicted utilization of Zone 1 (see Fig 2b in main article) would be indicative of neophilia, while higher than predicted utilization of Zone 4, alternatively lower than predicted utilization of Zone 2, would indicate neophobia.

Trial 1: 7 out of 90 individuals showed significant ($p < 0.05$) deviations from the expected zone utilization as predicted from random swimming pattern (5 fish classified as neophilic and 2 fish classified as neophobic). See Table S1A.

Trial 2: 5 out of 90 individuals showed significant deviations ($p < 0.05$) from the expected zone utilization as predicted from random swimming pattern (1 fish classified as neophilic, 4 fish classified as neophobic). See Table S1B.

In conclusion, relatively few fish showed any deviations from what would be expected from random swimming in the novel-object tests. No single fish was consistently classified as clearly neophobic or neophilic in both trials. Thus, we cannot assume that the fish reacted to the novel objects used in this study. The test is presented in the main article because it was part of the sequence of behavioural tests, and therefore should not be omitted from the methods.

Table S 1: Summary of the individuals classified as clearly neophobic or neophilic in either trial 1 (a) or trial 2 (b). A fish was regarded as clearly neophobic or neophilic if there was a significant deviation from expected zone utilization, as evaluated by a χ^2 –test (critical χ^2 – value, $P < 0.05 : 43$).

	Zone 1	Zone 2+3	Zone 4
Area (%)	14.8	$35.8 + 37.4$	12.0
Expected counts	4	22	4

A: Trial 1: Observed counts

ID	Zone 1	Zone 2+3	Zone 4	χ^2	Classification
8	22	8	0	94	Neophile
12	9	17	4	7	
31	21	9	0	84	Neophobe
41	0	10	20	75	Neophobe
51	8	14	8	11	
65	17	10	3	49	Neophile
85	13	9	8	32	
89	25	5	0	127	Neophile
104	0	14	16	43	Neophobe
108	15	10	5	37	
117	22	8	0	94	Neophile

B: Trial 2: Observed counts

ID	Zone 1	Zone 2+3	Zone 4	χ^2	Classification
8	2	10	18	57	Neophobe
12	0	13	17	50	Neophobe
31	5	18	7	3	
41	0	15	15	36	
51	16	10	4	43	Neophile
65	4	19	7	3	
85	4	8	18	58	Neophobe
89	7	17	6	4	
104	3	27	0	5	
108	4	10	16	43	Neophobe
117	6	10	14	33	

Size and growth: detailed statistical results

Size: wet mass (g) (GLMM)

The general effect of Treatment can be noted (significant contrasts: HL ¿ HH, HL ¿ LH, HL ¿ LL, all P ¡ 0.015), but is not particularly interesting in this experimental setting, as it depends largely on initial differences and subsequent assignment of food rations (see Fig. 1a in the main article). The general increase in mass over time (significant contrasts: Date 1 ¡ Date 3, P ¡ 0.001; Date 2 ¡ Date 3, P ¡ 0.001) is expected. The interaction term Treatment × Date was not significant at the 5% level, but showed a trend (P ¡ 0.1; Table S2). We use non-corrected pairwise contrast estimates to assess differences among groups at the different dates (Table S3). At the start of the experiment (Date 1) HH had, by chance, significantly lower mass than HL. At the switch of rations (Date 2) HH, LH, and LL had lower mass than HL. At the end of the experiment no treatments differed significantly. The groups becoming more similar (contrary to what would be expected) depended on initial differences. Significant differences are marked in bold in Table S3.

Table S 2: Statistics for main effects and interactions in the generalized linear mixed model (probability distribution: Gaussian; link function: identity) of wet mass over the experiment

Factor	F	df 1	df 2	*P*
Treatment	4.648	3	255	0.002
Date	14.331	2	255	<0.001
Treatment × Date	1.942	6	255	0.075

Table S 3: Pairwise contrast comparisons. *p*-values are not corrected for multiple comparisons (least significant difference significance level: 0.05

A: Date 1: Day 0 – Start of feeding treatments

	HH_J	HL_J	LH_J	LL_J	
HH_I		-0.05	-0.01	-0.05	**Contrast**
HL_I	**0.04**		0.04	0.01	**estimates**
LH_I	0.64	0.13		-0.04	*(I-J)*
LL_I	0.07	0.86	0.19		
		***p*-value**			

B: Date 2: Day 12 – Switch of rations

	HH_J	HL_J	LH_J	LL_J	
HH_I		-0.05	0.04	0.01	**Contrast**
HL_I	**0.03**		0.09	0.07	**estimates**
LH_I	0.06	**<0.01**		-0.03	*(I-J)*
LL_I	0.54	**<0.01**	0.23		
		***p*-value**			

Date 3: Day 34 – End of feeding treatments

	HH_J	HL_J	LH_J	LL_J	
HH_I		0.00	0.02	0.04	**Contrast**
HL_I	0.97		0.02	0.04	**estimates**
LH_I	0.38	0.36		0.02	*(I-J)*
LL_I	0.09	0.08	0.40		
		***p*-value**			

Growth rate: SGR_M, specific growth rate (wet mass, % per day) (GLMs)

Growth rates over Period 1 and Period 2 were analysed separately to include initial size (fork length) at the start of each period in the models. Both models were initially run with the interaction Treatment × Initial size. These interaction terms were subsequently removed as they were non-significant (Period 1: $p = 0.476$; Period 2: $p = 0.847$). Treatment had significant effects on growth rate in both periods (Table S4), following the expected patterns (see Fig. 1b in the main article). Body size (fork length) had significant effects on growth rate, with smaller fish growing faster than relatively larger fish.

Table S 4: Statistics for the generalized linear models (probability distribution: Gaussian; link function: identity) of specific growth rate over Period 1 (A, B) and Period 2 (C, D). *p*-values from pairwise comparisons (B, D) are not corrected for multiple comparisons (least significant difference significance level: 0.05). *** =< 0.001. EMM = estimated marginal means.

A: Period 1 – Main effect statistics

Factor	Wald χ^2	df	*p*
Treatment	145.198	3	<0.001
Initial size (9 Jun)[1]	56.749	1	<0.001

B: Pairwise comparisons of EMM – Period 1

	HH_J	HL_J	LH_J	LL_J	
HH_I		-0.001	0.018	0.017	**Mean diff.**
HL_I	0.76		0.018	0.018	*(I-J)*
LH_I	***	***		0.000	
LL_I	***	***	0.92		
		***p*-value**			

C: Period 2 – Main effect statistics

Factor	Wald χ^2	df	*p*
Treatment	112.238	3	<0.001
Initial size (21 Jun)[2]	38.597	1	<0.001

D: Pairwise comparisons of EMM – Period 2

	HH_J	HL_J	LH_J	LL_J	
HH_I		0.006	-0.004	0.003	**Mean diff.**
HL_I	***		-0.010	-0.003	*(I-J)*
LH_I	***	***		0.007	
LL_I	0.006	0.001	***		
		***p*-value**			

Total growth: absolute growth in wet mass (g) (GLM)

Absolute growth in wet mass (g) over the whole experiment was analyzed using initial size (fork length) as a covariate. The model was initially run with the interaction Treatment × Initial size. This interaction term was significant and retained in the model (Table S5). Treatment had significant effects on growth (Table S5; see also Fig. 1C in the main article). Initial body size (fork length) had a significant effect on growth, with smaller fish growing more than relatively larger fish.

Table S 5: Statistics for the generalized linear models (A) (probability distribution: Gaussian; link function: identity) of total growth in body wet mass (g). *p*-values from pairwise comparisons (*B*) are not corrected for multiple comparisons (least significant difference significance level: 0.05). *** = < 0.001. EMM = estimated marginal means.

A: Main effect statistics

Factor	Wald χ^2	df	*p*
Treatment	5.389	3	0.145
Initial size[3]	28.139	1	<0.001
Treatment × Initial size[4]	8.858	3	0.031

B: Pairwise comparisons of EMM

	HH_J	HL_J	LH_J	LL_J	
HH_I		0.045	0.033	0.081	**Mean diff.**
HL_I	***		-0.011	0.037	*(I-J)*
LH_I	***	0.24		0.048	
LL_I	***	***	***		
		***p*-value**			

[1]Parameter estimate: $B = -0.0019$ (95% CI: ±0.00048)

[2]Parameter estimate: $B = -0.0008$ (95% CI: ±0.00024)

[3]Parameter estimate: $B = -0.0088$ (95% CI: ±0.0046)

[4]Parameter estimates: HH × Initial size: B = 0.0070 (95% CI: ±0.0062); HL × Initial size: B = -0.0005 (95% CI: ±0.0065); LH × Initial size: B = 0.0053 (95% CI: ±0.0061); LL × Initial size: B = 0 (Redundant)

Size: fork length (mm) (GLMM)

The general effect of Treatment can be noted (HL > LH, HL > HH, both $p < 0.03$), but is not particularly interesting in this experimental setting, as it depends largely on initial differences and subsequent assignment of food rations (see Fig. 1d in the main article). The general increase in mass over time (significant contrasts: Date 1 < Date 3, $p < 0.001$; Date 2 < Date 3, $p < 0.001$) is expected. The interaction term Treatment × Date was not significant (Table S6). Nevertheless, we use non-corrected pairwise contrast estimates of Treatment × Date to assess differences among groups at the different dates (Table S7). The only difference in length detected in the pairwise contrasts was between HL > LH at Date 2. Significant differences are marked in bold in Table S7.

Table S 6: Statistics for main effects and interactions in the generalized linear mixed model (probability distribution: Gaussian; link function: identity) of fork length over the experiment.

Factor	F	df 1	df 2	*P*
Treatment	3.073	3	255	0.028
Date	16.836	2	255	<0.001
Treatment × Date	1.178	6	255	0.319

Table S 7: Pairwise contrast comparisons. *p*-values are not corrected for multiple comparisons (least significant difference significance level: 0.05)

A: Date 1: Day 0 – Start of feeding treatments

	HH_J	HL_J	LH_J	LL_J	
HH_I		-1.6	-0.3	-1.6	**Contrast**
HL_I	0.09		1.2	0.0	**estimates**
LH_I	0.72	0.18		-1.3	*(I-J)*
LL_I	0.09	0.98	0.18		
		***p*-value**			

B: Date 2: Day 12 – Switch of rations

	HH_J	HL_J	LH_J	LL_J	
HH_I		-1.6	0.7	-0.5	**Contrast**
HL_I	0.06		2.3	1.1	**estimates**
LH_I	0.44	**0.01**		-1.1	*(I-J)*
LL_I	0.58	0.19	0.19		
		***p*-value**			

Date 3: Day 34 – End of feeding treatments

	HH_J	HL_J	LH_J	LL_J	
HH_I		0.0	0.78	1.1	**Contrast**
HL_I	0.99		0.74	1.1	**estimates**
LH_I	0.34	0.33		0.6	*(I-J)*
LL_I	0.15	0.15	0.47		
		***p*-value**			

Growth rate: ΔL, absolute growth rate in fork length (mm per day) (GLMs)

Absolute growth rates in fork length (mm per day) over Period 1 and Period 2 were analyzed separately to include initial size (fork length) at the start of each period in the models. Both models were initially run with the interaction Treatment × Initial size. These interaction terms were subsequently removed as they were non-significant (Period 1: p =0.201; Period 2: p = 0.926). Treatment had significant effects on growth rate in both periods (Table S8), following the expected patterns (see Fig. 1e in the main article). Body size (fork length) had significant effects on growth rate, with smaller fish growing faster than relatively larger fish.

Table S 8: Statistics for the generalized linear models (probability distribution: Gaussian; link function: identity) of absolute growth rate (fork length, mm per day) over Period 1 (A, B) and Period 2 (C, D). p-values from pairwise comparisons (B, D) are not corrected for multiple comparisons (least significant difference significance level: 0.05). *** =< 0.001. EMM = estimated marginal means.

A: Period 1 – Main effect statistics

Factor	Wald χ^2	df	p
Treatment	158.668	3	<0.001
Initial size (9 Jun)[5]	33.17	1	<0.001

B: Pairwise comparisons of EMM – Period 1

	HH_J	HL_J	LH_J	LL_J	
HH_I		-0.014	0.081	0.082	**Mean**
HL_I	0.17		0.094	0.096	**diff.**
LH_I	***	***		0.002	*(I-J)*
LL_I	***	***	0.86		
		p-value			

C: Period 2 – Main effect statistics

Factor	Wald χ^2	df	p
Treatment	84.381	3	<0.001
Initial size (21 Jun)[6]	34.596	1	<0.001

D: Pairwise comparisons of EMM – Period 2

	HH_J	HL_J	LH_J	LL_J	
HH_I		0.062	0.008	0.068	**Mean**
HL_I	***		-0.054	0.007	**diff.**
LH_I	0.042	***		0.061	*(I-J)*
LL_I	***	0.48	***		
		p-value			

Total growth: absolute growth in fork length (mm) (GLM) Absolute growth in fork length over the whole experiment was analyzed using initial size (fork length) as a covariate. The model was initially run with the interaction Treatment × Initial size. This interaction term was subsequently removed as it was non-significant (p = 0.886). Treatment had significant effects on growth (Table S9; see also Fig. 1f in the main article). Initial body size (fork length) had significant effects on growth rate, with smaller fish growing more than relatively larger fish.

Table S 9: Statistics for the generalized linear models (A) (probability distribution: Gaussian; link function: identity) of total growth in fork length (mm). p-values from pairwise comparisons (B) are not corrected for multiple comparisons (least significant difference significance level: 0.05). *** =< 0.001.

A: Main effect statistics

Factor	Wald χ^2	df	p
Treatment	83.521	3	<0.001
Initial size[7]	60.740	1	<0.001

B: Pairwise comparisons of EMM

	HH_J	HL_J	LH_J	LL_J	
HH_I		1.20	0.98	2.33	**Mean**
HL_I	***		-0.22	1.13	**diff.**
LH_I	***	0.38		1.34	*(I-J)*
LL_I	***	***	***		
		p-value			

[5] Parameter estimate: $B = -0.0067$ (95% CI: ±0.00023)

[6] Parameter estimate: $B = -0.0069$ (95% CI: ±0.0023)

[7] Parameter estimate: $B = -0.23$ (95% CI: ±0.057)

Complementary analyses of behavior

Growth rate vs. behavior: analyses using growth rate as continuous factor

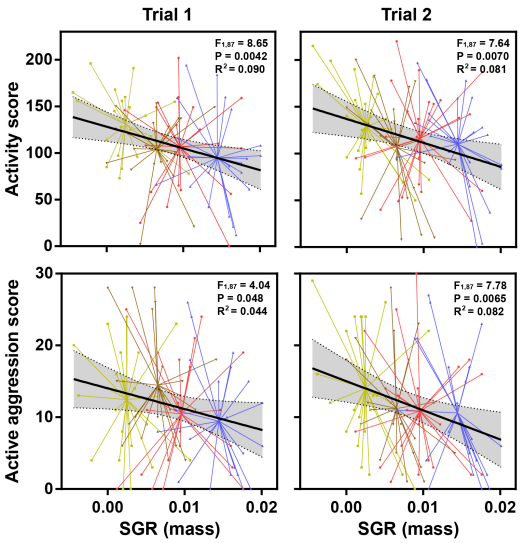


Figure S 2: Regression analyses of behavior using specific growth rate over days 13 - 34 (Period 2) of the experiment as independent variable. For activity and active aggression there were weak, but significant, general negative effects of growth rate Analyses for boldness scores were non-significant, and are not illustrated here (Trial 1: $F_{1,87} = 1.778$, $p = 0.186$, $R^2 = 0.009$; Trial 2: $F_{1,87} = 0.029$, $p = 0.866$, $R^2 = 0.0003$). Treatments are colored for illustrative purposes: green = HL, brown = LL, red = HH, blue = LH. Lines radiate out to data points from the centroid for each treatment. Grey-shaded areas show the 95% confidence intervals for the regression lines.

Mirror confrontation scores: total confrontation, passive- and active aggression

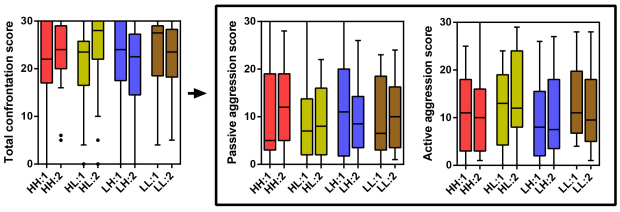


Figure S 3: Aggression levels for each treatment, each trial (denoted 1 and 2). Total confrontation score is the addition of passive and active aggression scores. Data is shown as Tukey boxplots, with box hinges representing first and third quartiles and the band within the box represents the second quartile (median). Whiskers represent the data within 1.5 interquartile range from the box hinges and dots represent data points more than 1.5 interquartile range away from the box hinges. Note the high level of total aggression in the total confrontation score; the majority of all fish are located within the confrontation zone, either passively displaying or actively swimming towards the mirror image at $> 66\%$ of all scoring moments.

Cluster assignment in relation to initial and final size

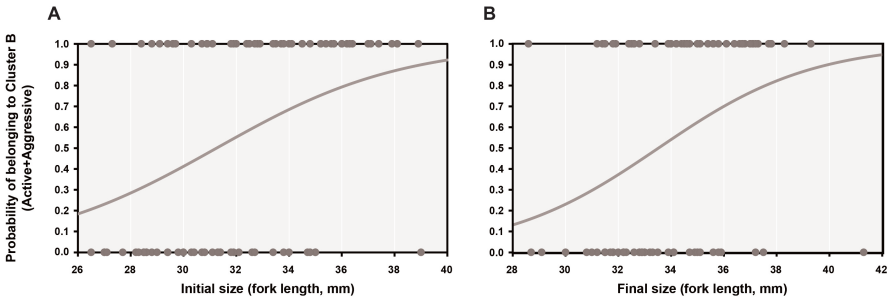


Figure S 4: Probabilities of being assigned to Cluster B (active and aggressive) as predicted by binary logistic GLM using initial size at the start of the experiment (A) and final size at the end of the feeding manipulations (B) as independent variables. Functions for the regression lines are: (A) $y = 1/(1 + e^{(8.845-0.283x)})$, and (B) $y = 1/(1 + e^{(11.464-0.342x)})$. Omnibus tests were significant for both models ($p < 0.001$).

Paper 5

Observation of egg consumption by parr in November

During recapture of STR in November we observed that many trout parr had unusually high stomach fullness. One such fish (91 mm fork length), not included in any treatment group, was sacrificed with a sharp blow to the head after being anaesthetised. Ten brown trout eggs were found in its stomach (Fig. S1).

Figure S 1: Brown trout parr (91 mm fork length) having consumed conspecific eggs during brown trout spawning in November (River Jörlandaån). The fish was anaesthetised and killed with a blow to the head prior to investigation of the stomach content.