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Survival of brown trout fry in nature: effects
of activity, body size and starvation

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The first year of life is one of the main survival bottlenecks for many fish species. Individual traits affecting survival can be morphological, physiological or behavioural. Body size, growth rate and activity have all been found to affect fitness in different organisms. However, the effects of these traits on fitness in natural conditions and for underyearlings are poorly investigated. In this study we attempted to induce compensatory growth in laboratory conditions in natural populations of brown trout fry (*Salmo trutta*). It was performed by exposing the fry to a period of restricted resources followed by a period of refeeding. Two behavioural trials were conducted on each individual where activity level was scored. All fish were subsequently released in their native stream and recaptured after a month to check for survival. We found that high individual activity level in an open field context increased the probability of survival under natural conditions. The importance of body size for survival decreased over time, and thus, with fish size. Full compensation was detected in body condition, while only partly compensation in weight and no compensation in length were detected during the experimental periods. Our results suggest that a brown trout fry's individual activity level is repeatable and can be an important trait for selection in nature. The instable interactions between activity and life-history traits indicate environmental effects on these interactions. Furthermore, if body size is not the only trait affecting survival, compensation in body structures may not be a fast response to increase fitness after a period of growth depression.

Nyckelord/Keyword:

Brown trout, fry, activity, survival, body size, compensatory growth, starvation

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1 Abstract

The first year of life is one of the main survival bottlenecks for many fish species. Individual traits affecting survival can be morphological, physiological or behavioural. Body size, growth rate and activity have all been found to affect fitness in different organisms. However, the effects of these traits on fitness in natural conditions and for underyearlings are poorly investigated. In this study we attempted to induce compensatory growth in laboratory conditions in natural populations of brown trout fry (*Salmo trutta*). It was performed by exposing the fry to a period of restricted resources followed by a period of refeeding. Two behavioural trials were conducted on each individual where activity level was scored. All fish were subsequently released in their native stream and recaptured after a month to check for survival. We found that high individual activity level in an open field context increased the probability of survival under natural conditions. The importance of body size for survival decreased over time, and thus, with fish size. Full compensation was detected in body condition, while only partly compensation in weight and no compensation in length were detected during the experimental periods. Our results suggest that a brown trout fry's individual activity level is repeatable and can be an important trait for selection in nature. The instable interactions between activity and life-history traits indicate environmental effects on these interactions. Furthermore, if body size is not the only trait affecting survival, compensation in body structures may not be a fast response to increase fitness after a period of growth depression.

2 Introduction

The juvenile life-stage is a major bottleneck for survival in many fish species (Mortensen 1977, Milner et al. 2003). Therefore, identifying individual traits affecting survival in this life-stage is crucial to understand the natural selection within populations. Recently the interactions between behavioural traits and life-history traits have gained increased attention (Stamps 2007, Biro & Stamps 2008, Reale et al. 2010). Surprisingly, there is a lack of studies in natural conditions investigating what individual traits, and how their interactions, can affect fitness.

Body size is one of the most important morphological traits affecting fitness in a wide variety of species (Peters 1983, Roff 1992, Stearns 1992). A large body size often gives an individual an advantage in

competition for territory, resources and mating. Thus, it could be expected that individuals grow as fast as physiologically possible to gain fitness advantages against conspecifics. In many organisms high growth rates have been found to come at a cost to fitness (Arendt 1997, Morgan & Metcalfe 2000, Metcalfe & Monaghan 2001, Dmitriev 2011). There is also extensive evidence of an optimal growth rate that is less than maximal. The costs of very high growth rates can affect the fitness of an individual through different traits. These can be morphological (Arendt & Wilson 2000, Robinson and Wardrop 2002), physiological (Stoks et al. 2006) and also behavioural (Houston et al. 1993, Nicieza & Metcalfe 1999, Sundström et al. 2005). Very high growth rates, in relation to normal growth trajectories, have been found in several organisms and contexts, for example after a period growth depression (Ali et al. 2003). Higher growth rates in this context is often referred to as compensatory (or catch-up) growth which occurs until normal growth trajectories are restored (Tanner 1963, Monteiro & Falconer 1966, Metcalfe & Monaghan 2001 & Ali et al. 2003). Compensatory growth has been found in all life-stages and in many organisms (Metcalfe and Monaghan 2001, Ali et al. 2003). If compensatory growth is a response to growth depression during the first year of life it could be one factor affecting survival. And if so, it is important to identify in what way high growth rates affect survival. The most studied vertebrate organism in the area of compensatory growth is fish (Ali et al. 2003). They make a suitable study object because of their indeterminate growth, which makes it possible to examine the compensatory effect in all life-stages.

One behavioural trait that may change as a response to higher growth rates is activity (Wieser et al. 1992, Ali et al. 2003, Dmitriev 2011). If activity is changed due to increased foraging rate (i.e. hyperphagia) it could be expected that these changes alters the growth-mortality trade-off (Sih et al. 2004, Biro et al. 2006, Stamps 2007, Biro & Stamps 2008). But there is an ongoing discussion among biologists whether an individual's behavioural traits are plastic and responds quickly to changes in ecological factors, or if they are intrinsic and highly canalized to resist such changes (Sih et al. 2004, Reale et al. 2010). As stated by Dingemanse & Wolf (2010) the reality is probably somewhere between the two theories. For example, Adriaenssens & Johnsson (2013) found that interindividual variation in activity level was consistent over time. But, activity level in general was increasing over the same time period under natural condition in juvenile brown trout (*Salmo trutta*). These results indicate that there are some changes, environmental or ontogenetic, which may affect an individual's activity level. Furthermore,

the same study found that the interindividual variation in activity was repeatable and where high activity increased the probability of survival within a population. These results questions one of the assumptions in the growth-mortality-trade-off-theory. Therefore, it is important to further investigate how interindividual variation in activity in relation to other fitness related traits affects survival in fish fry.

In this study we investigated if a brown trout fry's (*i*) individual activity level and body size affected the probability of survival under natural conditions. We also investigate if (*ii*) the fish changed their individual activity level in a short term perspective as a result of changes in resource availability, if (*iii*) a period of starvation followed by resumed feeding affected subsequent survival under natural conditions and if (*iv*) the fry were able to compensate in length, weight and condition after period of growth depression induced by restricted resources availability.

3 Material & methods

3.1 Summary

The study was conducted in 2013 and included two separate experiments run between June 11 and August 16, and between July 17 and September 19, respectively. In addition to different time periods, fish in the two experiments came from two different streams. Brown trout fry were caught and divided into two treatment groups, one control group (henceforth called "Control") and one starved-re-fed group (henceforth called "Restricted"). Due to minor differences between the two experiments they will be described one by one where only the changes in methods will be described for Stenungeån.

3.2 Norumsån

3.2.1 Catching and transporting

On June 11th, approximately 300 brown trout fry (0+) were caught in the small coastal stream Norumsån in western Sweden (58°2' N 11°49' E) by electric fishing, a widely used catch method known for its low-harm effect when properly applied (Arnekleiv et al. 2004). All fish were caught in a 50 m spawning section of the stream (mean width: 3.4 m, mean depth: 0.12 m). The fish were transported to the Department of Biological and Environmental Sciences (BioEnv) at the University of Gothenburg, in insulated plastic boxes filled with stream water.

3.2.2 Laboratory setup

On arrival to BioEnv (University of Gothenburg) a 200 l glass aquaria was used as acclimatization tank (holding tank) for all experimental fish. The holding tank was supplied with gravel, plastic plants, PVC pipes and PVC plates in order to provide shelter.

After tagging and the first weighing and photographing session (see below) the fish were moved to tanks where growth manipulation by resource restriction were to be conducted (experimental tanks). 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 compartments (each 50 cm long and 24 cm wide), hence in total 20 compartments. Each compartment was supplied with a 1 cm thick layer of sand (<0.5 mm) and ten rocks (5-10 cm in diameter) evenly spread across the bottom area. Water flow was approximately 1.1 l/min in each compartment and the outflow was located at the opposite short side from the inflow at a level maintaining the water depth at approximately 10 cm. Consequently, the water volume was held at approximately 12l in each compartment. Water temperature varied between 10.5 and 12.3 °C during the entire experimental period.

To minimize the risk for fungal infections (*Saprolegniasis*) which can be a severe problem in laboratory conditions (Shreier et al. 1996), fish were treated with salt water (salinity of 5 ‰) for one hour before fresh water flow was connected to the holding tank.

3.2.3 Tagging, weighing, photographing and measuring

Tagging was conducted one day after capture in the wild. All individuals were caught one by one, anaesthetized (0.5 mL 2-phenoxyethanol/l), and tagged with visible implant elastomers (Northwest Marine Technology, by Olsen & Vollestad 2001). Each individual was injected subcutaneously using a fine syringe. Different colour combinations were injected at two locations close to the anal fin and dorsal fin, respectively. This resulted in 20 different tagging combinations with 10 fish tagged with each combination.

Each individual was placed in a Petri dish filled with water. The Petri dish was placed on a light pad, and the fish was photographed on its dorsal and right side (Figure 1). Length was measured digitally and the photographs were also used to identify individuals with the tag colour combination by comparing parr markings, which are individually unique (Yagyū et al. 2007). Digital length measurement also minimized time in

air (above water) for the fish. After each fish was tagged as described above, wet weight was measured on a digital scale to the nearest centigram. The same procedure, except from the tagging, was repeated nine days after the first treatment period started, one day before the fish were released back into their native stream and one day after recapture.

Length was measured digitally (ImageJ 1.47t) to nearest 0.1 mm. All fish were measured twice and the mean of the two values was used in the analysis. A grid paper with 1mm squares was used as reference in each photo.



Figure 1. Photographs of the same individual of dorsal (left picture) and the right side (right picture) which were used for individual identification after recapture and to digitally measure length.

3.2.4 Experimental design and feeding schedule

Following tagging and the first session of measuring weight and length, fish were placed in groups of ten in each of the 20 tank compartment in order to simulate natural conditions and favour normal foraging patterns (Johnsson 2003). In order to avoid sampling bias fish were randomly distributed in to one of the two treatment groups (Control or Restricted) and among the 20 compartments. Only one treatment was present in each compartment. During the next five days, fish were acclimatized to the laboratory environment, i.e. experimental tank environment, and provided with frozen Chironomidae larvae once a day (Appendix 1). On the sixth and seventh day behaviour trials for all fish were conducted (see below). During the following nine days, a starvation treatment was performed where the Control group was fed to saturation while the Restricted group was fed the minimum amount of food needed to maintain body size and vital functions (Elliott 1994). During the treatment periods, food consisted of a mixture of frozen invertebrates, reflecting natural food items for stream living Salmonids.

3.2.5 Behavioural scoring

We performed open field tests, which is a well functioning method in terms of testing the activity of an individual and has shown reliable repeatability (Burns 2008). All individuals were tested twice; right before the first treatment period and a second time five or six days into the second treatment period. The ten open field tanks (scoring tanks) consisted of white plastic boxes (30cm high x 25.5 cm wide x 17 cm long) marked with numbers for later identification on video. Water depth was 5 cm and water temperature of was approximately 11-12.7 °C. All tanks were video recorded from above with webcams. Fish were moved from their treatment compartment by a hand net and subsequently placed one by one in 11 plastic cups filled with 2 dl water. When all fish in one compartment were caught they were moved to the scoring tanks at the same time. Individuals from the same treatment compartment were scored and recorded at the same time, one fish in each scoring tank. Five randomly selected compartments from each treatment were used on the first day and the other five on the second day. The same scoring order was used at both occasions. Fish from each compartment were scored for 30 minutes, whereupon each individual was identified with a UV-flashlight and returned to its original compartment. .

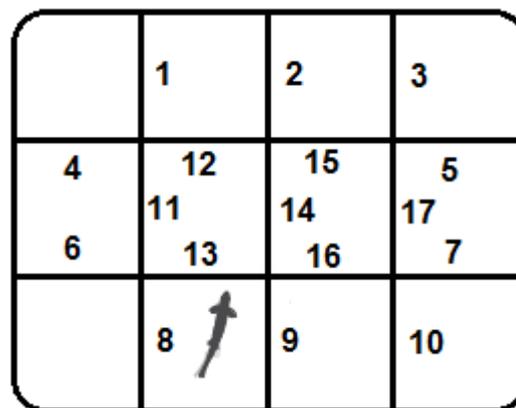


Figure 2. Illustration of behavioural scoring in an open field test. A total of 17 lines were used as activity references. Grid net was added on a computer screen and was not present during behavioural trials.

All video recordings were analysed in Adobe Premier Pro CS3 by adding a grid net (4x3 grids, dividing the open field tank into 64 mm x 57 mm areas) over each open field tank on the computer screen. Counting was done every time the whole body of an observed fish crossed a line of the grid (lines 1-17, Figure 1). The quantified variable “activity level” which was used in statistical analysis was defined by the number of grid lines

crossed during a 10 minute period. When a fish was presented to the new environment a common behavioural response was remaining immobile for a period of time (Näslund, personal comment, own observation). Counting started at first movement following this immobility period and continued for 10 minutes. To be able to analyze the difference in activity over the 10 minute period, summary notes were made after each minute.

3.2.6 Release and re-capture

At the end the laboratory period, July 12, all individuals were released in one batch into their native stream, in the same section (experimental section) as they were caught. The density of brown trout fry in Norumsån was very high (~23 fry/m³ at day of recapture). Therefore, to avoid a sudden change in territorial competition, the same number of fish that was to be released was caught from the stream and moved downstream where there was no possibility for re-colonization. After 34 days, re-capture was conducted over two days using three-pass electric fishing (Degerman & Sers 1999) to catch as many tagged individuals as possible and to estimate the total trout population size in the experimental section. In the buffer zones of the experimental section, 100 meters downstream and 50 meters upstream, fishing was repeated until one or less tagged fish were caught. The first day of fishing was conducted in the experimental section and the second day of fishing was conducted in the buffer zones. All untagged fry were weighed and measured (fork length) in order to estimate the natural size distribution in each stream. Furthermore, all caught parr, a potentially important predator on trout fry, were measured (fork length). All untagged fish were held in corves until fishing was finished and subsequently released within the experimental and buffer sections. The tagged fish were placed in a cooling box and transported back to BioEnv at the University of Gothenburg for weighting and photographing. All fish were killed with 2-phenoxyethanol (1 ml/l) and preserved for subsequent analyses.

3.2.7 Data handling and statistical methods

In order to investigate potential compensatory growth, the specific growth rate (growth in percent/day, SGR) was calculated for each individual in the variables length, weight and condition factor according to the formula:

$$SGR = \frac{\ln(x_{t_2} - x_{t_1})}{t_2 - t_1} \times 100$$

where x is the length, weight or condition factor at the different times t_1 and t_2 in days.

Condition factor (K) was calculated with the formula:

$$K = \frac{10^5 \times W}{L^3}$$

where W is the weight in gram and L is the standard length in mm (length from the nose to the start of the tail fin ray).

All statistical analyses were made in R Studio (R Core team, 2013). Student's T-test was used to analyse treatment effects on specific growth rate (SGR) in length and weight as well as condition factor (K) for the three different times of measuring. Individual consistency in activity between the two behavioural trials was analysed using ordinal linear regression. Ordinal linear regression was also used when analyzing associations between activity and the variables; length, weight, K . If associations between traits differed between treatments an ANCOVA was used to investigate regression line slope differences. All variables were tested for normality and equal variance.

A linear mixed model fitted with maximum likelihood and a random effect on the intercept (nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-113) was used to analyze the effect of treatment on change in activity over time. Treatment (2-level categorical) and time (2-level categorical) and their interaction were entered as fixed effects. In order to account for pseudoreplication because of measuring each individual twice, individual ID nested in time was added as a random effect. If significant results were discovered paired t-tests were used to identify the direction of the difference.

The effect on survival under natural conditions was analyzed using a generalized linear model (GLM). Fixed effects entered were; treatment (2-level categorical), activity at first behavioural trial (continuous) and standard length (continuous). Because of the risk of pseudoreplication caused by keeping several fish in each compartment a generalized linear mixed model (GLMM) with the same parameters as in the GLM was conducted, but with compartment (20-level categorical) added as random effect. This however did not affect the qualitative or quantitative results of the model. Thus compartment was dropped from the model and the

GLM was used. A first model also included interaction between all main effects. However, since no interactions showed significant results all interactions were dropped from the model.

3.2.8 Mortality

During the first experiment a total of 69 fish died between experimental start and release. This corresponds to a mortality rate of 1.3 % fish/day. No difference in mortality between treatments was found during the first experimental period where 17 and 16 fish died in Restricted and Control, respectively. Mortality differences were higher in the second period where 25 and 11 fish died in Restricted and Control, respectively. Mortality rates were similar to those found in natural populations of brown trout fry during the same period of the year (Mortensen 1977).

3.3 Stenungeån

3.3.1 Catching and transporting

On July 17th, approximately 200 brown trout fry (0+) were caught by electric fishing in a 140-meter section (mean width: 1.8 m, mean depth: 0.1 m) in the small coastal stream Stenungeån in western Sweden (N 58° 4', E 11° 52'). The fish were transported to the BioEnv at the University of Gothenburg, divided equally in three cooling boxes filled with stream water.

3.3.2 Accommodation and fish management

Methods were identical to experiment one.

3.3.3 Tagging, weighing, photographing and measuring

All individuals were caught one by one, anaesthetized (0.5 mL 2-phenoxyethanol/l), and tagged with visible implant elastomeres (fluorescent red, blue, yellow, and orange, Northwest Marine Technology, Olsen & Vollestad 2001). Elastomeres were injected with different colours under the skin close to the anal fin (for treatment identification), adipose fin (for individual identification) and dorsal fin (for individual identification) on each fish respectively. The tagging pattern resulted in one colour less used than in experiment one (pink was removed) and was changed due to the difficulty to separate red and pink.

3.3.4 Experimental design and feeding schedule

Fish were placed in groups of eight instead of ten, as in experiment one, due to lower density of fish in the stream and larger mean size of the fish.

3.3.5 Behavioural scoring

Methods were identical to experiment one.

3.3.6 Release and re-capture

At August 14, all fish were released divided in three batches (one every 40 meters) in the experimental section of Stenungeån. In Stenungeån the density of brown trout fry was much lower (~ 7 fry/m³ at recapture) than in Norumsån and were not located in a brown trout spawning area, which indicate that carrying capacity not is reached within this experimental section, therefore no fish were moved from the experimental area before release. After 37 days, re-capture and was conducted in the experimental section (the first day) and its buffer zones, i.e. 150 meters downstream and 50 meters upstream.

3.3.7 Data handling and statistical methods

Methods were identical to experiment one.

3.3.8 Mortality

During the second experiment, a total of 21 fish died between experimental start and release. This corresponds to a mortality rate of 0.5 %/day. Mortality differences between treatments were low during the first experiment period where 9 and 7 fish died in Restricted and Control, respectively. In contrast to the first experiment the mortality decreased drastically during the second experimental period where 4 and 1 fish died in Restricted and Control, respectively. Mortality rates were similar to those found in natural populations of brown trout fry during the same period of the year (Mortensen 1977).

3.3.9 Parasite infection

Parasite infection from the hookworm *Gyrodactylis derjavinii* was detected in both experiment on all fish and was present in both experimental streams. Because of problems with estimating parasite density from the photographs taken no reliable analyse on parasite effects on survival, growth and activity could be made. Furthermore, the effects of activity, size, growth rate and treatment on survival in both

experiments remain the same even if including parasite infection in the model.

4 Results

4.1 Recapture

In Norumsån, 40 out of 131 released fish (30%) survived the 36 day period in natural conditions. No statistically significant difference in recapture frequency was found between the two treatment groups (28% and 33% from the Restricted and Control treatment, respectively, $p > 0.1$).

In Stenungeån, 69 out of 139 (49.5 %) of the released fish were recaptured. Just as in Norumsån, no difference was detected in the recapture frequency between the treatments (55% and 43% from the Restricted and Control treatment, respectively).

4.2 Effects on survival

Activity (open field) was positively associated to the probability of survival under natural conditions in both Norumsån and Stenungeån (Table 1, Figure 4). Significant repeatability in activity level was found in both treatment groups in both streams (Figure 3).

Standard length was positively associated with probability of survival in the stream in Norumsån but not in Stenungeån (Table 1, Figure 5). Table 2 summarizes all statistical results.

Table 1. Summary of GLM 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 on at least a 5% confidence level.

	<i>N</i>	Dependent factor	Fixed effects	Estimate	z-value	p
Norumsån	131	Survival	Treatment	0.411	0.950	0.330
			Activity level	0.003	2.268	0.023
			Length	0.116	2.722	0.006
Stenungeån	137	Survival	Treatment	0.361	1.533	0.097
			Activity level	0.002	2.631	0.015
			Length	0.005	0.135	0.900

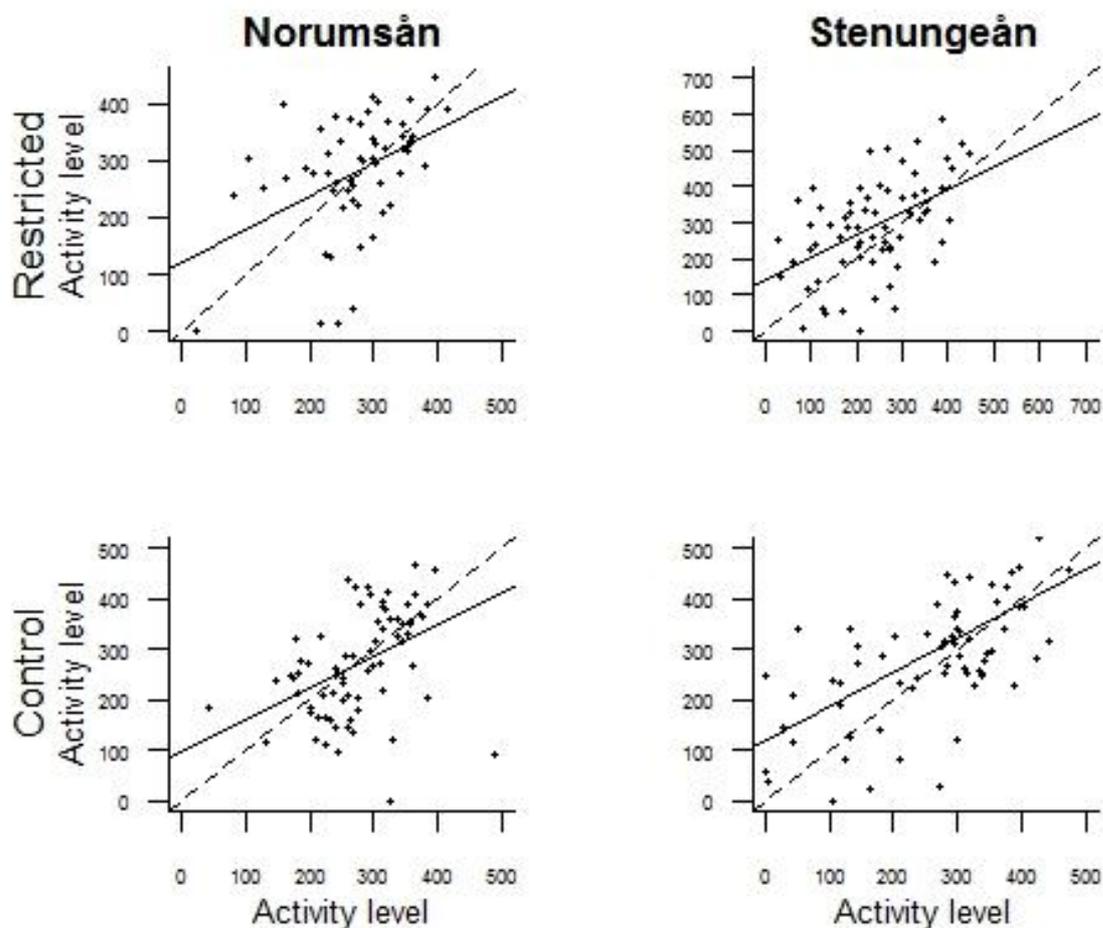


Figure 3. Correlation in activity level between the first (x-axis) and second (y-axis) behavioural trial for each individual in the two treatment groups Control and Restricted in Norumsån (Norumsån) and Stenungeån (Stenungeån). Solid lines represent fitted regression lines where significant correlation was found in all four cases (Norumsån; Control: $R^2=0.19$, $p=0.0004$; Restricted: $R^2=0.21$, $p<0.0001$. Stenungeån; Control: $R^2=0.25$, $p<0.0001$; Restricted: $R^2=0.37$, $p<0.0001$). Dashed lines represent 1:1 behaviour between the first and second trials.

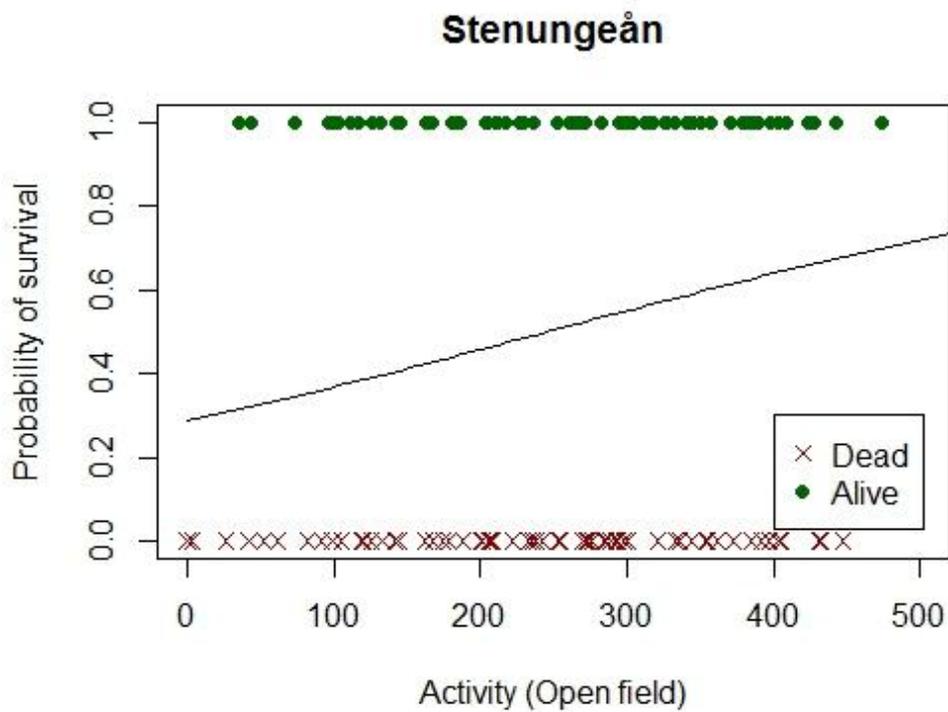
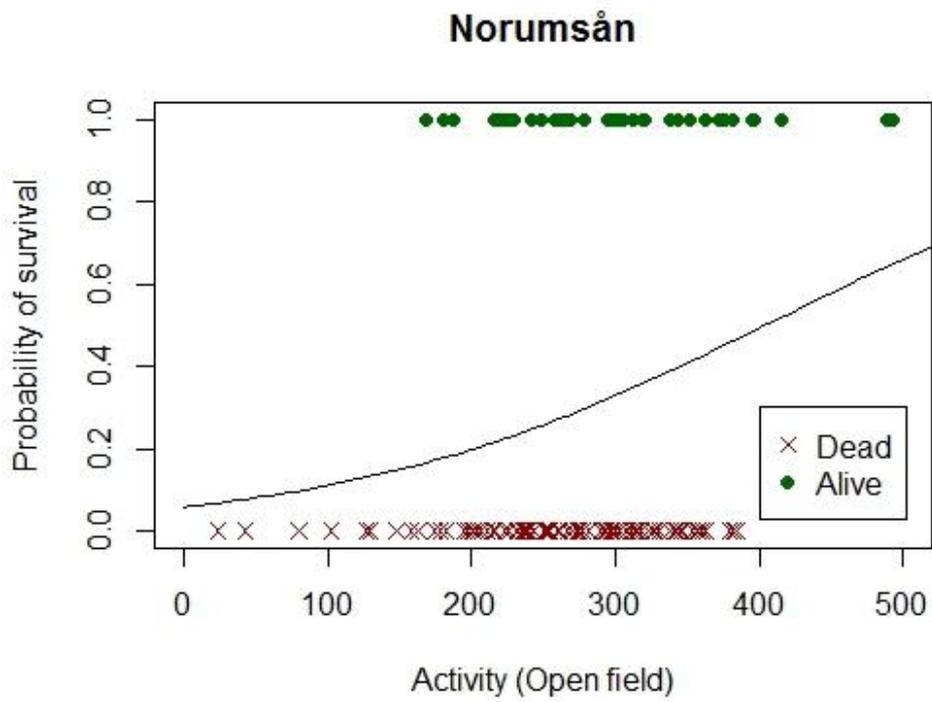


Figure 4. Predicted probability of survival for the brown trout fry (by GLM) depending on activity level in Norumsån and Stenungeån. Plots on the top and bottom represent survivors and dead fish, respectively.

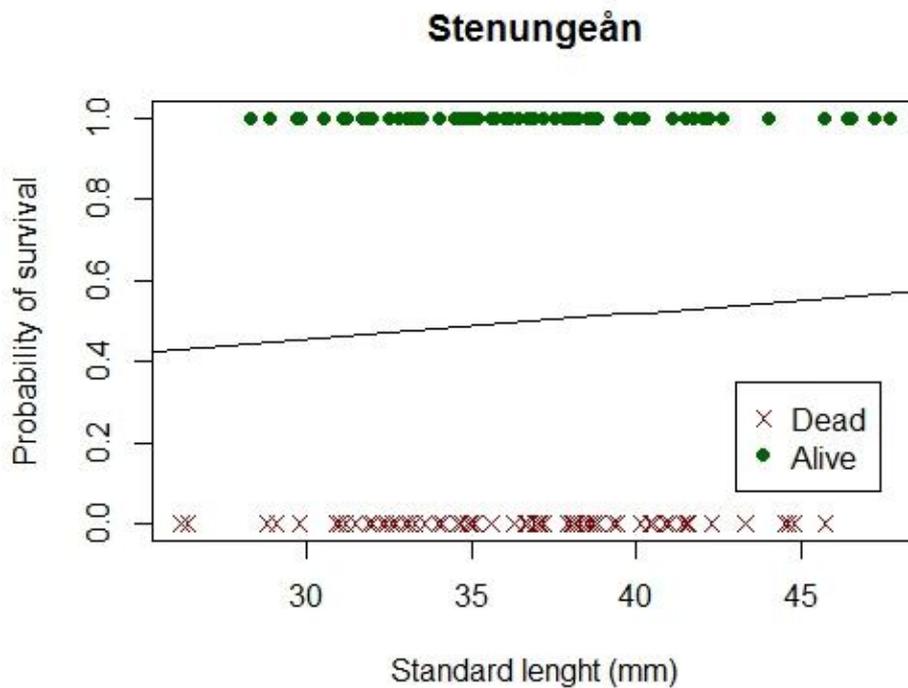
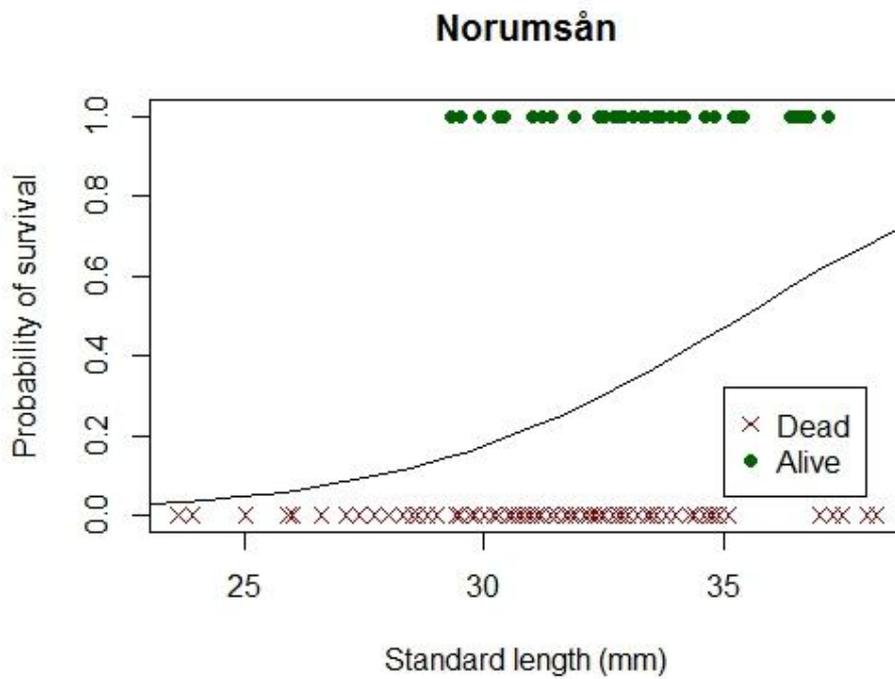


Figure 5. Predicted probability of survival for the brown trout fry (by GLM) depending on standard length in Norumsån and Stenungeån, respectively. Plots on the top and bottom represent survivors and dead fish, respectively. Association in Stenungeån is not statistically significant.

4.3 Changes in activity

In Stenungeån the overall activity level (both treatments included) increased over time in laboratory conditions ($F_{1;138} = 18.21$ $p < 0.0001$). No change in activity level was found between the two trials in Norumsån.

There was no effect of treatment on change in activity level over time in any of the two stream populations (Norumsån: $F_{1;129} = 0.64$; $p = 0.42$, Stenungeån: $F_{1;138} = 1.92$, $p = 0.17$). Table 2 summarizes all statistical results.

4.4 Growth trajectories

Body condition was fully recovered during the refeeding period in both the Norumsån-experiment ($t = 9.26$, $p < 0.0001$) and Stenungeån-experiment ($t = 11.01$, $p < 0.0001$) (Figure 6). During the periods in natural conditions no statistically significant difference in condition factor was found between treatment groups.

The specific growth rate in weight during the refeeding period was higher in the Restricted group than in the Control group in both Norumsån ($t = -7.88$, $p < 0.0001$) and Stenungeån ($t = -4.72$, $p = 0.0004$) (Figure 6). During the period in natural conditions no statistically significant effect of feeding treatment was found in specific growth rate for weight.

No compensatory growth in length was found during the refeeding period in any of the streams (Figure 6). During the periods in natural conditions no statistically significant effect of feeding treatment was found in specific growth rate in length. Table 2 summarizes all statistical results.

4.5 Associations between traits

Fish in the restricted group showed a positive association between activity level and specific growth rate in length during the refeeding period in both Norumsån ($R^2 = 0.19$, $p = 0.0003$) and Stenungeån ($R^2 = 0.10$, $p = 0.004$). This association was not found in the Control groups. The association was statistically significantly different between treatment groups in Norumsån ($F = 5.997$, $p = 0.016$) but not in Stenungeån ($F = 0.932$, $p = 0.34$) (Table 2).

Length and activity showed a weak positive association, both before experimental start ($R^2 = 0.08$, $p = 0.0003$) and during refeeding ($R^2 = 0.1$, $p = 0.0002$) in Stenungeån. In Norumsån such positive association was only found in the restricted group during refeeding ($R^2 = 0.32$, $p > 0.0001$).

There was no association between length and growth rate during any periods of the experiment. Table 2 summarizes all statistical results.

Body condition was not associated with activity level during any parts of the experiment in any of the two experiments.

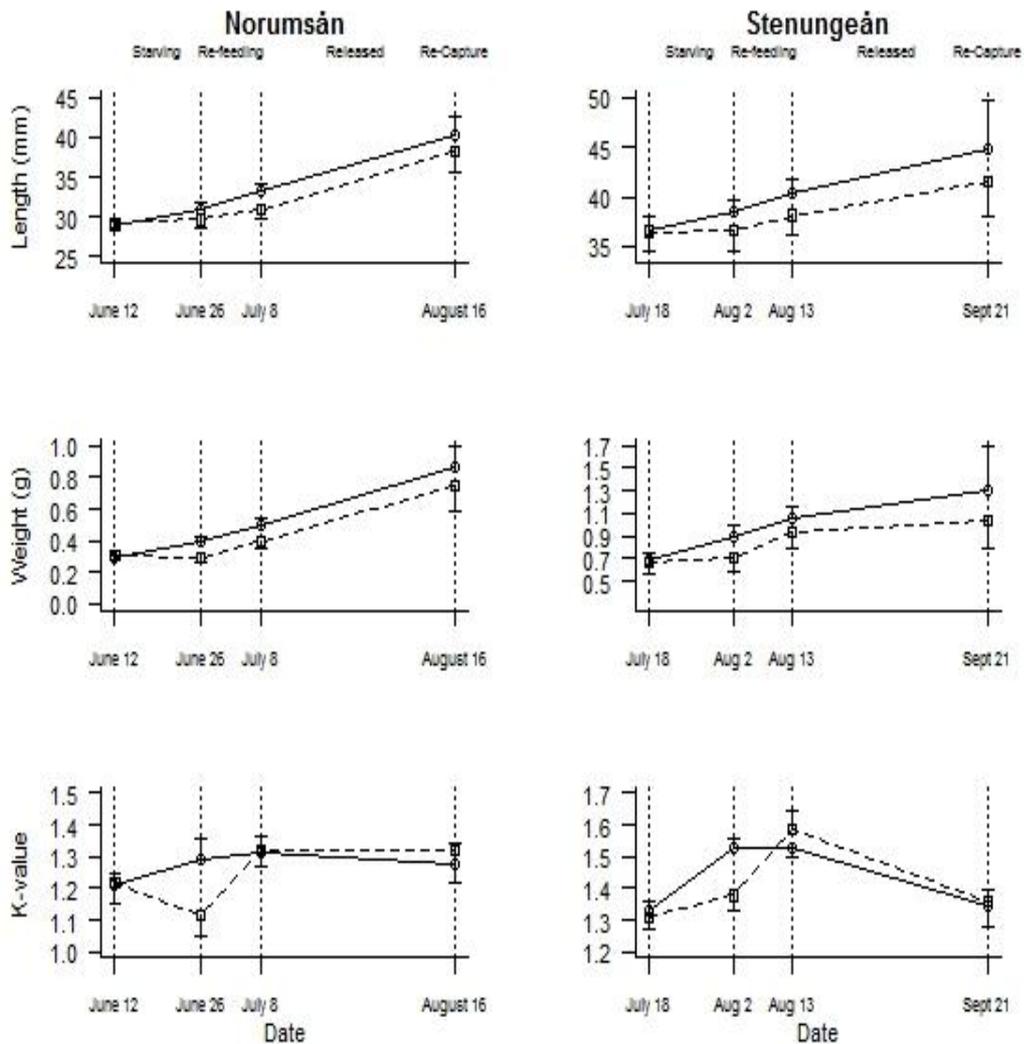


Figure 6. Mean weight, length and body condition (K-value) for the two treatments Control (circles) and Restricted (squares) at the four different measuring sessions in Norumsån and 2 (Stenungeån). Solid lines (Control) and dashed lines (Restricted) show growth trajectories. Error bars show standard deviation (95% CI).

Table 2. Summary of statistic results in Norumsån and Stenungeån. "Yes" indicates statistically significant effect ($p < 0.05$). "Positive" indicate the direction of association. Differences between treatment groups are mentioned.

		Norumsån	Stenungeån
	Period	Prob. of survival	Prob. of survival
Activity	In nature	Yes, Positive	Yes, Positive
Length	In nature	Yes, Positive	No
Condition factor	In nature	No	No
Treatment	In nature	No	No
		Compensation	Compensation
Length	Refeeding	No	No
	In nature	No	No
Weight	Refeeding	Partly	Partly
	In nature	No	No
Condition factor	Refeeding	Yes	Yes
	In nature	--	--
		Correlations between traits	Correlation between traits
Activity Trial 1 vs. Activity Trial 2	Experimental start vs. refeeding	Yes, Positive	Yes, positive
Activity vs. Length	Experimental start	No	Yes, positive
	Refeeding	Only restricted, positive	Yes, positive
Activity vs. Growth rate	Experimental start	--	--
	Refeeding	Only restricted, positive	Only restricted, positive
Activity vs. Condition factor	Experimental start	No	No
	Refeeding	No	No
Length vs. Growth rate	Experimental start	--	--
	Refeeding	No	No
	In nature	No	No

5 Discussion

Our results give further evidence to the recent findings (see Adriaenssens & Johnsson 2013) that interindividual difference in activity level is consistent and result in a higher probability of survival in natural conditions for more active individuals of underyearling brown trout fry. These results indicate the presence of long term consistent behavioural variation within a population, which could affect natural selection. The importance of body size for an individual's chance of survival in nature decreased over time, and thus, with increasing fry size. The consistent effect of activity on survival together with the change in importance of body size can help explain the compensation pattern in body structures. Our results show that individuals exposed to period of restricted resource availability followed by refeeding compensated in body condition and partly in weight but not in length. A fast retention of body condition can be important for the fitness of the fish, while fast growth in body size, i.e. length, may not be needed to increase probability of survival.

5.1 Behaviour as part of the pace-of-life strategy

A recent hypothesis argues for the inclusion of behavioural traits and behavioural syndromes to a general life-history framework, i.e. the pace-of-life strategy (Reale et al. 2010). This hypothesis predicts that the emergence of stable behavioural traits is caused by genetic connections with life-history traits. We did not investigate genetic associations between traits. However, we found evidence based on phenotypic traits that the associations between life-history -and behavioural traits are not stable. For example, the association between activity and the life-history traits, body size and growth rate, was inconsistent and changed in different environmental situations and also due to the growth history of the fish. This is in line with the results of several recent studies (Dingemans et al. 2004, Meekan et al. 2010, Adriaenssens & Johnsson 2011, Höjesjö et al. 2011). Furthermore, a recent study on the western stutter-trilling cricket (*Gryllus integer*), where both phenotypic and genetic associations were investigated, indicated inconsistency in the associations between traits (Niemelä et al. 2013). Such results indicate that the association between behaviour and life-history traits are more complex than predicted by the pace-of-life strategy hypothesis (Adriaenssens & Johnsson 2009). This also shows the importance of investigating associations in natural conditions where environmental fluctuation is present.

5.2 Activity

Activity has been found to be positively associated with boldness and exploration tendency to form a behavioural syndrome (proactive personality) in several contexts and organisms (Mikheev & Andreev 1993, Burns 2008). It has been argued that a proactive personality alters a growth-mortality trade-off, where more active individuals suffers increased mortality, mainly as a result of increased exposure to predation (e.g Biro et al. 2006, Stamps 2007). However, our results suggest the opposite, where high activity level results in higher probability of survival in both experimental streams. There are some indications on how an individual's activity level can affect its chance of survival.

One of the most common reasons for mortality in underyearling brown trout is starvation (Elliott 1994, Kennedy et al. 2008). Therefore, efficient foraging is especially important for fish fry in order to increase the chance of survival. High activity is often associated with aggressive behaviour (Adriaenssens & Johnsson 2013). Therefore high activity can be an advantage in territorial fights, which can increase feeding rates (Gross-Custard et al. 1984). High activity can also decrease the risk of predation. For example, in a recent study examining the survival of Trinidad guppies (*Poecelia reticulata*) more active individual survived longer than less active conspecifics when exposed to a predator (Smith & Blumstein 2010). However, whether it is an ability of escaping the predator or a predator-prey interaction, i.e. driven by the unprofitable prey hypothesis (Leal 1999), is unclear.

Even though activity level was found to be important for the probability of survival in our study, there was a high interindividual variation in both our experimental populations. If high activity is strongly connected to higher probability of survival in all environmental contexts this variation is expected to be much lower. Instead, this variation can probably be explained by different directions on selection pressure on a trait in different environmental situations (Höjesjö et al. 2004, Dingemanse et al. 2004). For example, Dingemanse et al (2004) found that selection pressure on stable heritable traits changed due to environmental fluctuation within a natural bird population. In small stream resident populations of brown trout, environmental selection pressures can be expected to fluctuate, even between patches within the stream (Townsend 1989). Predator pressure and resource availability is two factors which is known to fluctuate (Lancaster 1996). Since the activity of an individual was found to be consistent in relation to conspecifics, selection on

activity level could differ with fluctuation in these two factors. Thus, if predator pressure is high, high activity can be favourable (as found by Smith & Blumstein 2010). Another potentially important environmental factor is the fluctuation in resource availability. High activity has been found to be associated with a higher metabolic rate (Careau et al. 2011) which subsequently result in a higher energy demand. If resource availability is too low, a more active lifestyle could be too costly, and ultimately lead to starvation. For a population living in habitats with high fluctuation in environmental factors in each generation it is therefore not surprising that a large variation in a fitness related trait as activity is maintained (Conrad et al. 2011).

5.3 Body size

The body size was important for survival only in the first of our two experiments. The first experiment was conducted approximately one month earlier than the second. The decreasing importance of body size over time, and thus, with fish size indicates a decreased selection pressure on this trait. Fish fry is sensitive so predation, but with increasing size there are a decreasing number of potential predators. Predators can be excluded for example by their gape limitation (Godin 1997). Furthermore, as the relative size of energy storage increase with body size, larger fish have a more resistant buffer system to mortality by starvation (Post & Parkinson 2001).

5.4 Compensatory growth

The fry were able to fully compensate in body condition, partially compensate in weight but not to compensate at all in length. This pattern, where structural compensation lags behind compensation in body condition is consistent with earlier laboratory and field studies (e.g Broekhuizen et al. 1994, Jobling 2002, Johnsson & Bohlin 2006). The same lack of compensation in length as found in our study has been previously shown for brown trout fry. For example, Alvarez and Nicieza (2005) conducted a field study where no evidence for structural compensatory growth over a two month period could be found. However, this might not be enough to make conclusions about the presence or absence of compensation. Johnsson & Bohlin (2006) found structural compensation in the same species, but not until after five months. Fast compensation in body structure may not be possible because, for fry, constantly high growth rates are present. The lack of difference in growth rates between refeeding period and the period under natural conditions in our study indicate such growth levels. However, our results also indicate

another interesting explanation. A behavioural trait was found to be the most important trait affecting survival in nature. At the same time body size was found to decrease in importance over time, and thus, with increasing fish size. We could therefore hypothesize that compensation in body structures may not be a priority for the fish. Due to the costs with high growth rate, the risk of growing too fast may be higher than the risk of having a smaller body size. The body size may be of importance for fitness later in life, for example during migratory and mating stages (Elliot 1994). Thus, a slower compensation process where full compensation in body structures is first shown later in life can be more efficient.

Again, we have to consider the environmental factors, which have been found to affect the magnitude of compensation. For example, compensatory growth has been found to be both density dependent (Sundström et al 2013) and affected by the presence of predators (Capellan & Nicieza 2007). The fry density in our streams differed significantly (~ 23 fry/m³ in Norumsån vs. ~ 7 fry/m³ in Stenungeån). However, since there were no indications on a lower compensation effect on Norumsån, density should not be a main restricting factor. Compensation restrains due to high predator pressure can possibly be explained by restricted foraging intensity of the prey in order to avoid predation (Capellan & Nicieza 2007). However, we do not have sufficient data on the total number of predators affecting fry in the streams in order to examine this effect.

6 Conclusions

Probability of survival in nature for underyearling brown trout is affected by individual traits that vary within a population. We found evidence for a consistent individual activity level that was positively associated with survival both at different time periods and in different streams. In fish fry, high activity may instead be of great importance for efficient foraging tactics and predator avoidance. The varying association between activity and life-history traits indicate an effect on these associations from environmental factors. Thus, the associations between traits are more complex than presented by the pace-of-life-strategy hypothesis.

Fish length was positively associated with survival in one of the experiments. This was during the first experimental period which indicates a shift in selection pressure on body size. A potential

explanation is that increasing fish size decreases the number of potential predators.

Fish that experienced growth depression were not able to compensate in body structure. Since the importance of body size for survival decreased over time compensation might not be an efficient strategy for the fry due to the cost of higher growth rates. Furthermore the growth rate for the fry is already very high, possibly close to maximum, which makes growth compensation a much longer process.

7 Societal & ethical considerations

Fish populations are declining all across the globe, both in freshwater systems and in the oceans. Information on how natural selection in these populations is affected by the environment is crucial to be able to predict changes in population size. This project investigated natural selection processes in natural conditions. Our results increase the body of knowledge of the connections between fitness related traits within fish populations. Knowledge in how natural selection within and between populations works is an important knowledge for conservation managements to maintain sustainable populations.

Since live animals were handled and killed during the experiment we had to be careful in our planning. The healths of the fish were observed every day during the experiment. Fish that were observed to be sick in laboratory were killed immediately to decrease suffering. All fish were anaesthetised during when body measures were taken and tagging was conducted. Salt treatments were conducted at arrival to laboratory in order to decrease risk of fungi infections. Fish were killed with a high dose of anaesthetic which results in low suffering. We killed all remaining fish at the end of the experiment in order to use them for a subsequent brain analysis, which will be used in an upcoming study.

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10 Appendix

Table 1. Feeding amount and mixture during the laboratory period for Norumsån and Stenungeån, respectively.

Experiment	Date	Period/event	Diet		Amount of food (in percent of total body weight)		Feeding time, Control		Feeding time, Restricted	
			Bloodworms 100%	Bloodworms 50% Tubifex 20 % White mosquito 15 % Black mosquito 15 % Cyklops 10%	Restricted	Control	Morning	Afternoon	Morning	Afternoon
1	June 11	Capture	X		10	10	X		X	
1	June 12	Tagging, weighing photographing	X		10	10	X		X	
1	June13-June 15	Acclimatization	X		10	10	X		X	
1	June 16	Behavioural trials	X		10	10	X		X	
1	June 17	Behavioural trials	X		10	10	X		X	
1	June 18- June 26	Treatment period 1		X	2	20	X	X	X	
1	June 27	Tagging, weighing photographing		X	15	15		X		X
1	June 28-July 2	Treatment period 2		X	30	30	X	X	X	X
1	July 3	Behavioural trials		X	15	15		X		X
1	July 4	Behavioural trials		X	15	15		X		X
1	July 5- July 7	Treatment period 2 cont.		X	30	30	X	X	X	X
1	July 8	Tagging, weighing photographing		X	15	15		X		X
<hr/>										
2	July 17	Capture	X		10	10	X		X	
2	July 18	Tagging, weighing photographing	X		10	10	X		X	
2	July 19-July 21	Acclimatization	X		10	10	X		X	
2	July 22	Behavioural trials	X		10	10	X		X	
2	July 23	Behavioural trials	X		10	10	X		X	
2	July 24-August 1	Treatment period 1		X	2	20	X	X	X	
2	02-aug	Tagging, weighing photographing		X	15	15		X		X
2	August 3-7	Treatment period 2		X	30	30	X	X	X	X
2	08-aug	Behavioural trials		X	15	15		X		X
2	09-aug	Behavioural trials		X	15	15		X		X
2	August 10-12	Treatment period 2 cont.		X	30	30	X	X	X	X
2	13-aug	Tagging, weighing photographing		X	15	15		X		X