

Research

Behavioral and Growth Responses to Predation Threat in Wild and Sea-Ranched Brown Trout (*Salmo Trutta*):An Experiment with a Grey Heron Dummy

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Summary

Sea-ranching of salmonid fishes are common on the northern hemisphere as a way to counteract declining populations due to dam constructions or other anthropogenic factors that negatively affects the fishes. However, many studies have revealed that the wild form is superior under natural condition. Anti-predator responses, social behavior, and growth have so far mostly been investigated separately. In this study we investigated the behavioral and growth responses to a predator threat in wild and sea-ranched brown trout and their reciprocal crosses. Half of the groups of 12 fish (three of each cross-type) were exposed to a heron dummy (as a simulated predator) attack on five of 11 days. We found that wild fish was the only cross-type that responded to the treatment, displaying lower specific growth and lower total food intake. However, by the experiment's end, the wild fish had RNA levels equaling those of the other cross-types, indicating that they had habituated to the predation risk. The differences in response might be because the phenotypic stock reflects genetic differences between wild- and hatchery-born fish that recurrently develop within single-year classes, but that are counteracted by strong gene flow preventing cumulative differentiation over generations. Alternatively, the results of this and similar studies could indicate that the observed phenotypic differences represent non-genetic maternal effects. Whatever the reason, the consequences for wild salmonid populations of the release of hatchery-reared fish are speculative. However, the performance in the wild of fish of hatchery background may be constrained by changes in anti-predator behavior leading to initially high mortality rates when stocked.

Abstract

Sea-ranching of salmonid fishes are common on the northern hemisphere as a way to counteract declining populations due to dam constructions or other anthropogenic factors that negatively affects the fishes. However, many studies have revealed that the wild form

is superior under natural condition. Here we investigated the anti-predator responses of wild and sea-ranched brown trout and their reciprocal crosses. Half of the groups of 12 fish (three of each cross-type) were exposed to a predator (i.e., a heron dummy) on 5 of 11 days. We found that wild fish was the only cross-type that responded to the treatment, displaying lower specific growth and lower total food intake. However, by the experiment's end, the wild fish had RNA levels equaling those of the other cross-types, indicating that they had habituated to the predation risk. The differences in response might be because the phenotypic stock reflects genetic differences between wild- and hatchery-born fish that recurrently develop within single-year classes, but that are counteracted by strong gene flow preventing cumulative differentiation over generations. Alternatively, the results of this and similar studies could indicate that the observed phenotypic differences represent non-genetic maternal effects. Whatever the reason, the consequences for wild salmonid populations of the release of hatchery-reared fish are speculative. However, the performance in the wild of fish of hatchery background may be constrained by

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changes in anti-predator behavior leading to initially high mortality rates when stocked.

Keywords: Sea-Ranching; Brown Trout; *Salmo Trutta*; Growth; Anti-Predator Behavior

Introduction

The salmonid mariculture and restoration carried out in many areas of Europe and North America have led to an increasing number of escaped or deliberately released fish. This has occurred to such an extent that in certain locations the numbers of hatchery-reared fish approach or even exceed those of the wild fish [1,2,3]. Due to the specific conditions of the hatchery environment under which fish are reared (i.e., high density, absence of breeding competition, and absence of predators), important alterations can occur in the fish over generations. These can affect diverse characteristics at the phenotypic and genetic levels, therefore also influencing the behavioral performance of the salmonids in nature. Substantial research has demonstrated that the domestication [4] of anadromous salmonid populations results in a certain phenotypic divergence of the domesticated strains from the wild forms [5,6]. Several studies have also found evidence that environmental rearing conditions can influence diverse characteristics of juvenile salmonids [7,8,9], such as anti-predator behavior [10]. These studies suggest a genetic basis for some behavioral differences between hatchery-reared and wild fish [11].

In addition, many studies have revealed that the wild form is superior under natural conditions. For example: hatchery males partook in fewer spawning than wild males [5,12] hatchery fish produced fewer smolt offspring than wild fish [13] hatchery fish stayed for a shorter period of time in the river than wild fish, and a larger proportion of the hatchery fish returned to sea without having spawned [14] survival of the progeny of farmed salmon to the smolt stage was significantly lower than that of wild salmon [15], predation had a more significant impact on the survival of hatchery-produced coho salmon than on the survival of wild coho salmon [16] wild egg-to-smolt survival was higher than hatchery egg-to-smolt survival [17]. In addition, Akari et al [18] showed that domestication reduce subsequent reproductive capabilities by similar to 40% per captive-reared generation when fish are moved to natural environments. However, the superiority of the wild fish does not seem sufficient to eliminate the threat to wild populations of ecological interference or hybridization posed by escaped or released hatchery-reared fish [19]. The interactions between the two forms could seriously affect the ecological performance of wild populations and, due to interbreeding, threaten future generations [15].

In earlier studies, we compared the behavior of wild and sea-ranched

brown trout from River Dalälven (Sweden). Anti-predator responses [20,21], social behavior, and growth [22] have so far been investigated separately; these studies, along with others examining Atlantic salmon [23] as well as brown trout from River Dalälven [24,25,26], have identified differences between the wild and sea-ranched strains. Nevertheless, the results also indicate that the crosses of wild and sea-ranched fish may perform well, due to the wild population having been invaded by hatchery-reared fish for many years [27]. Thus, the differences found in earlier studies are likely due to divergence in genetic composition derived during one generation [27,28].

An alternative, or complementary, view is that the observed differences are due to parental effects. Such effects, commonly occurring through environmentally mediated maternal effects, are often adaptive [29]. Mothers may increase offspring fitness through mechanisms such as investment of resources in propagules [30] and choice of favorable egg-laying sites [31,32]. Environmental conditions experienced by the mother may affect offspring performance for several generations [33] and environmental variation may play a prominent role in the expression of maternal effects [34]. Therefore, when studying population differentiation using common environmental or reciprocal transplant approaches, differences that appear to result from genetic differentiation may actually relate to maternal contributions [35]. One way to overcome this problem is to raise offspring under common environmental conditions for a few generations to eliminate environmentally induced effects. However, in animals with long generation times, such as most salmonid fishes, this may not be feasible. Another way to approach the problem is to carry out reciprocal crosses between populations to evaluate the relative importance of genetic and maternal contributions. This appears to be relevant to at least some salmonids, in which egg size is negatively associated with early maternal growth, as occurs in hatcheries [36,37,38].

In this study, we examine growth and social behavior in the absence or presence of predation threat. The main aim was to assess the genetic and/or environmental impact on various phenotypic characteristics, such as growth, social behavior, and anti-predator behavior, using four crosses of sea-run brown trout (*Salmo trutta* L.).

Materials and Methods

Background and Strains Used

The experiment was carried out at SLU's fishery research station in Älvkarleby, situated on the River Delavan in central Sweden. Diadromous fish are prevented from following their natural migration route by the hydropower dam at Älvkarleby. Adult salmon and brown trout migrating upstream are caught, using a trapping cage, and transported to a sorting hall, where they are kept and used for

artificial breeding. Two strains of anadromous brown trout occurring in the river today both evolved from the same population. One strain was established in 1967, when a large number of trout were caught and used in an artificial breeding program for sea ranching. This sea-ranched (S) strain has since been kept separated from other strains for approximately 10 generations. The released offspring of S trout are marked by cutting off the left pelvic fin, so the wild (W) trout are identified by having both pelvic fins intact [39]. All the hatchery-reared fish are released as smolts (age 2+).

Crossing Procedure

In the spawning season [40], brown trout females (20 wild and 20

sea-ranched) and 40 males (20 wild and 20 sea-ranched) were used to create families of four cross-types (see Table 2), i.e. ten families of each cross-type, all representing full-sib. The eggs of half of the families (the largest females, i.e. thus which had most eggs) were further divided into two batches, giving 60 groups. These groups were reared separately in 0.5 × 1.0 × 1.0 m tanks under normal hatchery conditions, except for one factor; the groups were moved two times between the tanks during their first six month after hatching. This was done in order to reduce tank effect. The juveniles from these groups were used in the experiments. The egg size (after swelling) was measured using a special V-creased 250-mm ruler: eggs are lined up in the V-shaped crease of the ruler to the required length, and their size is calculated [38].

Table 1. Means and standard errors for the two variables differing significantly between the crosses (see Table 4). Means denoted with the same letter are not significantly different at the 5% level. Sample size = 18 in all cases (mean values for strain and replicate were used).

Cross-type (female × male)	Dominance index (log-transformed)	Specific growth rate
Sea-ranched × Sea-ranched	0.718 ± 0.048 ^{ab}	-0.103 ± 0.028 ^a
Sea-ranched × Wild	0.843 ± 0.049 ^b	-0.212 ± 0.030 ^b
Wild × Sea-ranched	0.586 ± 0.047 ^a	-0.243 ± 0.031 ^b
Wild × Wild	0.696 ± 0.048 ^{ab}	-0.194 ± 0.027 ^{ab}

Table 2. Outline of the procedure and observations made during the experiment.

F = Food provided; P = Predator dummy presented

Day	Morning observations		Afternoon observations	
	With predator	Without predator	With predator	Without predator
-2	Marking	Marking	Marking	Marking
-1	Acclimation	Acclimation	Acclimation	Acclimation
0	Acclimation	Acclimation	Acclimation	Acclimation
1	5min+F+5min	5min+F+5min	F+10min	F+10min
2	F+10min	F+10min	5min+F+5min	5min+F+5min
3	5min+F+5min	5min+F+5min	F+10min	F+10min
4	F+10min	F+10min	5min+F+5min	5min+F+5min
5	5min+F+P+5min	5min+F+5min	5min+F+5min	5min+F+5min
6	No obs.	No obs.	No obs.	No obs.
7	No obs.	No obs.	No obs.	No obs.
8	5min+F+P+5min	5min+F+5min	F+10min	F+10min
9	5min+F+5min	5min+F+5min	F+5min+P+5min	F+10min
10	F+5min+P+5min	F+10min	5min+F+5min	5min+F+5min
11	5min+F+P+5min	5min+F+5min	Terminated	Terminated

Sampling and Marking of Fish

The fish used in the experiment were fry 5-6 months old. The experiment consisted of three 13-day trials; six replicates per trial gave 18 replicates in total, half with a heron dummy and half without. For each trial two fry were randomly sampled from 48 holding tanks and from 12 holding tanks four fish were randomly sampled; half of the fish from each holding tank were put in the tank with the heron dummy and the other half in the tank without the dummy. Each replicate involved 12 fish, i.e. 72 fish in each trial for a total of 216 fish. The fish were anaesthetized with 0.5mLL^{-1} 2-phenoxyethanol. When anesthetized, the body length and weight of the fish were measured, and they were marked by dorsal freeze-branding with one of 12 combinations of dots, so we could distinguish the fish during the experiments. Freeze-branding is a method often used to mark fish. At no time after branding was any gross tissue damage associated with the marking, as also noted in other studies [41]. Studies of behavior rarely observe the branding as negatively affecting the fish in terms of reduced appetite, wounds, infections, or increased mortality [42].

After branding, the animals were placed in the experimental tanks. As mentioned above, the four crosses used in the experiment were mixed in each replicate, so we had twelve fish, three from each crossing, but from 12 different holding tanks.

Experimental Procedure

The experimental setup consisted of three dual experimental tanks ($82 \times 56 \times 20$ cm). The fish in each dual tank came from the same holding tank. After placing all the fish in the dual tanks, they were allowed to acclimatize for 48h, during which the fish were not fed. Groundwater, $6.8-7.5^\circ\text{C}$, was added to the tanks to a depth of approximately 15 cm, and the natural photoperiod was simulated. After acclimation, the observation period started. We moved the heron dummy from one end of the dual tanks to the other after each trial, in order to minimize possible tank effects. The observations were made twice a day, in morning and afternoon. The dummy predator was not presented until the seventh day. The fish were fed frozen chironomid larvae, which thawed in the water. The experimental setup is shown in Figure 1.

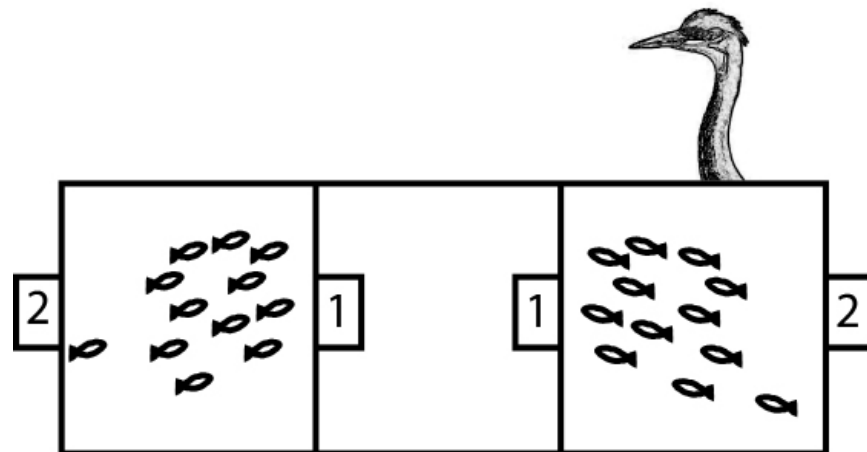


Figure 1.

Figure 1. Schematic view of the experimental set up. The experimental tanks were initially long tanks being 300×56 cm. Those tanks were divided into three sections where the sections in the ends measured 56×56 cm. Three such dual experimental tanks were used in each trial, in total six replicates per trial. The fish were placed in the sections at the ends, the middle section were not used. At one of the sections a heron dummy were placed. 1 = water inlet, 2 = water outlet.

Two kind of observations were made, behavioral and activity observations. The behavioral observations were made according to the 5- and 10-min periods listed in Table 1. In the behavioral observations we noted: (1) *number of food items* (chironomid larvae) taken, and (2) *number of aggressive interactions*, such as display, circling, charging, chasing, ramming, and biting, as well as which individuals won or

lost each interaction; The aggressive behaviors and how we assigned winners and losers are listed in Table 3. The levels of activity observed were freezing, feeding, holding low, holding high, swimming, and fighting, which were recorded for every fish about one hour after the behavioral observations. The levels of activity are described in Table 4. Each trial lasted 13 days, and observations were made for 9 days.

The trial was terminated on the afternoon of day 14. The fry were removed from the tanks and euthanized with an overdose of 2-phenoxyethanol. Final total body length and weight were measured, and the fish were placed individually in plastic boxes to be frozen. These fish were later used for measuring the amount of RNA in muscle (m_{gg}-1). Because RNA level reflects protein synthesis intensity, it provides useful information about the instantaneous growth rate

[43,44,45], whereas the specific growth rate (see below) is a measure of the growth throughout the experiment.

The experimental work, which complied with the standards and procedures stipulated by the Swedish Ministry of Agriculture, was conducted under license no. 34 3632/92.

Data Treatment and Statistical Analysis

The variables considered in these analyses were number of initiated aggressive attacks, dominance index, percent of time being active, total number of food items taken, specific growth rate, and mg RNA per g muscle weight. The sources of variation were crossing, predator, and interaction between crossing and predator. Specific growth rates [46] were calculated using the formula $G = \log(W_1/W_0) \times 100/\text{days}$, W_0 being the initial weight and W_1 the final weight at the end of the trial period (in days). Since growth rates are known to exhibit an allometric relationship, growth rates were adjusted as follows: $G_{ADJ} = G_m + (b \times \log(W_G) - b \times \log(W_A))$, where b is a constant (0.308 for brown trout), W_G is the geometric mean body mass of individual fish before and after the growth period, and W_A is the mean initial body mass [47]. Dominance index was according to a method described by Boyd and Silk [46]; this index is based on proportion won interactions. It gives low scores to dominant individuals (good fighters) and high scores to subordinate individual (bad fighters). Proportion of time being active: holding low and freezing were regarded as inactive

behavior and the remaining behaviors as active. Thus, we could calculate proportion of time being active as (number of observation each individual were active) (total number of observations for each individual).

For each trial, the variables were adjusted using the residuals from linear regressions, initial length being the independent variable (this does not apply for growth rate, which was adjusted as shown above). From these adjusted values, the mean values for each crossing and replicate were calculated, and these mean values were used in the subsequent analyses. The observations on the activity levels were also analyzed separately. Proportion of time for each behavior listed in Table 3 were calculated as (number of observation each individual performed that behavior) (total number of observations for each individual). These values were adjusted for initial length as described above and the data analyzed using MANOVA [48]. For feeding rate (i.e., number of food items taken), we calculated the mean numbers of food items taken per day for each crossing and replicate; these values were used for a repeated ANOVA. To evaluate overall differences and similarities between the crosses, canonical discriminate analysis was applied, both to the whole dataset and separately to replicates with and without the predator dummy. Because six variables were considered in the analyses, the levels of significance were corrected for multiple comparisons using the Šidák method [49], and all p-values presented below have been corrected. All the data were analyzed using SAS statistical software.

Table 3. Aggressive behaviors of brown trout juveniles observed in the experiments.

Behavior	Description
Display	One fish swam up alongside another fish usually holding the head slightly higher than the caudal peduncle. The attacking fish moved slowly sideways against the other fish. The other fish could either respond by behaving in the same way or by leaving the spot where the interaction took place. The fish leaving the spot were regarded as loser and the other fish as winner.
Charge	The attacking fish swam against another fish (head directed towards the lateral side of the other fish) but there was no physical contact between the two fishes. The attacked fish could either respond by leaving the spot or by making an evasive maneuver and make a counterattack. The fish leaving the spot were regarded as loser and the other fish as winner.
Ram	As charge, by the attacking fish hit the other fish with its head (nose), but no biting could be observed (usually the attacking fish had closed mouth). The attacked fish could either respond by leaving the spot or by making an evasive maneuver and make a counterattack. The fish leaving the spot were regarded as loser and the other fish as winner.
Circling	Usually started with display, charge or ram, but the attacked fish did not left the spot and did not strike back using the same kind of behavior. The two fishes started to chase each other's tail, usually swimming in rather slow speed. The fish that left the area were regarded as loser and the other as winner.
Bite	The attacking fish bite the attacked fish. The attacked fish could either respond by leaving the spot or by making an evasive maneuver and make a counterattack. The fish leaving the spot were regarded as loser and the other fish as winner.
Chase	This kind of behavior took place when a fish left the spot of the interaction, the winner did not stayed at the spot but swam after the loser and made short quick charges.

Results

Immediate Effects of the Predator Dummy

There was no overall difference in numbers of aggressive interactions between the first five minute and the last five minutes of observation

($F_{1,127}=0.01$, $p=0.904$), but there was an overall effect of predator dummy ($F_{1,127}=6.81$, $p=0.010$) and way of providing food and releasing predator dummy ($F_{2,127}=12.01$, $p<0.001$; see Figure 2), and the interaction between the three factors were also significant ($F_{3,127}=6.50$, $p<0.001$). Initially strain was included in the model, but was not significant ($F_{3,127}=0.81$, $p=0.489$) and was therefore excluded from the model.

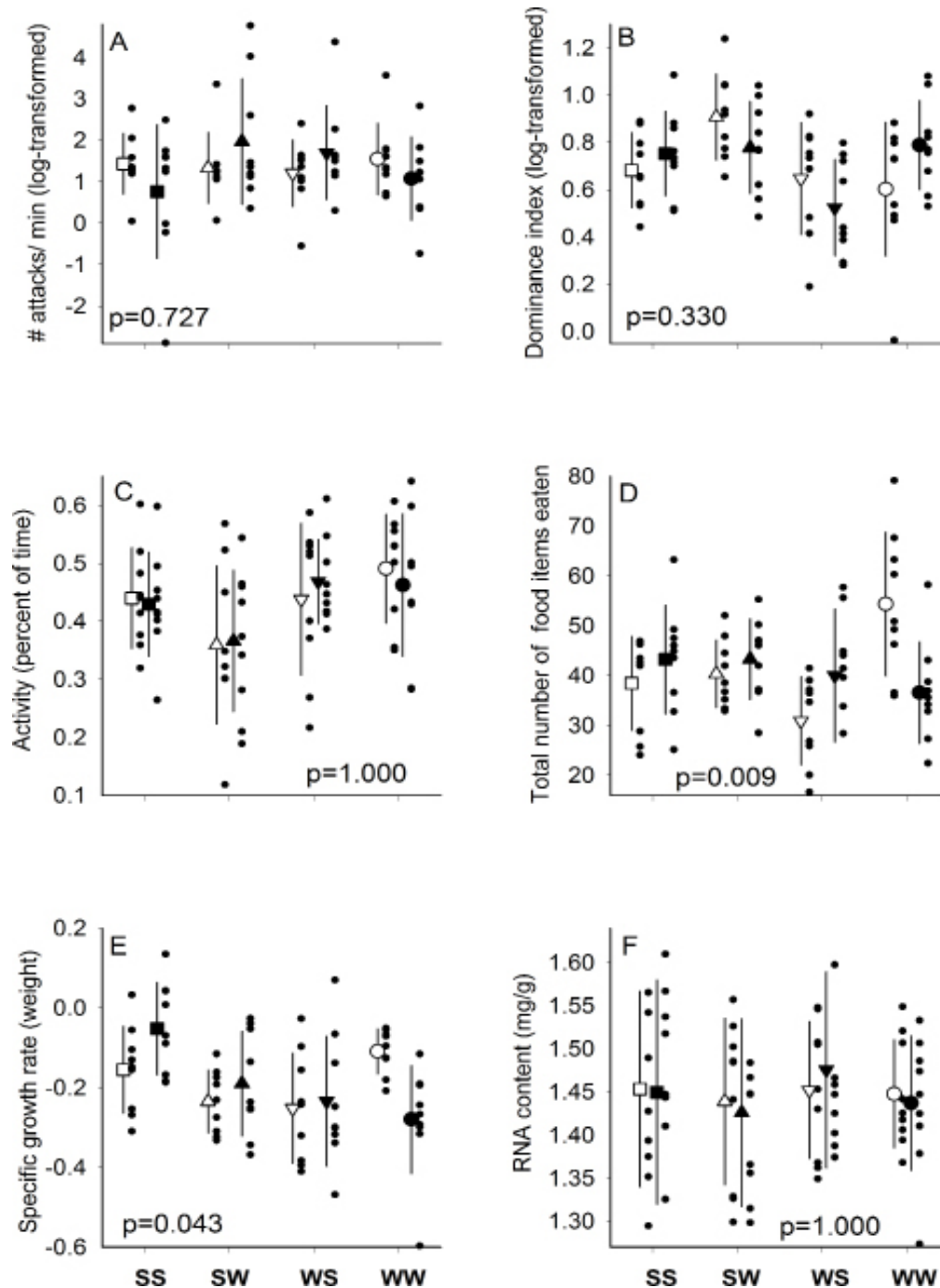


Figure 2.

Figure 2. Responses in six variables to the heron dummy. Filled symbols represent values for replicates in which a heron dummy was used and open symbols indicate that no heron dummy was used. The significant values in the figures refer to the interaction between predator and strain.

There was difference in numbers of food items eaten between the first five minute and the last five minutes of observation ($F_{1,127}=107.1$, $p<0.001$), there was also an overall effect of predator dummy ($F_{1,127}=6.33$, $p=0.013$) and way of providing food and releasing predator dummy ($F_{2,127}=72.01$, $p<0.001$; see Figure 2), and the interaction between the three factors were also significant ($F_{3,127}=330.9$, $p<0.001$). Initially strain was included in the model, but was not significant ($F_{3,127}=0.33$, $p=0.801$) and was therefore excluded from the model. As can be seen in Figure 2, the predator caused an almost total inhibition of number of food items eaten. In the cases where the predator dummy were not released or not used at all the tendency was the number of aggressive interactions increased during the last five minutes, i.e. when the food was provided. In the cases when the predator dummy was released the feeding rate was higher before the dummy was released, i.e. the fish ate the 'left-over's' from previous feeding time. In the cases when no predator dummy was released the fish ate almost all chironomid larvae within 10 minutes.

Morning's Afternoon

If just the 10-minute observations was regarded there were no differences in number food items taken (time of day: $F_{1,127}=2.15$, $p=0.153$; predator: $F_{1,127}=0.91$, $p=0.341$; strain: $F_{3,127}=0.25$, $p=0.862$; predator strain: $F_{3,127}=0.56$, $p=0.643$; time of day strain: $F_{3,127}=0.12$, $p=0.951$). Number of food items taken was log-transformed prior to analyses. In addition there were no differences in number of aggressive interactions (time of day: $F_{1,127}=2.15$, $p=0.153$; predator: $F_{1,127}=2.62$, $p=0.115$; strain: $F_{3,127}=2.04$, $p=0.128$; predator strain:

$F_{3,127}=0.49$, $p=0.694$; time of day strain: $F_{3,127}=1.96$, $p=0.139$). Thus, for the analyses of overall effects of predator dummy, morning and afternoon records are summed.

Overall Effects of Predator Dummy

The food rations given to the fish were calculated to result in no overall growth in any of the replicates, i.e., there should be competition for food. However, the specific growth rate was slightly negative (-0.189 ± 0.301 , $t=9.16$, $p<0.001$, $n=216$). Egg size did not differ between females of wild and sea-ranched origin (wild: 5.69 ± 0.178 mm, $n=20$; sea-ranched: 5.62 ± 0.163 mm, $n=20$; $t=1.02$, $p=0.232$; least-square means, corrected for weight before stripping).

If all six variables were accounted for, crosses were found to differ overall (MANOVA: Wilk's $\lambda=0.444$, $F_{18,167.36}=3.09$, $p<0.001$) but the predator dummy had no overall effect (MANOVA: Wilk's $\lambda=0.988$, $F_{6,59}=0.10$, $p=1.000$). The interaction between predator and crosses was significant (MANOVA: Wilk's $\lambda=0.565$, $F_{18,167.36}=2.08$, $p=0.0499$). As can be seen in Figure 3 and Table 5, the crosses did not differ in number of attacks, activity, food items taken, or muscle RNA content. However, the crosses differed in dominance index and specific growth rate (Table 1). WS fish had a significantly lower dominance index (i.e., were more dominant) than did SW fish, and SS fish had a higher growth rate than did SW and WS fish. In none of the recorded variables was any overall effect of the predator dummy noted (Table 5). The interaction between predator and crosses, however, was significant for two of the variables, i.e., total number of food items taken and specific growth rate (Table 4). Both these interactions could be accounted for by the response to the heron dummy among the fish of wild origin (WW), which ate less ($t=3.58$, $p=0.0041$) and had a lower growth rate ($t=3.01$, $p=0.022$) in replicates with the predator dummy than in replicates without.

Table 4. Activity behaviors of brown trout juveniles observed in the experiments.

Behavior	Description
Freezing	The fish lay motionless on the bottom of the aquarium, most of the individuals ventral side (the belly) resting towards the bottom and the fish usually had a darker coloration than when being more active.
Holding low	The fish lay more or less motionless on the bottom of the aquarium, but only the fins (dorsal, anal, pectoral and/or pelvic) were touching the bottom. The fish also made small (less than one cm) bonzes up and down.
Holding high	The fish kept still in the water current.
Feeding	The fish ate chironomid larvae.
Swimming	The fish moved (swam) in the aquarium.
Fighting	The fish were attacked by another fish or attacked another fish in the aquarium. (See Table 2)

Table 5. Results of analyses of variance of the six variables noted in the experiment examining juvenile responses to predation threat. “Crossing” refers to wild and sea-ranched trout from River Dalälven and their reciprocal crosses. “Predator” indicates that either a predator dummy either was or was not presented to the fish.

Variable	Source of variation	F value	d.f.	Level of sign.
Number of attacks	Crossing	0.81	3,71	1.00
	Predator	0.10	1,71	1.00
	Crossing × Predator	1.61	3,71	0.73
Dominance index	Crossing	4.73	3,71	0.03
	Predator	0.23	1,71	1.00
	Crossing × Predator	2.53	3,71	0.33
Activity	Crossing	3.67	3,71	0.10
	Predator	0.19	1,71	1.00
	Crossing × Predator	0.24	3,71	1.00
No. food items taken	Crossing	2.79	3,71	0.26
	Predator	0.11	1,71	1.00
	Crossing × Predator	5.83	3,71	0.01
Specific growth rate	Crossing	4.49	3,71	0.04
	Predator	0.09	1,71	1.00
	Crossing × Predator	4.37	3,71	0.04
RNA/muscle weight	Crossing	0.32	3,71	1.00
	Predator	0.12	1,71	1.00
	Crossing × Predator	0.13	3,71	1.00

Figure 3. Responses in feeding rate to the heron dummy. Mean values for feeding rate (no. of food items taken day⁻¹) for each cross. Filled symbols represent values for replicates in which a heron dummy was used and open symbols indicate that no heron dummy was used. The lower values on day 11 are due to the lack of afternoon observations (see Table 2).

Regarding the feeding rate, the results indicate no overall difference between the crosses (Wilk’s $\lambda = 0.623$; d.f. =24,160.118;F=1,18;p=0.27), although the feeding rate was lower in the replicates with the heron dummy (Wilk’s $\lambda = 0.515$;d.f. =8,55;F=6,61;p<0.001; see Figure 3).

Time had an overall effect on food intake (repeated ANOVA: $F_{8,384} = 14.77$, p<0.001) and the interaction between time and predator

was also significant (repeated ANOVA: $F_{24,384} = 6.37$, p<0.001). However, the remaining interactions were not significant, i.e., time crosses (repeated ANOVA: $F_{72,384} = 0.60$, p=0.996) and time crosses predator (repeated ANOVA: $F_{72,384} = 0.70$, p=0.964). If each cross was analyzed separately, only the WS cross did not display any predator effect (time predator) on food intake (SS: $F_{8,96} = 2.66$, p=0.0035; SW: $F_{8,96} = 2.02$, p=0.018; WS: $F_{8,96} = 1.25$, p=0.290; WW: $F_{8,96} = 2.74$, p=0.0023). However, the WW cross was the only one that displayed a lower overall food intake value due to predator dummy attacks (repeated ANOVA: $F_{5,12} = 3.50$, p=0.0035, between subject effects).

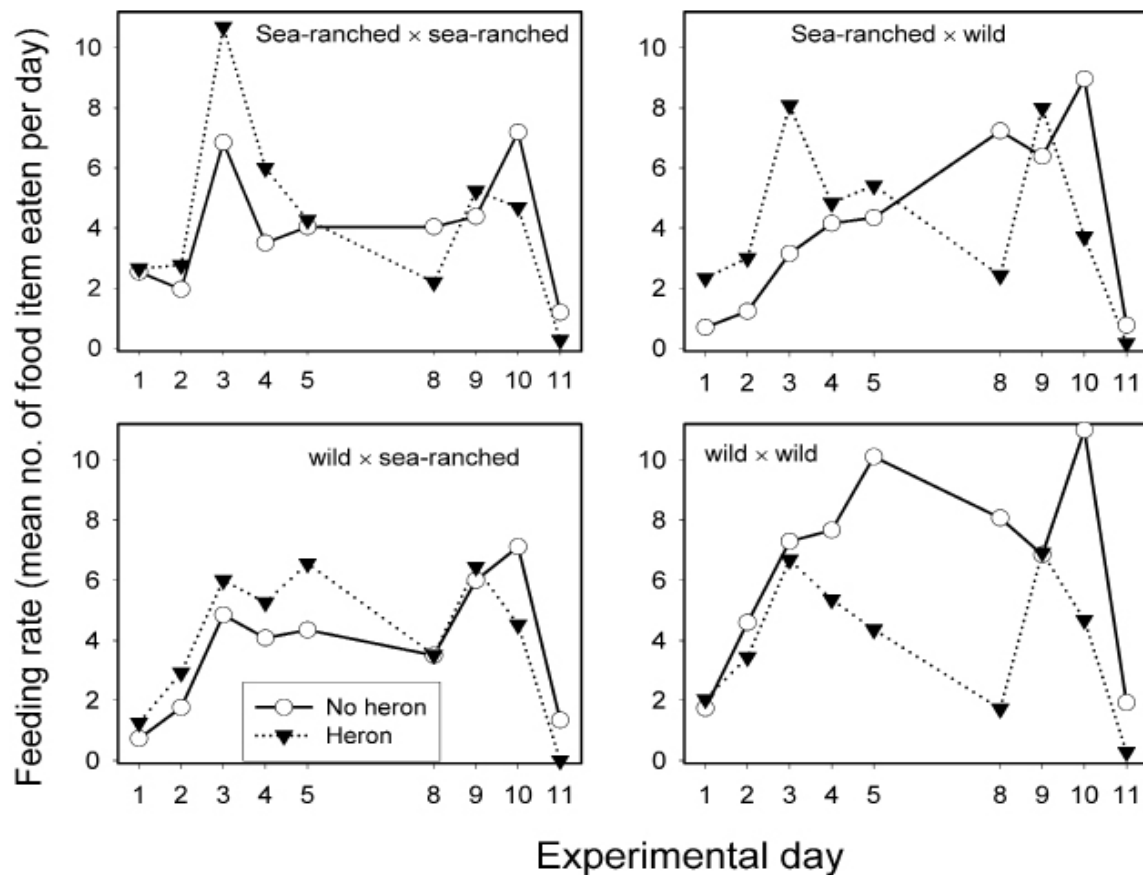


Figure 3.

Discussion

The results of the present study indicate that the hatchery selection had, at least initially during the experiment, a significant effect on the anti-predator performance of juvenile brown trout. This is consistent with studies of wild steelhead trout and wild-domesticated hybrids [10]. Fry of wild origin displayed a strong anti-predator response, evidenced by a high decrease in food intake and specific growth rate. In our study, the crosses between sea-ranched- and wild-origin fish, as well as the pure sea-ranched-origin fish, did not display any significant response when the predator was present. It can therefore be inferred that the background of the juvenile fish is important to their behavioral performance. The reduced anti-predator response in domesticated salmonids might be due to relaxed selection for trade-offs between foraging and predator avoidance in the hatcheries [50], so the differences in anti-predator response between crosses may have a genetic background. Genetic divergence between hatchery and wild populations might be expected for two main reasons: the use of small effective population sizes to initiate hatchery stocks, and the novel hatchery environment [51]. Other studies, using wild and hatchery-reared salmonids, suggest that size selection in hatcheries may favor risk-prone foragers [52] and that, due to rearing in the absence

of predators, the tendency of the juvenile to forage under predator risk increased, because they do not learn how to avoid predators while foraging. In such situations, an inherited adaptive response to predator presence is important. Domestication may reduce this inherited response, impairing the genetic variation of domesticated salmonids, and perhaps favoring risk-taking behavior, which leads to an increasing susceptibility to predation [53]. However, our results also indicate that the predation threat had no overall effect, and that the wild fish perform as well as do the crosses in terms of RNA levels. As the RNA levels reflect the growth on a much smaller time scale than does the specific growth rate, this implies that the wild fish were habituated to the situation. In addition, as can be seen in Figure 3, the food intake at the end of the experiment was equal between replicates with and without predator dummy exposure. Such a learning process likely works in the other direction as well: naive fish might improve their predator avoidance ability after stocking [54,55] but the benefits gained by experience may be limited by genetically determined predator avoidance capabilities [11]. Our study also indicate that lack of difference between strains in immediate response to a predator might not necessary mean that the strains differ in other respects, such as more long-term responses in growth rate.

Sea-ranched trout from River Dalälven reportedly have higher growth potential [24,25,26,56], are more prone to risk-taking [18,54], and display less pronounced physiological stress responses [57] than wild fish from the same river. However, a recent study did not reveal any genetic differentiation between the wild and sea-ranched trout, noting that the gene flow from sea-ranched to wild trout is probably considerable [27]. An explanation for the contradictory results might be that the phenotypic stock divergence reported in previous laboratory studies reflects genetic differences between fish born in the wild and in hatcheries that recurrently develop within single-year classes but that are counteracted by strong gene flow preventing cumulative differentiation over generations. Assuming additive genetic variance for the traits under study and a marked difference in selective regimes between wild and hatchery environments, some degree of genetic divergence may be generated from the egg to adult stage within a year class, even when most of the wild trout have parents born in the hatchery [26]. In this way, comparisons of offspring derived from returning sea-ranched and wild adults may reveal trait mean differences despite a high level of gene flow.

Another explanation for the contradictory results of the present versus previous studies may be that the observed phenotypic differences represent non-genetic maternal effects caused, for example, by egg-size differences among wild and sea-ranched females [54]. However, in the present study, the mothers' characteristics (such as egg size) had no significant effect on the variables studied here. Furthermore, the crosses having wild or sea-ranched mothers did not differ in such a way that maternal effects were suggested. In some cases, the SS and WW fish were more similar to each other than to their crosses. Although we did not find any maternal effect on the offspring, such an explanation cannot be ruled out as this and previous experiments have not been designed to allow discrimination between maternal effects and other sources of variation.

Conclusion

The consequences for wild salmonid populations of the release of hatchery-reared fish are speculative, but the performance in the wild of fish of hatchery background may be constrained by behavioral changes in anti-predator behavior, which might impair their fitness [54]. If so, fish raised in hatcheries for several generations might, when not stocked as eggs but as smolts, initially experience high mortality rates.

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