Behavourial conservation in hatchery-reared brown trout

Phenotypic effects of controlled crossbreeding with wild fish
BEHAVIOURAL CONSERVATION IN HATCHERY-READED BROWN TROUT

PHENOTYPIC EFFECTS OF CONTROLLED CROSSBREEDING WITH WILD FISH
Nico Alioravainen

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To my grandparents
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ABSTRACT

Human activities have a major impact on river ecosystems worldwide. To compensate for the loss of natural habitats, massive stocking programs have been built to maintain fish stocks. The lack of natural reproduction and natural selection have led into another increasing threat to our fish stocks: domestication. Considering the ecological and economic value of brown trout as well as the other salmonid stocks, there is an urgent need for better fisheries management and research for fisheries-induced evolution, but also for study of behavioural conservation. Indeed, behaviour has been a long-neglected trait that can have direct effects on individual survival and fitness. While stocking programs have focused on numbers of stocked individuals, and maintenance of genetic diversity, the local adaptations and behavioural diversity have not been among main interests. This has led to domestication and low stocking success making the expensive stocking programs ineffectual.

In this thesis, I focus on behaviour and behavioural conservation. I study how a brown trout strain, reared in the hatchery over multiple generations, differs in behavioural diversity from wild brown trout. By controlled crossbreeding between these two strains, I aim to rewild the local hatchery broodstock and thus save the last remaining adfluvial brown trout population in Oulujärvi watershed. I show that hatchery strain brown trout juveniles differ from wild strain fish in their acute post-release behavioural response and disperse rapidly downstream after release.
Hatchery-strain fish show higher risk-taking and exploration behaviour than wild crosses, which will likely increase their mortality in the wild. The relative vulnerability to angling may cause diverged within-strain phenotypic behavioural types in hatchery and wild strain fish. Results with wild strain fish support the idea that fishing targets certain behavioural types, which may result in increased timidity in the population.

Admixing genes of locally adapted wild fish with the hatchery fish could enhance stocking results by increasing the number of adaptive behavioural phenotypes. A rewilding project may not only save the last remaining lake-run brown trout in Oulujoki watershed but also provide an example of how hybridization may conserve the evolvability of the population.

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CAB Thesaurus: animal behaviour; wildlife conservation; wildlife management; endangered species; fisheries; fishery management; Salmonidae; brown trout; hatcheries; domestication; crossbreeding; hybridization; wild strains; genetic variation; genetic diversity; phenotypes; phenotype variation; predation; survival; angling

Yleinen suomalainen ontologia: eläinten käyttäytyminen; kalakantojen hoito; uhanalaiset eläimet; lajiensuojelu; kalanviljely; taimen; järviilaimen; risteytys; luonnonvaraiset eläimet; geneettinen monimuotoisuus; geneettinen muuntelu; fenotyyppi; saalistus; selviytyminen; virkistyskalastus; urheilukalastus
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nent.

Oulu, 14th September 2020
Nico Alioravainen
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>KFRS</td>
<td>Kainuu Fisheries Research Station</td>
</tr>
<tr>
<td>OUV</td>
<td>Oulujärvi watercourse brown trout (hatchery) broodstock</td>
</tr>
<tr>
<td>RAU</td>
<td>Rautalampi watercourse brown trout (hatchery) broodstock</td>
</tr>
<tr>
<td>KIT</td>
<td>Kitkajärvi watercourse brown trout (hatchery) broodstock</td>
</tr>
<tr>
<td>VAA</td>
<td>Vaarainjoki brown trout broodstock</td>
</tr>
<tr>
<td>HV</td>
<td>High vulnerability to angling</td>
</tr>
<tr>
<td>LV</td>
<td>Low vulnerability to angling</td>
</tr>
<tr>
<td>PIT</td>
<td>Passive integrated transponder</td>
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## GLOSSARY

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Alevin</td>
<td>A newly hatched salmonid still carrying the yolk sac</td>
</tr>
<tr>
<td>Broodstock</td>
<td>A parental stock that is permanently farmed in captivity and used to reproduce next generation</td>
</tr>
<tr>
<td>Fry</td>
<td>A fish larva that has consumed the yolk</td>
</tr>
<tr>
<td>Hybrid</td>
<td>An offspring from genetically divergent parents</td>
</tr>
<tr>
<td>Milt</td>
<td>Sperm of male fish</td>
</tr>
<tr>
<td>Parr</td>
<td>A riverine juvenile of trout</td>
</tr>
<tr>
<td>Roe</td>
<td>Eggs of female fish</td>
</tr>
<tr>
<td>Strain</td>
<td>Genetically diverged hatchery stocks</td>
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman Numerals I-III.


The publications are reproduced at the end of the thesis with the permission from their copyright holders *Boreal Environment Research Publishing Board* (II) and *Canadian Science Publishing* (I, III).
AUTHOR’S CONTRIBUTION

I) I, along with co-authors designed the experiments, collected, and analysed the data and was responsible for the writing of the article.

II) I analysed the data and was responsible for the writing of the article.

III) I planned the behavioural experiments with AV and collected the behavioural data. I analysed the data and was responsible for the writing of the article.
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Brown trout (*Salmo trutta* Linnaeus, 1758), living in freshwaters south of the latitude of 67°00’, is listed as endangered (EN) in Finland (Hyvärinen et al. 2019). Natural reproduction of migratory brown trout is threatened nearly everywhere in Fennoscandia due to human activities in freshwaters: damming, overfishing, intensive land use and habitat loss (Syrjänen et al. 2017). Brown trout can be differentiated in four types based on their life-history strategies: resident, potamodromous, anadromous, and adfluvial (as special case of potamodromous) (Elliott 1994), and the migratory types are in most critical danger. Attempts have been made to compensate for the collapse of the wild, naturally reproducing migratory populations by intensive stocking of hatchery-reared brown trout, but natural selection and broodstock renewing with wild fish have been virtually absent (Syrjänen et al. 2017). In Finland, the first hatchery practises in 1920s used spawners from various watercourses and populations were usually mixed, leading to losses of local adaptations (Syrjänen et al. 2017).

Stocking hatchery-reared fish has been a common practice to compensate for the loss of stocks and restore populations (Cowx 1994). Stocking programs typically enhance the catches in fisheries, but are ineffective to support naturally reproducing populations due to the low fitness of hatchery-reared fish in the wild (Araki et al. 2008). As found in Atlantic salmon (*Salmo salar*), supportive releases have had no effect on recruitment despite the intensive conservational stocking over a decade (Glover et al. 2018). Captive breeding leads to unintended domestication already in very few generations (Araki et al. 2007; Christie et al. 2012, 2016; Mäkinen et al. 2015), and may induce an inevitable loss of adaptations to the wild (Lorenzen et al. 2012). Captive breeding programs vary in their capability to conserve genetic diversity (Fraser 2008), but many of the stocks have been reared in captivity over decades virtually without any natural selection – especially in Finland (Syrjänen et al. 2017). Whilst hatchery breeding typically aims to maintain genetic diversity of broodstocks, maintenance of local adaptations has often been neglected (Neff et al. 2011). The idea of population enhancement by supportive stocking is to overcome the natural survival bottleneck that occurs in the beginning of the natural life cycle of fish (Aprahamian et al. 2003). Egg and fry mortality in nature can be nearly twenty times higher than in hatchery conditions, where over 90% of eggs and fry may survive (Reisenbichler et al. 2004). Anyhow, this relaxed selection maintains artificially high genotypic diversity, because the survival bottleneck in nature is important for the
population, as only the fittest of individuals survive and hence may reproduce and pass their genes to the following generations.

Now that the nursery and spawning habitats have been widely restored, there is yet another threat before brown trout can reproduce in the wild and form natural breeding populations: domestication. Domestication may manifest in adaptation to artificial hatchery environment and reduced ability to survive in the wild. Thus, the challenge is how to better match the hatchery-reared fish with the local environmental requirements. However, it is not yet clear how to solve the trade-off between genetic diversity and maintenance of local adaptation when the aim is to reintroduce a naturally reproducing population (Houde et al. 2011). The idea of evolutionary rescue introduces the notion that the extinction of declining populations can be prevented by increasing the frequency of adaptive alleles in the population (Carlson et al. 2014; Hamilton and Miller 2016). For instance, the low frequency of adaptive alleles in a domesticated population can be increased by admixing genes from the wild population using controlled crossbreeding programs (Hamilton and Miller 2016). Thereby, genetic admixing is expected to increase the fitness of introduced individuals in the environment that is low matching to broodstock, e.g. domesticated fish in the wild.

1.1 BEHAVIOURAL CONSERVATION—“REWILDING” DOMESTICATED STRAINS

Individual behavioural differences are maintained by natural selection. They influence population dynamics and have eco-evolutionary consequences (Conrad et al. 2011; Wolf and Weissing 2012; Mittelbach et al. 2014). Captive rearing may reduce heterogeneity of behavioural types and the behavioural repertoire of an individual, resulting in negative fitness effects in the wild (McDougall et al. 2006). Thereby, captive breeding programs should aim to maintain behavioural variation in order to provide successful reintroduction when a large number of captive reared individuals are released into the wild (Merrick and Koprowski 2017). Reintroduction success at the individual level depends on how quickly the introduced individuals learn to e.g. find food and avoid predation, but in the captivity such behavioural skills are not necessities. The more generations the captive rearing has continued for, the greater the contemporary evolutionary shift in the behavioural traits can be between natural and captive reared populations (Merrick and Koprowski 2017).
‘Rewilding’ is originally an exact scientific term meaning connecting wilderness areas with corridors, but has been later adopted as a plastic word to refer to anything that aims to “make things wild again” (Jørgensen 2015). This plastic usage of the term ‘rewilding’ can benefit communication with stakeholders, as it can make the complex scientific discourse about the importance of local adaptations and hatchery-induced selection easier to understand. Hence, I adopt this wider usage of rewilding and use it here to refer to an assisted gene flow from the local wild to the hatchery stock in order to improve the evolvability of adfluvial brown trout population. This kind of assisted evolutionary rescue by admixing wild fish with hatchery broodstock aims to increase behavioural heterogeneity and survival of the juvenile fish that are used for enhancement stocking to support the local brown trout population. Hybridization between hatchery broodstock and wild population may release domesticated hatchery stock from its adaptive limits, when frequency of adaptive alleles increases (Carlson et al. 2014; Hamilton and Miller 2016).

Local adaptation is a genotype by environment pattern in which the genotypes get a fitness benefit in their local environment but potentially get a disadvantage in a foreign environment (Kawecki and Ebert 2004). Bringing the pre-existing adaptations from a genetically and environmentally close population into a hatchery population has been found more effective than an investment on the adaptive potential, i.e. maximising the genetic diversity (Houde et al. 2015). The individual behavioural differences can be key for a population to survive. Controlled crossbreeding of hatchery broodstocks with locally caught wild fish might provide a solution to reinforce also behavioural adaptations that are essential for survival in nature.

1.2 BEHAVIOUR OF HATCHERY-REARED FISH

Behaviour in general is plastic, and hatchery rearing induces changes in behavioural development through gene–environment interactions (Johnsson et al. 2014). Rearing under culture practices and conditions, including artificial breeding and simplified environment, differs drastically from the natural environment of salmonids, and affects the expression of multiple genes (Christie et al. 2016). When a natural life cycle is precluded, selection does not act to increase local adaptation but favours phenotypes that are the most fit in the hatchery environment. Adaptations to hatchery conditions result in domestication process through selective breeding, and impaired natural and sexual selection (Lorenzen et al. 2012). Even though hatchery
practises aim to conserve genetic diversity (Fraser 2008), the artificial environment and the lack of natural selection produces phenotypes that are less adapted to nature (Araki et al. 2008; Fraser et al. 2011; Christie et al. 2012; Kekäläinen et al. 2013).

Because the experienced environment is greatly different in captivity from what it is in the wild, a rapid change occurs in behaviour (Huntingford 2004). The divergence between hatchery and wild behavioural phenotypes may also arise due to differential survival of behavioural phenotypes within a single generation, and selection for inherited behavioural traits over generations (Huntingford 2004). The lack of mate choice and the aim to maximise the number of offspring, along with unintended selection for large individuals that they produce more and larger eggs, and to minimise the egg and fry mortality by providing stable, crowded predator-free conditions without strong competition for food resources, enable the appearance of “artificial” behavioural phenotypes. Offspring that survive in hatchery when food is unlimited can be very different from those that must compete for food in the wild (Glover et al. 2004). Thereby, if the assemblage of survivors is different in hatchery from what it would have been in natural environment, it may result in high mortality when hatchery reared fish are released into the wild (Huntingford 2004).

When released into the wild, hatchery-reared parr displace themselves downstream more likely than wild parr immediately after release (Jørgensen and Berg 1991). The larger the number of fish released simultaneously into the wild, the more they tend to disperse downstream (Brunsdon et al. 2017). Congruently, low stocking density has been linked to better survival (McMenemy 1995). As an adaptation to crowded rearing conditions, hatchery-bred fish may display impaired territorial (Fenderson and Carpenter 1971) and unnatural schooling behaviour (Ruzzante 1994) that potentially results in downstream dispersal. The cost of territorialism in high density may exceed the benefits, and hence reduce agonistic behaviour with a consequent survival cost (Bohlin et al. 2002).

After the release, hatchery-reared fish may display maladaptive activity patterns that cause fitness consequences in nature (Metcalfe et al. 1999). In the wild, brown trout, as other fishes, follow a circadian rhythm. Feeding rates are low at night when visibility is low and at midday when predation risk and light intensity are high (Hoar 1942). Circadian rhythmicity is an adaptation to environmental selection pressures driving salmonids to crepuscular foraging activity (Hoar 1942). In hatcheries, such rhythmicity might be lost due to constant foraging opportunities in the absence of predators.

Domestication has a negative impact on innate predator avoidance (Berejikian 1995). The loss of learned and intrinsic antipredator responses increases the
predation risk of hatchery-reared brown trout (Álvarez and Nicieza 2003). Hatchery rearing may even unintentionally favour high growth rates because fast growing individuals grow bigger in the hatchery conditions and hence produce more eggs than slow growing individuals within the cohort (Heath et al. 2003). Fast growth can be linked to high risk-taking that increases predation risk of domesticated fish in the wild (Biro et al. 2004).

Natural predation is not the only force that selects behavioural traits in the natural populations (Leclerc et al. 2017). Effectively, human harvesting exceeds natural predation in magnitude in harvested wild populations (Darimont et al. 2009) and may conflict with natural selection resulting in increase of unnatural phenotypes (Allendorf and Hard 2009). In fish populations, such unnatural phenotypes can face increased predation risk, and hence the total mortality may increase (Jørgensen and Holt 2013). Hook and line catching is the sole or dominant method of exploiting fish stocks in most freshwater habitats where salmonids are distributed (Almodovar and Nicola 2004; Cooke and Cowx 2004). Approximately one out of ten people participates in recreational fishing in Western world countries (Arlinghaus et al. 2015), and in Fennoscandia, the participation rate is estimated to be even higher, ranging from 1/4 to 1/3 of the people (Toivonen et al. 2000). The role of recreational fishing cannot be excluded as it mainly targets mature fish or fish near maturation, and often with the best reproduction potential (Cooke and Cowx 2004; Sutter et al. 2012). When catchability relies on the active role of the fish, i.e. as in passive gear fishing or catching with hooks, the individual behavioural type substantially determines the individual vulnerability, i.e. the probability to be captured (Klefoth et al. 2017; Lennox et al. 2017). Especially brown trout stocking in Finland largely benefits the recreational freshwater fisheries. Vulnerability to fishing varies naturally among individuals, but hatchery reared fish are found to be more vulnerable than wild fish (Mezzera and Largiader 2001). Especially, certain behavioural traits associated with hatchery selection such as high boldness (Biro and Post 2008) and high exploration (Härkönen et al. 2014) increase the individual’s risk of being captured by fishing. Domestication may increase overall vulnerability to fishing, but it is expected to depend on capture method: wild fish are likely easier to catch with natural baits, whilst domesticated fish are more vulnerable when artificial baits (Klefoth et al. 2013) or passive gear like gillnets (Biro and Post 2008) are being used. Bold or active behaviour of hatchery fish could be linked to higher energy demands to reach high growth rates, but they may also display impaired foraging behaviour on wild prey which drives them to feed on easy baits (Klefoth et al. 2013, 2017; Härkönen et al. 2014; Lennox et al. 2017).
1.3 ON STUDYING ADAPTIVE BEHAVIOURS AND ARTIFICIAL ENVIRONMENTS

Individuals can express different behavioural types that define the consistent individual behavioural responses over time and context (Mittelbach et al. 2014). When multiple individual behavioural traits correlate, they form a pattern called behavioural syndrome (Réale et al. 2007). Although the behaviour of individuals is very plastic over time and space, there are consistent differences in responses between individuals that may be adaptive, because between-individual behavioural differences affect survival in the wild (Moiron et al. 2019). Some individuals may be more plastic than others, which in stochastic or changing conditions may be advantageous, but stereotypic responses may be favoured under stable conditions e.g. in hatcheries (Niemelä et al. 2013; Mittelbach et al. 2014).

Rearing conditions in hatcheries are much simplified compared to natural environments, which favours stereotypic behaviours over plasticity, because there is no need for an ability to respond to changing conditions (Niemelä et al. 2013). Therefore, unintended hatchery selection directly narrows the range of behavioural variation (Johnsson et al. 2014), which may result for example in increased boldness (Sundström et al. 2004). Behaviour that is advantageous in the hatchery environment is less likely beneficial in the wild, but it is also questionable how well behavioural tests in a familiar and/or artificial environment predict individual behaviour, performance and survival in the wild after all (Niemelä and Dingemanse 2014). Whilst laboratory set-ups enable management of environmental factors, they may be insufficient to reveal the behavioural repertoire of an individual. On the other hand, field experiments lack control over variables, making it difficult to discover associations between behavioural responses and environmental and/or genetic factors. For a holistic view, it is necessary to combine laboratory, mesocosm and natural experiments possibly considering also temporal variation in individual behaviour (Johnsson and Näslund 2018). Previous studies in brown trout have shown that, to discover individual behavioural differences, it is necessary to take full diel cycles (Závorka et al. 2016) and test environment (Näslund et al. 2015) into account.
1.4 OULUJÄRVI BROWN TROUT

Oulujoki catchment is a 22,841.4 square kilometre area located in northern central Finland. The main lake, Oulujärvi (Fig. 1) discharges to Bothnian Bay via 106 km long Oulujoki river. Because of intensive damming (18 hydropower dams) and juvenile habitat loss in Oulujoki watershed, adfluvial and anadromous brown trout have virtually gone extinct in the wild and exist only because of stocking programs (Hurme 1969). Nowadays, the brown trout population of Lake Oulujärvi is a combination of migrating populations from tributaries Kongasjoki and Varisjoki which have been captured for hatchery rearing in the 1960s and last replenished with wild fish in 1970s. Based on the genetic similarity of these populations (Hyvärinen et al. 1996), they most likely originate from the same migrating population from Alanteenjoki river (Fig. 1) which dried out after Varisjoki river erupted in the mid-1800s due to human activities. Before the uncontrolled eruption of Varisjoki, water from Kivesjärvi discharged to Oulujärvi via Alanteenjoki river. Thus, the migrating trout spawning in Kongasjoki river began to use Varisjoki (Fig. 1) instead of Alanteenjoki on their smolt migration to Oulujärvi (Hurme 1969). This population was the last remaining migratory population within the watershed above Oulujärvi, and it has been reared in the hatchery from the 1970s (Salojärvi et al. 1981). Varisjoki and Kongasjoki broodstocks were pooled in 2000, and since then the formed broodstock has been reared in captivity (Hyvärinen et al. 1996). In total, 51 males and 51 females (1♀×1♂) were used to produce the first generation of the new Oulujärvi (further OUV) broodstock that is nowadays used to reintroduce adfluvial brown trout to Oulujärvi system. The broodstock can be considered as a “mixed type” stock in wild–domesticated continuum of Lorenzen et al. (2012), because it has maintained genetic diversity and reared only for conservation purposes, but suffers low survival in the wild probably due to domestication and consequent phenotypic mismatch with the river environment.

Vaarainjoki river is a small tributary that discharges to Lake Kivesjärvi (Fig. 1). There has been no fishing targeting salmonids after early 1990s, and it has a small but viable wild brown trout population that is genetically diverged from OUV broodstock (pairwise genetic difference between the populations $F_{ST} = 0.11$, (Lemopoulos et al. 2019a). The broodstock was established in 2010–2012, when mature adults ($n=144$) were captured during spawning by electrofishing, and since then reared in semi-natural hatchery pools. Vaarainjoki brown trout reach sexual maturity in the river at a small size (< 40 cm), and there is no evidence for lake
migrations, even though the occurrence of adfluvial brown trout in the population cannot be totally excluded.

Figure 1. Oulujärvi area and tributaries indicating original rivers of the OUV broodstock and Vaarainjoki population. Alanteenjoki (red) has dried out after the emergence of Varisjoki and no longer exists.

1.5 AIMs OF THE THESIS

My aim was to determine behavioural variation within hatchery-reared and wild brown trout and to study how controlled crossbreeding may benefit the hatchery broodstock to produce phenotypes that display more natural behavioural patterns. This is an attempt to save and rewild the only remaining migratory lake-dwelling brown trout population within the Oulujoki watershed that has been reared in
hatcheries over generations without the possibility of replenishing the broodstock with wild fish.

With a set of behavioural experiments, I have studied how the hatchery strain brown trout differ from the wild strain fish in acute post-release behaviour (I), which is a crucial period for survival. By recognising the essential adaptive behavioural traits, it may be possible to increase the frequency of such behavioural traits in the stocked fish by controlled assisted gene flow from the wild fish. I also study experimentally how behavioural differences between pure hatchery strain fish and crossbred fish may further cause survival differences (II). Since hatchery-rearing is formerly linked to increased vulnerability to fishing, I study how hatchery and wild strain juveniles differ in their response to parental angling selection (III). I seek a behavioural and ecologically relevant explanation for why the stocking of captive-reared fish may fail. My experiments focus on behaviours that essentially affect individual survival (Moiron et al. 2019) and especially during the early life survival bottleneck (Näslund et al. 2017).

By using multi-faceted approaches to test individual behaviour, we were able to draw an overall picture of phenotypic variation within and between hatchery and wild fish. Further, this will enable us to target domestication issues using controlled crossbreeding programs and possibly other solutions that may help to rewild hatchery stocks, e.g. environmental enrichment (see Karvonen et al. 2016).
2 MATERIALS AND METHODS

2.1 FISH MAINTENANCE AND REARING PROCEDURES

All the experiments and fish maintenance in this thesis took place in facilities of National Resources Institute of Finland’s Kainuu Fisheries Research Station (KFRS, Fig. 2, www.kfrs.fi) in Paltamo (64°16’34”N. 27°12’18”E). The station has facilities of the first water for aquatic ecological and management-related research in laboratory and in semi-natural experimental streams. The numerous varieties of standard hatchery and semi-natural rearing tanks enable breeding fish experimentally to target research questions in ecologically relevant quantities. The water in the fish tanks in the KFRS comes from the adjacent Lake Kivesjärvi (Fig. 1), and thus the temperature and oxygen levels vary naturally according to the ambient conditions. Light conditions are set to follow the natural circadian rhythm. Both, hatchery OUV broodstock and wild Vaarainjoki river (VAA) fish were maintained at KFRS for at least a year prior to the experimental breeding. OUV fish were kept in two 70 m² standard concrete rearing tanks and VAA fish in two 50 m² gravel-bottomed rearing tanks, divided in two size groups to avoid interspecific cannibalism and ensure right diet composition for differently sized fish.

Figure 2. Kainuu Fisheries Research Station from above. Varisjoki flows in the bottom right corner. Photo taken by Marco Blixt

For taking measurements and individual tagging, the fish were anaesthetised by using 40 mg l⁻¹ benzocaine solution. We tagged fish by following a procedure
where we first pierce a 2 mm incision on the ventral side posterior to the pelvic fin and then insert the PIT-tag gently into the body cavity.

### 2.1.1 Breeding design

The controlled captive breeding (in I and III) was based on replicated fully factorial 3 females × 3 males breeding matrix design to maximise the genetic diversity in F1-generation. Different cohorts were also mixed to avoid inbreeding within OUV hatchery strain.

To study transgenerational behavioural effects of parental vulnerability to angling (in III), we performed experimental fly fishing on OUV and VAA broodstocks. Angling trials were performed in two rounds: the initial sorting of fish to vulnerable (captured) and non-vulnerable (not captured) was performed between 4 June 2015 and 23 June 2015, and the once-captured hatchery fish were fished again between 6 July 2015 and 8 July 2015. Angling was performed directly in the 50 –75 m² rearing ponds by an experienced fly fisher (mostly A. Vainikka) using fly fishing. We used fly patterns that did not resemble any natural food items of brown trout to confirm that we imposed selection on vulnerability to angling with artificial lures instead of capacity to forage on natural food items. The angling times were not systematic due to practical constraints but represented well the usual times when brown trout fishing takes place in real recreational fisheries (8:13–21:38). In any angling session, an angler fished a pond until a fish took the fly or five minutes passed. If no fish were captured within five minutes, the angling was continued an hour later at the earliest.

We crossbred parent fish within vulnerability groups within strain to create selection lines based on their vulnerability to angling. Within hatchery highly vulnerable, HV and low vulnerable, LV and wild HV and LV groups, three males were crossed with three females in all combinations, and the matrices were replicated three times resulting in total 18 parent fish and 27 half-sub families in each four selection lines (OUV HV & LV and VAA HV & LV). The hybrid wild × hatchery strain in both male × female combinations was created by breeding two matrices with wild females and hatchery males, and two matrices with wild males and hatchery females. Parent fish were randomized a priori from the HV and LV fish shoals, but due to logistic constraints and maturity status of the randomized fish, brood fish were occasionally replaced with other randomly dip-netted fish.
From each family, we took a sample of 100 eggs for further rearing under standard hatchery conditions and observed egg mortality until hatching. The eggs were incubated until hatching in randomised floating incubation tubes (Ø=11cm, 100 egg/tube, one tube per half-sib family) placed in a 0.4 m² fiberglass tanks. Soon after hatching, we randomly dip-netted a target sample of 25 alevin from each family and pooled them to replicated half-sib groups to maximise genetic heterogeneity (n = 225 individuals). When 25 fish were not available from a specific family (problem especially with the replicate pool formed last), the missing fish we taken equally from all the other families with an excess number of alevin. The mixed half-sib family groups were reared in 20 dark green fiberglass hatchery tanks (0.4 m²) and fed *ad libitum* with commercial fish food until the behavioural tests in the summer 2016.

For the stocking experiment (I), angling selection lines were pooled within strains in order to simplify the study design and because the angling selection was not considered relevant in light of the study question.

In the second chapter (II), brown trout females (n = 5) from the OUV broodstock that represented 3th–4th generation hatchery fish were crossed with males (n = 5) from four different broodstocks: i) OUV (control), ii) VAA that represented a local wild brown trout population, and two distinct hatchery strains that were from iii) Rautalampi broodstock and iv) Kitkajärvi broodstock (Ågren *et al.* 2019) The Rautalampi watercourse broodstock (5th or 6th generation hatchery fish, RAU hereafter) had been commonly used for stocking across the watercourse despite it originating from central Finland. RAU brown trout represents a fast-growing strain that is originally an adfluvial population with an ancestral connection to the Baltic Sea. Genetic distance (FST) between OUV and RAU was 0.073 (M.-L. Koljonen and J. Koskenniemi, unpublished data 2016 based on 16 microsatellite markers). The Kitkajärvi broodstock is an adfluvial, upstream-migrating first-generation hatchery stock (KIT), originating from River Kitkajoki above the Jyrävä waterfall. The river system belongs to Oulankajoki-Koutajoki watercourse located in north-eastern Finland, and has an ancestral connection to the White Sea (Lemopoulos *et al.* 2017).

Nonlocal hatchery broodstock fish RAU and KIT were not reared in the KFRS, but the milt from males representing these strains was brought from another National Resources Institute of Finland’s hatchery in Taivalkoski. The artificial fertilizations were performed on 12 October 2011 at KFRS by producing 25 half-sib families per crossing group (OUV×OUV, OUV×VAA, OUV×RAU and OUV×KIT).
2.1.2 PIT-telemetry system and individual identification

Radio-frequency identification (RFID) methods enable tracking of individual movements without disturbance within the area that is equipped with suitable remote reader antennas. To identify the fish individually, we used 12 mm half-duplex passive integrated transponder tags (HDX RFID PIT tags, manufactured by Oregon RFID, or Texas Instruments Inc.). Half-duplex PIT-tags enable us not only to identify fish ID, but also to follow their movements in natural scale environments, because the RFID reader for reading them can be a fairly simple coil of insulated wire that can be placed for example under the water. When a tagged individual swims through an antenna loop, the reader generates short magnetic pulses that wirelessly charge a capacitor inside the HDX PIT-tag that is inserted inside the individual. The passive tag stores the power when it gets activated, and when the charge field turns off, the tag sends the tag ID code back to the reader (“HDX | Oregon RFID”). Since the charge field is pulsed, HDX readers require only little power, which enabled us to use the very high scan rate of 9 per second that minimises the risk of missed passes through the loop. We used a self-made stationary antenna system in the experimental streams or pools as described in Janhunen et al. (2011). The insulated copper wire coils were covered with a 320-mm PE-tube (in II), or with a garden hose (in I), and placed in the water (Figs 3 & 4) or simply coiled around the fibreglass channel (in I). Each antenna was connected to a tuning module which in turn was connected to a reader (or multiplexer system in indoors experiment in I) with a twin-axial cable. Readers were connected to computer(s) with RS-232 cable through 8 × RS port adapter. We used TIRIS datalogger programme (Citius Solutions Oy, Kajaani, Finland) to configure ASCII data to computers simultaneously from 4–8 antennas to each depending on the experiment. When an individual is moving through the antenna coil, the system creates a date-time-ID timestamp of detection from each antenna. In the beginning of the experiments, each antenna was tuned for a similar reading range (30–40 cm).

Antenna-specific ASCII-data were configured to 1-second resolution using software PIT-data (www.pitdata.net). Only a reading from a different location than the previous reading was considered as a movement (in I & II).
2.2 EXPERIMENTAL DESIGN

The set of experiments in this thesis was aimed at discovering the range of phenotypic behavioural variation within hatchery and wild fish. Crossbreeding experiments were performed to study how admixing of genotypes affect behaviour and survival in conditions that mimicked natural stocking context, practices and environment.

2.2.1 Stocking experiments (I)

In September 2016, trout were PIT-tagged and rearing was continued in two 3.2 m$^2$ standard rearing tanks where OUV, VAA and their crosses were pooled together. The fish density was 450 individuals per tank and fish were fed ad libitum with commercial dry feeds using automated feeders.

2.2.1.1 Behavioural experiment in artificial channels

To study individual behavioural reactions in a group context, we performed a small-scale behavioural test in artificial indoor flow-through channels (length 6 m, width 0.4 m, depth 0.2 m, flowrate 1.60 L s$^{-1}$) that aim to mimic headwater stream conditions. The channels had a thin layer of added gravel (appr. grain size 50mm) and larger shelter stones (ca. Ø=200 mm) in the bottom. In both ends of the channel, there was a metal grid (mesh Ø=5 mm). Each channel was equipped with four PIT-antenna coils connected to the multiplexer system covering half a metre each to track individual movements.

In each trial, we released 12 fish ($n_{\text{strain}} = 4$) to acclimate in a sub-section separated with metal grid (mesh Ø=5 mm) in the lower end of each channel ($n = 4$) for 48 h before releasing them to explore the whole channel freely for five days (120 h). Hybrids were equally taken from hatchery × wild sire—dam and dam—sire crosses but considered as one group in the further analyses. Similarly, we sampled fish from HV and LV selection lines within strains, but lines were further pooled within the strain. The fish were not fed during the acclimation or the trial but natural food coming with the inflowing water was present. Altogether, we ran five consecutive trial periods testing 240 individuals. We measured individual total antenna by-passes per hour and per day for five consecutive days.
2.2.1.2 Behavioural experiment in semi-natural streams

The second phase of the stocking experiment was performed in eight seminatural streams (Fig. 3) that presented a typical environment of brown trout parr. Previously tested fish were randomly divided into two density treatments (high = 48 fish/stream, and low = 12 fish/stream) in equal amounts from each strain. To minimize stress upon final release, the fish were fasted for one day before they were first released to the flow-through fish chests. After 14.5–18h acclimation time in the chests, they were released into the stream on the following day (29 June 2018).

Fish movements were monitored for five consecutive days. We measured individual antenna by-passes per hour and per day, as well as the direction of the movement.

Figure 3. A schematic diagram of the seminatural streams. The thin blue arrow indicates the water inlet, and the thick blue arrow indicates the outlet. Metal grid (mesh size Ø = 5mm) prevents fish from escaping the riffle section to the sink which is in the middle of the pool. Green loops indicate the locations of the RFID antennas. Tanks consisted of a stream and a pool sections, and gravity-driven flow in the outer circle was adjusted to 40.5 L/s, and water depth to 0.30 m. The circular stream section was 26.15 m long (from the middle) and 1.5 m wide. The bottom of the riffle section was covered with gravel (appr. grain size 50mm) and CMU blocks were placed as shelter stones. Each stream was equipped with four RFID-antenna
loops to follow fish movements. Tanks were covered with a tent canvas to prevent avian predation and direct sunlight to heat up the water.

2.2.2 Predator exposure experiment and electrofishing procedure (II)

2.2.2.1 Predator exposure

We used northern pike (*Esox lucius* Linnaeus, 1758) as a piscivorous predator in an exposure experiment in order to reveal potential survival and antipredator behaviour differences among crossbred brown trout juveniles. Pike is known to be a significant threat for juvenile salmonids and especially detrimental to survival of stocked juveniles (Nilsson and Bronmark 2000; Jepsen *et al.* 2000; Kekäläinen *et al.* 2008). The wild origin pike used in this experiment were captured from Oulujärvi, but they were kept in captivity over five years. In the captivity they were fed with freshly killed or frozen natural prey fish including smelt and roach in order to maintain their predatory behaviour.

On 6 June 2013, the trout were exposed to pike predation for 20 days in semi-natural ponds without PIT-system or 46 days in ponds with PIT-system. We haphazardly dip-netted 10 trout per crossing group (OUV, OUV×VAA, OUV×RAU and OUV×KIT), and assigned them to eight pike predation ponds without PIT-antennas (*n* = 40 fish/pond). From pure OUV group and from OUV×VAA group we haphazardly dip-netted 25 trout from each group to each four pike predation ponds with PIT-antennas.

Each pond had a predator free stream section with gravel bed structure (*A* = 10.9 m², width 1.5 m, Fig. 4, eight ponds without and four ponds with PIT-system) and a pool section with a presence of two pike in each pool. This set-up mimicked a continuum of riffles and pools in a natural stream. Shallow and flowing riffle sections are typically safer for juvenile brown trout providing shelter, but food is potentially more abundant in the pool sections of the tributary, where predation risk is usually also higher.

During the experiment, a drift from the water supply and macroinvertebrate fauna from the gravel bed structure enabled brown trout parr to exploit natural food sources. To meet the ethical criteria of animal experiments, the pike were fed with dead roach twice a week to ensure their wellbeing. Brown trout juveniles were
stocked to the predator-free area and were not forced to swim to the predation area. They were able to swim back to the refuge area, and a relatively large pool section enabled prey to hide and escape predation also within the predation risk area.

In the end of the experiment, the pike were removed and checked for PIT-tags in the stomach with PIT-reader without sacrificing them. The PIT-tags were collected from the bottom of the tank, and all PIT-tags found in the pike area were assumed to be evacuated by pike, i.e. to represent trout killed by pike. PIT-tags that were found in the stream area were assumed to represent naturally dead trout. There was a slight possibility that few uncontrolled losses of tags occurred due to avian or mammalian predation, most likely by European herring gull (Larus argentatus) and American mink (Neovison vison). Due to uncertainty of the fate of the disappeared fish, they were not included in the analysis.

Automatic observations of PIT-tag recordings were used to calculate the first time that the trout entered the predator section (exploration), the last time that the trout entered both of the sections, the total time that the trout spent in the pike section (~boldness/risk taking), and total movements between the sections (activity). The fish were assumed to remain in the section specified by the last antenna until a new observation was made on the other antenna.

Figure 4. A schematic diagram of the experimental seminatural flow-through pools that mimic natural parr habitat used in the pike predation experiment. The white area indicates the predator-free section, and the grey area indicates the pool section. The black arrow points
water faucet and flow direction; the sink was in the middle of the pool. Total area of the pond was 50 m² (radii: outer 4.0 m, inner 2.5 m) and the water supply was based on gravity flow. The depth of the stream section was adjusted to ca. 0.3 m, and that of the pool section to 1.0 m. Movements between the sections were allowed through a plastic half-pipe channel, where a plastic-coated metal grid (mesh size = 45 mm) prevented predators from swimming to the stream section (indicated with a honeycomb in the figure). At both ends of the channel, there was a PIT-antenna loop to record brown trout movements between the sections. Plywood structure and metal grid (10 mm) at the other end of the riffle prevented fish movements past the antenna channel.

2.2.2.2 Stocking in the Varisjoki

The remaining tagged fish were released in a rapid section (Hotellinkoski) of Varisjoki river on 20 June 2013 between 12:00–14:30. Another rapid section (Myllykoski), located ca. 2 km upstream from the release site and the dam in the outlet of Lake Kivesjärvi were searched for PIT-tags using portable reader on 16 July 2013 and the release site on 23 July 2013. Hotellkoski area was electrofished on 24 July 2013 and Myllykoski on the following day. The rapids in Varisjoki were electrofished again in September 2013. All the captured fish were measured for length (to 1 mm) and weight (to 0.1 g).

2.2.3 Open field personality tests (III)

We performed experimental fly fishing on hatchery (OUV) and wild (VAA) adult brown trout to divide them based on their relative vulnerability to fly fishing within the broodstock strains. OUV and VAA fish were fished directly from their rearing ponds i.e. in a familiar environment. The fish were fed during the days of the angling trials to ensure that the angling selected for individual vulnerability rather than individual hunger level. Fly fishing is a common and popular method in recreational brown trout fisheries. Whilst it requires some talent from the angler, the catchability relies on the premise that the fish makes an active decision to accept the bait and take the hook. Thereby, fly fishing, or other similar hook and line methods may be considered selective with regard to behaviour (Lennox et al. 2017).

Open field behavioural tests are widely used to assess the individual behavioural response in a novel or familiar, but usually unnatural environment (Niemelä and Dingemanse 2014; Johnsson and Näslund 2018). The natural environmental factors are usually excluded, so that the test would reveal among and within individual behavioural variation that is independent of environmental effects. Here, we used
open field test to study exploration and boldness of juvenile fish in a novel environment.

In the open field test, the study individual was placed one at a time in a start box of a self-made emergence test tank (Fig. 5), that consisted of a darker-walled starting box, separated from the arena with a remote lift door, and a larger test arena. After a 2.5-minute acclimation period, the lift door was remotely pulled up, and the test individual could emerge into the arena. We used a software (AVBS software by A. Vainikka) to record the acclimatisation period, the time to emerge out from the start box, recorded line crossings based on observer clicks, and freezing time, i.e. the time that an individual spent motionless for more than one second. The total duration of each test was 10 minutes including the acclimatisation and 7.5 minutes of active swimming time for the fish. To evaluate the short-term repeatability within groups, each individual was tested twice. Both trials were performed on the same day, with approx. 5 hours in between the trials to let fish to recover. Altogether, we tested 287 individuals by sampling nine individuals from each rearing tank. The fish were fed during the experiment period, but not on the day prior to sampling.

Figure 5. A schematic diagram of the open arena (355 × 740 × 200 mm) that was used to assess individual behaviour of juvenile brown trout. Two lines drawn on the bottom of the test arena allowed the visual evaluation of movements, i.e. line crossings. Two rocks were attached to the bottom of the arena as shelters, and a mirror covered the back end of the arena.
2.3 COMPLIANCE WITH ETHICAL STANDARDS

Fish maintenance and all experiments took place at Finnish Natural Resources Institute’s Kainuu Fisheries Research Station in Paltamo, Finland (www.kfrs.fi) under a licence obtained from the National Animal Experiment Board in Finland (licence number ESAVI/2458/04.10.03/2011 in I and ESAVI/3443/04.10.07/2015 in II & III), and in compliance with ‘Guidelines for the treatment of animals in behavioural research and teaching’ (2012).
3 RESULTS AND DISCUSSION

3.1 DIFFERENCES IN ACUTE POST-RELEASE BEHAVIOUR BETWEEN HATCHERY, WILD AND CROSSBRED JUVENILES (I)

The experiment showed that post-release movements are mainly directed downstream from original stocking site. This result aligns with the predictions from Jørgensen and Berg (1991), and Brunsdon et al. (2017). Hatchery strain parr dispersed faster and further downstream than other strains indicating that it will likely increase their predation risk in the wild. Interestingly, low density even intensified downstream movement of hatchery strain fish in the semi-natural streams.

Hatchery, wild and crossbred fish equally obtained bimodal circadian activity patterns, but the daytime activity was higher in the hatchery strain fish and peaked during afternoon hours. This suggests that hatchery fish may demand more energy to reach high growth rate (Metcalfe et al. 1998) but it also might increase vulnerability to fishing due to high activity rates during common fishing hours, as activity increases fishing mortality (Biro and Post 2008; Biro and Sampson 2015).

Potential stress from stocking and novel environment with running water can trigger downstream dispersal in released fish. Releasing, or translocation in general, is a major human-induced environmental change, and dispersal can be an avoidance reaction to the novel environment (Sih et al. 2011). This is important to consider when developing soft release methods.

This detailed empirical evidence of post-release behaviour improves our understanding of the low success of captive-reared fish in the wild. Whilst exploration may increase predation risk (Hulthén et al. 2017) and vulnerability to fishing (Härkönen et al. 2014), it can facilitate habituation (Adriaenssens and Johnsson 2013; McCormick et al. 2018). Stocked wild fish are shown to establish territories faster after release, whereas hatchery fish may show unnecessary aggressions towards conspecifics and have issues with finding suitable habitat (Deverill et al. 1999). Limited food resources in enclosures may force individuals to continue searching habitat downstream (Grant and Kramer 1990; Grant et al. 2017).

Previous studies show that highest mortality occurs shortly after release (Berg and Jørgensen 1991; Thorstad et al. 2011), which, align with our results, highlight the importance of acute behavioural responses after release. Such adaptive behavioural
traits should therefore be favoured in hatchery programs in order to improve stocking success.

3.2 DIFFERENCES IN BEHAVIOUR AND SURVIVAL BETWEEN HATCHERY STRAIN AND CROSSBRED STRAINS UNDER PREDATION (II)

Avoiding predation requires more than a capability to freeze or escape when predator is noticed. Antipredator behaviour refers also the ability to avoid areas and times when the predation risk is high. We found that hatchery strain fish (OUV) made more visits and spent more time in the pool area where predators were present than crosses with wild fish (OUV × VAA) (Fig. 6). Emerging time to the predation risk area correlated positively with the mortality but was not a strain dependent behavioural measure. In general, predation was size-selective within the wild crosses and favoured larger individuals, but within OUV hatchery fish there were no clear indications of selection for size (Fig. 7). Crossing with non-local RAU or KIT hatchery strains reduced the survival in the predation experiment either directly or due to negatively size-dependent mortality (Fig. 7 & 8). The greatest difference in survival was between OUV × VAA and OUV × RAU fish, the latter suffering high mortality rates (Fig. 8).

Figure 6. Total time spent and visits in the predation indicating risk taking behaviour in OUV and OUV×VAA juveniles.
Size-dependency in predation mortality could have arisen via at least two mechanisms. First, large individuals may have had a better ability to escape the pike predation attacks (Nilsson 1999). Second, as suggested by the movement data, large individuals may have favoured the stream section and probably pushed small individuals to the pool area through aggressive encounters.

As numerous previous studies on various taxa (e.g. Quinn et al. 2012; Niemelä et al. 2012; Alós et al. 2012; David et al. 2014; Härkönen et al. 2014) have shown, predation targeted exploratory phenotypes also in our study.

Although the OUV × VAA crosses were slightly smaller at the release than OUV fish (mean ± s.d., 120.8 ± 13.5 mm and 123.2 ± 13.3 mm respectively), we did not observe any difference in recapture rate, or size dependent recapture probability (total recapture probability 0.18) in the river. Interestingly the size difference disappeared during the summer in the river within the recaptured fish (OUV × VAA: 144.0 ± 13.3 mm, and OUV: 145.1 ± 12.0 mm), suggesting a potential compensatory growth of wild crosses in the wild.

![Figure 7](image)

**Figure 7.** Initial total length of survived (circles) and predated (triangles) fish in predation experiment.

Our results suggested that anti-predator behaviour is intrinsic, and as such wild fish may benefit hatchery strain fish if used in controlled crossbreeding programs.

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Despite the lack of strong survival difference between OUV and OUV × VAA fish, the differences in risk taking behaviour and area preferences may indicate potential divergence in predation avoidance further. These behavioural differences may indicate either better intrinsic predator avoidance (Berejikian 1995) or differential habitat preferences, since pike predation is naturally higher in pool sections than in rapidly running riffles in rivers (Jepsen et al. 2000; Kekäläinen et al. 2008). Crossing hatchery and wild fish might result in reduced growth rate in the hybrid fish, but our results suggest that crosses can reach equal growth rates in the wild at juvenile stages, and hence reduce the risk of size-dependent mortality.

![Figure 8. Estimated mean survival rates of juvenile brown trout in predation experiment.](image)

Any crossing between hatchery populations cannot be recommended in order to improve survival based on our experiment. Crossing with wild local fish instead could improve behaviours that further may reduce mortality in the wild (Biro et al. 2004). This supports the earlier evidence of the importance of the genetic background that non-local hatchery strain can have lower fitness compared to local hatchery-reared fish in the wild (Araki et al. 2008).
3.3 BEHAVIOURAL DIFFERENCES IN RESPONSE TO PARENTAL ANGLING SELECTION BETWEEN HATCHERY AND WILD STRAIN JUVENILES (III)

We found that fly fishing may increase timidity in wild but not in hatchery brown trout (Fig. 9). The clear behavioural difference between hatchery HV and LV fish strengthens the idea that angling can transgenerationally affect behavioural phenotypes, but not necessarily manifest in similar and unambiguous patterns as previously hypothesised (Mezzera and Largiader 2001; Alós et al. 2012; Arlinghaus et al. 2017). Our study, however, did not support the prediction that increased timidity would associate with decreased somatic growth (Biro et al. 2004; Stamps 2007; Arlinghaus et al. 2017). Crossbred juveniles displayed behavioural response intermediate to those of the hatchery and wild juveniles. OUV juveniles were longer than VAA juveniles, but there was no size difference between angling selection lines within strains.

The stress tolerance might determine vulnerability to angling (Koeck et al. 2018), which may explain the counterintuitive behavioural response within hatchery strain.

![Figure 9](image-url)
juveniles. Based on the continuum of proactive–reactive ‘coping styles’ (Koolhaas et al. 1999), the response to stressful situations may manifest in avoidance or escape behaviour. It might explain the short emerging times in hatchery LV juveniles rather indicating high sensitivity to stress than boldness (Laskowski et al. 2016). Thereby, reactive individuals that are resistant to stress likely display relaxed behaviours in novel situations (Conrad et al. 2011; Laskowski et al. 2016). Fish can lose territorial behaviour and foraging ability on live prey due to hatchery-induced selection resulting in non-linear relationship between hatchery-induced selection on behaviour and vulnerability to angling (Tsuboi et al. 2018). The angling might have selected the less domesticated fish, which could explain why hatchery LV offspring emerged faster than HV fish in open arena test. Thus, the angling might have targeted on shy and less-aggressive individuals (Wilson et al. 2011) that accept fly as food.

Our study adds to the increasing body of evidence that harvesting reduces phenotypic diversity of populations. Boldness is an ecologically relevant and indicative behaviour that may reflect individual fitness in the wild (Mittelbach et al. 2014; Ballew et al. 2017). Individually assessed behaviour in a novel arena test has been shown to associate with boldness or activity in the wild (Závorka et al. 2015; Laskowski et al. 2016) and indicate the recapture probability (Näslund et al. 2017). An increase in timidity due to angling selection in wild fish may further result in decreased catchability and many ecological or even ecosystem-level changes (Arlinghaus et al. 2017), as boldness and/or exploration tendency may associate with vulnerability to natural predation (Biro et al. 2004; Hulthén et al. 2017). The juvenile fish that were determined as bold and exploratory here may further be more vulnerable to angling (Härkönen et al. 2014), hence resulting in increased frequency of timid phenotypes (Arlinghaus et al. 2017; Klefoth et al. 2017). If individual vulnerability to fishing associates with other behavioural traits, these behaviours can form a behavioural syndrome at the population level (Conrad et al. 2011). The more fish are harvested based on their boldness, for example, the shyer the remaining fish should be and also more and more difficult to catch (Uusi-Heikkilä et al. 2008). When these remaining fish reproduce, it can result in increased timidity in the next generation, hence narrowing the behavioural variation within the population (Arlinghaus et al. 2017; Diaz Pauli and Sih 2017).

Yet, it is notable that angling was not found to affect freezing tendency within the wild fish, perhaps because it is a vital response against predators (Petersson and Järvi 2006). To study ecosystem level consequences of the timidity syndrome, there is a need for long term field experiments targeting fishing-induced selection and its
evolutionary consequences, e.g. how timidity associates with life-history traits (Andersen et al. 2017).
4 CONCLUSION AND FUTURE DIRECTIONS

A decrease in genetic variation reduces evolvability of the population, which may have drastic consequences due to fisheries-induced selection and consequently for fitness in changing environment. Brown trout, amongst other salmonids, is a keystone species in lotic ecosystems in the Northern hemisphere (Huusko et al. 2017). Thus, changes in the behaviour of brown trout might affect the whole ecosystem via predator–prey dynamics. Research focusing on behavioural conservation and its importance to successful rewilding of domesticated hatchery stocks is of the utmost importance.

With a set of comparative behavioural experiments conducted in this thesis, I show that hatchery-reared trout, wild ones and their crosses differ in their average behavioural types at parr stage, which is a typical life cycle stage for releases. According to my findings, stocked hatchery fish may be under strong natural and fisheries-induced selection immediately after the release due to high number of individuals displaying maladaptive behavioural types. High risk-taking and downstream dispersal tendency may be beneficial to obtain high growth rates, but also expose to high risk of natural mortality, i.e. predation. Even though the results did not reveal major differences in survival in a short predation experiment, behavioural difference between hatchery fish and wild crosses may indicate forthcoming divergence in mortality rates when released into the wild (Biro et al. 2006). In particular, my results suggest that while angling may result in a timidity syndrome in wild fish, hatchery fish may become more sensitive to stress that occurs as restlessness in behavioural tests. More research is needed to discover whether domestication causes inability to forage on live prey which may cause lower vulnerability to angling. Altogether, my results indicate that the genetic components induced by hatchery selection explain part of the phenotypic mismatch resulting in poor stocking success, and assisted gene flow from wild fish might improve the survival of stocked fish.

My results highlight the importance of genotype–environment interactions contributing to behavioural traits with fitness consequences under different ecological contexts. Mixing locally adapted wild fish in the broodstock could rapidly mitigate some of the behavioural effects of hatchery selection, but more research is needed to confirm the fitness and productivity effects in the wild. My results endorse the importance of source population in breeding programs that aim to support reintroductions and natural reproduction (Houde et al. 2015). While hatchery
broodstock may still be genetically divergent, they may display maladaptive behavioural traits in the wild due to domestication effects. Lemopoulos et al. (2019) have shown that OUV and VAA strains differ in their migration tactics, the latter showing high tendency for residency. The following step is to study how migration behaviour manifests in crossbred individuals in F₁ but also further in following generations, as the idea is to rewild the adfluvial brown trout population. Furthermore, continuity of crossbreeding studies is of the utmost importance, because only introgression within crossbreds and between them and hatchery strain fish will display the long-term effects of evolutionary rescue and whether it is needed to be carried out repeatedly. On the other hand, possible outbreeding depression may only be revealed in the following generations as well.

It is yet unknown, how quickly behavioural diversity might be restored in a wild population after multigenerational human-induced selection. Long term studies are essential in order to discover how contemporary human-induced evolution affects evolvability in fish populations, as standing variation enables populations to further adapt to the changing environment. Rewilding the last remaining adfluvial brown trout in Oulujoki watershed is a valuable opportunity to study how hybridization and introgression may conserve evolvability of the endangered population. This project can have an extensive applied value for future restoration projects by guiding how active management benefits the evolving fish stocks. It shows how assisted gene flow from the wild to the captive-reared individuals can mitigate the loss of adaptive genotypes, and potentially increase introduction success by increasing adaptive behavioural traits in the introduced individuals. For example, fisheries stakeholders in the Oulujoki watershed have already agreed to protect original brown trout populations and abandon the stocking of foreign trout stocks based on our study results (I–III, Lemopoulos et al. 2019a, b; Ågren et al. 2019). Only local stocks and developed rewilded stocks will be used in future stockings within the watershed. In tributaries where genetically diverged populations are capable of reproducing naturally and successfully, stockings are terminated.
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Wild migratory brown trout (*Salmo trutta*) is endangered, and stocking success of hatchery-reared fish decreases due to domestication. In this thesis, the focus is on behavioural conservation and rewilding of the hatchery-reared migratory brown trout. Admixing genes of locally adapted wild fish with the hatchery fish could increase the number of adaptive behavioural phenotypes. A rewilding project provides an example of how hybridization may conserve the evolvability of the population.