Evaluating innate and learned determinants for improving antipredator behaviour of stocked fish

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Academic dissertation

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Evaluating innate and learned determinants for improving antipredator behaviour of stocked fish

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This thesis is based on the following articles:


These are referred to by their Roman numerals.
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Introduction

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1. Summary and outline

The main interest in my PhD research has been in studying antipredator behaviour of salmonid fish young and to examine the possibilities for improving the predator avoidance responses of hatchery reared fish prior their release into the wild. For this, I studied several different salmonid species, namely atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), Arctic charr (*Salvelinus alpinus*), European grayling (*Thymallus thymallus*), and whitefish (*Coregonus* sp.). Since Arctic charr were found to express clearly recognisable innate antipredator responses in my experimental system and also responded well to the applied treatments, many of the investigations under interest were completed with this species. All experiments were carried out in Saimaa Fisheries Research and Aquaculture station in Eastern Finland.

I proceeded from investigating the sophistication of innate ability to recognise variety of predator chemical cues (I) into examining the effect of experience on the development of avoidance responses (II, III). When studying learning, I supplied fish with chemical cues of predators in conjunction with different types of stimuli. Predator odours were namely introduced to charr young together with alarm chemicals of consumed conspecifics in predators’ diet, or in conjunction with disturbance pheromones from startled fish. Chemical cues were also accompanied with predator-experienced fish to promote social learning, or charr had experienced predators in a real encounter with them. Such arrangements enabled acquisition of predator information via social learning from experienced conspecifics or through direct individual experience.

With data from subsequent behavioural observations using chemical stimulus only, I could show how prey responsiveness towards predator odours was affected by prior experience of the predators. Social learning and direct individual experience both improved predator odour avoidance (II) and single conditioning with chemical cues of charr-fed predators even improved prey survival from an encounter with live predators (III). Successive introduction of predator odours without a real threat of capture did not habituate fish to the predator cues but instead further improved prey survival (III). In these behavioural tests I used completely predator-naïve prey fish as the controls.

I detected considerable individual variation in the innate ability of young fish to recognise and respond to olfactory cues of predators (I). Inspired by that discovery, I continued studying whether individual differences in innate ability to recognize chemical cues were associated with the differences in survival at a subsequent encounter with predators (IV). This association appeared to be positive. Finally, I wanted to reveal possible factors behind innate individual variation in risk-taking. I studied whether the genetic variability (heterozygosity) of brown trout young would correlate with their tendency to behave
recklessly under predation threat \((V)\). The results of this chapter are the first to demonstrate the significant positive association between greater genetic variability and willingness to expose oneself to predation threat. As such they provide one probable explanation for the inter-individual variation seen in predator avoidance behaviour.

In this introduction I will first review the current state of freshwater fisheries and explain why conservation based hatchery practises are needed. Then I am going to explain in more detail the problematic conditions prevailing in hatchery environment and lighten up why behavioural investigations on hatchery-bred fish are of any interest. I will introduce the concept of ‘antipredator behaviour’ and explain some of the ecological and evolutionary aspects that the use of olfaction and chemical cues has in predator avoidance. The term ‘life-skill training’ is illustrated with an introduction of methods suggested to be used for improving the survival skills of captive animals before their release into the wild. I will then supplement the text with my personal findings; adding also some new aspects to the discussion of the results in the Chapter reviews. This introduction as a whole is meant to give the reader more comprehensive background information of antipredator behaviour in salmonid fishes and its linkage to the environmental conditions of hatchery rearing. This will be helpful in realising the contact points between the presented research papers and the current interests in behavioural ecology as well as the needs of conservation biology.

2. Conservation rearing

2.1. Reasons and problems

Wild fish populations in general are decreasing dramatically (Hutchings & Reynolds 2004, FAO 2004). Freshwater species suffer heavily from biotope destruction through eutrophication and human constructions, non-adaptive cross-breeding with introduced strains of non-endemic individuals and toxic waste discharges. As an example, the global catches of wild Atlantic salmon have fallen by more than 80 per cent between 1970 and 2000 of which, habitat degradation, over-fishing and a rapid decline in sea stocks are primarily to blame (Young 2001). As a common conservation operation, endangered or economically important populations and species have been taken under preserving captive rearing and artificially propagated to produce individuals for reintroduction purposes (Olney et al. 1994). Conservation rearing is usually a society run activity that may take place in zoos, but for fishes the environment is most commonly a fish hatchery. The rearing methods used are basically the same that are applied in food fish production, yet adjusted to bring-up less but more healthy fish in lower densities. Also the population backgrounds are more carefully paid attention to in order to conserve genetic diversity and variability. The reared species vary geographically, yet salmonid fishes are possibly the most important group of species with their combined fisheries and recreational value (Andrews & Kaufman 1994).

Although the principle goal of conservation based hatchery programs is to produce fish that are as similar as possible to their wild conspecifics; both genetic and developmental divergence still often exists between hatchery and wild fish. Although the captive stocks of salmonid fish are usually large, all individuals may descent from a very small founding population which means that strong genetic drift might have taken place already at the setoff of the stock (e.g., Dannewitz 2003). Genetic variability then continues to diminish in captive breeding through accelerated inbreeding and genetic drift (Wang & Ryman 2001, Dushesne & Bernatchez 2002). The supplementation of captive stocks with new wild-captured individuals should be helpful in diminishing the genetic drift and evolutionary differentiation. In principle only small amount of migration is needed to counteract genetic drift in a large population (Conner & Hartl 2004). Yet, since the founding populations of captive stocks are usually small, even continual introduction of wild animals into a captive stock may not be enough to compensate for the negative effects that supportive breeding has on the prerequisites needed for the life in the wild (Petersson et al. 2004).
Hatchery rearing typically promotes changes in phenotypic characteristics that are associated with reduced fitness and survival in natural habitat (e.g., Fleming et al. 1996, Reisenbichler & Rubin 1999, Johnsson et al. 2001). Accordingly, less than 5% of the billions of hatchery reared salmonids released into the wild every year survive to the adulthood (Brown & Day 2002). This survival is especially low considering the large size at which they are released.

Post-release predation has turned out to be a major source of the post-stocking mortality in fish (review by Brown & Day 2002). Since many of the predators are size selective, it is well realised that the larger the stocking size of fish, the better their survival from predation should be (e.g., Hyvärinen & Vehanen 2004). The trend has thus been to grow and release larger individuals; fish of one to three years old. This is, however, economically unprofitable since the period of rapid growth then takes place in hatchery instead of nature. The ecological disadvantage of this practise is that the period of life when fish are supposed to come accustomed to e.g., feeding and predator avoidance in the wild, is now spent in hatchery. Despite the increasing number of stocked fish the proportion of fish surviving however keeps decreasing (Brown & Day 2002). This has made the captive breeding for stock enhancement purposes less effective for freshwater fish, than for other animal groups (Collares-Pereira & Cowx 2004). More generally, the whole suitability of captive-bred animals for release has been much under critical consideration (review by Maynard et al. 1995; Waples 1999; Mathews et al. 2005).

**2.2.1 Hatchery selection**

"Since the time of Darwin, it has been widely conceded that the heavy loss of young animals and plants is largely beneficial to the species". (Wales 1954)

For highly fecund species such as salmonid fish, the natural survival rate of juveniles in the wild is very low. Generally, ca. 10% of salmonid fish larvae survives their first year in the wild; on the other hand, in hatchery the figure can be even 90%. Survival in captivity has to be kept notably higher than in the wild for simply making the management profitable. Because the mortalities are so different in artificial versus natural environments, negative effects are bound to arise within the captive stocks (Reisenbichler & Rubin 1999, Ford 2002). Although hatcheries differ by their characteristics there are similar elements in selection pressures that are often referred to as ‘hatchery selection’. For instance, when hatchery born fish are used as parents in artificial propagation, there is no natural selection for antipredator behaviour, or other fitness characters among the individuals that get to reproduce (Berejikian 1995, Johnsson et al. 1996). Matrix crossing can typically be used for maximising genetic variability of the reproducing captive stock. The non-existence of mate choice among breeding individuals, however, is thought to have accumulative effects on fitness; yet not much is known about this. For fish born in a hatchery, the selection pressures can be very different to nature, even selecting for individuals that would be selected against in the wild. Hatchery selection for survival skills needed in the wild may thus be either relaxed or directed (Endler 1986, Price 1999).

Not surprisingly, the selection in hatcheries has been shown to reduce fitness and survival of fish after their release to the wild (e.g., Fleming et al. 1996, Olla et al. 1998, Kostow 2004). Behaviour has been shown to be one of the first traits that can get altered by hatchery selection (e.g., Sundström et al. 2004); even from the first hatchery generation onward (Kostow 2004, Hirvonen et al. unpub. results). Consequently it has been argued whether the usefulness of the captive stocks to conservation purposes might actually be limited to only first few captive generations (Ford 2002). Important inference can be drawn also from the study of Koskinen et al. (2002) demonstrating how the evolution of fish life-history traits can be considerably faster than the evolution of neutral markers in the genome. Selection in hatcheries could thus change the properties of captive fish long before
genetic divergence to wild fish by genetic drift becomes evident. Possibly the most striking demonstration of how fast direct selection can create an evolutionary change in animal behaviour comes from the studies of D. Belyaev showing the enormously rapid domestication of silver fox (*Vulpes vulpes*) in captivity (Trut 2001).

One of the main dissimilarities between the wild and captive environments is the total lack of predators in the hatchery. The absence of predator induced selection has been reported to cause reduced ability of fish to respond to cues from their natural predators (Metcalfe 1990, Olla *et al.* 1994, Johnsson *et al.* 1996). Also other fitness-related changes such as increased aggressiveness may appear under relaxed predation pressure (Wright & Huntingford 1993, Wang & White 1994, Sundström *et al.* 2004). A common phenomenon under hatchery selection is the intentional or unintentional acceleration of growth rate in salmonid fish. Hatchery selection may thus be also human driven for instance in aiming at producing larger smolts at younger age. However, this protocol has in particular proven to drive hatchery-reared fish phenotypically divergent from wild fish by the time of the release (Kostow 2004). Also, e.g., Johnsson (1993) showed how the selection for large body size under relaxed predation pressure increased the tendency of juvenile rainbow trout to forage under threat of predation.

### 2.2.2 Developmental discrepancy

The important life-skill of avoiding predators not only gets dampened over generations of captive selection (Sundström *et al.* 2004), but also fails to develop normally since animals lack all early-life experiences of predators. A vast majority of captive fishes are released without any prior experience of their natural predators (Olla & Davis 1989, Olla *et al.* 1998). The lack of experience together with the phenotypic divergence to wild conspecifics may lead to rapid post-stocking mortality at the release site (e.g., Howell 1994, Olla *et al.* 1994, 1998). The relative effects of prior selection and the unnatural growth environment may be hard to tell apart but it is clear that the predator avoidance skills of fish can improve by experience (e.g., Järvi & Uglem 1993, Chivers & Smith 1994, Berejikian 1995, Mirza & Chivers, 2000). Salmonid young can learn to avoid predators very rapidly, already after witnessing few attacks on conspecifics (Olla & Davis 1989, Berejikian 1995, II), or even through a single introduction of predator odours and alarm cues from conspecifics (Berejikian *et al.* 1999, III). In nature, the innate ability of prey to avoid predators gets boosted by the inevitable encounters with predators (Kieffer & Colgan 1992, Fuiman & Magurran 1994, Kelley & Magurran 2003). After surviving the first encounter prey should already have substantially better changes to escape the next attack.

Interestingly, the human disturbance that nearly all captive animals are exposed to, can sometimes be viewed as repeated predation-free predator stimulus that could also lower animals’ response threshold to sudden interferences (Beale & Monaghan 2004). Furthermore, chronic and high elevation of stress hormones following environmental disturbances such as human presence have shown to lead to poor learning abilities with laboratory animals (Mendl *et al.* 2004). On the contrary, domestication process has been shown to attenuate the stress responses of fish through alterations in brain neurotransmission (Lepage *et al.* 2000).

### 2.3. Stocking of fish

“It would be better economy to kill a large number of fry at start rather than spend money feeding them, rearing them, ... and then turning them loose in a stream for nature to eliminate.”

(Miller 1954)

#### 2.3.1. Success of reintroductions

If stocking fish from a captive population is aimed at supporting the natural reproduction in the wild population, the survival of released fish to reproductive age should be imperative. Even when releases of fish simply intend to support the commercial fisheries (e.g., sea-
ranching of Atlantic salmon), the immediate post-release survival of fish should like-wise be essential. Mortality rates, however, can be high after release, often due to heavy predation (e.g., Miller 1954, Wales 1954, Howell 1994, Shively et al. 1996, Olla et al. 1994, 1998, Jepsen et al. 1998, Blaxter 2000, Svasand et al. 2000, Brown & Day 2002). The poor survival is commonly thought to result from captive animals’ impaired behavioural skills (e.g., Sutherland 1998, Price 1999, Knight 2001, Mathews et al. 2005). Yet, this is usually compensated simply by releasing more fish. In addition to life-skill training in hatchery, even rather simple ‘soft-release’ procedures such as the use of enclosures (e.g., Jonsson et al. 1999) could presumably improve the post-release survival by letting fish to adjust to the new environment (reviewed by Brown & Day 2002); yet they are rarely been used.

2.3.2 Interactions with wild fish

In addition to the effects that the captive rearing has on viability of the released individuals, also the interactions with the native population of the release area have to be acknowledged. There is a good chance that the released fish will compete with the native fish as well as get to interbreed with them. The fact whether the released fish still maintain adequate genetic analogy with the original wild population should thus be of major relevance. Hatchery salmonids have repeatedly been demonstrated to be more aggressive than their wild conspecifics (e.g. Metcalfe 1986, Swain & Riddell 1990, Mesa 1991, Deverill 1999). Thus the fact whether they are able to out-compete wild fish with normal growth rates has created concern (Johnsson & Bjornsson 2001, Reinhardt et al. 2001). However, direct observations of competition between hatchery and wild fish, or proper tests of their competitive abilities are as yet rare (review by Weber & Fausch 2003).

If the original genetic make-up is distorted or the released fish are not indigenous to the release site it can be harmful for the wild population when introduced and wild strains get to interbreed (Gross 1998, Youngson & Verspoor 1998). Supplementation releases of hatchery-reared juveniles can for instance generate significant changes in the life history traits of naturally spawning populations (Unwin & Glove 1997) and reduction in natural reproduction (Nickelson et al. 1986). When hatchery and wild fish have genetic differences in aggressive behaviour (Swain & Riddell 1990; Einum & Fleming 1997) there is danger that also the progeny of the hatchery fish continues to compete with fry of the native strain. There is, however, also evidence for reduced reproductive success of hatchery reared fish breeding in the wild (Chilcote et al. 1986, Leider et al. 1990, Kostow et al. 2003), probably due to low egg-to-smolt survival (Kostow 2004). This may reflect relaxed selection pressures in hatcheries (Lynch & O’Hely 2001).

3. Antipredator behaviour

3.1. Selection of defences

Predation is a strong selective force since it can have the most dramatic effect on the fitness of an animal. In order to defend themselves from predators, prey have evolved numerous adaptations; allowing them to reduce the probability of being detected, to escape, to repulse the predators, or to signal repulsiveness (Fuiman & Magurran 1994, Kats & Dill 1998). These adaptations may be morphological (e.g., armour, spines, and claws), physiological (e.g., distastefulness, toxins, and electric organs), life historical (e.g., earlier or delayed hatching or maturation and emergence) or behavioural (e.g., biting, immobility, aggregation, and changes in feeding) (Brodie et al. 1991, Smith 1997, Kats & Dill 1998, Kusch & Chivers 2004). The most abundant groups of fishes (e.g., salmonids and clupeids) lack obvious morphological defences against large predators (Smith 1997). Their defences are largely behavioural, and consist of recognising potential predators and avoiding encounters with them (predator avoidance behaviour, Brodie et al. 1991); as well as increasing the probability of survival when perceived by a predator (antipredator behaviour, Brodie et al. 1991). Quite frequently the term
‘antipredator behaviour’ is used more broadly to describe all behavioural means prey uses for lowering the risk of getting caught. This is how it has been used for most of this introduction as well.

Prey may respond behaviourally to increased predator pressure for instance by moving away from the predator’s expected foraging grounds (Greenberg 1994, Wisenden et al. 1995), by schooling (Magurran 1990a, Pitcher & Parrish 1993), by reducing their activity (‘freezing’, Järvi & Uglem 1993, Smith 1997, Brown & Smith 1998), and by taking cover (Rehnberg & Schreck 1987, Kats & Dill 1998). In addition, ‘predator inspection’ may be used for gathering information of the predator’s motivation (Pitcher 1992) and ‘startle responses’ (i.e., ‘skitters’ Krause 1993) may be used for lowering the probability of capture.

### 3.2. Population differences

Avoiding predators entails costs. Change of habitat or reduced activity to avoid predators can mean reduced opportunities to feed and mate (Lima & Dill 1990, Magurran 1999). Predator avoidance can also affect diet composition and be sub-optimal for growth as shown for instance in lake trout fry (*Salvelinus namaycush*) (McDonald et al. 1992). In nature predators soon select reckless fish that feed regardless of hazard and specifically the risk balancers should succeed best (Pitcher & Parrish 1993). The predation pressure at each locality is thus reflected in the strength of intrinsic antipredator responsiveness of its prey fish. Experimental manipulations have furthermore confirmed that population variation of antipredator behaviour may arise as a consequence of selection exerted by predators (Endler 1986, Reznick et al. 1990, Magurran 1999). There is indeed evidence that the strength of predator avoidance has a genetic component (e.g. Giles 1984, Magurran 1990, Magurran & Seghers 1990a,b). Good examples of variation in antipredator behaviour between populations have been documented e.g., from arthropods (Riechert & Hedrick 1990), amphibians (Storfer & Sih 1998), reptiles (Brodie 1989), and freshwater fishes (e.g., Bell & Foster 1994, Magurran 1999, Vilhunen 2000).

### 3.3. Chemical cues

Chemical cues and their importance in predator avoidance have received great interest for quite some time (Schutz 1956, Pfeiffer 1962, Hara 1986), and the evidence is still vastly growing (e.g., Brown 2003, Darwish et al. 2005, Schoepffner & Relyea 2005, I-V). Chemosensory mediated antipredator behaviours seem to be common in every prey organism group (review by Kats & Dill 1998). The chemical senses of fishes are particularly highly developed and can mediate various behaviours (Hara 1986, Brown 2003). Individual’s ability to recognise and respond to different chemical cues can for instance affect its growth, survival and reproductive success (Liley 1982). Recognition of olfactory cues in salmonids can influence their prey selection (Bres 1989) as well as mate recognition (Brown & Brown 1992, Brown & Brown 1993a,b). Furthermore, juvenile salmon can use chemical cues for differentiating between their kin and non-kin in competitive interactions and so determine how aggressive they are towards each other (Griffiths & Armstrong 2000). Similar capabilities for sibling recognition have been found in Arctic char (Olsen et al. 1998).

Generally, chemically induced or mediated adaptations are especially important for fish living in turbid water where the visual perception of predators is low (Kats & Dill 1998). Truly remarkable sensitivity has, however, developed also to salmonid fish that live mostly in clear waters. The most astonishing example of olfactory recognition comes in the form of salmon migrations from sea back to their natal streams (homing phenomenon, Hara 1986). Odour possessing molecules and pheromones can have extremely low molecular weight and may travel in water for considerable distances. Chemical cues of predators (i.e., ‘kairomones’, Schoepffner & Relyea 2005) mediate many induced defences and can be perceived in very small concentrations by prey (Smith 1992). Consequently they allow early detection of predators which is the
key factor in successful avoidance for many prey (Kats & Dill 1998). Fish young may also discriminate between concentrations of predator odour (Mirza & Chiwers 2003a) and can respond threat-sensitively to changes in it (Ferrari et al. in press).

Chemical cues of predators may function as releasing stimuli that trigger innate ability for antipredator behaviour, without actual learning taking place (Suboski 1988, Suboski & Templeton 1989). ‘Alarm cues’ are components of either injured prey that get into the water via rupture of specialized dermal club cells (Pfeiffer 1977), or pheromones that prey may secrete under increased threat (i.e., ‘disturbance signals’, Brown 2003). These chemical signals can enable an alarm response (i.e., ‘Schreckstoff’, Pfeiffer 1963) to develop also towards previously neutral stimulus (‘releaser-induced recognition learning’, Suboski 1990, 1992, Chiwers & Smith 1994). Some prey species have thus been found to react also to cues from non-predatory species (Keefe 1992), but only after conditioning with alarm cues.

3.4. Individual variation in antipredator behaviour

It is well-acknowledged that animals within a species or a population can differ for their behavioural phenotype, yet its importance in fish behaviour has received wider attention only recently (Wilson et al. 1993, Coleman & Wilson 1998, Fuiman & Cowan 2003, Bell 2005) (but see also Huntingford 1976a, b). Of the highly fecund fish species only a notably small minority of individuals are known to survive the larval period (Bailey & Houde 1989) with predation as the main selective agent. It also appears that even small differences between individuals’ characters may have considerable input to their survival probability (Crowder et al. 1992, Heath & Gallego 1997, Fuiman & Cowan 2003). The recent view is that such behavioural differences among individuals may be relatively fixed and reflect ‘behavioural identities’ that derive from different behavioural traits being inherently correlated within individuals (Drent et al. 2003, Dall et al. 2004).

Behavioural variation can be quantitative such as inter-individual variability in recovery times from a predator attack found in three-spine sticklebacks (Huntingford et al. 1994). Qualitative variation can again arise from different types of behavioural strategies adopted by individuals (Noakes 1993). This has sometimes been interpreted as adaptive heterogeneity that could restrain predators of foreseeing the prey’s next move (e.g., Tulley & Huntingford 1987). There can also simply be differences in individuals’ perception skills that can create variation in behaviour (Kusch et al. 2004). As mentioned earlier, rearing animals over generations in captivity can lower their responsiveness towards their natural predators (Metcalfe 1990, Olla et al. 1994, Johnsson et al. 1996). There are now more precise indications that captive breeding could generate changes in the frequencies of individuals that are either responsive or non-responsive to a threat (McPhee 2003). So, instead of behavioural variation left unreported, it would be interesting to see authors focus more on pinpointing the extent of individual variation observed in fitness-related behavioural characters within wild and captive populations. Recently it has been demonstrated that salmonid fishes can have substantial variation in life-skills also among different families (Dannewitz 2003, Laakkonen & Hirvonen submitted) which naturally should account for some of the variation observed between individuals in a random sample.

4. Antipredator training

4.1. The history

Thompson (1966) demonstrated in his PhD research how antipredator conditioning can improve the post-release survival of hatchery bred salmon. The studies of Suboski & Templeton (1989) and Olla & Davis (1989) were the first to break the news to a wider audience, suggesting that hatchery reared fish could be ‘trained’ in predator avoidance behaviour to improve their survival skills. To date there are already several

4.2. Different methods

Since fish in hatcheries lack all experiences of predators, introduction of predator stimuli can aim at triggering the existing innate antipredator responses of prey before their release into the wild. As predators are then encountered in the wild, innate avoidance responses are already set to work from the previous experience given to them prior to the release. As captive rearing can entail lowered ability to recognize and respond to natural predators, classic conditioning may be used to compensate it. Conditioning is a straightforward method where subjects are presented with a conditional factor (i.e., predator odour) that they get conditioned to by a simultaneous introduction of an unconditional stimulus. Such unconditional stimulus is typically an alarm cue from consumed, damaged, or disturbed prey (e.g., Suboski 1988, Brown & Smith 1998, III). Other unconditional stimuli have included anything from short electric shocks to predator attacks and alarming behaviour of conspecifics which can produce social learning (II). Even a simple exposure to caged predators may be able to improve the survival of salmonids (Maynard et al. 2001).

Overall, antipredator training ought to improve the immediate post-release survival of individuals as compared to an unfortunate scheme where most of the individuals are soon caught independent of their underlying biological differences. After surviving the critical first few days, other life-skill training methods such as introduction of live prey prior to release could help fish to get accustomed to wild habitat more rapidly.

4.3. Social learning

It has been shown that fishes are able to pass on predator information to each other (‘social learning’ reviewed by Kelley & Magurran 2003). Making use of this ability has been suggested as a possible mean to enable ‘en masse’ training of large units of captive fish (reviewed by Brown & Laland 2001). The great advantage of utilising social learning in antipredator training would be that only a smaller proportion of the stock would need to be trained with a more direct exposure to predators and these experienced fish could then be used in passing on the information to larger groups of predator-naïve conspecifics. Although the social transmission of predator information can succeed in captive environment (e.g., Suboski 1988, Suboski & Templeton 1989, Mirza & Chivers 2000, Kelley et al. 2003, Griffin & Bennett 2005, Ferrari et al. in press, II), the expected survival advantage to socially learned fish is yet very poorly examined. It has been suggested that if we supply life-skill training for some of the fish in the hatchery, social learning among the trained and naïve individuals could also continue to take place after their release into the wild. Predator-naïve fish have, at least, been shown to survive better in the company of predator-experienced individuals (Patten 1977, Sugita 1980).

4.4. Habitat enrichment

Habitat enrichment can refer to all improvements of captive environment that make it less artificial by provision of some of the key elements that natural environment possess but that are usually missing from the captivity. Introduction of predator cues can thus be viewed as one type of ‘habitat enrichment’ method as it provides fish with natural cues of their predators that are lacking from hatchery environment (e.g., Berejikian et al. 1999). Another major sphere of life-skills training in fish is the attempt to improve foraging skills in hatchery (e.g., Stradmeyer & Thorpe 1987, Maynard et al. 1996b, Brown et al. 2003). The provision of individuals with experience of catching live prey is thus another step forward
in trying to enrich the captive habitat with wild-like elements. Inability to feed on natural prey may lead to starvation after stocking (Erbsb & Haase 1983).

A variety of non-targeted behavioural modifications can arise simply from altering the physical aspects of the habitat. Examples include lowering the rearing density, increasing the physical complexity of the rearing vessels, and altering the water flow. Enriched rearing environments have already been shown to improve foraging behaviour (Brown et al. 2003) and the post-release survival of hatchery fish (e.g., Maynard et al. 1996a, Ward & Hilwig 2004). The ability of fish to take cover, flee, hide using cryptic substrates, perceive predator cues, recognise and catch natural food, and to practise social interactions in natural scale are all spheres under particular interest. More general goals have been improvements in perception skills and learning ability. Most likely due to a structurally extremely simple, stimulus poor environment, hatchery reared fish have been shown to suffer from abnormal brain development (Marchetti & Nevitt 2003). Exposure to enriched environment has, however, been shown to have several positive effects on brain structure and function with laboratory rodents (Naka et al. 2002), which for instance improve spatial learning (Lee et al. 2003, and references therein).

5. Chapter reviews

All data in the common garden experiments were attained by direct observations of fish behaviour in aquaria or two-choice maze.

I Innate anti-predator responses of Arctic charr (Salvelinus alpinus) depend on predator species and their diet

I measured the innate antipredator responses of predator-naive Arctic charr young in response to chemical cues of either large brown trout or pikeperch (Sander lucioperca) that were either food-deprived or had been fed charr or grayling. The behaviour of the test fish receiving the predator odours I then compared to the behaviour of control fish that was recorded in the absence of the predator odours.

Chapter I demonstrates how the recognition of predator odours (kairomones) by prey fish can have a strong and sophisticated innate element to it. The results suggest that innate responses of prey fish to chemical cues of predators can show discrimination between predator species as well as predator individuals with different diets (see also Keefe 1992). The strength of avoidance responses even shows risk-sensibility since predator diet of test fish conspecifics was responded strongest. This is in contrary to somewhat prevalent suggestions that chemical recognition of predators would need experience to develop unlike the recognition of visual cues (e.g., Chivers & Smith 1994, Chivers et al. 1995, Mathis et al. 1996, Brown & Smith 1998, Mirza & Chivers 2000, Kelley & Magurran 2003). However, also results supporting the present findings do exist (Hirvonen et al. 2000, Berejikian et al. 2003, Hawkins et al. 2004, Darwish et al. 2005).

Of the two different predators used in here, the chemical cues from large brown trout were more strongly responded to than the odours emitted from pikeperch. It could be presumed that brown trout generally inhabit more similar environment with charr in Lake Saimaa than pikeperch do. Since brown trout and pikeperch are both actively cruising foragers, it may nevertheless be expected that charr has a history of encountering cues of the both predator species. Accordingly, also the kairomones of pikeperch alone elicited innate freezing response in some of the tested charr as compared to control fish that showed no signs of freezing. If a predator species feeds consistently on the individuals of the prey population in the wild, it is advantageous to avoid all predators of that kind (Lima & Dill 1990, Lima 1998). However, when predation pressure is uncertain the benefits of high odour discrimination ability can be used to detect whether the current dietary habits from its faeces. This helps in avoiding unnecessary avoidance actions (Chivers & Mirza 2001b) that can reduce opportunities for to feed and mate (Lima 1998). Lake Saimaa is rich in predator and prey species, and the conspecific
alarm cues in the predators’ faeces should elicit strongest avoidance responses in prey fish. Saimaa Arctic charr young behaved in line with this hypothesis and responded most strongly to odours of predators with a charr diet, as compared to predators with heterospecific prey diet, or predators having no current fish diet.

Chapter I furthermore reports of individual variation in antipredator responsiveness which has already been treated earlier in this introduction. Some of the variation in the level of antipredator responses seems to be due to complete inability of some of the individuals to recognize or respond to the encountered cue. However, the lack of a behavioural response may not mean always inability to recognize the cue (Rehnberg & Schreck 1987, Brown et al. 2001). Namely, individuals can differ by their response threshold so that e.g., predator odour concentration that is needed to launch avoidance response could vary between individuals (Mirza & Chivers 2003, Kusch et al. 2004).

The research on the odour recognition abilities has already advanced further from my findings in I, now able to demonstrate in reptiles how genetic relatedness of the recipient prey with the remains of an individual in the predator’s faeces can affect the strength of the avoidance response (Sullivan et al. 2005). In theory, some of the individual variation seen in behavioural response to a conspecific odour in the predator diet could thus be due to differences in relatedness between the prey individuals. In studying chemically induced defences it has been most common to use alarm cues of crushed prey (reviewed by Chivers & Smith 1998) instead of the prey whole-body content in predators’ faeces that also include the kairomones (i.e., predator odours). The results of Schoepnner & Relyea (2005) now interestingly suggest that the reliability of the threat that the cues from naturally consumed conspecifics signal to a recipient prey could be fundamentally different from the cues of artificially crushed individuals. Providing this is the case, the reliance to these different types of chemical cues may indeed have evolved differently which could explain the observations that report a lack of innate response to chemical cues of crushed prey.

II Less is more: social learning of predator recognition requires a low demonstrator to observer ratio in Arctic charr (Salvelinus alpinus)

Predator-experienced charr were formed by facing the fish with live predators. These fish were used as demonstrators (d) for predator-naïve observers fish (o) in three different ratios (4d:16o, 10d:10o, 16d:4o). To these mixed shoals of experienced and naïve fish I then introduced chemical cues of unfed predators. The observer fish were expected to get conditioned to the introduced predator odour by observing the behavioural response of the predator-experienced demonstrators. Whether social learning occurred was verified by comparing the behaviour of the observer fish from the mixed shoals to the behaviour of still predator-naïve fish when these groups were again later introduced the same predator odours.

Recently it has been suggested that the cognitive abilities of fish are well beyond of what has been the prevailing supposition (Bshary et al. 2002, Brown 2004). Fish have been reported of being able to learn quickly from experiences, use tools, and have sophisticated social structures, and impressive long-term memories (Brown & Laland 2003, Brown 2004, Griffin 2004). Social learning (of foraging, antipredator behaviour, orientation, etc.) has had an unofficial ‘hotspot’ status in behavioural ecology (e.g., Bshary et al. 2002, Kelley et al. 2003, Brown 2004, Griffin & Bennett 2005). Social learning has also been suggested as a promising candidate to enable cost-effective life-skill training in hatchery raised fish prior their release (Brown & Laland 2001, Brown & Day 2002). Yet, some of the basic requisites of a successful information transfer have still remained untested. For instance the effect of the demonstrator to observer ratio on the efficiency of the learning has hardly even been acknowledged in the existing literature.

Actually, chapter II came up with surprising, yet convincing results: It showed how social acquisition of predator information among naïve observer fish can produce equally strong later
avoidance of predator cues as the direct individual experience of real predation. However, social learning was successful only in shoals where the predator-experienced demonstrator fish (d) were in minority (4d:16o). The observer fish in other treatment groups with larger proportion of demonstrators (10d:10o, 16d:4o) did not succeed in social learning of predator recognition.

Previous studies have suggested that social learning would instead be promoted by higher number of demonstrators (e.g., Sugita 1980, Lachlan et al. 1998, Brown & Warburton 1999). Yet, unlike in the present experiment, also the total group size was enlarged when increasing the demonstrator number. This might explain their results of better learning since increase of group size can in itself promote learning (see II). Since the behaviour of the demonstrator and observer fish could not be observed in the present experiment while they were together, the details of information transfer remain still in the mist until further research is conducted. Other findings however imply that visual and possibly lateral line (Patten 1977, Magurran & Higham 1988, Ryer & Olla 1991, Smith 1992, Krause 1993, Ferrari et al. in press) signalling could be the means of information transfer. The present study showed that pheromones from startled predator-experienced fish alone (i.e., chemical disturbance signals, Brown 2003) failed in promoting learning among the predator-naïve fish.

The conclusion of the chapter II is that at least not in every case would the higher proportion of demonstrators improve social learning. To be able to explain why smaller proportion of tutoring fish was better for social learning, direct observations of the learning event ought to be conducted. I am convinced that the details of the demonstrator behaviour in the company observer fish could determine the successfulness of the social learning. This view is supported by a recent study of Ferrari et al. (in press) showing how the quality of the demonstrator fish behaviour correlates with the learning ability of the observer fish. My believe is that the details of the demonstrator behaviour could depend on the fact whether these predator-experienced fish are in the majority or in the minority of the group when the predator odours are introduced to them. If the behaviour of the demonstrators could so differ according to different group dynamics, this could explain the successfulness of the information transfer from demonstrators to observer fish; provided that the phenomenon observed by Ferrari et al. (in press) holds true.

Certainly the present results do not present an exhaustive understanding of the demonstrator-observer dynamics necessary for social learning; they simply demonstrate the importance of further research on the subject that is especially welcome before any large scale life-skill training applications utilizing social learning are launched. The present results are also still among the very first to demonstrate successful social learning of predator recognition in salmonid fish; an especially important taxonomic group within conservation rearing. The importance of demonstrator to observer ratio for social learning now calls for testing across different fish species and circumstances. The results of the present chapter also challenge the validity of making presumptions of the efficiency of the social learning in predator avoidance concept based on results from other animal groups and other type of social behaviours such as foraging (see II, discussion).

III Repeated antipredator conditioning: a pathway to habituation or to better avoidance?

I introduced Arctic charr juveniles odours of charfed predators either once of repeatedly for four times. I simultaneously monitored the change in individual behaviour through the successive conditioning and finally tested the survival of all fish including still predator-naïve control fish in staged encounters with live predators.

Chapter III disentangles two utterly interesting questions that relate closely to the usage of antipredator conditioning as a method of life-skill training for captive fishes. First of all it adds to the few published studies that have actually tested how the trained fish survive from predation as compared to untrained naïves
(Berejikian *et al.* 1999, Maynard *et al.* 2001, Mirza & Chivers 2001, Gazdewich & Chivers 2002, Schlechte *et al.* 2005). Furthermore, it gives an important example of what can be gained from repeating the antipredator conditioning. Namely, as the successive chemical conditioning here did not include any real threat of capture, the behavioural responses might have been expected to even attenuate through repeated introduction of the stimulus (e.g., risk allocation hypothesis, Lima & Bednekoff 1999). Yet, a single 6-min conditioning with chemical cues of charr-fed predators improved charr survival in predation trials as compared to predator-naïve conspecifics. In addition, the results revealed positive signs of avoidance learning through repeated introduction of predator odours. Not only had the spatial avoidance of the predator odour got stronger in prey (tested in two-choice maze), but also the actual survival was further improved as compared to singly trained fish.

There are only a handful of studies within the last 30 years testing the effects of repeated stimulus presentation for fish learning, with mixed outcomes of learning or habituation (Dill 1974, Garcia *et al.* 1992, Järvi & Uglem 1993, Jachner 1997, Berejikian *et al.* 2003, Hawkins *et al.* 2004). According to my results the chemical cues from predators with a test fish diet seem to create a relatively strong conditioning effect for prey, whereas for instance the use of model predators seem not to create learning or even entails a risk of habituation (e.g., Kanayama 1968, Fraser 1974, Magurran 1990, Van Heezik *et al.* 1999).

The successfulness of antipredator learning in terms of subsequent prey behaviour and their survival advantage seem to vary also according to the test environment characteristics (Shalter 1984, Welch & Colgan 1990, Berejikian *et al.* 1999, Gazdewich & Chivers 2002). The question thus remains whether the enhanced survival has a chance to get manifested also in the wild after the release? The change in prey behaviour that here prolonged their survival after training was the improved spatial avoidance of predators (III). This I find very encouraging, since my personal view is that conditioning hatchery fish with chemical cues of predators should most importantly be manifested as an improved tendency to spatially avoid areas with these chemical cues. In the wild the spatial avoidance can expected to be more extensive than in a restricted test aquarium and the survival advantage of the trained prey could thus hopefully persist. Berejikian *et al.* (1999) had actually observed better survival for the trained fish after the release to the wild although no improved survival had been observed in a semi-natural test environment.

**IV Natural born survivors: innate responsiveness to predators is associated with survival despite divergent learning opportunities**

I investigated the possible association between individuals’ innate responsiveness towards predator odours and their subsequent survival from predation. I first recorded the innate behavioural responses of charr towards predator chemical cues and then exposed the same fish to predation at a later date. Half of the fish had been presented with the same predator odours for three more times before the survival test to induce learning. This enabled studying whether divergent early-life learning opportunities of the same predator cues would change the relationship between individuals’ innate predator recognition skills and later survival. Such empirical work in fish has not been published before, nor has the basic idea received much attention in the study of inter-individual differences of behaviour.

The results showed a positive association between innate odour recognition skills and subsequent survival. Namely, individuals that possessed higher intrinsic recognition skills to chemical cues did better also in the predation trials. The relationship was of the same nature also in the learning treatment suggesting that learning did not interfere with innate inter-individual differences in predator avoidance skills. The inferences of the result to the scheme of what happens to the inter-individual behavioural differences as a result of learning are rather interesting. Namely, the different scenarios include at least the options introduced as Fig. 1.

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If the present study would have showed an effect from learning treatment to the relationship between the innate level of antipredator behaviour and later survival, scenarios in Fig. 1a or Fig. 1b would have come into question. When the results of the III and IV are pulled together it may be concluded that early-life learning can improve the average survival of fish (III); yet maintaining the innately determined inter-individual differences since the improvement from experience seems to benefit all individuals relatively to the same extent (IV, Fig. 1c).

The positive association found here between the antipredator behaviour measured in two different situations and environments could possibly arise from a within-individual behavioural correlation (Van Oers et al. 2004, Sih et al. 2004a). This fascinating idea of behavioural correlations has recently received great attention in literature (‘behavioural syndromes’, Sih et al. 2004 a,b). It is grounded on a conception that individual personalities and behavioural types (Drent et al. 2003, Dall et al. 2004) could be results of rather fixed associations between different behaviours or same type of behaviour expressed over varying occasions.

Chapter V examines the reasons behind innate determination of differences in antipredator skills. Individuals may differ considerably in their behaviour (Wilson 1998, Reale et al. 2000, Brick & Jakobsson 2002, van Oers et al. 2005) and variation in boldness in the vicinity of predators is expected to reflect different behavioural identities arising from within-individual behavioural correlations. The correlations of, for instance, aggressiveness and predator avoidance may be non-adaptive and thus referred as behavioural syndromes (Sih et

Figure 1. Hypothetic illustration with three possible scenarios of the effect that learning could have on the relative strength of predator avoidance among individuals with different levels of innate responsiveness: a = individuals with poorer innate skills catch up which evens out the innate differences, b = innate skills and individual learning rates are positively correlated which increases the behavioural differences, c = improvement from learning is the same for everyone and any individual differences remain fairly steady.

The bold and the variable: fish with high heterozygosity act recklessly in the vicinity of predators

I measured the behaviour of brown trout young twice, with and without the presence of chemical cues from predatory burbot (Lota lota). The intra-individual genetic variability (heterozygosity) of the trout were later analysed using microsatellite loci and its association to the observed behaviours was examined.

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al. 2004a,b). More generally, the inter-individual variation in behaviour is expected to have genetic basis (Dall et al. 2004), yet the genetic properties producing these differences are still poorly discovered.

The results of $V$ revealed positive association between intra-individual heterozygosity and reckless behaviour under increased threat of predation. More heterozygous individuals took greater risks by sustaining higher activity and by continuing feeding in the presence of predator chemical cues. In contrary, no association of individual heterozygosity and brown trout behaviour were observed in the absence of predation threat. The possibilities of why the more heterozygous individual may have acted more risk-prone have been discussed further in $V$. The results of chapter $V$ are the first to demonstrate the association between genetic variability and antipredator behaviour in any animal species.

The results suggest that increased risk of predation could elicit individual differences in genome construction to be manifested in fish behaviour. The fact whether the higher tendency to take risks among the genetically more variable brown trout would also lead to lower survival from predation remains to be tested. Since the association between heterozygosity and antipredator responsiveness was found across all three brown trout populations including wild and hatchery backgrounds, suggests that the phenomena could be of more general nature at least in this species.

The relationship found here between heterozygosity and boldness under predation threat is interesting to interpret within the concept of hatchery rearing since captive rearing usually generates changes in the genetic variability of fish. Namely, as the setoff of a captive stock is usually done with only a limited number of individuals the genetic variation can get reduced and will continue to do so over generations in captive breeding (e.g., Fleming & Einum 1997, Crozier 1998). This means that more fearful individuals should be found within hatchery stocks. On the other hand, it has been shown that hatchery selection could favour fish with higher metabolic rates (Metcalfe et al. 1995, Yamamoto et al. 1998) that also tend to be the more aggressive and dominant individuals (Lahti et al. 2002). There are indications that these dominant phenotypes would also possess higher heterozygosity (Metcalfe 1995, Metcalfe 1998, Tiira et al. 2005). Although the dominant and bold phenotypes could get selected for by the selection occurring in hatcheries (e.g., Johnsson et al. 1996, Sundström et al. 2004) their reckless behaviour in front of predators should lead to lower survival in the wild (e.g., Johnsson & Abrahams 1991, Mesa 1991, Johnsson 1993, Dellefors & Johnsson 1995).

The selection favouring different behavioural phenotypes could so vary between different environmental conditions. For instance, Biro et al. (2004) showed how differences in predation pressure between wild environments accounted for the opportunities of fish with high growth rates to thrive. Similarly, Alvarez & Nicieza (2005) demonstrated how metabolic rate can be positively correlated with individual growth rate in captive habitat, yet metabolic rate appeared to have a negative correlation or no relationship at all with growth and survival in natural streams.

The above suggests that there is a complex relationship to be found among heterozygosity, boldness, and environmental variability. The variable conditions among and within hatcheries, and the unpredictability of natural habitats over the years means that the advantages of being more heterozygous entailed with higher metabolic rate, earlier hatching, and risk-prone behaviour should not be constant.

The amount of individual’s genetic variability can be one of the factors determining its personality and tendency to respond to predator threat, but most probably not the only one. For instance, the genetic variability of Saimaa Arctic charr (I-IV) is very low (Primmer et al. 1999), yet there are distinct individual differences in innate responsiveness to predator cues. To disentangle the question whether the actual predator recognition skills of the more heterozygous individuals are weaker, or are they simply aware of the risk but continue to behave risk-prone (see V), we need further studies. It may also pay off
to study the basic physiological responses of fish (e.g., heart and opercular rates, Rehnberg & Schreck 1987, Höjesjö et al. 1999, Johnsson et al. 2001, Hawkins et al. 2004) to enlighten this matter.

6. Future perspectives and implications for management

Common to all the life-skill training and habitat enrichment studies is that they are highly applicable from their principles (Brown & Day 2002) and draw public attention (see Randerson 2001), yet very few applications still exist at the hatchery level. My view is that this is mainly due to a lack of information transfer between the behavioural ecologists working on the subject and the fish management people. This is a problem of research culture that should be dealt with. Also primarily due to a lack of information there can be some preconceived attitude amongst hatchery managers towards life-skill training. Quite understandably, they seem to feel that securing the in-culture welfare of the animals is their top priority. The general outlook on the subject is, however, vastly moving to the direction that the poor post-release survival of fish should not be inevitable. The more radical views consider releasing fish with poor survival skills even unethical.

One of the future challenges in antipredator training is to develop ecologically meaningful and widely applicable methods. One of the most important aspects is to take into account the fact that most of the natural habitats are multi-predator communities. Training fish to avoid one type of predator may not help it avoid another species (Darwish et al. 2005). Theoretically, generalization to avoid all large fish after learning to avoid one is probably not an evolutionary viable trait due to the accompanying disadvantages of loosing opportunities for feeding etc. Darwish et al. (2005), however, showed how prey fish may be able to acquire predator recognition from chemical cues of multiple predator species simultaneously and gain a subsequent survival advantage from it.

Different species as well as fish of different age and developmental stages may, nevertheless, have different responses to hatchery environment and life-skill training (see e.g., Swain & Riddell 1990, Berejikian et al. 1996) and may require different type of enrichment methods. The results of III suggest that improved survival of the trained fish may be gained by generating changes in different areas of their behaviour. Namely, mechanically chasing fish young around in a fluviarium seemed to improve their antipredator responses, presumably by lowering their reaction threshold or enhancing the spurt velocity or both. Introduction of predator chemical cues made fish spatially avoid such cues in the future which can be considered a change in preventive predator avoidance behaviour. The inference from this is that both predator avoidance behaviour as well as antipredator behaviour (see 3.1.) of fish could possibly be improved by training. Independent of the properties of the release site and the details of the expected predation pressure, it may pay of to try to incorporate these two aspects into training methodology.

Incorporating also other nature-like elements into rearing habitat, or simply constructing more versatile surroundings, could possibly increase the general adaptability of the fish. Adjusting to the wild environment, where prerequisites for survival are suddenly drastically different, could then be easier. Adding variability into captive habitat would presumably alter also the selection in hatchery, making it not to favour one type of phenotype only.

Also the in-culture welfare consequences of treating fish with predator odours or other nature-like elements need to be investigated. Evoking chronic stress with severe treatments could predispose fish to increased mortality. On the other hand, experiencing acute stress in hatchery from having to face e.g., predator cues, variable food distribution and composition, higher water currents, and more complex habitat could reduce the presumable post-release stress when the same elements are subsequently experienced in the wild (e.g., Järvi 1990, Shepherdson 1994, Wallace 2000, Ward & Hilwig 2004, Schlechte et al. 2005). For instance, antipredator conditioning no doubt causes momentary stress...
or even limited mortality among the animals, yet enhances the later ability to evade these predators (e.g., Maynard et al. 2001, II, III).

The idea of increasing the structural complexity of the tanks is received with great suspicion by the management people. This is due to reduced in-culture health and survival that are expected to entail. Its effects to the hygiene and disease outbreaks would have to be properly studied. The research programme of Natural Rearing Enhancement System (NATURES), however, showed how rearing chinook and steelhead salmon fry in enriched tanks and in semi-natural raceways produced more competitive fish that had also better post-release survival without any impacts on their in-culture well-being (Maynard et al. 2001).

Using guidelines shown by the results of smaller scale investigations such as the present thesis, the details of the most efficient methods for actual training on a hatchery scale will hopefully be soon revealed. Few unsuccessful large scale projects with inadequate training methodology could at worst bury, at least locally, the whole concept of life-skills training as proven to be non-effective. Thus, it is to be hoped that the best possible knowledge and experience on the subject would be gathered together when designing any large scale experiments. Definitely more live predation trials, and proper release & recapture experiments are needed to verify the survival advantages of conditioning prey to recognize and avoid their natural predators since overall the existing results are still mixed (Berejikian et al. 1999, Mirza & Chivers 2001, Gazdewich & Chivers 2002, Mirza & Chivers 2002, 2003b).

All experiments in the current thesis have been conducted in hatchery facilities using mostly fish of captive stocks. Accordingly, the interpretation of the current results in the broader concept of behavioural ecology of fishes cannot be made without restrictions. The observed behaviours describe the innate character, learning capacities and survival properties of artificially propagated fish. Such fish have already been to some extent under unnatural selection and developmental environment which can have an effect on their behaviour. Nevertheless, as the biomass of for instance cultured Atlantic salmon already exceeds that of the wild populations (Gross 1998), the studies done in aquaculture habitat should make sense. Sometimes observations in natural environment are even restrained because the wild individuals are so few in number or rarely encountered and captive rearing of such populations has become a normal procedure. This is exactly the case for instance with Saimaa Arctic charr. Saimaa is the largest lake in Finland and behavioural observations of this rare species are very challenging to conduct in the wild.

Based on my experiments it appears that fish can improve their predator recognition and avoidance skills by experiencing predators directly in a physical contact, but equally well from chemical cues alone. Results such as the ones in chapter I provide excellent grounds for the use of chemical cues since the studied prey population possessed a sophisticated ability to discriminate between different odours. Social learning of predator avoidance can be very successful in char, yet the learning appears to be extremely sensitive of the prevailing conditions such as the demonstrator to observer ratio. From the viewpoint of life-skill training, conditioning fish to perform stronger predator avoidance could be achieved with least effort and casualties by chemically mediated training methods. However, the relative effectiveness of different training methods still calls for further research. Furthermore, the use of multiple predator cues could be expected to have an additive effect on the strength of the prey avoidance response (Smith & Belk 2001, Amo et al. 2004).

Individual variation in responding to threat has to be acknowledged since studying the population averages do not tell about the frequencies of responsive and non-responsive individuals. This may influence the learning ability of the population and should affect the choice of the most appropriate training method. Based on my results in V, the intra-individual genetic variability appears as a plausible character contributing to inter-individual variation in behaviour under predation threat. How the changes in heterozygosity due to captive breeding affect the boldness of the individuals
thus requires further notice. Although the individual differences in predator avoidance so seem to be related to underlying differences in their genome, learning appears to promote the average survival of all the fish.

To my mind it would be ecologically most advisable for the fish to be released at early life; during a period when natural selection is at its strongest. This would place them under the same selection regimes with their wild conspecifics i.e., also restrain or diminish the post-release effects of hatchery selection in the wild. In general, wild fish should be considered more valuable than artificially propagated hatchery fish and thus for instance fish introductions should be designed with care. Captive breeding should also continue to be viewed only as means for buying time for in situ conservation of species and their natural habitats. Thus, simultaneously with improving the conditions of supportive breeding all necessary re-establishment measures should also be addressed in trying to return the essential physical and ecological prerequisites for naturally reproducing populations to manage in the wild.

It would be easy to say that producing less but ecologically more viable fish would mean better economics. Yet the more realistic view is that to date neither the scientists nor the management personnel have enough information for releasing a protocol of methods that can be used for bringing up large quantities of trained fish. Luckily, the knowledge is accumulating rapidly partly due to overwhelming interest in the subject. My optimistic view is that at a long run this will help us to come up with knowledge of how to cost/effectively produce fish with improved post-release performance. As a consequence less fish would need to be released in total. This would not only be making reintroduction programs more profitable but it would also be more ethical, as well as diminish the negative interactions with the naturally reproducing populations.

How the ability of fish to learn in a hatchery transforms into improved survival also in the wild is yet to be accurately proven. For getting resources and funds to this kind of future experiments that may also require more extensive efforts, we first need convincing results from smaller scale investigations; and as such behavioural studies like the present thesis could be considered essential.

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