Reproductive traits are strong determinants of fitness and are therefore subject to particularly intense selection. The interplay between natural and sexual selection shapes the evolution of sex-specific characteristics that ultimately determine the breeding success of individuals. This thesis addresses some fundamental questions regarding the selective factors in traits that may be of importance for the reproductive biology of Arctic charr, an externally fertilizing fish species with a non-resource-based mating system.
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Phenotypic and genetic variations in reproduction-associated traits in Arctic charr, Salvelinus alpinus L.

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ABSTRACT

Reproductive traits are strong determinants of fitness and are therefore likely to be subject to particularly intense selection. The interplay between natural and sexual selection shapes the evolution of sex-specific characteristics that ultimately determine the breeding success of individuals, i.e. the ability to pass on their genes to the next generation. In order to understand these patterns better, in this thesis, I have addressed some fundamental questions regarding the genetic and phenotypic aspects of traits that may be of importance for reproductive biology. As a model species, I used the Arctic charr, *Salvelinus alpinus*, an externally fertilizing fish with a non-resource-based mating system.

A comparison of external body shapes between three ecologically distinctive charr populations revealed significant genetically determined differences both outside and during the reproductive season. Sexually matured fish, however, exhibited uniform patterns of sexual dimorphism among the populations. The males possessed a more robust (deeper) body profile, larger head dimensions and longer pectoral fins than females. Such relatively enlarged features of males presumably reflect their more intense intra-sexual breeding competition compared to females.

Controlled breeding experiments indicated that embryo survival and larval post-hatching size are largely attributable to maternal effects. Some of the variations were also explained by independent male effects and the genetic interactions between parents, suggesting that selection during the early stages of development can simultaneously target both intrinsically high-quality and compatible genotypes. The present results also show that increased investment by both sexes at the gamete level can contribute to developmental success. In males, sperm swimming speed was positively associated with progeny viability (consistent with the ‘good sperm’ hypothesis), whereas in females the positive effects were mediated through increased and less variable egg size. Polyandrous mating as a result of
sperm competition improved the average survival rate and increased yolk size among offspring above the average values obtained from single matings. Thus, besides showing pre-mating selectivity, females may incur indirect fitness benefits by exposing the eggs to multiple males in a given spawning event. Instead, I found no evidence of consistent viability differences between half-sibling progenies when the charr females were paired with the males they preferred compared to those they did not prefer in a preceding, dichotomous ‘free choice’ test.

The degree of carotenoid-based breeding colouration proved to be a potential indicator of reproductive quality (fertility) within both sexes. In accordance with the phenotype-linked fertility hypothesis, I observed a positive link between male redness and sperm velocity. Thus, brightly coloured males may advertise their efficient fertilization capability to females. Nevertheless, differences in the ornamental expression of male charr did not translate into differences in offspring early fitness traits. In contrast, the intensity of colouration in females was negatively related to their fecundity and brood viability, which refers to a significant trade-off between offspring production and ornamentation. These findings do not support the direct (mutual) selection hypothesis as an explanation for the existence of carotenoid-based breeding displays in both sexes. Instead, the ornamental colouration in female charr might have evolved as a genetic correlative trait resulting from sexual selection on male colouration. However, varying female preferences, selection on genetically compatible males and cryptic female choice may also weaken the directional selection on male colouration and thus maintain its genetic variation within populations of Arctic charr.

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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–VI. In addition, some unpublished results are presented in the summary.


The publications are reprinted with permissions from Wiley-Blackwell (papers I, II, IV and VI), BioMed Central Ltd (III) and Springer (V).
In papers I and IV, I was mainly responsible for data handling and analysis, and writing. In paper III, I took part in planning, data collection and analysis, and writing. For the papers II, V and VI, I collaborated with co-authors in planning and data collection, and was mainly responsible for the data analysis and writing.
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1 Introduction

“Sexual selection has given the most brilliant colours, elegant patterns, and other ornaments to the males, and sometimes to both sexes of many birds, butterflies and other animals.”
Charles R. Darwin, The Origin of Species (1872)

1.1 SEXUAL SELECTION

1.1.1 Framework and definition
In a variety of animal species, males and females exhibit a pronounced divergence in their outward appearance during the breeding season, involving changes in body morphology, colouration and mating behaviour. To explain the evolution of such elaborated structures and actions, i.e. secondary sexual traits, Darwin (1871) originally introduced the concept of sexual selection as distinct from his natural selection theory. Nowadays, sexual selection is considered a highly multidimensional process, which not only encompasses the existence of elaborate mating displays but also a considerably broader range of traits and patterns associated with reproduction.

Basically, in all sexually reproducing organisms, the interplay between natural and sexual selection has resulted in a complex set of adaptive traits that determine the ability of individuals to transfer their genes to the next generations (i.e. their relative fitness in a population). As far as the success in competition for mates or matings is dependent on the expression of specific characteristics, these characteristics are subject to sexual selection (Andersson 1994). In other words, sexual selection arises when certain individuals gain a reproductive advantage over other individuals of the same sex. It is often a directional rather than a stabilizing evolutionary force and can operate through two different ways: choice of mating partners or intra-sexual competition for mating opportunities (Andersson 1994;
Jennions and Kokko 2010). These main selection mechanisms may further appear in multiple pre- and post-copulatory forms and occur simultaneously (or successively) in either concert or conflict (Andersson and Simmons 2006). Males are usually, but not always, under the strongest sexual selection due to their potentially higher reproductive rate – a primary consequence of the reduced energetic investment in offspring through gamete production (anisogamy) and parental care (Trivers 1972; Clutton-Brock and Vincent 1991; Clutton-Brock 2007).

The disparity of reproductive inputs between the sexes also largely explains why females are generally more selective of their mates than males. In addition, the relative number of sexually active males is often larger than that of receptive females, resulting in greater variations in the mating success of males (e.g. leks; Höglund and Alatalo 1995). Within the limits of intra-sexual competition (and sperm depletion), however, males may often maximize their reproductive success by obtaining as many additional partners as possible (Bateman 1948; Reynolds 1996). For females, instead, it may be more worthwhile investing in the quality rather than the quantity of offspring. Therefore, the potential fitness advantages from acquiring multiple matings (polyandry) have remained less unambiguous (Jennions and Petrie 2000). The differences in sex-specific strategies concerning optimal reproduction can generate sexual conflicts between the sexes. Sexual conflicts can have substantial impacts on the variation in sexual selection as they may lead to a co-evolutionary arms race or, in some cases, constrain the evolution of a trait that is favoured in one sex but selected against in another (i.e. sexually antagonistic selection; Fricke et al. 2010).

1.1.2 Maintaining mechanisms
Several theories, collectively referred to as indicator models of sexual selection (Andersson 1994), have been proposed for the basis of an idea that female behaviour can direct mating by favouring males with elaborate ornamental traits. Interestingly, in some systems, the directional mate choice may have arisen entirely from non-mating contexts, representing a by-product of
the pre-existing sensory bias, for example, towards food items of specific colours (Endler and Basolo 1998). In contrast, the Fisherian ‘runaway’ selection theory (Fisher 1930) suggests that both expression of the male trait and female preference for it are heritable and become linked over successive generations, resulting in their mutual reinforcement throughout the population. In other words, attractive males will produce attractive sons, and attractiveness can simultaneously be genetically correlated with female choice behaviour. This process may ultimately lead to the proliferation of both the trait and the preference well beyond their initial forms. However, the development and maintenance of most sexually selected traits are believed to involve significant costs (Iwasa et al. 1991; Cotton et al. 2004). This is because these traits may otherwise be maladaptive in terms of natural selection (e.g. Stuart-Fox and Ord 2004; Hamon and Foote 2005). Zahavi’s (1975, 1977) classical handicap hypothesis predicts that the preference by females for elaborately ornamented males has indeed evolved specifically for that reason: the handicapping ornaments that lessen the changes of these males surviving are likely to function as honest signals of male quality in a range of circumstances. Zahavi’s handicap principle has since played a crucial role in placing the idea of sexual signals indicating fitness components other than attractiveness on a steady footing. On the other hand, the runaway selection and the handicap theory may represent different ends of the same sexual selection continuum rather than being strictly alternative evolutionary mechanisms (Kokko et al. 2002).

Hamilton and Zuk (1982) subsequently brought up the potential importance of parasites in the process of sexual selection. Accordingly, costly secondary sexual traits could signal genetic resistance against the predominant parasites. Variation in these traits would then be maintained by cycles of host-parasite co-evolution. In accordance with the handicap principle, less infected and more parasite-tolerant individuals are capable of producing more elaborate sexual traits than more infected individuals because the expression of such traits is dependent on health and vigour. As a mechanistic extension to
Hamilton and Zuk’s (1982) theory, Folstad and Karter (1992) further presented the immunocompetence handicap hypothesis (ICHH). The ICHH rests on the assumption that the expression of sexually selected traits is constrained by an androgen-mediated trade-off with immune functions. More specifically, testosterone (or other such hormones) is known to stimulate the development and maintenance of secondary sexual traits, but it is simultaneously associated with immuno-suppressive effects (Folstad and Karter 1992). However, this immunosuppression can also be adaptive in some cases (see Wedekind and Folstad 1994). Nevertheless, only healthy males can elevate their steroid hormones to superior levels and tolerate the costs of decreased immunological defence, thus making testosterone-dependent sexual traits honest signals of quality. Some support for the prediction of the ICHH has been found in many species, especially among teleost fishes (Taskinen and Kortet 2002; Kortet et al. 2003).

The concrete fitness benefits derived by females from being promiscuous and choosing particular males have received a great deal of attention. In resource-based mating systems, selection may favour mating preferences towards males that are more fertile or afford other direct benefits, such as access to high-quality territories, protection from predators, nutrients and paternal care (Andersson 1994; Møller and Jennions 2001). However, in resource-free mating systems, in particular, selective mating is likely to be adaptive as a consequence of indirect benefits: the offspring only inherit genes that promote their survival and reproductive success. Female mate choice is then expected to be based on male traits that reveal heritable components of fitness (‘good genes’), or, alternatively, non-additive genetic benefits (genetic compatibility) (Zeh and Zeh 1996; Neff and Pitcher 2005; Nordeide 2007; Puurtinen et al. 2009). In mating systems where ‘good genes’-based selection is prevalent, females typically express a consistent preference for males with elaborate ornamental traits. Some additive genetic effects of such males are advantageous to all females, independent of the maternal genome (Hunt et al. 2004). In contrast, genetic compatibility differs from mate choice for
‘good genes’ in that the fitness of the offspring varies depending on how well a male’s genetic make-up matches the female’s own genotype (Tregenza and Wedell 2000; Mays and Hill 2004). Parental genetic compatibility often improves fitness by increasing the degree of genetic heterozygosity among the offspring; this is a likely underlying mechanism, for example, in the special case of inbreeding avoidance (Kempenaers 2007).

Whether or not the genetic benefits are large enough to overcome the various costs related to mate choice has been widely questioned (Kirkpatrick and Barton 1997; but see Head et al. 2005). Alatalo et al. (1998) suggested that, in fact, in many mating systems the costs of any choice may be so small that female choice for honestly signalling males evolves even with minute benefits in offspring viability. Furthermore, on an evolutionary time scale even relatively minor fitness benefits can maintain the preference of females for sexually selected male traits and lead to considerable adaptive consequences (Alatalo et al. 1998; Møller and Alatalo 1999). Nevertheless, it remains contentious how directional female choice for ornamented males is maintained over time in the absence of direct fitness benefits, given that any unanimous mate preference should erode the underlying additive genetic variation and thereby eliminate, over time, the benefits of choice (Fromhage et al. 2009). As one attempt to resolve this theoretical problem known as the ‘lek paradox’ suggested, a strong simultaneous selection for non-additive benefits (genetic compatibility) may weaken the directional selection on male secondary sexual traits (Colegrave et al. 2002; Neff and Pitcher 2005), thus maintaining the genetic variation in both ornamental display traits and the associated fitness traits they signal. Moreover, it is worth noting that Hamilton and Zuk’s (1982) idea about everlasting cycles between hosts and parasites can potentially explain the lek paradox.

When females engage in multiple matings sexual selection may also continue in the post-copulatory arena via sperm competition and cryptic female choice (Birkhead and Pizzari 2002). As with pre-copulatory sexual selection, these two analogous mechanisms of selection (cf. male-male competition
and female choice) are nowadays recognized as common and strong evolutionary forces; they generate selection on ejaculate traits and thereby determine the relative reproductive success of males by limiting their access to the females’ eggs (Pitnick and Hosken 2010). Since sperm competition and cryptic female choice (or sperm-egg interactions) occur concurrently, they cannot be discriminated as alternative processes giving rise to differential male fertilization success (Eberhard 1996). The precise interaction between pre- and post-copulatory selection has remained unclear (Andersson and Simmons 2006), and, depending on the species and population, these two episodes of selection can either reinforce each other or work in opposing directions (e.g. Evans et al. 2003; Evans 2010).

Selection may favour polyandry in so much as the competitive fertilization environment and the females’ cryptic choice result in the best sperm fertilizing the eggs (Eberhard 1996). In addition to genetic incompatibility avoidance (e.g. Jennions and Petrie 2000; Colegrave et al. 2002; Pryke et al. 2010), there are two other potential explanations for the post-copulatory sexual selection for genetic benefits. According to the ‘good sperm’ hypothesis (i.e. the post-copulatory analogy of the good genes model for female choice evolution), females might receive indirect fitness benefits through positive co-variation of sperm competitive ability and offspring viability (Yasui 1997). In contrast, the sexually selected sperm hypothesis (or ‘sexy sperm’ hypothesis) predicts that males who have high fertilization success produce male offspring that are successful in sperm competition (i.e. directly comparable to the pre-copulatory ‘runaway’ selection; Keller and Reeve 1995).

1.2 SECONDARY SEXUAL TRAITS

1.2.1 Morphological features
Body size and shape are in many ways tightly bound to an individual’s performance, and thus, they may be targets for a combination of various sex- and population-specific selective pressures. The occurrence of sexual dimorphism in physical
body dimensions is a widespread phenomenon throughout the animal kingdom and it comprises a significant part of biological diversity (Andersson 1994; Delph 2005). A basic knowledge of the patterns involved with sexual dimorphism is therefore useful for understanding the ecological, behavioural, and life history characteristics of a given species, as well as for making morphological comparisons between populations.

Sexually dimorphic traits are generally associated with causes that restrict the reproductive success of animals. As females and males differ, by definition, in their reproductive roles, the inter-sexual differences in the selective pressures can ultimately result in the evolution of sexual dimorphism in morphological traits that lack a direct link to reproduction (Andersson 1994). Males are often larger than females and possess elaborate structures that may serve as important signals in attracting mating partners and deterring rivals. Some of the most exaggerated traits, such as antlers, horns and tusks, are exclusively valuable as weapons (armaments) in agonistic male-male encounters. In addition to intra-sexual competition, sexual divergence in external body structures can be directed by the preference of one sex for particular traits of the other sex (Andersson 1994), or it can evolve due to ecological (niche) differentiation between the sexes (Slatkin 1984; Butler et al. 2007).

Regardless of the underlying mechanisms, populations of the same species can greatly vary in the extent and form of sexual dimorphism in energetically (or otherwise) costly morphological traits, depending on the evolutionary interaction or compromise between natural and sexual selection (Blair et al. 1993; Hendry et al. 2006; Aguirre and Akinpelu 2010). Likewise, variability in the expression of secondary sexual traits can also be considerable within populations, with some individuals having relatively larger secondary sexual characters for their absolute size than others. This may fit the idea that most secondary sexual traits are condition-dependent and, hence, that investment in them varies among individuals in relation to their ability to bear the costs of developing and maintaining these traits (Zahavi 1975, 1977; Jennions et al. 2001).
1.2.2. Carotenoid-based ornaments

Carotenoid-derived yellow-red integumentary pigmentation are among the most conspicuous sexual signals and they have often been shown to serve as targets of directional mate preference (Møller et al. 2000). However, carotenoids are not exclusively used to attract mates, but they also have a number of important biological functions, including roles in the immune system and as antioxidants for detoxifying free radicals (Olson and Owens 1998; Blount et al. 2003; McGraw 2005; but see Pérez-Rodríguez 2009). It has been long thought that the high costs (or even a handicap aspect) associated with carotenoid ornaments render them reliable indicators of condition and/or health status (Olson and Owens 1998). Because animals cannot synthesize carotenoids by themselves (de novo), but have to obtain them from dietary sources, carotenoid acquisition, assimilation and conversion are potentially limiting factors for the expression of carotenoid-based ornaments (Grether et al. 1999; McGraw and Hill 2001; Hill et al. 2002; McGraw et al. 2005b; Fitze et al. 2007). Furthermore, animals may face a physiological compromise in carotenoid allocation, and ornamental colouration may be to the detriment of self-maintenance needs (McGraw 2005).

The proposition that carotenoid-based sexual traits are maintained by a trade-off between ornamentation and the immune/detoxification system has also received support from many correlative studies (Skarstein and Folstad 1996; Brawner et al. 2000; McGraw and Ardia 2003; Saks et al. 2003; Alonso-Alvarez et al. 2004; Dawson and Bortolotti 2006; Mougeot et al. 2007, 2010; Baeta et al. 2008). Accordingly, the more intensely coloured individuals, who can afford to invest more carotenoids in signalling, are likely to have a greater capacity to resist parasites, diseases and oxidative stress (Lozano 1994; von Schantz et al. 1999). However, some recent studies suggested that the immunostimulatory effects of carotenoids are not necessarily mediated by their antioxidant properties (Pérez-Rodríguez et al. 2008; Pérez-Rodríguez 2009). Furthermore, it has been suggested that carotenoid-based sexual traits may rather advertise the availability of many other non-pigmentary antioxidants (including various vitamins, antioxidant enzymes
and minerals) that might protect the carotenoids themselves from harmful oxidative effects, thus making them more accessible for ornamental purposes (Hartley and Kennedy 2004; Bertrand et al. 2006; Pike et al. 2007; Pérez et al. 2008).

In brightly coloured males, the enhanced ability to combat oxidative stress may further mitigate deleterious oxidative damage to their sperm, and so be directly related to their functional fertility (Blount et al. 2001; Helfenstein et al. 2010). Likewise, as far as the intense carotenoid-based ornamentation reflects low immune activity within the body, the sperm cells of colourful individuals should be less exposed to autoimmune attacks (Folstad and Skarstein 1997). An increasing number of studies have provided evidence for a connection between carotenoid-based sexual signals and fertilization capacity (or, rather by proxy, ejaculate characteristics related to it) (Evans et al. 2003; Peters et al. 2004; Locatello et al. 2006; Pitcher et al. 2007; Pike et al. 2009; Helfenstein et al. 2010; but see Evans 2010). These findings lend support to a more generalized phenotype-linked fertility hypothesis, which predicts that secondary sexual ornaments would reliably advertise male functional fertility (Sheldon 1994). Such a measure of male reproductive quality can be equated with the direct benefits of mate choice, even in species with no resource-based mating systems (Chargé et al. 2010). Furthermore, empirical evidence from a few studies on fish support the view that females can also obtain indirect genetic benefits from basing their choice of males on their carotenoid-based colour patterns (Barber et al. 2001; Evans et al. 2004; Eilertsen et al. 2009).

The evolution of carotenoid-based sexual traits is of particular interest in species where both males and females are ornamented. This raises an intriguing question of whether the information content and/or the underlying selection mechanisms of carotenoid ornaments are similar in both sexes. However, females greatly differ from males in that they are not only constrained to allocate carotenoids to their own needs, but also to their offspring (Blount et al. 2000, 2002). Developing embryos are particularly dependent on maternally derived yolk carotenoids because their rapid metabolism incurs high rates of
free radical production, and their tissues are rich in unsaturated lipids that are susceptible to free radical attack (Blount et al. 2000; Surai et al. 2001). By investing high concentrations of carotenoids in the nourishing yolk, females may provide a high antioxidant defence to offspring and thereby significantly contribute to their fitness (McGraw et al. 2005a). Hence, the occurrence of sexual dimorphism in carotenoid-based colours (females are usually less intensely coloured than males) could reflect inter-sexual differences in the compromise between natural and sexual selection. Depending on the mating system, ornamental female breeding colours may either have an adaptive signal function in mate choice (i.e. they provide a cue of good female quality; Siefferman and Hill 2005; Gladbach et al. 2010) or, alternatively, they may have evolved as a result of non-adaptive genetic correlation through the sexual selection on male colours (Lande 1980; Kraaijeveld et al. 2007). Giving some support to the latter, a negative relationship between the females’ carotenoid-based ornamentation and the amount of carotenoids in their gonads was demonstrated in two species of fish (Nordeide et al. 2006, 2008).

1.3 OBJECTIVES OF THE STUDY

The present work focused on the phenotypic variation in primary and secondary sexual traits, and on some of the selective factors that may be of importance for the reproductive biology of an externally fertilizing (fish) species with a non-resource-based mating system. I used different populations of Arctic charr, Salvelinus alpinus L., 1758, as a model in the experiments of this thesis.

The specific aims of this thesis were:

- to investigate whether or not the three populations of Arctic charr, representing distinct geographical origins and ecological forms, and measured at different life-history stages, differ in respect to external morphological features when reared in a common environment, and, in particular,
to characterize the general patterns of sexual dimorphism within populations (I).

- To examine the information content of carotenoid-based ornamentation in the mating system of the species. That is, whether the expression of breeding colouration reflects aspects of individuals’ phenotypic (reproductive)/genetic quality in either or both sexes (indicator models of sexual selection; II, V, VI).

- To study whether polyandrous mating as a result of sperm competition (III) and/or pre-spawning mate preference (VI) yields indirect fitness consequences for female charr in the form of the offspring’s increased developmental success, and, furthermore, to check whether or not a male’s sperm quality correlates with the early success of his offspring (the ‘good sperm’ hypothesis; III).

- To study the relative importance of distinct parental effects (underlying sources of phenotypic variation) in the early fitness traits of offspring under two environmental conditions (IV).
2 Materials and methods

2.1 STUDY MODEL: THE ARCTIC CHARR

The Arctic charr (family Salmonidae) is the world’s most northern freshwater fish with both anadromous and resident forms (Johnson 1980). The species is characterized by an extensive variability in its phenotypic expression and ecology (Klemetsen et al. 2003b). Throughout their Holarctic range, Arctic charr occur as numerous distinct (allopatric) populations and they also form coexisting (sympatric) morphotypes that show divergence in habitat use, diet, growth patterns, morphology, colouration and life history traits (Jonsson and Jonsson 2001). Although this intra-specific polymorphism undoubtedly involves a significant amount of genetically determined differences (Wilson et al. 2004), most of it appears to be environmentally induced and is thus attributable to phenotypic plasticity (e.g. Nordeng 1983; Hindar and Jonsson 1993; Janhunen et al. 2010). Nevertheless, Arctic charr are considered poorly competitive in inter-specific interactions and their environmental requirements are demanding (Johnson 1980; Johnston 2002). In particular, this species exhibits a highly restricted variation in terms of thermal adaptation (Lyytikäinen et al. 1997; Elliott and Klemetsen 2002; Larsson et al. 2005), being both ecologically and energetically adapted to low water temperatures (Klemetsen et al. 2003a; Larsson and Berglund 2005). Arctic charr are capable of feeding and growing at temperatures very close to 0 °C (Brännås and Wiklund 1992; Siikavuopio et al. 2010), and growth efficiency is maximized when environmental temperatures stay below 10 °C (Larsson and Berglund 2005). Low temperature is particularly beneficial to early developmental stages with respect to survivorship and efficient utilization of yolk reserves (de March 1995; Bebak et al. 2000; paper IV).
Arctic charr usually spawn in the autumn and they have a lek-like breeding system, where both females and males mate multiple times. Like other salmonids, charr do not provide parental care; the eggs are hidden in the spawning substrate (gravel and/or stones) and thereafter left unguarded to develop over winter (Sigurjónsdóttir and Gunnarson 1989). At the onset of the breeding season, reproductively active males gather on spawning grounds where they compete intensely whilst waiting for the arrival of females (Figenschou et al. 2004). The colouration of the fish intensifies in both sexes and males, in particular, appear to undergo certain external changes in body shape (paper I). Larger and more aggressive males tend to establish and defend the best spawning territories, which are frequently visited by the spawning females (Fabricius and Gustafson 1954; Sigurjónsdóttir and Gunnarson 1989). Dominant males generally adopt a guarding position in the vicinity of females and chase away approaching rivals. Smaller, subordinate males, in contrast, circle nearby and attempt to steal fertilizations from the guarding males by rushing into spawning acts and releasing their milt (Fabricius 1953; Sigurjónsdóttir and Gunnarson 1989). Due to the high frequency of simultaneous parasitic spawnings, sperm competition is likely to be an important intra-sexual selective force in charr (Taborsky 1998). Sperm swimming speed is presumably a prime component in the determination of male fertility and sperm competitive ability (Liljedal et al. 2008), and it is known to vary among males in relation to their social status (Rudolfsen et al. 2006; Haugland et al. 2009).

Along with the overall phenotypic variability, an elaborate carotenoid-based spawning dress makes Arctic charr a specifically intriguing vertebrate model for studying mate choice and polyandrous behaviour. Both sexes can develop strong abdominal colouration, though males are generally more ornamented than females (i.e. a sexually dichromatic trait; Skarstein and Folstad 1996; see also Fig. 3 in the Results and discussion section). However, there can be a large variation in colour intensity and shades within both sexes. The considerable differences in the red-coloured pigmentation of various
populations of charr, both in the wild and under culture, refer to a strong genetic component in pigment uptake and allocation (Elvingson and Nilsson 1994). The predominant carotenoids responsible for the pigmentation of Arctic charr are astaxanthin and its metabolites, which primarily accumulate in muscles, skin and fins (Scalia et al. 1989). The carotenoid reserves in the muscles are redistributed to the gonads, skin and fins in maturing females and to the skin and fins in maturing males (Hatlen et al. 1996). Dietary carotenoid availability may be a key determinant in the carotenoid reserves of individuals and ultimately in their ability to provision these resources to different tissues (Shahidi et al. 1993). Natural sources of astaxanthin are various invertebrates such as amphipods, copepods and insects. Commercial, pelleted salmon food is supplemented with synthetically produced astaxanthin.

Figenschou (2010) recently reported that charr males whose immune system was experimentally activated allocated less of their body carotenoids into ornamental pigmentation when compared to the control males. Furthermore, a negative correlation between the redness of the belly and lymphocyte counts was shown in both sexes (Skarstein and Folstad 1996), suggesting that immunological costs may be involved, at least to some extent, in the development of ornaments, in accordance with the immunocompetence hypothesis. In a recent study on wild-caught female charr, however, no relationship was found between the red intensity of the belly/fins and potential condition-dependent parameters (leucocytes, condition factor and parasitism) (Nordeide et al. 2008).

It is presumable that the carotenoid-based skin colouration of both sexes of Arctic charr reveals information that could be of use in mate choice. Because males only appear to provide ejaculate to females during spawning and no parental care, directional female mate choice can be expected to be primarily based on genetic benefits according to the intrinsic male quality hypothesis (Møller and Alatalo 1999). Reciprocally, it is possible that males would assess female quality on the basis of carotenoid colouration as well (Nordeide et al. 2008). Nevertheless, little is known about the importance of breeding
colours in the sexual behaviour of Arctic charr, or about the ultimate causes that promote the development of these traits in both sexes.

On the whole, the Arctic charr is an excellent object of research in terms of sexual selection due to their external fertilization, large family sizes (high fecundity), and a multiform, non-resource-based mating system. In addition, they can be artificially bred (in vitro) and reared in moderate densities under hatchery conditions, which also enables controlled studies regarding the indirect parental influences on offspring. This thesis was primarily conducted with the cultivated brood stocks originating from two Finnish lake-resident populations representing large-growing, predatory forms: Lake Inarinjärvi (I–III, VI) charr and Lake Saimaa (Kuolimo region; I, IV and V) charr. Both populations have experienced dramatic declines in abundance over the last few decades, and the Finnish Game and Fisheries Research Institute (FGFRI) began cultivating them for preservation and restocking purposes (Kallio-Nyberg and Koljonen 1991). The population from Lake Saimaa, in particular, is currently critically endangered and relies almost completely on supportive breeding for its existence (Urho et al. 2010). The third hatchery-reared strain used in this work (I), Lake Karhunpesälampi charr, lives as a stunted population in a small lake in the Posio region. All of the studied populations are both geographically and genetically highly distinct (paper I, Fig. 1; Primmer et al. 1999, 2000).

2.2 GENERAL STUDY DESIGNS

All of the studies presented in this thesis are experimental in nature, and were carried out under standardized hatchery conditions at four different aquaculture stations of the FGFRI over the years 2001–2010. The main research questions, materials and methods are briefly summarized below (including Table 1). More detailed descriptions are presented in the individual articles.
### Table 1. Summary of the experimental designs of the studies in this thesis.

<table>
<thead>
<tr>
<th>Main research topic</th>
<th>Description of the experiment</th>
<th>Measured traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper I: How does the general morphology vary among the three Finnish Arctic charr populations at their two life-history stages?</td>
<td>A common garden study; Morphological measurements from the samples in spring (age 3+) and autumn (age 4+); Population differences and sexual dimorphism</td>
<td>Body size; 28 morphological distances</td>
</tr>
<tr>
<td>Paper II: Do breeding colouration and some aspects of sperm quality interrelate with each other among the male charr?</td>
<td>Phenotypic measurements of the males sampled from F1 and F2 hatchery generations; Relationship between colouration and various ejaculate traits</td>
<td>Body size*, abdominal redness, spermatocrit, sperm velocity and longevity</td>
</tr>
<tr>
<td>Paper III: Does polyandry yield indirect fitness benefits to Arctic charr females? Is sperm quality associated with offspring quality?</td>
<td>Monandrous (single males) and polyandrous (sperm competition of two males) fertilizations; Offspring quality differences between the fertilization treatments</td>
<td>Body size* of parental fish; Spermatocrit and sperm velocity; Embryo survival to eyed stage; Larval mass, length and yolk volume</td>
</tr>
<tr>
<td>Paper IV: How do parental effects vary in early developmental traits under favourable and stressful environmental conditions?</td>
<td>A factorial breeding design; Egg incubation at two water temperatures (2 and 7 °C); Sources of genetic and phenotypic variation in offspring traits</td>
<td>Body size* of parental fish; Female-specific egg mass; Embryo survival to eyed stage; Larval length and yolk volume</td>
</tr>
<tr>
<td>Paper V: Does breeding ornamentation signal individuals’ genetic variation or reproductive quality?</td>
<td>Maternal half-sibling families; Male parents ranked bright to pale individuals; Relationship between colouration and offspring quality in both sexes</td>
<td>Body size*, abdominal redness and microsatellite variation of parental fish; Female-specific egg size and GSI†; Embryo survival to eyed stage; Larval length and yolk volume</td>
</tr>
<tr>
<td>Paper VI: Is female mate preference or parental breeding ornamentation related to the early viability of offspring?</td>
<td>PIT system in mate choice trials; Males ranked by their colouration; Maternal half-sib families; Relationship of female mate preference and parental colouration to progeny survival</td>
<td>Female mate preference (association time); Body size* and abdominal redness of parental fish; Embryo survival to eyed stage</td>
</tr>
</tbody>
</table>

* Body size involves the measurements of total length and mass
† Gonadosomatic index of females
2.2.1 Phenotypic measurements of adult fish

In each study of this thesis, the experimental fish were sedated before any further handling using a buffered tricaine methane sulphonate (MS-222) anaesthetic solution. In addition, as a routine operation, the fish were always measured for their total length and body mass. These data are not provided in all articles.

The morphometric characters describing external body shape were measured and analysed for the three charr populations at their two different life stages and at different times of year (spring 2003 and autumn 2004) (I). The conventional measurements were taken from digital photographs, including 28 distances between the standardized anatomical landmarks, i.e. a truss network to which standard length and pectoral fin length were added (Strauss and Bookstein 1982). The fish had been maintained from fertilization onwards under similar hatchery conditions at the Taivalkoski Game and Fisheries station (Janhunen et al. 2010), which ensured constant rearing environment for the different populations.

The abdominal colouration of the reproductively active fish was assessed in three experiments which were carried out at FGFRI’s premises in Taivalkoski (II), Enonkoski (V) and Paltamo (VI) in the autumn of 2002, 2007 and 2009, respectively. Calculated definitions for the degree of redness were determined on the basis of numerical parameters derived from either the digital photographs or directly from the surface of fish using a graphical user interface (InFotonics Center®, University of Eastern Finland, Joensuu, Finland) or a handheld colourimeter (Minolta CR-10; Konica Minolta Sensing Americas Inc., NJ, USA), respectively. The colour measurements were conducted on the left flank from one or two skin areas by applying either the RGB (red, green, blue; Stevens et al. 2007) (II) or CIE L*a*b* colour systems (CIE 1986) (V, VI).

The quality of sperm in charr males (Lake Inarinjärvi population) was investigated in relation to their body size, breeding colouration, hatchery background and offspring early viability (II, III). Sperm quality was described by the
spermatocrit (*i.e.* the proportion of sperm in a given volume of semen) and a few measures of sperm motility. The different sperm motility parameters were quantified using a computer-assisted sperm analysis (CASA) system which allows a rapid and exact analysis of various useful components of motility (Rurangwa *et al.* 2004). Sperm samples were activated in either water (II) or ovarian fluid (III) and their movement was recorded onto videotape for 40 seconds using a CCD B/W camera (Sony XC-ST50CE PAL, Tokyo, Japan) coupled to a microscope (Olympus CH30, Tokyo, Japan). The obtained videotapes were later analysed by CEROS computer software (Hamilton Thorne Research, Beverly, MA, USA). The motility variables used in the final analyses were the mean curvilinear velocity (VCL) and sperm longevity (percentage of motile cells 40 s after activation) (II), and the mean straight line velocity (VSL) (III).

The egg production potential of females (fecundity) was described using the gonadosomatic index (GSI), which is the ratio of total egg mass to body mass (V). From the samples of fertilized and water-hardened eggs, the individual egg sizes were determined by dry mass (IV) or, alternatively, by measuring the maximum diameter from digital photographs using the graphic software Image-Pro PLUS 3.0 (Media Cybernetics Inc., Silver Spring, MD, USA) (V).

### 2.2.2 Microsatellite typing
The level of assumed neutral microsatellite variation in the Lake Saimaa charr (*n* = 90) was assessed using 11 microsatellite markers (V). The polymerase chain reaction (PCR) was performed in two 10 μl multiplexes using QIAGEN Multiplex PCR Kit. To ensure the addition of an A-overhang to each PCR fragment by Taq polymerase, a GTTT-tail was added to each reverse (3’) primer (Brownstein *et al.* 1996). In addition, the forward (5’) primer of each locus was end-labelled with a fluorescent dye. Microsatellites were genotyped using the methods described by Vähä *et al.* (2007).
2.2.3 Breeding experiments
In the controlled crossings of brood fish (III–VI), the stripped batches of eggs from females were divided into two or more equally sized portions which were then artificially (in vitro) fertilized with milt from different males. Each family (female-male combination) was further divided into two to four incubation replicates. There were some differences in the applied (non-competitive) fertilization techniques between the experiments. In the fertilization trials of paper III, the amount of sperm was adjusted for each male according to the recorded spermatoctrit levels, so that an approximately equal number of sperm cells was used in all fertilizations. The pipetted milt droplets from each male were activated in the same volume of water and the mixtures of water and milt were then poured over each replicated egg batch. In the three other experiments (IV–VI), the milt was used randomly instead and ‘in excess’ to secure maximal fertilization rates in each family. Here, the ejaculates of the males were stripped straight onto larger egg portions and some water was quickly added afterwards. The family-specific replicates were formed by random sampling of eggs only after the fertilizations had occurred.

The incubations took place in family-specific containers (i.e. partitioned incubation trays (III) or free-floating cylinders (IV–VI) placed under running water) from which dead eggs were regularly counted and removed. Rearing temperatures were either constant (IV) or they followed the variations of the ambient water systems (Fig. 1).
Figure 1. Water temperatures during the experimental periods at the fisheries research stations of Taivalkoski (paper III), Enonkoski (paper V) and Paltamo (paper VI).

In each experiment, the survival (or mortality) to eyed-embryo stage (i.e. the proportion of remaining individuals from the initial number of eggs per replicate/family) was used as a primary measure of progeny quality. Furthermore, larval post-hatching body length and yolk-sac volume were used as additional components of fitness (III–V). These size measurements were determined from the digitally photographed samples using Image-Pro PLUS 3.0. In paper III, in addition, individual body masses were directly determined from the samples of newly-hatched fish.

For paper IV, a cross-classified breeding design (North Carolina II) was employed to specifically yield estimates for different parental influences. That is, the phenotypic data from the progenies of Lake Saimaa char were combined with information on relatedness and the observed variations in trait expressions were partitioned into effects attributable to different measurable factors, i.e. additive and non-additive genetic effects, as well as maternal effects. Furthermore, by exposing the experimental families to two constant environmental treatments, I also aimed to investigate whether or not the relative magnitudes of these underlying causal sources of variation differed between favourable (2 °C) and stressful (7 °C)
incubation temperatures. This may allow us to further elucidate whether or not there is any environmental dependence in sexual selection targeting either specific female-male crosses (‘compatible genes’) or intrinsically high-quality mating partners (‘good genes’), as well as to see, in a more general sense, the population’s selective responses and adaptability in the face of a novel environmental challenge.

In the other three breeding experiments (III, V, VI), the interest was, in particular, to test for the indirect paternally derived effects on reproductive success. In order to do this, the potentially confounding effects arising from maternal effects were controlled for by using half-sibling designs. The *a priori* expectations from these studies were that females who mated either polyandrously (*i.e.* exposing their eggs to the sperm competition of two rival males) or with a brightly/more intensely coloured male would gain fitness advantages, resulting on average in more viable and/or larger offspring compared to matings with single or less colourful males, respectively. In competitive mating trials, the mixtures of sperm from ten male pairs were used to fertilize the eggs of three females (III). Comparison groups were established from the progenies sired by the same males in a non-competitive situation. The sperm numbers were kept equal between both the fertilization treatments and the competing males. Furthermore, in the non-competitive (monandrous) matings, the progeny traits were examined in regard to the size category of the males (small *vs.* large) and sperm velocity.
3 Results and discussion

3.1 POPULATION- AND SEX-SPECIFIC DIFFERENCES IN BODY MORPHOLOGY (I)

Natural and sexual selection are often regarded as opposing evolutionary forces, with outcomes in the expression of phenotypic traits that can be highly divergent. Owing to their great functional significance, the morphological traits of animals, in particular, are not only prone to reflect adaptation to local environmental conditions (i.e. the obvious requirements of survival), but also the effects of intra-sexual competition and mate choice. In paper I, the general (external) morphological variations in the three Finnish Arctic charr populations were characterized at two life history stages, outside and during the breeding season. The populations represent the extremes of environmental growth potential in the wild (i.e. one stunted and two large-growing predatory charr). However, because the study fish were reared in a standardized hatchery environment, most of the detected within- and between-population size and shape variations were expected to be a result of genetically determined effects (see also Janhunen et al. 2010).

Prior to the spawning period, the studied charr populations (age 3+) exhibited highly divergent morphologies, presumably reflecting their different life-styles in the wild (i.e. habitat use, swimming ability and trophic ecology). However, significant convergence in the expression of morphological traits was found among the populations as a consequence of sexual maturation (age 4+). The mature males had more robust bodies (deeper lateral profile), relatively larger head dimensions (including mouth size) and longer pectoral fins than the mature females. The observed sexual dimorphism is consistent with other findings for salmonid species and is presumably explained by the different reproductive roles occupied by the sexes (Fleming and Gross 1994; Casselman and Schulte-Hostedde 2004; Fleming...
and Reynolds 2004; Monet et al. 2006). Also, in the case of Arctic charr, intra-sexual breeding competition in the form of agonistic behaviours is intense among the males (Fabricius and Gustafson 1954; Sigurjónsdóttir and Gunnarson 1989). Moreover, males are likely to maximize their reproductive success by seeking, courting and guarding with multiple mates, whereas females may benefit more from gamete production and expending energy to maximize the viability of their offspring (i.e. females possess a lower potential reproductive rate). It seems likely that the developmental changes in the head and external body structures are primarily favoured by intra-sexual selection: these traits are likely to enhance the acquisition of mating opportunities among males as they function as specialized weapons/shield in fighting, block the access of rivals to spawning females, increase speed and manoeuvrability and serve as a display of status and condition (Fleming and Gross 1994; Quinn and Foote 1994; Kokita and Mizota 2002). Furthermore, enlarged male structures may act as amplifiers, reinforcing the perceptiveness of some mating signals such as contrasting colour patterns and courtship rates (and vice versa) (Hasson 1997).

It is intriguing to notice that sexual selection has consistently favoured the persistence of certain sexual differences, irrespective of the differences under other selection pressures experienced by the populations in their natural environments. This also suggests that the occurrence of sexual dimorphism is a primitive feature that is maintained in ecologically diverse charr populations. In comparison with natural selection, sexual selection acting on (male) morphological traits can thus be viewed as a more unidirectional process across populations. However, given that the development and maintenance of most secondary sexual traits incur significant costs (Zahavi 1975; Cotton et al. 2004), and, furthermore, since Arctic charr individuals can remain reproductively active for a period of 15 years (Johnson 1980), the energetic investments during a given breeding season should be carefully traded off against future possibilities for reproduction. Therefore, the balancing patterns of natural and sexual selection can be expected to vary between
populations living in distinct habitats that subject them to different selection regimes.

1.1 SEXUAL SELECTION AND SPERM QUALITY (II, III)

3.2.1 Phenotype-linked fertility and ‘good sperm’ hypotheses
In resource-free mating systems females are often thought to solely derive indirect benefits that can result either from the intrinsic genetic quality of males or from interactions between maternal and paternal haplotypes. However, it has been suggested that the preferred males (such as those with elaborate secondary sexual traits), under these circumstances, would also directly benefit the female by increasing her fecundity through higher fertilization success (Sheldon 1994). Consistent with the phenotype-linked fertility hypothesis, the results presented in paper II illustrate a potential link between male ornamentation and sperm quality when the confounding effect of body size is statistically controlled (see also Pitcher et al. 2007). This finding is consequential as sperm swimming speed, in particular, is known to be a determining component of the competitive ability of sperm in Arctic charr (Liljedal et al. 2008), and it also largely contributes to fertilization success in other externally fertilizing species (Levitan 2000; Gage et al. 2004; Rudolfsen et al. 2008).

It is likely, however, that some of the positive associations between the intensity of the red breeding colouration and sperm velocity are contorted by the competitive interactions between males (see Liljedal et al. 2008; Pitcher et al. 2009). Differences in both sperm velocity and density have been found in relation to the dominant status of charr males: less competitive, subordinate individuals (which may be smaller and less colourful) are capable of rapidly compensating for their disfavoured reproductive role by increasing the investment in sperm quality (Liljedal and Folstad 2003; Rudolfsen et al. 2006). In another experiment, for example, I found that the small-sized males produced, on average, faster swimming sperm than the larger males (III). In Arctic charr, post-spawning sexual selection due to intense sperm competition has presumably imposed a
particularly strong selection on the quality of sperm motility, or on energetics (ATP synthesis), as inferred from the positive inter-dependence between sperm swimming velocity and sperm longevity (II; see also Kortet et al. 2004; Fitzpatrick et al. 2009; Pitcher et al. 2009). However, it is also conceivable that the pre- and post-spawning mechanisms of selection in this species are, to some extent, positively associated; that is, competitive fertilization success favours highly ornamented, attractive males. Such a mutually reinforcing pattern may arise, for example, when the pre- and post-copulatory traits reflect a male’s underlying condition (Simmons and Kotiaho 2002; Chargé et al. 2010).

It has been suggested that male secondary sexual traits may have evolved through female choice as indicators of the trade-off between ejaculate quality and immunological activity (Folstad and Skarstein 1997; Hillgarth et al. 1997; see also Chargé et al. 2010). Both support and disagreement over this view have been gained from studies on Arctic charr (Måsvær et al. 2004; Figenschou 2010). On the other hand, the observed positive relationship between carotenoid-based colouration and average sperm velocity (II) might also be mediated through the males’ capacity to protect their sperm (and the substrates responsible for ornamental pigmentation) from oxidative stress (Blount et al. 2001; Helfenstein et al. 2010). Then, females choosing to spawn with more colourful males would gain a direct fitness benefit by fertilizing their eggs with less oxidatively damaged sperm, thereby lowering the risk of infertility (Tremellen 2008; Velando et al. 2008). Two recent studies on fish showed that males whose diet was supplemented with carotenoids (astaxanthin) had a higher fertilization success than males with a carotenoid-limited diet (Ahmadi et al. 2006; Pike et al. 2009). Correspondingly, individuals with a superior ability to acquire or assimilate carotenoids from a common diet may be able to simultaneously increase both their breeding colouration and functional fertility. There could also be some non-pigmentary antioxidants (e.g. Pike et al. 2007) involved in providing the mechanistic link between sexual ornamentation and sperm quality metrics in Arctic charr.
When studying post-copulatory sexual selection, and in particular, the genetic basis of sperm competitive ability, the implications of male effects on embryo viability can be important (García-Gonzáles 2008). For example, oxidative damage to sperm DNA has been found to translate into deleterious mutations in the zygote (Tremellen 2008). Besides avoiding infertility directly, females would thus also gain indirect fitness benefits by producing offspring with a higher viability. Indeed, our findings from the non-competitive in vitro fertilization trials lend support to this prediction in accordance with the ‘good sperm’ hypothesis (Yasui 1997): sperm quality may be determined, to some extent, by differences in the intrinsic quality of males (III). Given that variations in resistance to oxidative stress may underlie variations in survival ability (Monaghan et al. 2009), a male’s heritable ability to resist oxidative stress offers a potential explanation for our finding in which sperm quality and offspring viability were associated. However, it is beyond the scope of this thesis to discuss the underlying mechanism(s) in any further detail, and more experimental studies are needed to establish the causes linking sperm quality to both male carotenoid-based colouration and offspring fitness in the Arctic charr.

3.2.2 Benefits of polyandry (sperm competition)
Both the phenotype-linked fertility hypothesis and the ‘good sperm’ hypothesis suggest that females are selected to be promiscuous (Sheldon 1994; Yasui 1997). By mating with more than one male, females may increase the likelihood of their eggs being fertilized by males who are genetically superior or compatible (García-Gonzáles and Simmons 2005). Alternatively, sperm competition could lead to a situation where only the highest quality sperm cells within each ejaculate become selected. I, together with my collaborators, demonstrated in paper III that paternally transmitted genetic (and potential non-genetic) benefits are likely to increase, on average, the fitness of polyandrous females. Embryo mortality during incubation was lower and post-hatching yolk reserves (but not body length) were larger among offspring from polyandrous matings relative
to those from monandrous matings. In our study, however, polyandry was caused by simultaneous sperm competition among male pairs. This essentially differs from the situation where females pursue extra-pair matings by actively changing their partner between different spawning acts (i.e. genetic bet-hedging; Yasui 1998). Thus, one could presume at first glance that the indirect benefits arising from sperm competition are solely side effects, rather than a consequence of sexual selection acting on the females themselves. However, the case is not necessarily that simple. It is possible, for example, that females could facilitate sperm competition by preferentially spawning in the presence of several males, as was shown for the European bitterling, *Rhodeus sericeus* (Candolin and Reynolds 2002).

Although in externally fertilizing fish species the females may often be unable to determine how many males take part in a single spawning act, they may not completely lose their control over the eggs after oviposition. In Arctic charr, for example, the effect of ovarian fluid on sperm velocity is highly dependent on the particular female-male combination (Urbach *et al.* 2005; III), suggesting that cryptic female choice, i.e. discrimination among mates at the gamete level, is an important post-spawning mechanism influencing the relative fertilization success of males under sperm competition (Skarstein *et al.* 2005b; Liljedal *et al.* 2008; Rosengrave *et al.* 2009). Conceivably, cryptic choice could also contribute to differential fertilization by sperm that produce more viable embryos, thereby reducing incubation failure.

### 3.2 Parental Effects in the Early Fitness Traits of Offspring (III–VI)

#### 3.3.1 Genetic and environmental sources of variation

The genetics of embryonic development are likely to be of crucial importance in fish owing to their high early mortality and the strong relationship between morphological traits (e.g. body size and remaining yolk reserves) and fitness during this period (Einum and Fleming 2000). It is likely that any
phenotypic variation present in early life stages experiences selection at an intensity greater than that at any later life stages. Furthermore, early developmental stages provide a reasonable period in which to investigate and understand the effects of parental background on fitness-related traits, since possible confounding effects due to social interactions, including density-dependent competition for food, can be ruled out.

The results of the present fertilization experiments clearly show that a large proportion of parentally induced variation in the early developmental success of Arctic charr offspring stems from maternal effects (III–VI). In contrast, the independent effects of sires are considerably weaker, though not totally absent. The strong female influences observed are consistent with a large body of work on other salmonid species (Heath et al. 1999; Nagler et al. 2000; Vøllestad and Lillehammer 2000; Berg et al. 2001; Pakkasmaa and Jones 2002) and can result from both genetic and non-genetic components (i.e. variations in egg size and composition). Figure 2 indicates that the ability of females to produce eggs of a more uniform size (lower coefficient of variation in egg diameter), in particular, can translate into the increased survival of progeny. This is most likely due to the capacity of females to more evenly distribute the essential yolk components among ova.

\[
\begin{align*}
A & \quad B \\
\text{Embryo survival} & \quad \text{CV of egg diameter}
\end{align*}
\]

\begin{align*}
A & \quad B \\
\text{Mean egg diameter (mm)} & \quad 0.015 \ 0.020 \ 0.025 \ 0.030 \ 0.035 \ 0.040 \ 0.045 \ 0.050 \ 0.055 \\
\text{CV of egg diameter} & \quad 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4
\end{align*}

\text{Figure 2. Linear regressions in which the arcsine square-root transformed embryo survivals to eyed stage are regressed on (A) the female-specific means (}r^2 = 0.059, n = 30, P = 0.195\text{) and (B) coefficients of variation of egg diameter (}r^2 = 0.164, n = 30, P = 0.027\text{). Egg sample size per female is 220. Survival values represent the total means obtained from two maternal half-sib families (paper V). M. Janhunen (unpublished data).}
The adaptive significance of maternal effects has only been recognized quite recently (Mousseau and Fox 1998; McAdam et al. 2002). Maternal effects have also been shown to contribute to several fitness components in salmonids (Hutchings 1991; Ojanguren et al. 1996; Einum and Fleming 1999, 2000; Einum 2003). Nevertheless, from the standpoint of quantitative genetics, maternal effects have been traditionally treated as a source of confusion, preventing the precise estimation of genetic parameters in early phenotypic traits. In the closely related brook trout, *Salvelinus fontinalis*, the ontogenetic decrease in predominant maternal effects coupled with concurrent increases in paternal effects have been shown to be most pronounced at the transition point between endogenous and external feeding (Perry et al. 2004).

In paper IV, a cross-classified breeding design was employed to specifically disentangle the sources of genetic and environmental variances in three fitness-related traits: survival to eyed stage, larval post-hatching length and yolk size. Here, the contributions of additive, maternal and non-additive components to total phenotypic variances were estimated on the basis of the observed male, female and male × female effects. Besides referring to strong maternal effects, the results in paper IV also reveal significant interactions between parental genotypes. This indicates that genetic compatibility plays an important role in offspring viability and growth during the early stages of development in the Arctic charr (see also Pakkasmaa et al. 2006). The non-additive genetic component tends to be larger for the traits closely related to fitness (Crnokrak and Roff 1995), whereas the heritability estimates are generally low (Mousseau and Roff 1987; Carlson and Seamons 2008). However, fitness traits may harbour moderate levels of additive genetic variation due to the multiple genes affecting them (Merilä and Sheldon 1999). The fact that I found a significant heritability estimate based on the sire component of variance in larval post-hatching length implies that this early life history trait, in particular, is open for direct selection (IV).

Paper IV further demonstrates how the parental effects on embryonic viability and growth may vary within a charr
population in two environments of a different quality, i.e. at low (favourable) and abnormally high (stressful) temperatures. Considering the occurrence of critical developmental events in the embryos (e.g. organogenesis), which are absent or not as comprehensively present in later life history stages, the sensitivity of early developmental stages to any environmentally induced adverse effects is not a surprise. Even though early developmental performance is highly influenced by the thermal environment, genotypes appeared to buffer differently against its effects. Nevertheless, the obtained results suggest that both the additive genetic contribution of the sires and maternal effects diminish when Arctic charr offspring are subjected to thermal stress. When conditions are unfavourable, sensitivity to environmentally induced variation tends to increase due to impaired canalization, decreasing the importance of heritable and maternal components as a source of adaptive fitness variation (Hoffmann and Merilä 1999; Charmantier and Garant 2005). In contrast, the relative proportion of dominance variation in larval size traits was found to substantially increase at the stressful temperature (IV), suggesting that the significance of genetic compatibility for embryonic growth may be more pronounced under adverse environmental conditions. Because families clearly differ in their responses to different thermal regimes, exposure to increased temperatures is likely to strengthen the selection pressure on certain genotypes, thereby leading to reduced genetic diversity within a population. Generally speaking, a more comprehensive understanding of the changes involved in parental effects would be necessary in order to evaluate the implications of, and adaptations to, natural and human-induced environmental changes.

As with any quantitative genetic study, the results obtained in paper IV cannot be generalized across populations, and, also, some additive genetic variations may have been lost due to hatchery rearing (Carlson and Seamons 2008). For example, the fact that no male effect (additive genetic variation) could be found on embryonic survival rates measured at the eyed stage of development (IV) seems to be in disagreement with the
finding of paper III (i.e. males with a higher sperm velocity sired offspring with a lower rate of mortality). On the other hand, this apparent discrepancy may not only be explained by the different origins of the study populations, but there might also be some causes inherent to the experimental procedures, such as differences in the fertilization techniques applied. In the fertilization trials of paper III, the amount of sperm was equalized among the males, whereas in the three other breeding experiments (IV–VI), sperm was used excessively in order to ensure maximal fertilization rates in each mating pair. It is possible that the observed variations in the numbers of eggs that reached the eyed-ova stage in paper III involved, to a greater extent, differences in fertilization success. For example, in their recent mating trials on sea urchins, *Heliocidaris erythrogramma*, Evans et al. (2007) controlled the sperm densities among the males and found that fertilization rates, which were influenced by intrinsic male and female effects, were not associated with subsequent embryo viability, which, in turn, was strongly influenced by interacting paternal effects.

A strong current debate prevails over the net fitness consequences of sexual selection by female mate choice, especially regarding animal species with non-resource-based mating systems. The present findings suggest that the genetic architecture of early survival and growth in Arctic charr is intricate (IV), and that optimal mate selection based on both additive and non-additive genetic effects may significantly contribute to these traits. Hence, the pre-spawning preference of females for certain mating partners (i.e. intrinsically good-quality and/or genetically compatible males) could also be expected to yield indirect fitness benefits that will manifest themselves in enhanced fertilization success and offspring viability. When specifically testing whether or not the offspring survivorship would be higher when females were paired with males they supposedly preferred in a simple ‘free choice’ test (i.e. without social constraints) compared to males they did not prefer, no statistical difference was found in this respect (VI). Although the variation arising from maternal effects was considerable, there were also significant effects of male identity.
on offspring viability, suggesting that females could have perhaps done better, on average, by consistently choosing a proper alternative. On the other hand, our inability to detect differences in viability between the half-sib families sired by preferred and non-preferred males could be partly due to the small sample size ($n = 12$), as well as to the somewhat conservative experimental design; the females were only allowed to discriminate between two males and any physical interaction within these triplets of fish was prevented. Furthermore, it remains unknown to what extent female preference in such a dichotomous choice experiment predicts sexual preference and thus the final mate choice decision in charr.

3.3.2 The role of ornamental colouration
The presence of additive genetic variation, especially with respect to larval post-hatching length (IV), suggests that paternally derived ‘good genes’ can be of major biological importance under favourable environmental conditions. Nevertheless, our experiment with maternal half-sibships indicated that the genetic variation related to offspring early developmental traits is not consistently associated with the degree of male ornamental skin colouration (V, VI): on average, bright-coloured males did not produce more viable or better growing offspring when compared with their pale-coloured counterparts. This is also partly contradictory to the recent findings by Eilertsen et al. (2009) and indicates that paternal redness cannot be seen as a general predictor for the early quality of offspring in Arctic charr.

When both selection mechanisms (i.e. ‘good genes’ and ‘compatible genes’) operate in parallel, selection for genetic compatibility can undermine the directional selection on male secondary sexual traits (Colegrave et al. 2002). This should further weaken the inter-relationship between ornamental expression and heritable quality (Rudolfsen et al. 2005; Pitcher and Neff 2007; Kekäläinen et al. 2010). Moreover, because carotenoid-based colouration can have a size- or condition-dependent expression in males (II; Barber et al. 2000; McGraw et
al. 2005b; Sparkes et al. 2008), being influenced not only by genetic quality but also by certain environmental factors, the correlation between male redness and offspring quality may not occur across all individuals. For example, in their comparable half-sib study on Arctic charr, Eilertsen et al. (2009) concluded that the effect of paternal colouration on offspring post-hatching length interacts with male body size; the effect was only present for smaller males. Figure 3 presents the unpublished results from the colour and size measurements that I took from every sexually matured individual in a rearing lot (a cultivated brood stock) over two consecutive breeding seasons. It shows that the relationships between carotenoid-based colouration and fish growth and, in particular, body condition (an indirect measure of a fish’s health and nutritional status) may also apply to Arctic charr, although they are most likely dependent on both the age and the sex of the fish.

In the present half-sib studies (V, VI), some of the potentially confounding effects (age, body size and rearing environment) were controlled between males in each half-sibling family pair, and thus the within-pair differences in colouration were most likely to be genetic (such as intrinsic differences in the capacity to assimilate carotenoids (astaxanthin) from food and mobilize them further for ornamental and self-maintenance purposes). However, in cultivated fish the causes of variation in colouration may be somewhat different from those in the environments where the signalling system has evolved. In wild charr populations the costs of ornamental development may also be directly related to carotenoid acquisition, as many of the carotenoid-carrying crustaceans serve as intermediate hosts to common fish parasites (Skarstein and Folstad 1996; Skarstein et al. 2005a). Likewise, carotenoid availability in the diet of cultivated fish is likely to be different from those present in nature.
Figure 3. The relationships of abdominal red colouration (chroma, $C^*$) with (A) total fish length and (B) condition factor (Fulton’s $K$; $100\times g/cm^3$) among the sexually mature Lake Saimaa Arctic charr in two successive years. Colouration value is an average derived from the three replicate measurements of two locations (i.e. behind the tip of the pectoral fin and above the anal fin). The only statistically significant correlation was found between the redness and condition among five-year-old males ($Pearson’s r = 0.358, n = 65, P = 0.003$). M. Janhunen (unpublished data).

The strength of the present breeding studies undoubtedly lies in the consistency of circumstances across individuals and families. Controlled hatchery experiments enabled us to control for unknown environmental variation, which may influence both the parents themselves and their progeny. Nevertheless, one could speculate whether or not the genetic benefits linked to male colouration are largely context-dependent and therefore were not revealed under our experimental conditions (see e.g. Welch 2003). Inferring from the results of paper IV, however, it seems unlikely that the ‘good genes’ effects due to sires would have become more obvious under more challenging conditions,
in particular where the survival change of lower quality embryos is notably smaller.

It has been proposed that the expression of some ornamental traits may not be associated with specific beneficial alleles but rather that it reflects the overall genetic diversity and/or inbreeding level of individuals (the ‘good-genes-as-heterozygosity’ hypothesis; Brown 1997) – either directly or indirectly through associations between heterozygosity and other fitness parameters (e.g. disease resistance; Reid et al. 2005). This would further offer an alternative solution for the debate on how continuous directional mating with elaborately ornamented males can contribute to the persistence of genetic variation within populations (Neff and Pitcher 2008). Using seven polymorphic microsatellite markers in our Arctic charr data, I found no evidence that the intensity of the red breeding colouration would reflect the degree of individual genetic variability in either of the sexes (V). Thus, the directional mating preference for this ornamental trait may not yield genetic benefits in terms of producing more outbred/genetically diverse offspring (Neff and Pitcher 2008). On the other hand, it is difficult to assess for certain how well the estimators based on a relatively small number of microsatellite loci actually reflect the genome-wide variability of individuals (Slate et al. 2004).

Our findings from the dichotomous mate choice trials (VI) indicate that female charr do not consistently bias their preference towards more colourful males, but there may also be some other phenotypic criteria involved in the assessment of potential partners (including the cues that reveal information about genetic compatibility). Different females often prioritize different male signals, thereby focusing on different aspects of mate quality (Lehtonen and Lindström 2008; Lehtonen et al. 2010). As already noted above, a considerable part of the variation in female preference may be explained by the pursuit of non-additive genetic benefits (genetic compatibility). Furthermore, male attractiveness (and hence female choosiness) may be largely dependent on a particular environmental context (Qvarnström 2001; Suk and Chloe 2008), and such an adaptive variation in mate preferences is also apt to maintain, over time,
the permanent genetic variation in male sexual traits. For example, in guppies, *Poecilia reticulata*, the preference of females for male carotenoid-based colouration increases as carotenoid intake (availability) declines (Grether et al. 2005). Correspondingly, the responsiveness of our study females to the carotenoid ornamentation of males may have decreased in a culture environment where the supply of carotenoid-rich food was constantly abundant.

The central feature of the present results is the pervasive role of maternal effects as a determinant of variation in progeny traits. The findings further indicate that the red spawning dress of females can also be an informative trait when it comes to the reproductive quality of individuals. Indeed, female carotenoid-based colouration was found to negatively correlate with both egg production (relative gonad mass) and offspring viability (V, VI). Thus, the investment of females in elaborate sexual ornamentation appears to occur at the expense of fertility. Confirming the reliability of this assumption, a similar negative relationship between female colour intensity and offspring early viability was found in two different Arctic char populations. In oviparous animals like fish, carotenoids are known to be a resource that is largely responsible for non-genetic maternal effects (egg quality) (Craik 1985; Blount et al. 2000). As a consequence, there is likely to be a strong selection pressure among females to store large amounts of valuable carotenoid pigments in the eggs, and this might constrain their ability to develop as intense a breeding colouration as is found in males. Maternal provisioning of carotenoids to eggs has been linked to various fitness consequences, such as enhanced fertilization and hatching success, and juvenile resistance to diseases and oxidative stress (Palace et al. 1998; Ahmadi et al. 2006; Sawanboonchun et al. 2008; Tyndale et al. 2008). There are also some experimental evidences available to show that carotenoid availability imposes the trade-off between female ornamentation and egg quality or egg number (Nordeide et al. 2006; Morales et al. 2009). The results in paper V support the idea that fecundity costs of sexually selected traits may limit the elaboration of ornamental traits in females (Fitzpatrick et al. 1995; Chenoweth
et al. 2006), although the existence of any trade-off between ornamentation and eggs cannot be definitely concluded and more research on this topic is needed.

A key prediction of the direct (mutual) selection hypothesis is that the same secondary sexual traits in both sexes convey information about some aspect of individual quality, and, consequently, are a result of assortative mating patterns (Kraaijeveld et al. 2007; Baldauf et al. 2009). Because charr males seem to gain no apparent benefits from their choice of more colourful females, the direct selection by males seems to be an incorrect explanation for the existence of female ornamentation in this species (see also Nordeide et al. 2008). Instead, the present findings, together with sexual dichromatism (i.e. female charr have on average a less intense colouration when compared to males), leave open an alternative possibility that female ornamentation has rather evolved as a non-adaptive, genetically correlated trait resulting from direct sexual selection on males (Lande 1980; Kraaijeveld et al. 2007). This view is also supported by the notion that the strength of carotenoid-based colouration is likely a reliable measure of body condition only in males (Fig. 3; Nordeide et al. 2008). Conceivably, the carotenoid-based ornamentation of Arctic charr could even be subject to sexual conflict (or to sexually antagonistic selection; Wedell et al. 2006), being selectively favoured in males but selected against in females.
4 Conclusions and future prospects

This thesis has mainly highlighted some evolutionary perspectives, in particular regarding the reproductive characteristics in an externally fertilizing fish with no parental care. Within the species, different forms of selection seem to have created a complex set of phenotypic adaptations that coordinate and constrain the breeding attempts of individuals. During their spawning season, Arctic charr further exhibit pronounced sexual dimorphism, for example, in terms of external morphology (I) and colouration (V). Basically, this phenotypic divergence is likely to reflect differences in the intensity of sexual selection between the sexes as well as differences in the trade-off between natural and sexual selection. The more exaggerated secondary sexual traits of males are consistent with the greater variation of reproductive success usually observed in that sex.

The mutual, yet sexually dichromatic expression of the carotenoid-based ornamentation can be indicative, to some extent, of individual reproductive quality (fertility) in both males and females. Nonetheless, the relationship between the primary and secondary sexual traits (i.e. the information content of the carotenoid colouration) appears to be opposing between the sexes: colourful males may advertise their good sperm quality and thus fertilizing ability (II), whilst in females the intense ornamental colouration may rather be a sign of decreased investment in offspring production (low number and quality of eggs; V, VI). Arctic charr offers a potential example of resource-free mating systems where direct fertility benefits for the females might drive, at least in part, mate choice based on male ornamentation. In other words, by assessing male breeding colouration, female charr could gather reliable information on
the current phenotypic condition of potential mates and improve their reproductive success through efficient fertilization. Instead, the observed negative association between female colouration and reproductive quality is in disagreement with the predictions of the direct (mutual) selection hypothesis, and rather lends support to the assumption that the fecundity costs involved in sexually selected traits may, in some cases, constrain the degree of elaboration of ornamental traits (and the intensity of sexual selection) in females.

The present breeding studies indicate that offspring viability and post-hatching size as integral parts of the overall developmental success of Arctic charr are complex quantitative traits that rely on both the independent effects of mating partners and their reciprocal genetic interactions. This conforms to the increasing notion that sexual selection, in its various forms, can simultaneously facilitate both the intrinsically high-quality and compatible genes (III, IV, VI). The relevance of genetic compatibility effects in offspring fitness may weaken directional selection on male carotenoid-based colouration, thus preventing the exhaustion of genetic variation in this ornamental trait (i.e. one possible explanation for the lek paradox).

Furthermore, the results do not support the conjecture that differences in the ornamental expression of male charr would be consistently translated into differences in offspring quality, as measured by their early developmental performance (V, VI). On the other hand, since a male’s sperm motility appears to positively relate to his offspring viability (III), we cannot totally exclude the possibility that the directional mate preference for certain phenotypic correlates (e.g. a combination of small body size and relatively bright colouration) could yield observable indirect (genetic) benefits as well. Even though pre-mating selective mechanisms are likely to play an important role in terms of the offsprings’ early performance, our experimental approach to show this did not produce an expected result: females accrued no indirect viability benefits for their offspring when they were paired with the male for which they showed a pre-spawning preference (VI). Instead, female charr increased
their fitness by exposing their eggs to multiple males in a given spawning event (III); such a polyandrous mating due to sperm competition may raise the mean reproductive output of females above the average value obtained from single matings.

Finally, the findings of this work have addressed some fundamental questions regarding the genetic and phenotypic aspects of sexual (and natural) selection in Arctic charr, but, at the same time, they have raised several new intriguing research questions. A multitude of selective processes can account for the evolutionary trajectories of male and female reproductive traits (and the co-evolution between them), including various natural and sexual selection regimes, life-history trade-offs and genotype-environment interactions. However, the single mechanisms are not easily distinguishable, and we still have a highly limited knowledge of their relative importance for the adaptability of populations. Referring to the findings of paper I, for example, it would be interesting to discover with subsequent studies to what extent sexual dimorphism in morphological features varies across environments that differ in respect of resource availability (phenotypic plasticity and population-specific reaction norms). Further investigations are also required to gain a deeper understanding about the actual role of other visual, assumedly sexually selected traits (such as carotenoid-based ornamentation) in the breeding behaviour of Arctic charr, and about the underlying causes that mainly promote the evolution and maintenance of these traits in either or both sexes (female mate choice vs. male-male competition). The predictions based on the intrinsic male quality also need more empirical support from the breeding experiments that can identify and unequivocally separate the fertilization success of males from their ability to influence the development of embryos. When evaluating the genetic basis of mate choice and other forms of selection, considerations should also be extended to multiple components of offspring fitness. It remains to be examined, for example, whether paternal (and maternal) sexual colouration functions as an indicator of additive genetic variation in disease resistance, health and condition, or whether it is associated with some other fitness consequences that are progressive and only
manifest themselves in the later life history stages of offspring. To better understand the selective pressures on reproductive success, mating success and the patterns of sexual selection in this species, these questions would be worth addressing in the future.
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Reproductive traits are strong determinants of fitness and are therefore subject to particularly intense selection. The interplay between natural and sexual selection shapes the evolution of sex-specific characteristics that ultimately determine the breeding success of individuals. This thesis addresses some fundamental questions regarding the selective factors in traits that may be of importance for the reproductive biology of Arctic charr, an externally fertilizing fish species with a non-resource-based mating system.