FORESTRY AND NATURAL SCIENCES

Matti Janhunen

Phenotypic and genetic variations in reproduction-associated traits in Arctic charr, Salvelinus alpinus

UNIVERSITY OF
EASTERN FINLAND

MATTI JANHUNEN

Phenotypic and genetic variations in reproduction-associated traits in Arctic charr, Salvelinus alpinus L.

Academic Dissertation
To be presented by permission of the Faculty on Sciences and Forestry for public
examination in the Auditorium N100 in Natura Building at the University of Eastern
Finland, Joensuu, on April, 29, 2011, at 12 o'clock noon.

Department of Biology

Author's address: Finnish Game and Fisheries Research Institute
Joensuu Game and Fisheries Research
Yliopistokatu 6
80100 JOENSUU
FINLAND
email: matti.janhunen@rktl.fi

Supervisors: Professor Raine Kortet, Ph.D.
University of Eastern Finland
Department of Biology
P.O.Box 111
80101 JOENSUU
FINLAND
email: raine.kortet@uef.fi

Research Manager Nina Peuhkuri, Ph.D.
Finnish Game and Fisheries Research Institute
Viikinkaari 4
P.O.Box 2
00791 HELSINKI
FINLAND
email: nina.peuhkuri@rktl.fi

Senior Fisheries Biologist Jorma Piironen, Ph.D.
Finnish Game and Fisheries Research Institute
Joensuu Game and Fisheries Research
Yliopistokatu 6
80100 JOENSUU
FINLAND
email: jorma.piironen@rktl.fi

Reviewers: Senior Lecturer Iain Barber, Ph.D.
University of Leicester
Department of Biology
Adrian Building
LE1 7RH LEICESTER
UNITED KINGDOM
email: ib50@le.ac.uk

Assistant Professor Ola Svensson, Ph.D.
University of Gothenburg
Department of Zoology
P.O.Box 463
40530 GOTHENBURG
SWEDEN
email: ola.svensson@zool.gu.se

Opponent: Professor Jarle Tryti Nordeide, Ph.D.
University of Nordland
Faculty of Biosciences and Aquaculture
P.O.Box 1490
8049 BODØ
NORWAY
email: jarle.nordeide@uin.no

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.

Acknowledgements

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.

I   Janhunen M, Peuhkuri N and Piironen J. Morphological variability among three geographically distinct Arctic charr (*Salvelinus alpinus* L.) populations reared in a common hatchery environment. *Ecology of Freshwater Fish 19: 108–116, 2009.*

II   Janhunen M, Rudolfsen G, Kekäläinen J, Figenschou L, Peuhkuri N and Kortet R. Spawning colouration and sperm quality in a large lake population of Arctic charr (Salmonidae: *Salvelinus alpinus* L.). *Biological Journal of the Linnean Society 98: 794–802, 2009.*

III   Kekäläinen J, Rudolfsen G, Janhunen M, Figenschou L, Peuhkuri N, Tamper N and Kortet R. Genetic and potential non-genetic benefits increase offspring fitness of polyandrous females in non-resource based mating system. *BMC Evolutionary Biology 10: 20, 2010.*

IV   Janhunen M, Piironen J and Peuhkuri N. Parental effects on embryonic viability and growth in Arctic charr *Salvelinus alpinus* at two incubation temperatures. *Journal of Fish Biology 76: 2558–2570, 2010.*

V   Janhunen M, Peuhkuri N, Primmer CR, Kolari I and Piironen J. Does breeding ornamentation signal genetic quality in Arctic charr, *Salvelinus alpinus*? *Evolutionary Biology 38: 68–78, 2011.*

VI   Janhunen M, Kekäläinen J, Kortet R, Hyvärinen P and Piironen J. No evidence for an indirect benefit from female mate preference in Arctic charr, *Salvelinus alpinus*, but female ornamentation decreases offspring viability. *Biological Journal of the Linnean Society, 2011.* In press.

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.

Contents

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 binge 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 pigmentations 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 (Brännäs 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, un 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.

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

* 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 spermatocrit 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).
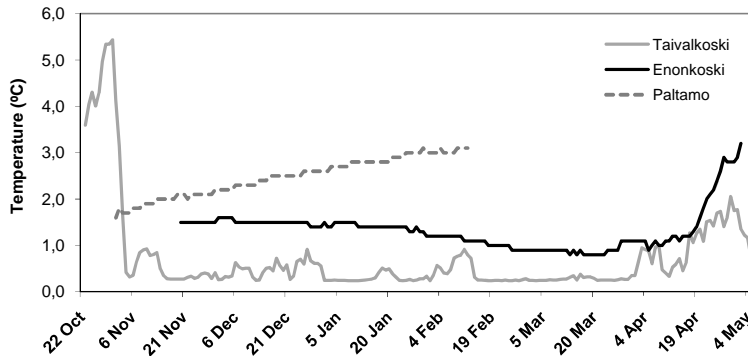
Figure 1. *Water temperatures during the experimental periods at the fisheries research stations of Taivalkoski (paper III), Enonkoski (paper V) and Paltamo (paper VI).*

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 charr 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 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.
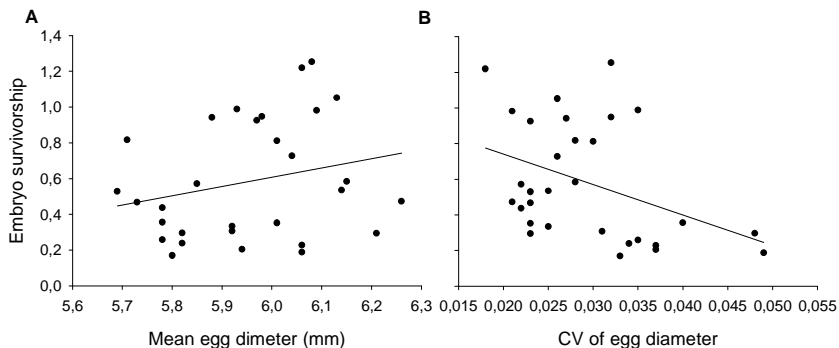
Figure 2. Linear regressions in which the arcsine square-root transformed embryo survivals to eyed stage are regressed on (A) the female-specific means ($r^2 = 0.059$, $n = 30$, $P = 0.195$) and (B) coefficients of variation of egg diameter ($r^2 = 0.164$, $n = 30$, $P = 0.027$). Egg sample size per 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.
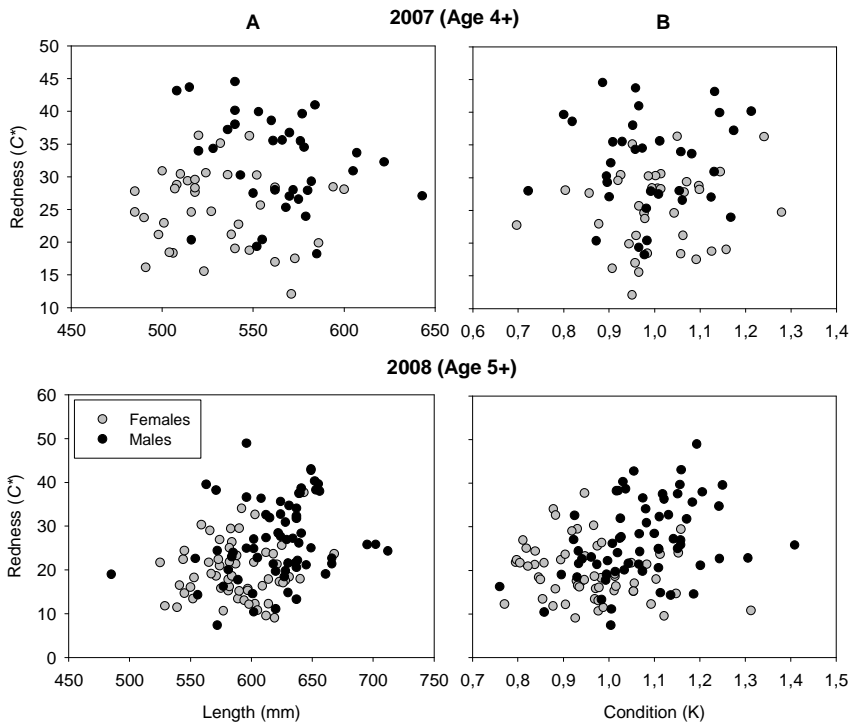
Figure 3. The relationships of abdominal red colouration (chroma, C*) with (A) total fish length and (B) condition factor (Fulton's K; $100 \times g \times cm^{-3}$) among the sexually mature Lake Saimaa Arctic charr in two successive years. Colouration value is an average derived from the three replicate 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 charr 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.

REFERENCES

Aguirre WE and Akinpelu O 2010. Sexual dimorphism of head morphology in three-spined stickleback *Gasterosteus aculeatus*. *J Fish Biol* 77:802–821.

Ahmadi MR, BazyarAA, Safi S, Ytrestøyl T and Bjerkeng B 2006. Effects of dietary astaxanthin supplementation on reproductive characteristics of rainbow trout (*Oncorhynchus mykiss*). *J Appl Ichthyol* 22:388–394.

Alatalo RV, Kotiaho J, Mappes J and Parri S 1998. Mate choice for offspring performance: major benefits or minor costs? *Proc R Soc B* 265:2297–2301.

Alonso-Alvarez C, Bertrand S, Devevey G, Gaillard M, Prost J, Faivre B and Sorci G 2004. An experimental test of dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *Am Nat* 164:651–659.

Andersson M 1994. Sexual selection. Princeton University Press, Princeton, USA.

Andersson M and Simmons LW 2006. Sexual selection and mate choice. *Trends Ecol Evol* 21:296–302.

Baeta R, Faivre B, Motreuil S, Gaillard M and Moreau J 2008. Carotenoid trade-off between parasitic resistance and sexual display: an experimental study in the blackbird (*Turdus merula*). *Proc R Soc B* 275:427–434.

Baldauf SA, Kullmann H, Schroth SH, Thünken T and Bakker TCM 2009. You can't always get what you want: size assortative mating by mutual mate choice as a resolution of sexual conflict. *BMC Evol Biol* 9:129.

Barber I, Arnott SA, Braithwaite VA, Andrew J, Mullen W and Huntingford FA 2000. Carotenoid-based sexual colouration and body condition in nesting male sticklebacks. *J Fish Biol* 57:777–790.

Barber I, Arnott SA, Braithwaite V, Andrew J and Huntingford FA 2001. Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proc R Soc B* 268:71–76.

Bateman A J 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2:349–368.

Bebak J, Hankins JA and Summerfelt ST 2000. Effect of water temperature on survival of eyed eggs and alevins of Arctic char. *N Am J Aquacult* 62:139–143.

Berg OK, Hendry AP, Svendsen B, Bech C, Arnekleiv JV and Lohrmann A 2001. Maternal provisioning of offspring and the use

of those resources during ontogeny: variation within and between Atlantic salmon families. *Funct Ecol* 15:13–23.

Bertrand S, Faivre B and Sorci G 2006. Do carotenoid-based sexual traits signal the availability of non-pigmentary antioxidants? *J Exp Biol* 209:4414–4419.

Birkhead TR and Pizzari T 2002. Postcopulatory sexual selection. *Nat Rev Gen* 3:262–273.

Blair GR, Rogers DE and Quinn TP 1993. Variation in life history characteristics and morphology of sockeye salmon in the Kvichak River system, Bristol Bay, Alaska. *Trans Am Fish Soc* 122:550–559.

Blount JD, Houston DC and Møller AP 2000. Why egg yolk is yellow? *Trends Ecol Evol* 15:47–49.

Blount JD, Møller AP and Houston DC 2001. Antioxidants, showy males, and sperm quality. *Ecol Lett* 4:393–396.

Blount JD, Surai PF, Nager RG, Houston DC, Møller AP, Trewby ML and Kennedy MW 2002. Carotenoids and egg quality in the lesser blackbacked gull *Larus fuscus*: a supplemental feeding study of maternal effects. *Proc R Soc B* 269:29–36.

Blount JD, Metcalfe NB, Arnold KE, Surai PF, Devevey GL and Monaghan P 2003. Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch. *Proc R Soc B* 270:1691–1696.

Brawner WR, Hill GE and Sundermann CA 2000. Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *Auk* 117:952–963.

Brown JL 1997. A theory of mate choice based on heterozygosity. *Behav Ecol* 8:60–65.

Brownstein MJ, Carpten JD and Smith JR 1996. Modulation of non-templated nucleotide addition by taq DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques* 20:1004–1010.

Brännäs E and Wiklund B-S 1992. Low temperature growth potential of Arctic charr and rainbow trout. *Nord J Freshw Res* 67:77–81.

Butler MA, Sawyer SA and Losos JB 2007. Sexual dimorphism and adaptive radiation in *Anolis* lizards. *Nature* 447:202–205.

Candolin U and Reynolds JD 2002. Why do males tolerate sneakers? Tests with the European bitterling, *Rhodeus sericeus*. *Behav Ecol Sociobiol* 51:146–152.

Carlson SM and Seamons TR 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. *Evol Appl* 1:222–238.

Casselman SJ and Schulte-Hostedde AI 2004. Reproductive roles predict sexual dimorphism in internal and external morphology of lake whitefish, *Coregonus clupeaformis*. *Ecol Freshw Fish* 13:217–222.

Chargé R, Jalme MS, Lacroix F, Cadet A and Sorci G 2010. Male health status, signalled by courtship display, reveals ejaculate quality and hatching success in a lekking species. *J Anim Ecol* 79:843–850.

Charmantier A and Garant D 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proc R Soc B* 272:1415–1425.

CIE (International Commission on Illumination) 1986. Colorimetry. CIE Publication No. 15.2., Vienna.

Crnokrak P and Roff DA 1995. Dominance variance: associations with selection and fitness. *Heredity* 75:530–540.

Chenoweth SF, Doughty P and Kokko H 2006. Can non-directional male mating preferences facilitate honest female ornamentation? *Ecol Lett* 9:179–184.

Clutton-Brock TH and Vincent ACJ 1991. Sexual selection and the potential reproductive rates of males and females. *Nature* 351:58–60.

Clutton-Brock T 2007. Sexual selection in males and females. *Science* 318:1882–1885.

Colegrave N, Kotiaho JS and Tomkins JL 2002. Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. *Evol Ecol Res* 4:911–917.

Cotton S, Fowler K and Pomiankowski A 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc R Soc B* 271:771–783.

Craik JCA 1985. Egg quality and egg pigment content in salmonid fishes. *Aquaculture* 47:61–88.

Darwin C 1871. The descent of man and selection in relation to sex. Murray, London, UK.

Dawson RD and Bortolotti GR 2006. Carotenoid-dependent colouration of male American kestrels predicts ability to reduce parasitic infections. *Naturwissenschaften* 93:597–602.

Delph LF 2005. Processes that constrain and facilitate the evolution of sexual dimorphism. *Am Nat* (Suppl.) 166:S1–S4.

de March BGE 1995. Effects of incubation temperature on the hatching success of Arctic char eggs. *Prog Fish-Cult* 57:132–136.

Eberhard WG 1996. Female control: sexual selection by cryptic female choice. Princeton Univ Press, Princeton, USA.

Eilertsen EM, Bårdsen B-J, Liljedal S, Rudolfsen G and Folstad I 2009. Experimental evidence for paternal effects on offspring growth rate in Arctic charr (*Salvelinus alpinus*). *Proc R Soc B* 276:129–136.

Einum S 2003. Atlantic salmon growth in strongly food-limited environments: Effects of egg size and paternal phenotype. *Environ Biol Fishes* 67:263–268.

Einum S and Fleming IA 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proc R Soc B* 266:2095–2100.

Einum S and Fleming IA 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution* 54:628–639.

Elliott JM and Klemetsen A 2002. The upper critical thermal limits for alevins of Arctic charr from a Norwegian lake north of the Arctic circle. *J Fish Biol* 60:1338–1341.

Elvingson P and Nilsson J 1994. Phenotypic and genetic parameters of body and compositional traits in Arctic charr, *Salvelinus alpinus* (L.). *Aquacult Res* 25:677–685.

Endler JA and Basolo AL 1998. Sensory ecology, receiver biases and sexual selection. *Trends Ecol Evol* 13:415–420.

Evans JP 2010. Quantitative genetic evidence that males trade attractiveness for ejaculate quality in guppies. *Proc R Soc B* 277:3195–3201.

Evans JP, Zane L, Francescato S and Pilastro A 2003. Directional postcopulatory sexual selection revealed by artificial insemination. *Nature* 421:360–363.

Evans JP, Garzía-Gonzáles F and Marshall DJ 2007. Sources of genetic and phenotypic variance in fertilization rates and larval traits in a sea urchin. *Evolution* 61:2832:2838.

Evans JP, Kelley JL, Bisazza A, Finazzo E and Pilastro A 2004. Sire attractiveness influences offspring performance in guppies. *Proc R Soc B* 271:2035–2042.

Fabricius E 1953. Aquarium observations on the spawning behavior of the char, *Salmo alpinus*. *Rep Inst Freshw Res Drottningholm* 34:14–48.

Fabricius E and Gustafson K 1954. Further aquarium observations on the spawning behaviour of the char, *Salmo alpinus*. *Rep Inst Freshw Res Drottningholm* 35:58–104.

Figenschou L 2010. Reproductive behaviour and sex trait allocations in an external fertilizer- the Charr (*Salvelinus alpinus*). PhD Thesis, University of Tromsø, Norway.

Figenschou L, Folstad I and Liljedal S 2004. Lek fidelity of male Arctic charr. *Can J Zool* 82:1278–1284.

Fisher RA 1930. The genetical theory of natural selection. Clarendon Press, Oxford, UK.

Fitze PS, Tschirren B, Gasparini J and Richner H. 2007. Carotenoid-based plumage colors and immune function: is there a trade-off for rare carotenoids? *Am Nat* 169:S137–S144.

Fitzpatrick S, Berglund A and Rosenqvist G 1995. Ornaments or offspring: costs to reproductive success restrict sexual selection processes. *Biol J Linn Soc* 55:251–260.

Fitzpatrick JL, Montgomerie R, Desjardins JK, Stiver KA, Kolm N and Balshine S 2009. Female promiscuity promotes the evolution of faster sperm in cichlid fishes. *Pros Natl Acad Sci USA* 106:1128–1132.

Fleming IA and Gross MR 1994. Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* 48:637–657.

Fleming IA and Reynolds JD 2004. Salmonid breeding systems. In: Hendry AP and Stearns SC (eds). Evolution illuminated: salmon and their relatives. Oxford Univ Press, New York, USA, pp. 264–294.

Folstad I and Karter AJ 1992. Parasites, bright males, and the immunocompetence handicap. *Am Nat* 139:603–622.

Folstad I and Skarstein F 1997. Is male germ line control creating avenues for female choice? *Behav Ecol* 8:109–112.

Fricke C, Bretman A and Chapman T 2010. Sexual conflict. In: Westneat DF and Fox CW (eds). Evolutionary behavioural ecology. Oxford Univ Press, New York, USA, pp. 400–415.

Fromhage L, Kokko H and Reid JM 2009. Evolution of mate choice for genome-wide heterozygosity. *Evolution* 63:684–694.

Gage MJG, Macfarlane CP, Yeates S, Ward RG, Searle JB and Parker GA 2004. Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. *Curr Biol* 14:44–47.

García-Gonzáles F 2008. Male genetic quality and the inequality between paternity success and fertilization success: consequences for studies of sperm competition and the evolution of polyandry. *Evolution* 62:1653–1665.

García-Gonzáles F and Simmons LW 2005. The evolution of polyandry: intrinsic sire effects contribute to embryo viability. *J Evol Biol* 18:1097–1103.

Gladbach A, Gladbach DJ, Kempenaers B and Quillfeldt P 2010. Female-specific colouration, carotenoids and reproductive investment in a dichromatic species, the upland goose *Chloephaga picta leucoptera*. *Behav Ecol Sociobiol* 64:1779–1789.

Grether GF, Hudon J and Millie DF 1999. Carotenoid limitation of sexual colouration along an environmental gradient in guppies. *Proc R Soc B* 266:1317–1322.

Grether G F, Kolluru GR, Rodd FH, de la Cerda J and Shimazaki K 2005. Carotenoid availability affects the development of a colour-based mate preference and the sensory bias to which it is genetically linked. *Proc R Soc B* 272:2181–2188.

Hamilton WD and Zuk M 1982. Heritable true fitness and bright birds: a role for parasites? *Science* 218:384–387.

Hamon TR and Foote CJ 2005. Concurrent natural and sexual selection in wild male sockeye salmon, *Oncorhynchus nerka*. *Evolution* 59:1104–1118.

Hartley RC and Kennedy MW 2004. Are carotenoids a red herring in sexual display? *Trends Ecol Evol* 19:353–354.

Hasson O 1997. Towards a general theory of biological signaling. *J Theor Biol* 185:139–156.

Hatlen B, Arnesen AM and Jobling M 1996. Muscle carotenoid concentrations in sexually maturing and immature Arctic charr, *Salvelinus alpinus* (L.). *Aquacult Nutr* 2:207–212.

Head ML, Hunt J, Jennions MD and Brooks R 2005. The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol* 3:e33

Heath DD, Fox CW and Heath JW 1999. Maternal effects on offspring size: variation through early development of Chinook salmon. *Evolution* 53:1605–1611.

Helfenstein F, Losdat S, Møller AP, Blount JD and Richner H 2010. Sperm of colourful males are better protected against oxidative stress. *Ecol Lett* 13:213–222.

Hendry AP, Kelly ML, Kinnison MT and Reznick DN 2006. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *J Evol Biol* 19:741–754.

Hill GE, Inouye CY and Montgomerie R 2002. Dietary carotenoids predict plumage colouration in wild House Finches. *Proc R Soc B* 262:1119–1124.

Hillgarth N, Ramenofsky M and Wingfield J 1997. Testosterone and sexual selection. *Behav Ecol* 8:108–109.

Hindar K and Jonsson B 1993. Ecological polymorphism in Arctic charr. *Biol J Linn Soc* 48:63–74.

Hoffmann AA and Merilä J 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol Evol* 14:96–101.

Hunt J, Bussière L, Jennions MD and Brooks R 2004. What is genetic quality? *Trends Ecol Evol* 19:329–333.

Haugland T, Rudolfsen G, Figenschou L and Folstad I 2009. Sperm velocity and its relation to social status in Arctic charr (*Salvelinus alpinus*). *Anim Reprod Sci* 115:231–237.

Hutchings JA 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution* 45:1162–1168.

Höglund J and Alatalo RV 1995. Leks. Princeton University Press, Princeton, USA.

Iwasa Y, Pomiankowski A and Nee S 1991. The evolution of costly male preferences. II. The 'handicap' principle. *Evolution* 45:1431–1442.

Janhunen M, Peuhkuri N and Piironen J 2010. A comparison of growth patterns between a stunted and two large predatory Arctic charr populations under identical hatchery conditions. *Environ Biol Fishes* 87:113–121.

Jennions MD and Petrie M 2000. Why do females mate multiply? A review of the genetic benefits. *Biol Rev* 75:21–64.

Jennions MD and Kokko H 2010. Sexual selection. In: Westneat DF and Fox CW (eds). Evolutionary behavioural ecology. Oxford Univ Press, New York, USA, pp. 343–364.

Jennions MD, Møller AP and Petrie M 2001. Sexually selected traits and adult survival: a meta-analysis. *Quart Rev Biol* 76:3–36.

Johnson L 1980. The Arctic charr, *Salvelinus alpinus*. In Balon EK (ed). Charrs: salmonid fishes of the genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, Netherlands, pp. 15–98.

Johnston G 2002. Arctic charr aquaculture. Wiley-Blackwell, Cornwall, UK.

Jonsson B and Jonsson N 2001. Polymorphism and speciation in Arctic charr. *J Fish Biol* 58:605–638.

Kallio-Nyberg I, Koljonen M-L 1991. The Finnish char (*Salvelinus alpinus*) stock register. *Finnish Fish Res* 12:77–82.

Kekäläinen J, Huuskonen H, Tuomaala M and Kortet R 2010. Both male and female sexual ornaments reflect offspring performance in a fish. *Evolution* 64:3149–3157.

Keller L and Reeve HK 1995. Why do females mate with multiple males? The sexually selected sperm hypothesis. *Adv Study Behav* 24:291–315.

Kempenaers B 2007. Mate choice and genetic quality: a review of the heterozygosity theory. *Adv Stud Behav* 37:189–278.

Kirkpatrick M and Barton NH 1997. The strength of indirect selection on female mating preferences. *Proc Natl Acad Sci USA* 94:1282–1286.

Klemetsen A, Knudsen R, Staldvik FJ and Amundsen PA 2003a. Habitat, diet and food assimilation of Arctic charr under the winter ice in two subarctic lakes. *J Fish Biol* 62:1082–1098.

Klemetsen A, Amundsen PA, Dempson JB, Jonsson B, O'Connel MF and Mortensen E 2003b. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L., and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecol Freshw Fish* 12:1–59.

Kokita T and Mizota T 2002. Male secondary sexual traits are hydrodynamic devices for enhancing swimming performance in a monogamous filefish *Paramonacanthus japonicus*. *J Ethol* 20:35–42.

Kokko H, Brooks R, McNamara JM and Houston AI 2002. The sexual selection continuum. *Proc R Soc B* 269:1331–1340.

Kortet R, Vainikka A, Rantala MJ and Taskinen J 2004. Sperm quality, secondary sexual characters and parasitism in roach (*Rutilus rutilus* L.). *Biol J Linn Soc* 81:111–117.

Kortet R, Vainikka A, Rantala MJ, Jokinen I and Taskinen J 2003. Sexual ornamentation, androgens and papillomatosis in male roach (*Rutilus rutilus*). *Evol Ecol Res* 5:411–419.

Kraaijeveld K, Kraaijeveld-Smit FJL and Komdeur J 2007. The evolution of mutual ornamentation. *Anim Behav* 74:657–677.

Lande R 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–305.

Larsson S and Berglund I 2005. The effect of temperature on the energetic growth efficiency of Arctic charr (*Salvelinus alpinus* L.) from four Swedish populations. *J Therm Biol* 30:29–36.

Larsson S, Forseth T, Berglund I, Jensen AJ, Näslund I and Elliott JM 2005. Thermal adaptation of Arctic charr: experimental studies of growth in eleven charr populations from Sweden, Norway and Britain. *Freshw Biol* 50:353–368.

Lehtonen TK and Lindström K 2008. Repeatability of mating preferences in the sand goby. *Anim Behav* 75:55–61.

Lehtonen TK, Wong BBM and Lindström K 2010. Fluctuating mate preferences in a marine fish. *Biol Lett* 6:21–23.

Levitan DR 2000. Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. *Proc R Soc B* 267:531–534.

Liljedal S and Folstad I 2003. Milt quality, parasites, and immune function in dominant and subordinate Arctic charr. *Can J Zool* 81:221–227.

Liljedal S, Rudolfsen G and Folstad I 2008. Factors predicting male fertilization success in an external fertilizer. *Behav Ecol Sociobiol* 62:1805–1811.

Locatello L, Rasotto MB, Evans JP and Pilastro A 2006. Colourful male guppies produce faster and more viable sperm. *J Evol Biol* 19:1595–1602.

Lozano GA 1994. Carotenoids, parasites, and sexual selection. *Oikos* 70:309–311.

Lynch M and Walsh B 1998. Genetics and analysis of quantitative traits. Sinauer Associates Inc., Sunderland, USA.

Lyytikäinen T, Koskela J and Rissanen I 1997. Thermal resistance and upper lethal temperatures of underyearling Lake Inari Arctic charr. *J Fish Biol* 51:515–525.

Mays HL and Hill GE 2004. Choosing mates: good genes *vs.* genes that are a good fit. *Trends Ecol Evol* 19:554–559.

McAdam AG, Boutin S, Réale D and Berteaux D 2002. Maternal effects and the potential for evolution in a natural population of animals. *Evolution* 56:846–851.

McGraw KJ 2005. The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? *Anim Behav* 69:757–764.

McGraw KJ and Ardia DR 2003. Carotenoids, immunocompetence, and the information content of sexual colours: an experimental test. *Am Nat* 162:704–712.

McGraw KJ and Hill GE 2001. Carotenoid access and intraspecific variation in plumage pigmentation in male American Goldfinches (*Carduelis tristis*) and Northern Cardinals (*Cardinalis cardinalis*). *Funct Ecol* 15:732–739.

McGraw KH, Adkins-Regan E and Parker RS 2005a. Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. *Naturwissenschaften* 92:375–380.

McGraw KJ, Hill GE and Parker RS 2005b. The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch, *Carduelis tristis*. *Anim Behav* 69:653–660.

Merilä J and Sheldon BC 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of idea. *Heredity* 83:103–109.

Monaghan P, Metcalfe NB and Torres R 2009. Oxidative stress as a mediator of life-history trade-offs: mechanisms, measurements and interpretation. *Ecol Lett* 12:75–92.

Monet G, Uyanik A and Champigneulle A 2006. Geometric morphometrics reveals sexual and genotypic dimorphisms in the brown trout. *Aquat Living Res* 19:47–57.

Morales J, Velando A and Torres R 2009. Fecundity compromises attractiveness when pigments are scarce. *Behav Ecol* 20:117–123.

Mougeot F, Pérez-Rodríguez L, Martínez-Padilla J, Leckie F and Redpath SM 2007. Parasites, testosterone and honest carotenoid-based signalling of health. *Funct Ecol* 21:886–898.

Mougeot F, Martínez-Padilla J, Bortolotti GR, Webster LMI and Piertney SB 2010. Physiological stress links parasites to carotenoid-based colour signals. *J Evol Biol* 23:643–650.

Mousseau TA and Roff DA 1987. Natural selection and the heritability of fitness components. *Heredity* 59:181–197.

Mousseau TA and Fox CW 1998. Maternal effects as adaptations. Oxford University Press, New York, USA.

Måsvær M, Liljedal S and Folstad I 2004. Are secondary sex traits, parasites and immunity related to variation in primary sex traits in the Arctic charr? *Proc R Soc B* (Suppl.) 271:S40–S42.

Møller AP and Alatalo RV 1999. Good-genes effects in sexual selection. *Proc R Soc B* 266:85–91.

Møller AP and Jennions MD 2001. How important are direct fitness benefits of sexual selection? *Naturwissenschaften* 88:401–415.

Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N and Surai PF 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poult Biol Rev* 11:137–159.

Nagler JJ, Parsons JE and Cloud JG 2000. Single pair mating indicates maternal effects on embryo survival in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 184:177–183.

Neff BD and Pitcher TE 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Mol Ecol* 14:19–38.

Neff BD and Pitcher TE 2008. Mate choice for non-additive genetic benefits: a resolution to the lek paradox. *J Theor Biol* 254:147–155.

Nordeng H 1983. Solution to the "char problem" based on Arctic char (*Salvelinus alpinus*) in Norway. *Can J Fish Aquat Sci* 40:1372–1387.

Nordeide JT 2007. Is there more in 'gamete quality' than quality of the gametes? A review of effects of female mate choice and genetic compatibility on offspring quality. *Aquacult Res* 38:1–16.

Nordeide JT, Rudolfsen G and Egeland ES 2006. Ornaments or offspring? Female sticklebacks (*Gasterosteus aculeatus* L.) trade off carotenoids between spines and eggs. *J Evol Biol* 19:431–439.

Nordeide JT, Mohus Å, Nicolaisen O, Volden R and Egeland ES 2008. Offspring or ornaments? Is carotenoid-based ornamentation in female Arctic charr, *Salvelinus alpinus* (L.), condition-dependent and traded off against offspring? *Ecol Freshw Fish* 17:328–339.

Ojanguren AF, Reyes-Gavilán FG and Braña F 1996. Effects of egg size on offspring development and fitness in brown trout, *Salmo trutta* L. *Aquaculture* 147:9–20.

Olson VA and Owens IPF 1998. Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol Evol* 13:510–514.

Pakkasmaa S and Jones M 2002. Individual-level analysis of early life history traits in hatchery-reared lake trout. *J Fish Biol* 60:218–225.

Pakkasmaa S, Penttinen O-P and Piironen J 2006. Metabolic rate of Arctic charr eggs depends on their parentage. *J Comp Physiol B* 176:387–391.

Palace VP, Brown SB, Baron CL, Fitzsimons J, Woodin B, Stegeman, JJ and Klaverkamp JF 1998. An evaluation of the relationships among oxidative stress, antioxidant vitamins and early mortality syndrome (EMS) of lake trout (*Salvelinus namaycush*) from Lake Ontario. *Aquat Toxicol* 43:195–208.

Pérez C, Lores M and Velando A 2008. Availability of nonpigmentary antioxidant affects red colouration in gulls. *Behav Ecol* 19:967–973.

Pérez-Rodríguez L 2009. Carotenoids in evolutionary ecology: re-evaluating the antioxidant role. *BioEssays* 31:1116–1126.

Pérez-Rodríguez L, Mougeot F, Alonso-Álvarez C, Bias J and Viñuela J 2008. Cell-mediated immune activation rapidly decreases plasma carotenoids but does not affect oxidative stress in red-legged partridges (*Alectoris rufa*). *J Exp Biol* 211:2155–2161.

Perry GML, Audet C, Laplatte B and Bernantchez L 2004. Shifting patterns in genetic control at the embryo-alevin boundary in brook charr. *Evolution* 58:2002–2012.

Peters A, Denk AG, Delhey K and Kempenaers B 2004. Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. *J Evol Biol* 17:1111–1120.

Pike TW, Blount JD, Lindström J and Metcalfe NB 2007. Availability of non-carotenoid antioxidants affects the expression of a carotenoid-based sexual ornament. *Biol Lett* 22:353–356.

Pike TW, Blount JD, Lindström J and Metcalfe NB 2009. Dietary carotenoid availability, sexual signalling and functional fertility in sticklebacks. *Biol Lett* 6:191–193.

Pitcher TE and Neff BD 2007. Genetic quality and offspring performance in Chinook salmon: implications for supportive breeding. *Conserv Genet* 8:607–616.

Pitcher TE, Rodd FH and Rowe L 2007. Sexual colouration and sperm traits in guppies. *J Fish Biol* 70:165–177.

Pitcher TE, Doucet SM, Beausoleil J-MJ and Hanley D 2009. Secondary sexual traits and sperm traits in coho salmon *Oncorhynchus kisutch*. *J Fish Biol* 74:1450–1461.

Pitnick S and Hosken D 2010. Postcopulatory sexual selection. In: Westneat DF and Fox CW (eds). Evolutionary behavioural ecology. Oxford Univ Press, New York, USA, pp. 379–399.

Primmer CR, Aho T, Piironen J, Estoup A, Cornuet J-M and Ranta E 1999. Microsatellite analysis of hatchery stocks and naturalpopulations of Arctic charr, *Salvelinus alpinus*, from the Nordic region: implications for conservation. *Hereditas* 130:277–289.

Primmer CR, Huttula E, Särkisaari P, Huusko A, Piironen J and Ranta E 2000. Genetic characterization of a potentially new population of Arctic charr, *Salvelinus alpinus*, from the Posio region. In Makkonen J (ed). Veden satoa 2000 – Riista ja kalatalouden tutkimuslaitoksen XXIV vesiviljelypäivät. Kala- ja riistaraportteja 180. Riista- ja kalatalouden tutkimuslaitos, Helsinki, pp. 37–38.

Pryke SR, Rollins LA and Griffith SC 2010. Females use multiple mating and genetically loaded sperm competition to target compatible genes. *Science* 329:964–967.

Puurtinen M, Ketola T and Kotiaho JS 2009. The good-genes and compatible-genes benefits of mate choice. *Am Nat* 174:741–751.

Quinn TP and Foote CJ 1994. The effects of body size and sexual dimorphism on the reproductive behavior of sockeye salmon, *Oncorhynchus nerka*. *Anim Behav* 48:751–761.

Qvarnström A 2001. Context-dependent genetic benefits from mate choice. *Trends Ecol Evol* 16:5–7.

Reid JM, Arcese P, Cassidy ALEV, Marr AB, Smith JNM and Keller LF 2005. Hamilton and Zuk meet heterozygosity? Song repertoire size signals inbreeding and immunity in song sparrows (*Melospiza melodia*). *Proc R Soc B* 272:481–487.

Reynolds JD 1996. Animal breeding systems. *Trends Ecol Evol* 11:68–72.

Rosengrave P, Gemmell NJ, Metcalf V, McBride K and Montgomerie R 2008. A mechanism for cryptic female choice in chinook salmon. *Behav Ecol* 19:1179–1185.

Rudolfsen G, Figenschou L, Folstad I and Kleven O 2008. Sperm velocity influence paternity in the Atlantic cod (*Gadus morhua* L.). *Aquacult Res* 39:212–216.

Rudolfsen G, Figenschou L, Folstad I, Nordeide JT and Søreng E 2005. Potential fitness benefits from mate selection in the Atlantic cod (*Gadus morhua*). *J Evol Biol* 18:172–179.

Rudolfsen G, Figenschou L, Folstad I, Tveiten H and Figenschou M 2006. Rapid adjustment of sperm characteristics in relation to social status. *Proc R Soc B* 273:325–332.

Rurangwa E, Kime DE, Ollevier F and Nash JP 2004. The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture* 234:1–28.

Saks L, Ots I and Hõrak P 2003. Carotenoid-based plumage colouration of male greenfinches reflects health and immunocompetence. *Oecologia* 134:301–307.

Sawanboonchun J, Roy WJ, Robertson DA and Bell JG 2008. The impact of dietary supplementation with astaxanthin on egg quality in Atlantic cod broodstock (*Gadus morhua*, L.). *Aquaculture* 283:97–101.

Scalia S, Isaksen M and Francis GW 1989. Carotenoids of the Arctic charr, *Salvelinus alpinus* (L.). *J Fish Biol* 34:969–970.

Shahidi F, Synowiecki J and Penney RW 1993. Pigmentation of Artic char (*Salvelinus alpinus*) by dietary carotenoids. *J Aquat Food Prod Tech* 2:99–115.

Sheldon BC 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. *Proc R Soc B* 257:25–30.

Siefferman L and Hill GE 2005. Evidence for sexual selection on structural plumage colouration in female eastern bluebirds (*Sialia sialis*). *Evolution* 59:1819–1828.

Sigurjónsdóttir H and Gunnarson K 1989. Alternative mating tactics of Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Environ Biol Fishes* 26:159–176.

Siikavuopio SI, Knudsen R and Amundsen PA 2010. Growth and mortality of Arctic charr and European whitefish reared at low temperatures. *Hydrobiologia* 650:255–263.

Simmons LW and Kotiaho JS 2002. Evolution of ejaculates: patterns of phenotypic and genotypic variation and condition dependence in sperm competition traits. *Evolution* 56:1622–1631.

Slate J, David P, Dodds KG, Veenvliet BA, Glass BC, Broad TE and McEwen JC 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical implications and empirical data. *Heredity* 93:255–265.

Slatkin M 1984. Ecological causes of sexual dimorphism. *Evolution* 38:622–630.

Skarstein F and Folstad I 1996. Sexual dichromatism and the immunocompetence handicap: an observational approach using Arctic charr. *Oikos* 76:359–367.

Skarstein F, Folstad I and Rønning HP 2005a. Spawning colouration, parasites and habitat selection in *Salvelinus alpinus*: initiating speciation by sexual selection? *J Fish Biol* 67:969–980.

Skarstein F, Folstad I, Liljedal S and Grahn M 2005b. MHC and fertilization success in the Arctic charr (*Salvelinus alpinus*). *Behav Ecol Sociobiol* 57:374–380.

Sparkes TC, Rush V and Foster SA 2008. Reproductive costs, condition and carotenoid-based colour in natural populations of threespine stickleback (*Gasterosteus aculeatus*). *Ecol Freshw Fish* 17:292–302.

Suk HY and Choe JC 2008. Dynamic female preference fo multiple signals in *Rhinogobius brunneus*. *Behav Ecol Sociobiol* 62:945–951.

Stevens M, Párraga A, Cuthill IC, Partridge JC and Troscianko T 2007. Using digital photography to study animal coloration. *Biol J Linn Soc* 90: 211–237.

Strauss RE and Bookstein FL 1982. The truss: body form reconstruction in morphometrics. *Syst Zool* 31:113–135.

Stuart-Fox DM and Ord TJ 2004. Sexual selection, natural selection and the evolution of dimorphic colouration and ornamentation in agamid lizards. *Proc R Soc B* 271:2249–2255.

Surai PF, Speake BK and Sparks NHC 2001. Carotenoids in avian nutrition and embryonic development. 2. Antioxidant properties and discrimination in embryonic tissues. *J Poultry Sci* 38:117–145.

Taborsky M 1998. Sperm competition in fish: 'bourgeois' males and parasitic spawning. Trends Ecol Evol 13:222–227.

Taskinen J and Kortet R 2002. Dead and alive parasites: sexual ornaments signal resistance in the male fish, *Rutilus rutilus*. *Evol Ecol Res* 4:919–929.

Tregenza T and Wedell N 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Mol Ecol* 9:1013–1027.

Tremellen K 2008. Oxidative stress and male infertility – a clinical perspective. *Hum Reprod Update* 14:243–258.

Trivers RL 1972. Parental investment and sexual selection. In: Campbell B (ed). Sexual selection and the descent of man. Aldine, Chicago, USA, pp. 136–179.

Tyndale ST, Letcher RJ, Heath JW and Heath DD 2008.Why are salmon eggs red? Egg carotenoids and early life survival of Chinook salmon (*Oncorhynchus tshawytscha*). *Evol Ecol Res* 10:1187–1199.

Urbach D, Folstad I and Rudolfsen G 2005. Effects of ovarian fluid on sperm velocity in Arctic charr (*Salvelinus alpinus*). *Behav Ecol Sociobiol* 57:438–444.

Urho L, Pennanen JT and Koljonen ML 2010. Fish. In: Rassi P, Hyvärinen E, Juslén A and Mannerkoski I (eds.). The 2010 red list

of Finnish species. Ministry Of The Environment & Finnish Environment Institute, Helsinki, pp. 336–343.

Velando A, Torres R and Alonso-Alvarez C 2008. Avoiding bad genes: oxidatively damaged DNA in germ line and mate choice. *Bioessays* 30:1212–1219.

von Schantz T, Bensch S, Grahn M, Hasselquist D and Wittzell H 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proc R Soc B* 266:1–12.

Vähä V, Erkinaro J, Niemelä E and Primmer CR 2007. Life-history and habitat features influence the within-river genetic structure of Atlantic salmon. *Mol Ecol* 16:2638–2654.

Vøllestad LA and Lillehammer T 2000. Individual variation in early life-history traits in brown trout. *Ecol Freshw Fish* 9:242–247.

Wedekind C and Folstad I 1994. Adaptive and non-adaptive immunosuppression by sex hormones. *Am Nat* 143: 936–938.

Wedell N, Kvarnemo C, Lessells CM and Tregenza T 2006. Sexual conflict and life histories. *Anim Behav* 71:999–1011.

Welch AM 2003. Genetic benefits of a female mating preference in gray tree frogs are context-dependent. *Evolution* 57:883–893.

Wilson AJ, Gíslason D, Skúlason S, Snorrason SS, Adams C, Alexander G, Danzmann RG and Ferguson MM 2004. Population genetic structure of Arctic Charr, *Salvelinus alpinus* from northwest Europe on large and small spatial scales. *Mol Ecol* 13:1129–1142.

Yasui Y 1997. A ''good-sperm'' model can explain the evolution of costly multiple mating by females. *Am Nat* 149:573–584.

Yasui Y 1998. The 'genetic benefits' of female multiple mating reconsidered. *Trends Ecol Evol* 13:246–250.

Zahavi A 1975. Mate selection: a selection for a handicap. *J Theor Biol* 53:205–214.

Zahavi A 1977. Cost of honesty (further remarks on handicap principle). *J Theor Biol* 67:603–605.

Zeh JA and Zeh DW 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proc R Soc B* 263:1711–1717.

Matti Janhunen

Phenotypic and genetic variations in reproduction-associated traits in Arctic charr, Salvelinus alpinus

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.

UNIVERSITY OF
EASTERN FINLAND