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Mechanisms of Honest Sexual  
Signalling and Life History  
Trade-offs in Three Cyprinid Fishes











## ABSTRACT

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Mechanisms of honest sexual signalling and life history trade-offs in three cyprinid fishes

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Yhteenveto: Rehellisen seksuaalisen signaloinnin ja elinkiertojen evoluution mekanismit kolmella särkikalalla

Diss.

Testosterone is known to enhance reproductive success, but has also been proposed to suppress immune defence. Prior to spawning, male roach (*Rutilus rutilus* L.) produce testosterone-associated breeding tubercles those have been found to signal parasite load and parasite resistance. Tench (*Tinca tinca* (L.)) is sexually dimorphic throughout the year, and in addition to their large pelvic fins males produce ventral protuberances of unknown function during breeding. The indicator value of male tench ornaments, immunosuppressivity of testosterone, cortisol and oestradiol in roach and tench, and the level of stress during spawning in roach were studied. A study of crucian carp (*Carassius carassius* (L.)) aimed to link individual's behavioural decisions to somatic growth, reproductive effort and future survival. In these studies, cortisol but neither testosterone nor oestradiol was found to have immunosuppressive effects. Neither did an immunological challenge decrease the concentration of testosterone. Although testosterone was positively related to the condition factor in tench, it was only weakly associated to the expression of male ornaments. High spawning-time prevalence of epidermal papillomatosis in male roach was suggested to result from sex-differences in spawning stress. Body depth gain in crucian carp was associated to growth in a manner dependent on predator presence, but the behavioural decisions of an individual did not correlate with measures of growth or reproductive effort. I suggest that stress steroids contribute to the honesty of sexual signalling in the studied cyprinids, whereas testosterone in both sexes may act as a condition-dependent hormone having a positive effect on sexual signalling and primary sexual traits, but being metabolically costly at high levels.

Keywords: *Carassius carassius*; freshwater; immune function; ornament; reproduction; *Rutilus rutilus*; sexual selection; *Tinca tinca*.

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers which will be referred to in the text by their Roman numerals (I-VI).

- I Vainikka, A., Jokinen, E.I., Kortet, R. & Taskinen, J. 2004. Gender- and season-dependent relationships between testosterone, oestradiol and immune functions in wild roach *Rutilus rutilus* L. *J. Fish Biol.* **64**: 227-240.
- II Vainikka, A., Kortet, R. & Taskinen, J. 2004. Epizootic cutaneous papillomatosis, cortisol and male ornamentation during and after breeding in roach *Rutilus rutilus*. *Dis. Aquat. Org.* **60**: 189-195.
- III Vainikka, A., Kortet, R., Paukku, S., Rantala, M.J. & Pirhonen, J. What do male tench, *Tinca tinca*, advertise with morphological ornaments? *Acta Ethol.* In press.
- IV Vainikka, A., Jokinen, E.I., Kortet, R., Paukku, S., Pirhonen, J., Rantala, M.J. & Taskinen, J. 2005: Effects of testosterone and  $\beta$ -glucan on immune functions in tench *Tinca tinca* (L.). *J. Fish Biol.* **66**: 348-361.
- V Vainikka, A., Löytynoja, K. & Jokinen, E.I.: *In vitro* effects of testosterone, oestradiol and cortisol on phagocytosis in tench, *Tinca tinca* (L.) and roach, *Rutilus rutilus* L. Manuscript.
- VI Vainikka, A., Pirhonen, J. & Ylönen, H.: Growth and behaviour as antipredatory adaptations in crucian carp, *Carassius carassius* (L.). Manuscript, under review for the *J. Fish Biol.*

## RESPONSIBILITIES OF ANSSI VAINIKKA IN THE ARTICLES OF THIS THESIS

- Paper I            The data collection was planned by J. Taskinen, E.I. Jokinen and R.Kortet. The analysis was planned by all the authors, and I was responsible for hormone and IgM assays, statistics and writing the draft of the article.
- Paper II            The study was planned and conducted by all the authors with the main responsibility being mine. I wrote the draft of the article.
- Paper III           The study was planned with the coauthors. S. Paukku was responsible for eyefluke counting, J. Pirhonen for technical facilities, E.I. Jokinen for immune methods, and R. Kortet helped in the laboratory and with the manuscript preparation. M.J. Rantala participated in writing the manuscript. I was mainly responsible for practical work and I wrote the draft of the article.
- Paper IV            The study was planned with M.J. Rantala and E.I. Jokinen. R. Kortet helped in catching fish and in the laboratory, S. Paukku in the sampling the fish, and the other co-authors helped with statistics and manuscript preparation. However, I was mainly responsible for all practical work. I wrote the draft of the article.
- Paper V            The study was planned with the coauthors. The laboratory work was performed mainly by me and Katja Löytynoja. I wrote the manuscript.
- Paper VI            I planned the study with H. Ylönen and conducted it with J. Pirhonen. All coauthors helped in manuscript writing although the main responsibility was mine.

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Anssi Vainikka

# 1 INTRODUCTION

## 1.1 Concepts of evolution

All living organisms carry genes that – according to current knowledge – have a common origin; here all current organisms are descendants of one ancestral “super-mother”. Each sexually produced individual, excluding identical siblings, represents a unique combination of these recipes of life, affected by recombination of genetic material from two parents, and randomly occurring errors, mutations. Life, as an endless loop of reproduction, evolves continuously by selecting successful individuals to form species, separate gene pools to be recombined or mutated by the individuals (Kutschera & Niklas 2004).

In the 18<sup>th</sup> century, species were thought to represent unchanging units of nature and a huge effort was made to classify species on the basis of their morphology. We now know that morphological similarities are either signs of a common evolutionary origin or common environmental factors that have led to analogical characters by a separate but parallel evolutionary route. In addition, modern evolutionary science has begun to understand the relationship between environmental factors and selective forces existing within species by genetic conflicts. These between-sexes and within-sexes forces, in addition to being responsible for sexual dimorphism (Rice 1984), can maintain reproductive isolation between diverging populations and lead to speciation (e.g. Panhuis et al. 2001, Boake 2002, Kirkpatrick & Ravinge 2002, Martin & Hosken 2003, Kirkpatrick & Nuismer 2004, Knight & Turner 2004).

## 1.2 Targets of selection

Darwin (1871) was the first to recognise sexual selection as separate from natural selection and as the cause for notable sexual dimorphism in some

species. For an individual to be able to reproduce, two stringent assumptions must be met: firstly to stay alive until reproduction, and secondly to find and seduce a mate. For these two purposes organisms sometimes use conflicting strategies: those used to attract or manipulate mates (such as the tail of a peacock, antlers of an elk or conspicuous colouration of a fish) are harmful and costly when trying to survive in a challenging biotic and abiotic environment. This leads to a conflict and some balancing point between natural and sexual selection (e.g. Breden & Stoner 1987).

To understand this conflict and other natural phenomena that seem maladaptive at first sight, we must be aware of the targets of selection. For example, killing of juveniles by dominant individuals in communally breeding animals seems wasting, but ultimately it appears to be adaptive (Johnstone & Cant 1999). Moreover, in this scenario, individuals should use non-conspicuous good genes advertisements to enable the best individuals to reproduce and speed adaptation of a species to its environment. Actually, Wedekind (1994) showed theoretically that such honest signalling without a cost, for example using chemical revelation of major histocompatibility complex (MHC) allele composition, could evolve via fitness benefits to individuals. However, nature does not have species-specific plans; rather selfish individuals compete with each other and viable individuals form viable species (Kutschera & Niklas 2004). In another view, the selection could be thought to work on genes, but the individual genes do not have other fitness functions than the individual they build with other genes in the interaction with environment. Thus, they are the phenotypically plastic individuals that are selected, but allele frequencies in populations evolve as a result of selection on individuals (Kutschera & Niklas 2004). Consequently, natural selection targets indirectly on populations and species by favouring allele compositions that keep both sexes of a species viable (selection on reproductive units). Keeping these ultimate mechanisms in mind, it is possible to understand further those natural phenomena that seem maladaptive.

### **1.3 Sexual selection and the paradox of good genes**

Sexual selection results from unequal mating success between individuals of the sex (usually males) that commonly has higher reproductive potential but invests less in rearing offspring. In many species, females base their mate choice on the expression of male secondary sexual characteristics (Andersson 1994) or on male success in male-male contests (Qvarnström & Forsgren 1998), where secondary sexual characters may also reflect dominance status over intra-sexual competitors (Berglund et al. 1996). Intrasexual competition using armaments like horns is a form of sexual selection involving not choice but sometimes dangerous rivalry among possible mates, of whom only the winners can usually reproduce (Berglund et al. 1996). In addition, hidden sexual selection

may occur in the form of sperm competition (Parker 1990, Ball & Parker 1996, Taborsky 1998) or by unequal mortality of embryo after fertilisation depending on the male identity. The most obvious sexually selected species, such as black grouse, have intensive and complex leks (e.g. Höglund et al. 1997, 2002), but some studies have suggested sexual selection by female choice to occur even in group spawning fishes (Wedekind 1996, Hutchings et al. 1999).

Existence of sex and sexual selection are thought to be adaptive (e.g. Andersson 1986, Iwasa & Pomiankowski 1999, Møller & Alatalo 1999, Rice & Chippindale 2001) by speeding evolution through recombination and mate choice for viability. However, males of many species have evolved a variety of ways to overcome female choice. The theory of sexual conflict (Rice 1984, 1992, Moore et al. 2000, Arnqvist & Rowe 2002, Chapman et al. 2003) refers to conflicting interests between the sexes; some genes that are beneficial to one sex can be deleterious to the other. Thus, sexual selection has actually two viewpoints: 1) acquisition of good genes for offspring and 2) avoidance of manipulative mates to increase fitness. To be maintained, benefits from sexual selection should be at least as high to one sex as the costs of sexual selection to the opposite sex (Kirkpatrick 1987).

When this viewpoint is taken into account, it is not always clear that females gain absolute net benefits by picking males with the most elaborated (manipulative) ornaments (e.g. Brooks 2000) as suggested by the 'traditional good genes' advocates (e.g. Andersson 1986, Hamilton & Zuk 1982, Folstad & Karter 1992). However, by being choosy and by avoiding the most manipulative males, females should be able to increase their fitness relative to other females (Moore et al. 2003). Thus, a very few species with totally random mating should exist. Moreover, by rejecting the possibility of parental care and by seeking new mates, males do not automatically increase their fitness. Females usually carry a higher cost of offspring production simply because males cannot be sure about their paternity and it is not evolutionarily worthwhile to rear foreign offspring (Kokko & Jennions 2003).

Sexual selection by female choice may be evolved and maintained by several mechanisms, including handicap models (Zahavi 1975, 1977, Iwasa et al. 1991), 'good resistance genes' models (Hamilton & Zuk 1982, Folstad & Karter 1992, Wedekind & Folstad 1994, Siva-Jothy & Skarstein 1998), the Fisherian model of sexy sons (Fisher 1930) and the sensory-bias hypothesis, where females prefer males because they resemble for example food (e.g. Basolo 1990, Rodd et al. 2002). In the current literature, one mechanism that has received rather little attention is 'the sex recognition' that may increase mating success of males that carry alleles that make a male look more male than the other males (Barlow 1998).

'Good genes' have long been thought to be adaptive in relation to natural selection (Zahavi 1975, Hamilton & Zuk 1982, Andersson 1986, Folstad & Karter 1992), but no a precise and comprehensive definition for 'good genes' has been given. Siva-Jothy & Skarstein (1998) suggested that the antigen pool available by major histocompatibility complex alleles drives sexual selection for parasite resistance. In many studies, some measures of immune function are used to

approximate the presence of 'good genes' (e.g. Rantala & Kortet 2003, 2004). This is justified because immune function, also in insects that lack MHC-genes, is assumed to include a heritable component.

In all cases, to be maintained a female preference should have a genetic basis, which indeed leads to the fact that Fisher's mechanism of coupled preference and the preferred trait is included in every mate choice model as detected also by the theoretical framework of Kokko et al. (2002). Eshel et al. (2002) showed theoretically that only models including a cost of signalling can explain the maintenance of sexual preferences and honest 'good genes' signalling in the long run (see also Johnstone & Grafen 1992, 1993). Thus, I define good genes to represent all genes that a) maintain sexual selection and b) have positive fitness effects in the sex showing stronger selectivity. However, an alternative strategy for females to acquire a good genetic match with a mate is mating with multiple males (Colegrave et al. 2002).

Despite continuous directional sexual selection for elaborate male traits, these traits show notable variation among individuals which is referred to as the 'paradox of the lek' (Kirkpatrick & Ryan 1991). Existence of variation in male traits is proposed to result from condition-dependence of signalling (Grafen 1990, Iwasa et al. 1991, Kotiaho et al. 2001). Condition among individuals varies due to quality-dependent costs associated with the development of ornamentation (Zahavi 1975, 1977), changing environmental factors and coevolving parasites (Hamilton & Zuk 1982). Thus, individuals carrying elaborated good gene advertisements are thought to be also in good condition.

#### **1.4 Sexual selection for parasite resistance?**

Hamilton & Zuk (1982) proposed that the expression of sexual signals indicates health, vigour and parasite resistance. According to their hypothesis, individuals with the most elaborated sexual ornaments should be subject to a wide variety of parasites but also be the most resistant to currently harmful parasites. Because immune function is assumed to include a heritable component (Hall et al. 2000, Cotter & Wilson 2002), females selecting highly-ornamented males should produce offspring that can resist current pathological threats. This would give an evolutionary advantage to individuals performing mate discrimination by honest ornamentation. According to Hamilton & Zuk (1982), this should be seen in variation between species so that the species or populations parasitized most heavily should have the strongest sexual ornamentation and thus sexual selection.

Folstad & Karter (1992) explained the positive relationship between the intensity of sexual signalling and parasite resistance by a proximate mechanism utilising the endocrine system. According to this immunocompetence handicap hypothesis (ICHH), testosterone or an analogous substance mediates a negative

feedback loop between sexual signals and parasite load: hormones responsible for the production of sexual signals suppress immune function making only the highly-resistant males successful in the crossfire of sexual and natural selection. Folstad & Karter's (1992) hypothesis is a mechanistic extension to Zahavi's handicap principle (1975, 1977) according to which a cost is associated to the development of honest ornamentation.

The ICHH predicts two important effects: (1) high parasite burden and nutritional deficits can decrease the concentration of male sex hormones; and (2) male sex hormones suppress immune function that is not genetically fixed. An important finding in this respect was the suggestion by Kurtz et al. (2004) that the non-specific immune system supports specific immune functions that are fixed by available MHC-alleles (Schmid-Hempel & Ebert 2003). Thus, individuals having a less optimal MHC-allele combination should possess stronger non-specific responses (Kurtz et al. 2004). In combination with the immunocompetence-handicap hypothesis, this suggests that testosterone or an analogous substance should suppress innate immune defence.

An important note here is that the immunocompetence-handicap hypothesis does not give a clear prediction on the relationship between parasite load and the extent of sexual signalling: the correlative relationship might be positive, but equally negative or neutral. Generally, however, the ICHH mechanism is thought to contribute to the Hamilton & Zuk (1982) hypothesis so that intensive signalling means fewer parasites (should actually relate only to the load of currently most harmful ones), although both positive and negative correlations between testosterone concentration and parasite load have been invoked to support the ICHH (Braude et al. 1999, Roberts et al. 2004).

The immunocompetence-handicap hypothesis has been criticised, and both the testosterone-dependency of sexual signalling and testosterone-induced immunosuppression have raised doubts (Roberts et al. 2004). Rather than immunosuppression, testosterone may cause redistribution of immune function (Braude et al. 1999), reallocation of resources (Wedekind & Folstad 1994) or indirect effects by corticosteroids (Braude et al. 1999). Moreover, the effects of testosterone may differ among vertebrate taxa (Roberts et al. 2004).

## **1.5 Is immunocompetence-handicap only a result of life-history trade-off?**

Because males can produce offspring with more than one female, but almost never being absolute sure about the paternity, males aim to maximize their mating rate and females their lifespan. Due to sex-specific life-history strategies (number of available female gametes restrains reproduction) the sexes may have different optimal adaptive peaks of growth rate or immunity. This ultimate strategic sex-difference in life-histories is known as Bateman's principle (Bateman 1948, Rolff 2002), but is also included in the theory of sexual

conflict (Rice 1984, 1992, Moore et al. 2000, Chapman et al. 2003). This could explain why males may have a lower immunity than females (Rolff 2002) or why many female fish grow larger than males. This also leads to mate choice and male-male competition, i.e. sexual selection and possibly to androgen-mediated handicaps in males (Zuk 1990, 1996, Klein 2000a, b, Moore et al. 2000).

Hypothetically, females might promote the evolution of an immunocompetence handicap to assure the honesty of male ornaments by male sex hormones, but avoiding the handicap themselves by lacking the functional hormone. Thus, an immunocompetence handicap might be mediated only by a hormone specific to males. However, males should develop counteradaptations and resist the negative effects of such a hormone, if such a handicap is not adaptive to them. An immunocompetence handicap might be adaptive for males if honestly signalling males lose more than cheating males gain in the case of cheating (number of cheating individuals in a population will decrease until extinction or to some balancing point between honest and dishonest males due to poor viability of cheating males). For mathematical considerations about male handicaps see for example Grafen (1990) or Eshel et al. (2002). Generally, however, male-specific handicap by androgen-induced immunosuppression is not seen as adaptive and the non-adaptivity of such suppression has been used to criticise the immunocompetence handicap hypothesis (e.g. Braude et al. 1999).

Reproduction-related traits can impair immune function (e.g. Rolff & Siva-Jothy 2002, Kilpimaa et al. 2003), i.e. cause immunocompetence-handicap. Current theories about the evolution of life-histories predict that animals allocate energy between reproduction and survival to maximize their reproductive value (Sheldon & Verhulst 1996, Norris & Evans 2000). Survival is mainly a function of immune defence, feeding and antipredatory adaptations, whereas reproduction is mainly a function of condition and success in sexual selection. Thus, testosterone-induced allocation of resources from immune defence to reproduction might be adaptive in males (Wedekind & Folstad 1994).

There is no single measure of immune defence, immunocompetence. Rather the immune system is a physiological complex having both adaptive and non-adaptive qualities that must meet the requirement of the pathogenic environment, stress, production of haploid gametes and the risk of autoimmune attacks (Ahmed et al. 1985, Braude et al. 1999). Although the maintenance and activation of immune defence have been shown to have ecologically meaningful costs (e.g. Ots et al. 2001, Colditz 2002, Bonneaud et al. 2003, Kilpimaa et al. 2003, Jacot et al. 2004, reviewed by Lochmiller & Deerenberg 2000), the costs of disruption in immune defence are likely even higher (Westneat & Birkhead 1998). Moreover, the costs of innate immune function may be higher than those of adaptive immune system (Råberg et al. 2001) making the costs of immune function dependent on individual's genetics (Kurtz et al. 2004, Schmid-Hempel & Ebert 2003), and thus supporting conditional handicap theories of honest sexual signalling.

Thus, the immunocompetence-handicap mechanism (Folstad & Karter 1992, Wedekind & Folstad 1994), including the possibility of adaptive



immunoredistribution rather than general suppression of immunocompetence (Råberg et al. 1998, Braude et al. 1999), can be seen as a mechanistic explanation of the life-history trade-off between reproduction and future survival. While increasing reproductive success male sex hormones divert resources from other life-history traits (such as some immune functions, growth etc.). When the effects of androgens on breeding and dominance behaviour (Kobayashi & Nakanishi 1999, Oliveira et al. 2001, Oliveira et al. 2002, Páll et al. 2002) and metabolic rate (Buchanan et al. 2001) are taken into account, it seems apparent that life-history trade-offs have a hormonal control.

This view does not support the original idea of immunosuppressive testosterone (Folstad & Karter 1992), but enlarges the conditional handicap theories by Zahavi (1975, 1977) by assuming testosterone or an analogous substance to have only a general cost (see also Westneat & Birkhead 1998). This 'hypothesis of endocrine-mediated honesty of sexual signalling' works if a hormone having a positive effect on sexual traits leads to any kind of cost of signalling (trade-off between reproduction and maintenance).

## 1.6 Possible life-history hormones

All higher animals consist of several subsystems, such as the locomotory system, digestive system, blood-vascular system, reproductive system, neural system etc. These subsystems communicate with each other not only by nerves but also by hormones. Thus, it is likely that there are genes analogous to homeotic genes (HOX genes) that do not build animals but regulate their further development by hormones. For example, it might be necessary to downregulate some other functions to save energy and resources for reproduction.

To have an evolutionary meaningful role in life-history regulation, a hormone has to have an effect on reproduction and some function of maintenance. Testosterone should meet these requirements for reasons described in earlier sections. In insects, juvenile hormone not specific to either sex has been shown to meet these requirements by increasing sexual signalling, suppressing immune function and by shortening lifespan (Herman & Tatar 2001, Rolff & Siva-Jothy 2002, Rantala et al. 2003b). There is also some evidence that the female sex hormone essential to female reproduction, oestradiol, can regulate life-history trade-offs. For example, in goldfish, oestradiol administration increased susceptibility to infestation by *Trypanosoma danilewskyi* Laveran & Mesnil (Wang & Belosevic 1994) suggesting a trade-off between reproduction and immune defence.

Stress is defined as a physiological state in which normal function is disturbed for some reason. Stress reaction provides a good example of how the endocrine system can help the whole organism to maintain homeostasis. In a stress reaction, both hypothalamic-pituitary-adrenal and sympathetic-adrenal-

medullary axes activate and cause release of resources such as glycogen to cope with the stressor (Padget & Gaser 2003).

Stress decreases the production of androgens with severe consequences for reproduction (Consten et al. 2002, Goos & Consten 2002). Cortisol may not act directly on the ovaria by inhibiting steroid production (Pankhurst et al. 1995), but an inhibiting role on 11-KT production by a direct route on Sertoli or germ cells is suggested to be present in males of common carp (*Cyprinus carpio* L.) (Goos & Consten 2002). Moreover, many immune system cells have receptors for cortisol, and at high levels cortisol can cause severe immunosuppression (e.g. Caruso & Lazard 1999, Haddy et al. 1999, Harris & Brid 2000, Davis et al. 2003, Padget & Gaser 2003). Thus, cortisol may be one of the most important hormones to regulate allocation of resources between different life-history traits in teleosts. Between-individual differences in stress responses might even explain the honesty of condition-dependent sexual signals (Buchanan 2000).

The ability to detect life-history trade-offs by correlative studies is limited, because the amount of available resources may be divided evenly between functions that ultimately are traded-off against each other (van Noordwijk & de Jong 1986). Thus, only experimental studies can confirm the existence of endocrine-mediated life-history trade-offs.

## 1.7 Life-history decisions and fitness under predation

Most studies of sexual selection do not take into account how sexually selected traits or their production affect the survival of an individual. Many fish populations are harvested intensively and it is known that heavy fishing pressure will lead to evolutionary decrease in the maturation age of fish (Barot et al. 2004). According to life-history theory (Roff 1992, Stearns 1992), animals have to balance their resources between reproduction and maintenance. However, little is known about how prey fish change their life-history decisions according to prevailing predation pressure and resulting behavioural changes. Generally, non-lethal effects of predation can have drastic effects on prey populations by restraining feeding and mating possibilities (e.g. Sinclair & Arcese 1995, Ruxton & Lima 1997, Kokko & Ruxton 2000, Carvalho & Del-Claro 2004).

Crucian carp (*Carassius carassius* (L.)) is a phenotypically plastic species: in multispecies communities including predatory fish the rare individuals are often large whereas individuals in dense monospecies populations are often stunted (Brönmark et al. 1995). When the amount of available food limits the growth of individuals, intraspecific competition for food may favour aggressive traits despite the energetic cost of being aggressive (Vøllestad & Quinn 2003). However, studies on dominance in cyprinids are very scarce (Rincó & Grossman 2001, Wedekind 1996, Kortet et al. 2004a).

Both somatic growth and dominance behaviour may be regulated partly by male sex hormones (Tveiten et al. 1998, Oliveira et al. 2001). Growth and resulting deep body morphology are important antipredatory adaptations in crucian carp (Nilsson et al. 1995). Thus, behavioural decisions of individuals in a shoal exposed to simulated predation risk should affect their growth and fitness (Lima & Dill 1990, Lima & Bednekoff 1999). In study VI, predation risk was simulated by both visual and olfactory presence of pike, a threat that is known to affect instantly the behaviour of perch (*Perca fluviatilis* L.) (Vainikka et al. 2005) and crucian carp (Pettersson et al. 2001).

## 1.8 Reproduction in the studied cyprinid fishes

The family Cyprinidae includes 2010 species in 210 genera. In this thesis, three species from separate genera have been studied: roach (*Rutilus rutilus* L.), tench (*Tinca tinca* L.) and crucian carp (*Carassius carassius* L.). Depending on the role of males in offspring rearing, females may gain different amount of resources from their mates. The studied fishes, in that respect, represent one end of the continuum: males give only their genes along with seminal products and possible resources in gametes to the offspring. This makes the chosen fishes excellent model organisms to study mechanisms and consequences of sexual selection (Amundsen 2003).

All the studied cyprinid fishes are poikilothermic and spawn in spring or summer when their metabolic rate is increasing. Their physiological state and immune functions are dependent on the ambient water temperature. They have external fertilisation, and they do not form clear spawning pairs or clear spawning territories. All three species spend their lives in small groups or shoals. The exact mating system of most cyprinids is unknown: Wedekind (1996) has shown sexual selection to occur in roach, but Breder & Rosen (1966) reviewed only suggestions for sexual dimorphism in tench. Most cyprinids, but tench, are sexually monomorphic, although males of many species like roach and crucian carp produce breeding tubercles prior to spawning.

Male sexual behaviour and spermatogenesis in teleosts are dependent on 11-ketotestosterone (11-KT) (Stacey & Kobayashi 1996, Takeo & Yamashita 2000) that is the major androgen in most teleosts fishes (Borg 1994). However, an important group in evolutionary studies, poecilids, may not have 11-androgens although there seems not to be a clear phylogenetic distinction between fishes with and without 11-androgens (Borg 1994). 11-KT is present in cyprinid females but at concentrations 10-100 times lower than those of testosterone (Lokman et al. 2002). 11-KT is able to cause masculinization during sex reversal in some species (Condeça & Canario 1999).

The main oestrogen in fish is 17 $\beta$ -oestradiol (Borg 1994) which can prevent the development of male gonadal tissue (Condeça & Canario 1999). Testosterone is present in both sexes of most teleosts at somewhat equal

concentrations (Borg 1994), and it is not able to cause masculinization in sexually undifferentiated genotypic female chinook salmon (*Oncorhynchus tshawytscha* Walbaum) (Piferrer et al. 1993). Although not strictly an androgen in teleosts, testosterone is an important precursor of oestradiol (Piferrer et al. 1993) and 11-KT (Lokman et al. 2002). Due to conversion of testosterone to oestradiol and other steroids, it is not clear whether testosterone has any independent effects by its own (Borg 1994).

Borg (1994) did not know receptors for 11-KT and androgen receptors of the testes of rainbow trout (*Oncorhynchus mykiss* Walbaum) have been found to be unable to distinguish testosterone from 11-ketotestosterone (Takeo & Yamashita 2000). This may suggest that these steroids have analogous effects in some fishes.

Because Kortet et al. (2003c) found that high plasma testosterone concentration of male roach was associated with elaborate breeding tubercle ornamentation and also with high risk from epidermal papillomatosis disease, there was reason to expect testosterone to have an evolutionary meaningful role in the possible ICHH-mechanism in roach. This suspicion was supported by experimental evidence that testosterone causes development of papillomata in *Catostomus commersoni* (Lacépède) (Premdas et al. 2001). Moreover, in the study of Kortet et al. (2003c), 11-ketotestosterone lacked a relationship with the epidermal papillomatosis disease, although the concentrations of testosterone and 11-KT correlated positively with each other ( $R = 0.49$ ).

In addition, several types of receptors, androgen-binding proteins and sex steroid-binding proteins show higher affinity for testosterone than for 11-KT (Borg 1994). However, free steroids bind more potently to classical steroid receptors than do protein-bound steroids, and the high affinity of steroid-binding proteins for testosterone may partly explain why 11-KT is often more effective than testosterone (Borg 1994).

## 1.9 Aims of the study

The aim of this thesis was to detect life-history trade-offs between predictors of survival and estimates of reproductive success, and to examine whether these trade-offs have a hormonal control. The existence and possible mechanisms of sexual selection in tench were explored. In roach, endocrinological mechanisms of honesty of sexual signalling were aimed to be revealed. To detect sex-dependent differences in life-history strategies, sexes were studied separately in all studies.

In paper I my aim was to examine whether testosterone correlates with the measures of immune defence, and whether these correlations show any seasonality. In paper II, my aim was to examine the role of stress in the previously detected relationship between the elaborate ornamentation of male roach and the high risk from papillomatosis (Kortet et al. 2003c). In study III I

tried to answer the question why the sexes of tench are morphologically dimorphic, and to test correlatively the predictions of the good resistance genes hypotheses. In paper IV and V, I examined experimentally the role of testosterone in the predicted immunocompetence-handicap mechanism (Folstad & Karter 1992). In study VI, my aim was to detect individual behaviours and predator-induced changes in life-history strategy that would increase the fitness of crucian carp. The size of gonads was used as a measure of reproductive effort and thus fitness.

Taken together, these studies aim (1) to explain the honesty of male roach ornaments by testing the predictions of the immunocompetencehandicap-hypothesis (Folstad & Karter 1992), (2) to examine the existence and mechanism of sexual selection in tench, and (3) to relate resource allocation between life-history traits to predation risk.

## 2 METHODS

### 2.1 Study species

In this thesis, three wild cyprinid species from separate genera were used as model organisms: the roach (I, II & V), tench (III-V) and crucian carp (VI). All of these species are common, widely distributed in Europe and Asia and native to Finland.

Roach males show some territoriality during spawning in intense lek-like shoals and females have preferences towards specific males, although parasitic spawnings are common and not avoided by females, making sperm competition obvious in this species (Wedekind 1996). Roach develop breeding tubercles as sexual ornaments (Wiley & Collette 1970). The breeding tubercles of roach indicate intraspecific dominance (Kortet et al. 2004a) and both parasite resistance (Taskinen & Kortet 2002) and parasite load (Wedekind 1992, Kortet & Taskinen 2004), partly as suggested by the Hamilton & Zuk (1982) hypothesis. Males having elaborated breeding tubercle ornamentation have longer-lived sperm than their less ornamented rivals (Kortet et al. 2004c). However, highly-ornamented males do not produce more viable offspring than their less-ornamented controls (Kortet et al. 2004b). Moreover, males resistant to *Rhipidocotyle campanula* Dujardin parasites produced offspring that were less viable in early development than the offspring from less resistant males (Kortet et al. 2004b). Highly-ornamented roach males have higher plasma testosterone and 11-ketotestosterone concentrations and they are more susceptible to virus-induced skin disease, epidermal papillomatosis than their low-ornamented specimens (Kortet et al. 2003c). Epidermal papillomatosis is more common in males than in females and its prevalence clearly peaks during the spawning season (Kortet et al. 2002). Roach is iteroparous, it undertakes spawning migrations (Mills 1991), and methods for immunological research on this species are well developed (e.g. Aaltonen et al. 1994, Salo et al. 1998).

Tench is a sexually dimorphic cyprinid: females are commonly larger than males, males have larger pectoral and pelvic fins than females, and the second fin ray of male pelvic fins is thick and bent (Linhart & Billard 1995). In addition,

during the breeding season males develop notable ventral protuberances (Breder & Rosen 1966). Tench usually spawn in groups of three, with two males competing for a female (Breder & Rosen 1966, Koli 1990). Male-specific characters of tench are not known to have any function (Breder & Rosen 1966).

Like roach, crucian carp males also develop breeding tubercles, but the mating system of the species has not been studied. Crucian carp are known for their exceptional ability to survive under anoxic winter conditions. Crucian carp has also been used intensively in studies concerning morphological antipredatory defence (e.g. Brönmark & Miner 1992, Holopainen et al. 1997), but less is known about the relationship between dominance, growth and the adaptivity of morphological antipredatory defence.

## 2.2 Assays of immune function

In studies I and III-V the ability of head kidney phagocytes to kill pathogens was assayed using the chemiluminescence technique (Scot & Klesius 1981). In studies III and IV the method was applied also to whole blood, when it gives information on both the relative count and efficiency of phagocytes.

Except for study V, the ability of tench blood phagocytes to produce reactive oxygen species was assayed at 24 °C with phorbol-12-myristate 13-acetate (PMA)-stimulated, luminol enhanced chemiluminescence method (Scot & Klesius 1981) using 10 µl of whole blood diluted in 40 µl of Na<sup>+</sup> and Ca<sup>2+</sup> free Hank's balanced salt solution on chemiluminescence plates (DYNEX Microlite™, Dynex Technologies, Chantilly, USA). A similar method was used for tench head kidney phagocytes, but instead of blood and saline, 50 µl cell suspension of 10<sup>6</sup> cells ml<sup>-1</sup> was used. In roach (I), a suspension of 10<sup>5</sup> cells ml<sup>-1</sup> and PMA concentration of 2 µg ml<sup>-1</sup> was used (Kortet et al. 2003a). In study V, chemiluminescence response was induced by latex beads without PMA, and a denser cell suspension of 2 \* 10<sup>6</sup> cells ml<sup>-1</sup> was used.

For extraction of tench phagocytes from the head kidney, the organ was collected, placed in an Eppendorf-tube with Hank's Na<sup>+</sup> and Ca<sup>2+</sup> free balanced salt solution and a drop of liquid heparin, and was homogenized through a nylon net (mesh size: 80 mesh) (Salo et al. 1998). The homogenate was layered on a two-step Percoll-gradient (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). After centrifugation (400 × g for 40 min) the cells were collected from the 1.040 (1.050 in V) - 1.090 g cm<sup>-3</sup> interphase (modified after Pedrera et al. 1993). The cells were washed once for 5 min at 400 × g and viable cells were enumerated using trypan blue exclusion as the criterion (10<sup>6</sup> cells ml<sup>-1</sup>). For roach cells in study I, centrifugation for 30 min (400 × g), and Percoll bilayer gradient consisting of 1.070 and 1.090 g cm<sup>-3</sup> layers was used. The roach cells were washed twice before counting cells as described for tench. In study V, roach cells were treated similarly as tench cells. Except for the study V, chemiluminescence values for blood and head kidney phagocytes are expressed

as peak rate of the reaction. In study V, chemiluminescence values are reported as the mean rate of reaction during 30 min.

Migration of roach head kidney phagocytes was assessed using a modified under-agarose technique (Nelson et al. 1975) as described by Salo et al. (1998). Casein (100 mg ml<sup>-1</sup>, Sigma Chemical Co., USA) was used as a chemoattractant, the cells were allowed to migrate in a humidified environment at 25°C for 3 h and then fixed overnight with methanol. The agarose was carefully removed and the slides with migrated cells were air-dried and stained with a haematological stain (Diff-Quick, Baxter Diagnostic AG, Germany). The distance (µm) that the leading front of cells migrated from the margin of the well towards the well containing casein and the distance the least migrated cells moved spontaneously away from the well were measured under a microscope equipped with an ocular micrometer. The difference between spontaneous and directed migration (chemotactic differential) was used in the analyses. Plasma IgM concentration in roach was determined by the method described by Aaltonen et al. (1994).

Plasma lysozyme activity of tench was determined using a modification of a technique used by Caruso & Lazard (1999). Plasma samples of 80 µl were mixed with 220 µl bacterial suspension (0.20 mg ml<sup>-1</sup> *Micrococcus lysodeikticus* (Sigma-Aldrich, Schnellendorf, Germany) in phosphate buffered saline, pH 6.2) on ELISA plates (Cliniplate EB, Labsystems, Finland) and the reduction in sample absorbance was measured spectrophotometrically at 492 nm after 1.5 hours at 24 °C (Multiskan, Flow Laboratories, UK) with correction being made for absorbance by plasma and pure bacterial suspensions. Lysozyme activities were near the detection limit of the method.

### 2.3 Examination of parasite infestations

The proportion of dead *Rhipidocotyle campanula* parasites among all *R. campanula* individuals in the roach gills was assessed (I) and used as a measure of resistance as described in Taskinen & Kortet (2002). The proportion was not defined if the total number of parasites was less than three. Infection of tench males with *Diplostomum sp.* and *Tylodelphys sp.* eye flukes in study III was examined by squashing the lens and vitreous body between two large glass plates followed by microscopic examination.

### 2.4 Manipulation of tench immune system

β-glucans, derived from yeasts and fungi, are polyglucoses consisting of a linear backbone of β-1,3 linked D-glucofuranosyl residues with varying degrees of branching from the C6 position (Robertsen 1999). Low concentrations of β-



glucans are effective stimulators of non-specific immune functions in fish (Santarém et al. 1997, Robertsen et al. 1999), and high concentrations may exhaust phagocytotic cells (Castro et al. 1999).  $\beta$ -glucan injection was prepared by sonicating 3.00 g of water-insoluble  $\beta$ -glucan powder (MacroGard AquaSol, Biotec Pharmacon ASA, Norway) in 20 ml  $\text{Na}^+$  and  $\text{Ca}^{2+}$  free sterile Hank's balanced salt solution (HBSS). Fish were injected intraperitoneally with  $\beta$ -glucan ( $150 \text{ mg kg}^{-1}$ ) in HBSS.

Testosterone (Fluka Chemie AG, Switzerland) was first dissolved in 95% ethanol (1:1) and then mixed with melted coconut butter. The concentration of testosterone in coconut butter was  $80 \text{ mg ml}^{-1}$ , and the used dose of liquid mixture was  $1 \text{ ml kg}^{-1}$ .

## 2.5 Hormone assays

Plasma hormone concentrations (I, II, III & IV) were determined using commercial radioimmunoassay-kits ( $^{125}\text{I}$  as a marker) according to the manufacturer's instructions (TESTO-CTK, DiaSorin, Italy, for testosterone, ESTR-CTK-4, DiaSorin, Italy, for  $17\beta$ -oestradiol and GammaCoat<sup>TM</sup>, DiaSorin, USA for cortisol). However, the sample volume for the roach testosterone analysis was decreased to  $30 \mu\text{l}$  and for the oestradiol analysis to  $10 \mu\text{l}$  following preliminary tests on roach plasma (See also Kortet et al., 2003c). In study IV, sample volume was doubled from that in study III, since male tench appeared to have very low testosterone concentration.

## 2.6 Quantification of sexual characters and condition

In study II, male roach were categorized into four groups according to the breeding tubercle ornamentation on their lateral sides (see Taskinen & Kortet 2002): (1) no-ornamentation, (2) very slight ornamentation, (3) clearly observed ornamentation and (4) very rough skin and easily seen breeding tubercles. The classification of fish on the basis of their breeding-tubercle ornamentation has been shown to be highly repeatable (Kortet et al. 2003c). In addition, the number of breeding tubercles on the front head of male roach was counted (see Kortet & Taskinen 2004).

In study III, the maximum width of the male tench belly was measured to the nearest mm to represent the width of ventral protuberances. Area of pelvic fins was measured to  $0.5 \text{ cm}^2$  by drawing the outline of the dissected fins on graph paper. Logarithmic regression ( $\text{Ln}(\text{feature size}) = a + b * \text{Ln}(\text{body mass without intestinal organs or total length})$ , where  $a$  and  $b$  are constants) was used for calculation of size-independent residuals (deviance from expected size for an individual) of the area of pelvic fins and the width of belly.

Length-independent condition factor for all fish was calculated using the equation,  $K = 100 \times M_T L_T^{-b}$  where the  $M_T$  is the total body mass in grams,  $L_T$  is the total body length in cm and  $b$  is the slope of a regression of  $\log_{10}(M_T)$  on  $\log_{10}(L_T)$  (Bolger & Connolly 1989).

## 2.7 Observations of behaviour

In study III, behavioural observations were conducted using two setups. In the first setup, I wanted to explore whether male-male dominance appears in tench and whether it is related to male quality. I allowed the fish (two males with a female) to settle in the tanks for four days, and then on days five (around dusk), six (around dusk) and seven (around dawn) they were observed for 30 min. Dusk and dawn are known to be the most active periods of tench (Perrow et al. 1996). I assumed that during the four days of acclimation the fish would establish a dominance hierarchy if they showed any pre-spawning dominance behaviour. After several days in tank conditions and in a small group of three fish, the fish were found relatively immobile and usually hiding under vegetation. Thus, only the locations of the males in the tank and their positions in relation to the female were recorded. Locations of males were classified as (1) next to the female (distance at maximum c. 20 cm) or (2) distant from the other fish and away from shelter (distance at least c. 100 cm). The 30 min observation period was divided into 5 min intervals, and a male was recorded to be at a specified location if it spent most ( $> 2.5$  min) of the time in that position. Therefore it was possible for the male to be in neither of these recorded positions. The male's behaviour was quantified using the number of 5 min periods near the female minus the number of periods alone. The use of locations to assess male behaviour was based on previous studies assessing dominance in fish (Bailey et al. 2000).

In the second setup, I wanted to explore whether females can differentiate males by their morphological characteristics. To test female preferences for male qualities, I used a dyadic setup, where the movements of a focal fish are observed during exposure to two male fish (e.g. Beeching & Hopp 1999, Bisazza & Pilastro 2000, Suk & Choe 2002). I performed thirteen preference tests. Each test lasted 24 h and was conducted in a tank (730 cm long x 47 cm wide tank, water depth 40 cm) divided into five compartments. The outermost internal divisions were constructed using wire net (15 mm mesh size) that allowed the focal and tested fish to see and smell each other. To quantify fish behaviour without direct observation and to minimize disturbance, we used computer-based automated passive integrated transponder (PIT)-technology.

In study VI, an analogous PIT-system was used to follow the movements of a crucian carp shoal. All fish were tagged with a passive integrated transponder (PIT-tag Destron 400 kHz) under anaesthesia prior to testing. PIT-readers were 50 cm long transparent acrylic tubes of 200 mm in diameter set a

transparent, waterproof, acrylic box (50 cm x 20 cm x 60 cm) 5 cm above the bottom (Pirhonen et al. 1998). Each tube was equipped with an antenna at both ends. Each time a fish swam through the PIT-antennae, two data points consisting of the antenna number, tag code, time and date were registered in a computer file. This allowed determination of the direction the fish was swimming. I tested the reliability of the ports by moving a PIT-tag through each port by hand, and the sensitivity of the antennae was maximised by adjusting their resistance using an adjustable potentiometer. Based on three randomly selected crucian carp from each observation period, the system successfully recorded 83.9 % (95 % C.I. 78.2 - 89.6 %) of all port by-passes (the following port signal was expected by the last).

## **2.8 Epidermal papillomatosis**

The intensity of papillomatosis in study II was determined by counting the number of scales covered by papillomas, since the outlines of scales were visible even when covered by the largest papillomas, which were up to 2 mm in thickness. Moreover, the number of scales in a fish is somewhat constant throughout life and thus represents a scalable measure of fish surface area. If the papilloma was in a location where scales do not exist, such as fins, the area covered by the tumors was estimated as the number of scales they would have covered in that particular fish individual.

## 3 RESULTS AND DISCUSSION

### 3.1 Steroids, immunity and immunocompetence handicap

Fish immune functions undergo remarkable seasonal changes in the number of lymphocytes in blood, in the size of immunological organs and in immunoglobulin concentration (e.g. Nelson & Demas 1996, Álvarez et al. 1998, Scapigliati et al. 1999, Thomas et al. 1999, Kortet et al. 2003a). Reproduction has been shown to impair immune function and increase parasitism in Arctic charr (*Salvelinus alpinus* (L.)) (Skarstein et al. 2001). Breeding-related immunosuppression is thought to result from high breeding-time concentration of androgens (Aida 1988, Folstad & Karter 1992, Suzuki et al. 1997, Hou et al. 1999a, b, Muñoz et al. 2000), although seasonal changes can also be induced by variation in water temperature and in the concentration of corticosteroids (Zapata et al. 1992, Collazos et al. 1994, Alcorn et al. 2002).

Although immunosuppressive effects of testosterone were thought to be obvious at least in mammals (Grossman 1985), the recent meta-analysis by Roberts et al. (2004) on the applicability of the immunocompetence-handicap hypothesis (Folstad & Karter 1992) suggests that in many species, testosterone may increase susceptibility to ectoparasites whereas direct effects on immune function do not exist. For example, in red-winged blackbirds (*Agelaius phoeniceus* L.) a clear absence of immune function response to testosterone has been reported (Hasselquist et al. 1999). In contrast, many studies in lizards clearly support the immunosuppressivity of testosterone (e.g. Saad et al. 1990, Klukowski & Nelson 2001). For a review of the effects of steroids on fish immune system see Harris & Bird (2000) and on different types of mammalian leukocytes see Miller & Hunt (1996).

Slater et al. (1995) characterized androgen receptors responsive to testosterone in rainbow trout (*Oncorhynchus mykiss* Walbaum) leukocytes, and suggested that they are an important link in the androgen-mediated immunosuppression. Since then, several studies have confirmed that testosterone has immunosuppressive effects in many salmonids (Buchmann 1997, Slater & Schreck 1997, Hou et al. 1999a, b). However, more recent studies

by Law et al. (2001) and Yamaguchi et al. (2001) suggested that such effects on non-specific immunity are not present in cyprinids. In contrast, testosterone has been found to suppress the production of specific antibodies *in vitro* in common carp (Saha et al. 2004). Moreover, Watanuki et al. (2002) found that 11-ketotestosterone and oestradiol suppressed phagocytosis, superoxide anion and nitric oxide production of common carp leukocytes *in vivo*. These results suggest that cyprinids and salmonids may differ in their responsiveness to testosterone.

Although the testosterone-dependency of breeding tubercles has not been studied experimentally, a correlative relationship has been revealed (Kortet et al. 2003c). Immune functions in roach show some sex-dependence and seasonal variation related to reproduction in May (Kortet et al. 2003a). In addition, testosterone has been associated with the peak occurrence of epidermal papillomatosis in spring (Kortet et al. 2002, 2003c). Thus, roach should be a good model species to test the existence of a testosterone-mediated immunocompetence-handicap mechanism. There are no experimental data on the relationship between androgens and male-specific characteristics in tench, but if steroids regulate life-history trade-offs between reproduction and immune defence, testosterone should suppress immune function also in tench.

In Paper I correlations of testosterone and oestradiol with several measures of immunity (differential and total leukocyte counts, IgM, chemiluminescence and migration of head kidney phagocytes and size of the spleen) and using condition factor as a partial correlate were studied five times during a year in two roach populations. No significant negative correlations were found. Instead, both testosterone and oestradiol correlated positively with parasite resistance, measured as the proportion of dead *Rhipidocotyle campanula* parasites. Moreover, testosterone was positively correlated with chemiluminescence response of head kidney phagocytes (a measure of their ability to kill phagocytosised particles) and total IgM concentration. Because condition factor was used as a partial correlate in these analyses, the results suggest that neither testosterone nor oestradiol suppress immune functions in roach.

The measures of immune defence used in the Paper I can be regarded as indices of both health and immunocompetence. Phagocytes form the major cell-mediated non-specific defence mechanism in fish (Secombes & Fletcher 1992), and chemiluminescence and chemotaxis are often used to assay the ability of phagocytes to kill pathogens (e.g. Collazos et al. 1995, Salo et al. 1998). For example, Hakoyama et al. (2001) found that the lower reduction of nitroblue tetrazolium (NBT) (the NBT test is analogous to a respiratory burst assay with the chemiluminescence method) in gynogenetic *Carassius auratus* (L.) was related to their lower resistance against parasites (*Metagonnimus* sp.; Trematoda). The spleen is involved in haematopoiesis, the clearance of pathogens and other foreign particles from the blood stream, and antibody-production (Dalmo et al. 1997, Manning 1994), and a large spleen is thought to indicate good condition and the ability to respond to infection (Wester et al. 1994). Concentrations of plasma immunoglobulins increase with infection or

exposure to parasites, but low concentrations may be indicative of a weakened immune system (Manning 1994). The total count of lymphocytes is an index used to assess health status (Wester et al. 1994) and differential counts of leukocytes are used to reveal possible infections, because the proportion of phagocytes usually increases with infection (Wester et al. 1994).

The correlative data on the immunological effects of testosterone were not explicit, so two further experiments were planned (Papers IV and V). The *in vivo* testosterone treatment did not suppress the lytic activity of plasma or the chemiluminescence response of whole blood or head kidney phagocytes in tench (Paper IV). However, the testosterone-treated fish lost more mass than the control fish during the experiment. The testosterone-induced decrease in body mass (65.2 g vs. 55.6 g) was probably caused by an increase in metabolic rate (Lynshiang & Gupta 2000, Ros et al. 2004), although the initial fish mass may have contributed to this result (length was used as a covariate). This suggests that testosterone might not suppress immune functions but may cause energetic demands by increasing basal metabolic rate (Buchanan et al. 2001).

Both  $\beta$ -glucan and testosterone administrations led to a decrease in the relative mass of the spleen, a measure which is known to have some indicative value of immunocompetence (Wester et al. 1994). Conversely, the spleen is known to have an important function in haematopoiesis (Dalmo et al. 1997), and the reduction in its size may indicate that repeated blood samples increased demand for new blood cells. Testosterone-induced lymphopenia of the spleen has been reported also in the turtle, *Mauremys caspica* Gemlin (Saad et al. 1991). However, some effects in this experiment might have been caused by stress, since stress is known to increase relative number of phagocytes in relation to lymphocytes (the effect that was detected as an increase in the chemiluminescence of blood).

In study V, the effects of testosterone, oestradiol and cortisol on the phagocytosis of head kidney phagocytes in both roach and tench were studied *in vitro*. Again, this experiment did not reveal immunosuppression caused either by testosterone or oestradiol. This experiment suffered from a decrease of responses by ETOH-vehicle but it clearly demonstrated that possible effects of testosterone are much weaker in magnitude than those of cortisol. Other studies on cyprinids (Law et al. 2001, Saha et al. 2003) support this view, although Yamaguchi et al. (2001) found that high concentrations of oestradiol suppressed phagocytosis in common carp.

The results of the *in vitro* experiment (V) clearly demonstrated that physiological levels of cortisol suppress the phagocytosis-induced respiratory burst response of both roach and tench phagocytes. Taken together, these results suggest that spawning stress, rather than testosterone or oestradiol, might explain the reproduction-related immunosuppression in general (Braude et al. 1999). Interestingly however, high breeding-time testosterone concentration in both sexes of roach may contribute to the breeding-related increase in the occurrence of epidermal papillomatosis (Kortet et al. 2002) since

testosterone is known to decrease production of specific antibodies in common carp (Saha et al. 2004).

Testosterone, however, is equally present in both sexes of cyprinids and at least some species of salmonids, and the major male sex hormone in teleosts is 11-ketotestosterone (Borg 1994, Pottinger & Carrick 2000). Thus, the lack of an effect on immune function by testosterone without experimental evidence for the effects of testosterone on sexual signalling does not conflict with the immunocompetence handicap hypothesis (Folstad & Kartet 1992): it just might be the wrong hormone to study. It is notable that in our previous study (Kortet et al. 2003c) concentrations of testosterone and 11-ketotestosterone were positively correlated, and the effects that were attributed to testosterone may actually have been due to 11-ketotestosterone.

### **3.2 Spawning stress and the occurrence of epidermal papillomatosis**

Epidermal papillomatosis is a skin disease, characterized by white, smooth, ovoid tumours on the skin, fins and lips of fish. It has been reported in several fish species (Bylund et al. 1980, Möller & Anders 1985, Lee & Whitfield 1992, Premdas et al. 1995, Mellergaard & Nielsen 1997, Kortet et al. 2002). Papillomatosis has been associated with herpes viruses or papilloma viruses in several fish species including koi carp (*C. carpio*), smelt (*Osmerus eperlanus* L.) and rainbow smelt (*O. mordax* Mitchill) (e.g. Hedrick et al. 1990, Lee & Whitfield 1992, Sano et al. 1993). Latent infectious agents of papillomatosis are suggested to be present in the population all the time, and only a part of the population may develop the disease with tumours (Lee & Whitfield 1992, Sano et al. 1993).

Both prevalence and intensity of epidermal papillomatosis increase during spawning period in roach and especially in males (Kortet et al. 2002). Moreover, Kortet et al. (2003c) associated occurrence of papillomatosis with high testosterone but not 11-ketotestosterone concentration. However, testosterone alone cannot explain the observed high prevalence of papillomatosis in males, since both sexes of roach have equal concentrations of testosterone as found in Paper I.

Paper II demonstrated spawning-time plasma cortisol concentrations nearly 20 times higher than previously reported for unstressed roach outside the breeding period (Pottinger et al. 1999). In addition, the study showed a rapid, statistically significant decrease of roach plasma cortisol concentrations to one third of the spawning-time values six days after spawning. These results suggested that roach are subject to severe stress during spawning. Furthermore, the stress may be stronger in males as suggested by their 1.25 times higher cortisol levels during spawning in comparison to females. Thus, a stress-based mechanism of reproduction-related immunosuppression may exist in roach.

The results in Paper II strongly suggested that spawning-related stress is associated to the occurrence of epidermal papillomatosis in roach. If testosterone-induced suppression of specific immune defence (Saha et al. 2004) explained the occurrence of epidermal papillomatosis, the disease should show high prevalence in both sexes already in early winter, when the testosterone concentration is increasing (Paper I).

Unfortunately, Paper II could not resolve whether high cortisol concentration was the cause or consequence of papillomatosis. However, plasma cortisol concentration did not correlate with the intensity of papillomatosis among the diseased fish, suggesting that high cortisol concentration most likely expose to papillomatosis, not *vice versa* (in the opposite case the severity of the disease should correlate with the level of stress). Moreover, the results of an earlier study indicated that papillomatosis does not significantly affect the survival of roach (Kortet et al. 2003b). The opposite hypothesis is supported by the decrease of hematocrit (proportion of red blood cells of blood volume) after spawning in diseased fish. Because the hematocrit has been used as an indicator of health (e.g. Munkittrick & Leatherland 1983, Kortet et al. 2003a), the result suggests that epidermal papillomatosis might have a physiological cost and lead to lower condition in diseased fish. However, this was not supported by the equal condition factors between healthy and diseased fish after the spawning or by the positive correlation between hematocrit and plasma cortisol concentration. Clearly, however, an experimental study is needed to examine whether stress stimulates papillomatosis in fish (unpublished data by Korkea-Aho, Taskinen & Vainikka suggest that stress really induces papillomatosis to erupt).

Neither the front head nor lateral breeding tubercle ornamentation was related to plasma cortisol concentration, although papilloma-diseased males had a higher cortisol concentration than healthy males. Kortet et al. (2003c) showed that elaborated breeding tubercle ornamentation was positively related to males' androgen concentrations and to severity of papillomatosis. Since cortisol is a potent immunosuppressor (e.g. Weyts et al. 1999, Davis et al. 2003, study V), the consequences of spawning stress might contribute to the honesty of sexual signalling in roach so that only genetically highly resistant males are able to carry the cost of both stress and elevated levels of androgens (c.f. Folstad & Karter 1992, Braude et al. 1999, Buchanan 2000).

### **3.3 Gender-differences in immune defence and parasitism**

In several vertebrate species, males have a weaker immune system than females (reviewed by Zuk & McKean 1996). Male-biased gender differences in parasitism and disease have been reported in fish (Reimchen & Nosil 2001, Kortet et al. 2002), but data concerning immune parameters of fish are scarce (Collazos et al. 1994, Kortet et al. 2003a). In Paper I, no clear gender differences



in immune and health parameters were found. The effects of testosterone were not dependent on the sex of tench in Paper IV. Similarly, study V did not detect any gender-differences either in the baseline reaction to phagocytosis or in the responsiveness of head kidney phagocytes to studied steroids.

Kortet et al. (2003a) showed that male roach have a larger spleen, higher haematocrit and marginally higher concentration of plasma IgM than females throughout the year. Also in Paper III, male tench were found to have larger spleens than females. Thus, it seems that cyprinid males have higher values of some haematological measures than females. However, these results suggest that clear differences in immune function between males and females in these species are unlikely.

During spawning, male roach engage in territorial behaviour, aggressive behaviours towards other males and courtship behaviours directed to females (Diamond 1985, Wedekind 1996). This may contribute to the higher cortisol concentration and stronger spawning stress in male than in female fish observed in Paper II. In this respect, it is important to note that ultimate ecological reasons might be evolutionarily more important in male-biased parasitism than endocrinological mechanisms *per se* (Reimchen & Nosil 2001).

### 3.4 Sexual advertisement of immune function

The correlative relationship between sexual signalling and immune function has been examined intensively and several studies, conducted mainly with insects, have found evidence for a positive association (e.g. Wedekind 1992, Lindström & Lundström 2000, Hine et al. 2002, Rantala et al. 2002, Taskinen & Kortet 2002, Rantala & Kortet 2003). However, it is not clear how the honesty of signalling is maintained. Empirical evidence suggests that condition may have a major impact both on immune function and sexual signalling (Rantala et al. 2003a), whereas activation of immune function may not decrease the concentration of testosterone (IV) or the attractiveness of male *Tenebrio molitor* L. pheromones (Vainikka et al. unpublished data). This would suggest that the relationship between sexual signals and parasites is not a plastic feedback loop in the sense meant by the immunocompetence-handicap hypothesis (Folstad & Karter 1992) but instead is dependent on individual genetics and condition.

Studies including methods of quantitative genetics or multigenerational observation of offspring fitness suggest that in some species individuals might gain a fitness advantage by choosing highly-ornamented mates (e.g. Boake 1985, Møller & Alatalo 1999, Wedekind et al. 2001). However, the number of offspring produced does not always translate to fitness: offspring from resistant parents may suffer high mortality in the juvenile phase but be more successful as adults (Barber et al. 2001, Kortet et al. 2004b). This, as well as advantages by choosing immunity, most probably have a heritable component: in other words, immunocompetence has to be heritable as shown, for example, in insects by

Cotter & Wilson (2002) and in humans by Hall et al. (2000). In this respect, the suggestion of Siva-Jothy & Skarstein (1998) might be valid: individuals choose for genetics (or for the resulting condition) but measures of immune function correlate with genetics or condition and supportive results for selection for immune function are found as by-products.

In roach, the number of front head tubercles correlated negatively with the intensity of papillomatosis (II) corresponding to earlier results of Kortet & Taskinen (2004). However, our recent study comparing the chemiluminescence response of head kidney phagocytes to latex beads between highly-ornamented and weakly-ornamented roach males (N = 10 per group) after spawning did not reveal differences (independent-samples t-test,  $t_{(8.74)} = -1.21$ ,  $P = 0.292$ , Löytynoja & Vainikka, unpublished data). Thus, parasite intensities might reveal genetic immunocompetence better than measures of non-specific immune function.

In tench, 73-79 % of the variation in the size of the male-specific characteristics was explained by the body mass without intestinal organs, whereas body length explained 66-76 % of the variation. The variation in the ornaments of male tench was rather small when compared to 20-80 % variation (SD, CV is approximately the same) for example in the sexual ornaments of feral guppies (*Poecilia reticulata* Peters) (Karino & Haijima 2001). This suggests that something other than sexual selection may be responsible for sexual dimorphism in tench.

Actually, no evidence for sexual selection was found either in male-male contests or in female preference tests: In male-male contests, no significant correlations were found between the behaviour of a male and the relative size of pelvic fins, relative width of the ventral protuberances, condition factor, plasma testosterone concentration, immunocompetence indexes and load of eyflukes, when these were analysed as differences within pairs. In female preference tests, no significant preferences towards males based on their qualities appeared.

Both the relative size of pelvic fins and the width of ventral protuberances showed correlation with relative body fatness supporting the idea of their condition-dependency. Plasma testosterone concentration was positively correlated with condition factor and size of the pelvic fins relative to length but not with the other parameters. The two principal components of immune function did not correlate with the condition factor, plasma testosterone concentration, GSI, the count of eyflukes or the relative sizes of pelvic fins and ventral protuberances.

These results based on behavioural observations conducted a few weeks prior to spawning do not fully exclude the possibility that sexual selection occurs in tench. Alatalo et al. (1998) concluded that sexual selection may be maintained if the cost of choice is minor eventhough the benefits are small. Thus, if females do not carry a cost by picking a male with large ornaments, sexual dimorphism is maintained without substantial genetic benefits. However, based on these results it seems that sexual dimorphism in tench most

likely facilitates sex recognition (Barlow & Siri 1997), but sexual selection for good resistance genes may be unlikely.

Tench is not the only sexually dimorphic fish without clear sexual selection: sexual dimorphism of orangethroat darter (*Etheostoma spectabile* Agassiz) was found not to relate to female mate choice (Pyron 1995). The relationship between sexual signals and mate choice in *Xiphophorus pygmaeus* Hubbs & Gordon was found to be negative (Morris et al. 1996). Moreover, female three-spined sticklebacks can relate their mate choice to their own MHC-genetics (Aeschlimann et al. 2003, Milinski 2003). Thus, mate choice strategy in tench might also be much more complex than simple choice for large ornaments. For example, Pinillos et al. (2003) showed that tench release and detect spawning pheromones. Moreover, the possibility that males use their ornaments somehow to manipulate females or other males during spawning should be studied.

### 3.5 Life-history traits in an interactive environment

The original aim of the Paper VI was to relate plasma testosterone concentration to individual behaviour and growth. Plasma samples were collected prior to the experiment and after the experiment but the samples were not analysed because the measures of growth or reproductive effort did not show clear correlations with the behavioural measures.

The relative mass of testes was almost significantly ( $P = 0.061$ ) correlated positively with the time spent near pike during food delivery times among males in the first period with pike. The respective time spent in the control patch was actually correlated negatively with gonad mass among males ( $P = 0.044$ ). Among females, a similar pattern was seen in the second period with pike. These correlations were not present in both periods when the pike was present, but the results suggest that taking higher risks might improve the reproductive potential in crucian carp exposed to a simulated predation risk.

The only significant correlation between behavioural measures and specific growth rate was found among females in the second period with pike, when the time spent next to pike during daytime was negatively correlated with specific growth rate ( $R = -0.454$ ,  $N = 23$ ,  $P = 0.030$ ). It is possible that a larger number of individuals may have yielded significant results, and further work is clearly needed to address this issue.

Overall, crucian carp transferred from a natural population to *ad libitum* food conditions increased their body mass by one third during the 69 days experiment. Both sexes increased their relative body height by about 10 %, but the rate of morphological change was not affected by the introduction of a pike. Change in body depth was correlated with overall growth rate in both sexes in the absence of predation risk. Interestingly, however, the positive correlation between growth rate and increase in relative body height disappeared in both

sexes after the pike was introduced. This suggests that under predation risk some fish can allocate more energy to the increase in height than to overall growth (see also Brönmark & Petterson 1994).

The introduction of the pike did not affect the rate of increase in condition factor, body mass or relative body depth. However, the effects of pike were difficult to separate from those caused by food manipulation or changes in time. This is because the study was not specifically planned to test whether the presence of the pike *per se* would affect the growth of the fish.

The original idea of the study was to induce dominance behaviour among a shoal of crucian carp by restricting feeding possibilities in the control end and forcing the (subordinate) fish to feed in a risky patch next to pike. However, no evidence was found that crucian carp formed dominance hierarchies based on their body size or growth rate. Instead, all fish compensated by feeding in the pike end during night. However, it is possible that some dominance appeared but that did not affect the feeding behaviour of the fish. For example, van der Veen (2002) found no evidence that dominance among yellowhammers (*Emberiza citronella* L.) could affect foraging delay after simulated predation risk.

Utne et al. (1997) reported that growth rate and maturity affected risk-taking behaviour in perch. In this study, fish size, gonad size or body depth did not relate to their risk-taking behaviour (presence next to pike). In a field study using divisions in a pond, Paszkowski et al. (1996) showed that the smallest crucian carp that were most vulnerable to predation by perch decreased their food intake due to predation-induced ecological competition between individuals. In this study, the only size-related effect was found in females, where originally large individuals developed relatively higher bodies than their smaller conspecifics. However, this might have been related to the higher overall growth rate in females than in males.

Paper VI demonstrated a change in the allocation of resources between growth and body height gain in crucian carp induced by predation risk. Because growth rate correlated negatively with gonad mass in both sexes, it is likely that the ambient level of predation risk can alter the balance between reproduction and growth simultaneously affecting the resulting body depth and thus the ability to avoid predation in crucian carp. Because our setup did not reveal significant correlations, the effects of predation on life-history decisions should be studied further using individual fish as independent replicates.

## 4 CONCLUSION

Current knowledge of the evolution of life-histories suggests that hormonal control plays an important role in the regulation of life-history trade-offs. In sexual selection theory, the honesty of sexual signals has been proposed to result from a handicap caused by their expression (Zahavi 1975, Grafen 1990, Iwasa et al. 1991). The extended immunocompetence-handicap hypothesis (Folstad & Karter 1992, Wedekind & Folstad 1994) suggests that male sex hormones are adaptively involved in the regulation of resources between secondary sexual traits and immune function. According to this hypothesis, high levels of male sex hormones needed for gonadal development (e.g. Schulz et al. 2000, Weltzien et al. 2002) and production of sexual signals cause immunological or metabolic costs.

In this thesis, I concentrated on the role of testosterone in the regulation of immune function in roach and tench (I, III, IV and V). In contrast to mammals, testosterone is equally present in both sexes of cyprinids, but several studies suggest that testosterone might contribute both to sexual signalling and breeding-related immunosuppression. To relate biotic environmental factors to life-history regulation, I examined the relationship between somatic growth, behaviour and reproductive effort in crucian carp with and without a predator (VI). To understand male-biased occurrence of a viral disease, epidermal papillomatosis in roach, I compared cortisol levels between healthy and diseased males and females during and after spawning (II).

Simultaneous changes in the immune function and hormonal state of roach were found, but no negative correlations between the measures of immune defence and testosterone or oestradiol concentrations were detected (I). Nor did the observations of tench (III) reveal negative correlations between testosterone concentration and immune function. Testosterone-implantation in tench (IV) confirmed those results, but by causing body mass loss, suggested that high circulating testosterone levels are energetically costly by increasing metabolic rate (c.f. Buchanan et al. 2001). This is supported by the positive correlation between condition and testosterone concentration (III). An *in vitro* experiment with testosterone, oestradiol and cortisol in tench and roach (V)

yielded findings that were in line with earlier results *in vivo*, but suggested that cortisol is a potent immunosuppressor in cyprinids (Fig. 1).

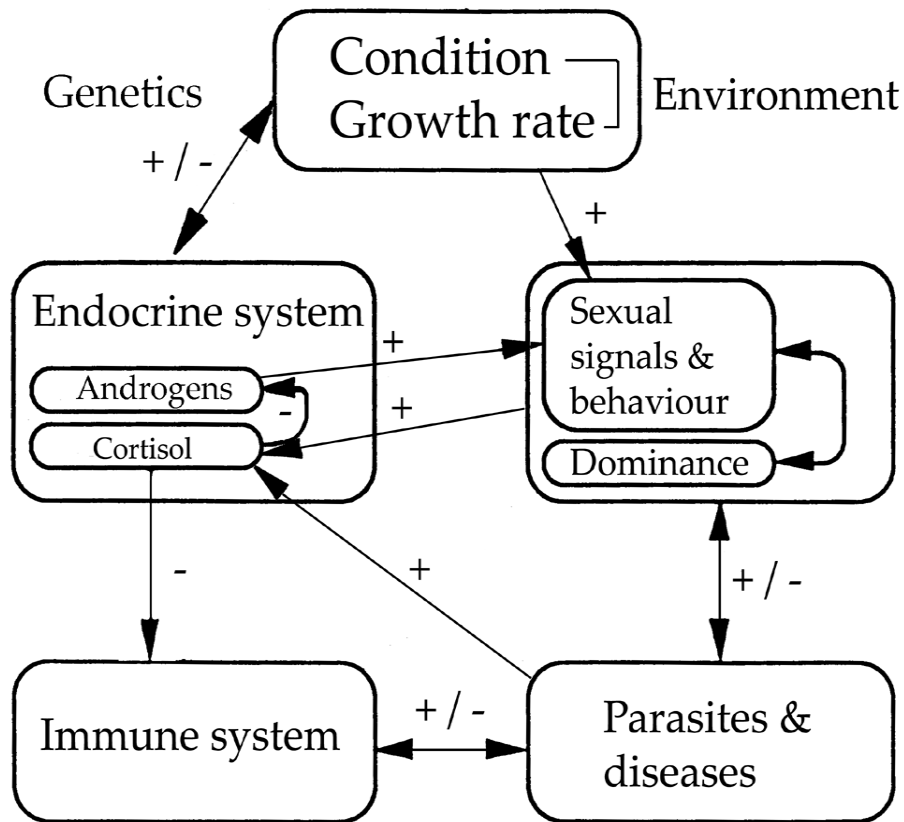


FIGURE 1 Environment - endocrine - immune system - parasites - sexual signalling feedback web (modified after Folstad & Karter 1992). Growth rate and condition are affected by individual genetic and environmental factors such as food availability and prevailing predation risk. Good condition is reflected in high androgen concentration which, along with condition-dependent factors, increases the level of sexual signalling. Controversially, androgens may increase basal metabolism affecting growth and condition. Sexual signals and vigour increase success in dominance contests, but sexual behaviour causes stress leading to immunosuppressive cortisol levels. Sexual signals also expose individuals to parasites by, for example, increasing surface area and behavioural encounters. Parasites may impair immune function by stress or activate it directly, whereas stress inhibits androgen production and thus decreases the level of sexual signalling.

Spawning is evidently more stressful to male than female roach (II): stress and stress hormones being a possible cause of male-biased occurrence of epidermal papillomatosis. Growth rate was traded-off against gonad size and body height-gain dependent on the risk of predation in crucian carp (VI). Thus, testosterone might regulate the allocation of resources between somatic growth and reproductive effort in relation to prevailing predation risk. Further studies should address this possibility.

Taken together, cortisol is likely to mediate immunocompetence-handicap either directly (Buchanan 2000) or in the interplay with androgens (Braude et al.

1999) (Fig. 1). Consten (2002) found that cortisol can decrease androgen synthesis in common carp (see also Haddy & Pankhurst 1999). Thus, stress hormones are likely to have an important role in reproduction and reproduction-related stress, possibly by contributing to the honesty of androgen-dependent traits in the studied cyprinids (Fig. 1).

In this thesis, the effect of testosterone on the antibody production was not tested experimentally. According to the Hamilton & Zuk (1982) hypothesis, males should advertise resistance to the currently most harmful parasites. Thus, it is possible that the predicted immunosuppression by testosterone (Folstad & Karter 1992) targets only some specific resistance mechanisms (Saha et al. 2004).

The original immunocompetence-handicap hypothesis of Folstad & Karter (1992) was proposed without concepts of sexual conflict theory and before recent advances in immunogenetics and knowledge of the effects of stress hormones. Current knowledge suggests that direct matching of MHC-genetics plays a more important role in sexual selection than selection for functional immunocompetence (Wedekind 1994, Siva-Jothy & Skarstein 1998).

If females promoted the evolution of immunocompetence-handicap in males by exposing males to male-specific hormone that suppresses the non-specific immune function, females would be able to choose genetically highly competent males by their sexual signals. Actually, the predictions of the immunocompetence-handicap hypothesis are supported by many studies in mammals and amphibians (Roberts et al. 2004). Cyprinid fishes, with their different testosterone-metabolism, offer an interesting model system, and the effects of the main teleost androgen, 11-ketotestosterone, should be studied for the case that immunosuppression in males is in the interest of females.

Recent advances in insect studies (Herman & Tatar 2001, Rantala et al. 2003) suggest that juvenile hormone not specific to either sex can affect longevity, sexual signalling and immune function. These results support the prediction of the immunocompetence-handicap hypothesis (Folstad & Karter 1992) even the hormone controlling the mechanism is common to both sexes. In cyprinids, testosterone might be an analogous non-sex-specific hormone that increases reproductive effort, but has costly effects by increasing metabolic rate and exposing fish to stressful behaviour and consequently to immunosuppressive stress hormones (Fig. 1).

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Everything did not always work out as planned: crucian carp and ruffe were not able to smell their mates as expected (at all!).  $\beta$ -glucan injection increased the metabolic rate of tench by 20 % but due to problems with the equipment, this result is mentioned only here. Since the equipment to measure basal metabolic rate did not work, a study was only planned to examine the effect of testosterone on basal metabolism. PIT-systems sometimes caused more stress than they gave data, but the two studies were still (successfully) conducted. However, data on sex recognition by female tench and data on mate choice by male tench were lost due to technical difficulties. Thanks to bacteria (or sometimes lack of water) both tench and crucian carp studies included lower number of fish than originally planned, and the study on the temporal stability of androgen levels within a male group failed. Due to lack of finance for material expenses, no studies on 11-ketotestosterone are included in this thesis. Still, I am happy to see this thesis finished and hopefully these open questions will be answered in the future.

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## YHTEENVETO

### Rehellisen seksuaalisen signaloinnin ja elinkiertojen evoluution mekanismit kolmella särkikalalla

Elinkiertopiirteiden evoluutiota koskevan nykytiedon mukaan hormoneilla on merkittävä rooli elinkiertopiirteiden välisten vaihtokauppatilanteiden säätelyssä. Seksuaalivalinnan teorian mukaan sekundääristen sukupuoliominaisuuksien tuottamisesta seuraa kustannuksia, jotka varmistavat näiden piirteiden kyvyn kertoa evolutiivisesti edullisista geneettisistä ominaisuuksista (Zahavi 1975, Grafen 1990, Iwasa et al. 1991). Laajennetun immuunihaittahypoteesin (Folstad & Karter 1992, Wedekind & Folstad 1994) mukaan koirassukupuolihormonit ohjaavat resurssien jakoa sukupuoliominaisuuksien ja immuunipuolustuksen välillä adaptiivisesti. Tämän teorian mukaan korkeita koirassukupuolihormonipitoisuuksia tarvitaan sukusolujen (esim. Schulz et al. 2000, Weltzien et al. 2002) ja seksuaaliornamenttien tuottamiseksi, mutta näiden piirteiden tuotosta on joko immunologinen tai metabolinen kustannus.

Keskityin väitöskirjassani testosteronin rooliin immuunipuolustuskyvyn säätelyssä särjellä ja suutarilla (I, III, IV ja V). Toisin kuin nisäkkäillä, testosteronia on särkikalalla yhtä paljon molemmilla sukupuolilla. Kuitenkin useat tutkimukset viittaisivat siihen, että testosteroni vaikuttaa sekä seksuaaliseen signalointiin että immuunipuolustuskykyyn. Bioottisten interaktioiden kytke-miseksi elinkierto-piirteiden välillä tapahtuvaan allokontiin, tutkin somaattisen kasvun, käyttäytymisen ja lisääntymispanostuksen välistä suhdetta ruutanalla hauen läsnä ollessa ja ilman sitä (VI). Selvittääkseni syitä epidermaalisen papil-lomatoosin runsaampaan esiintymiseen koirilla kuin naarailla, vertailin kortisoliarvoja terveiden ja sairaiden särkien välillä kudun aikana ja sen jälkeen (II).

Havaitsin yhtäaikaista muutoksia särjen immuunipuolustuskyvyssä ja hormoniprofiileissa, mutta negatiivisia korrelaatioita testosteronin tai oestra-diolin ja immuunipuolustuskykyä mittaavien parametrien välillä ei löytynyt (I). Myöskään korrelatiivinen suutariaineisto ei paljastanut negatiivisia yhteyksiä testosteronipitoisuuden ja immuunipuolustusmittareiden välillä (III). Testoste-ronin injektointi suutarilla ei myöskään johtanut immuunisupressioon, mutta aiheutti lievää somaattisen massan laskua (IV) viitaten siihen, että testosteronilla on vaikutuksia perusmetaboliatasoon (vrt. Buchanan et al. 2001). Tätä ideaa tuki myös havainto, jonka mukaan testosteronipitoisuus korreloi positiivisesti kuntokertoimen kanssa suutarilla (III). Suutarilla ja särjellä tehdyssä *in vitro*-kokeessa testosteronilla ja estradiolilla saatiin samansuuntaisia tuloksia kuin aikaisemmissa kokeissa (I, III, IV): immuunisupressiota ei havaittu. Kuitenkin kortisolin havaittiin voimakkaasti heikentävän molempien lajien fagosyyttien patogeenin tappokykyä (kuva 1). Tutkimus II osoitti stressiarvojen olevan koi-railla naaraita suurempia, mikä voi selittää koiraiden naaraita suurempaa altti-

utta sairastua epidermaaliseen papillomatoosiin. Kasvunopeus korreloi negatiivisesti gonadien koon kanssa, ja riippuen hauen läsnäolosta, joko positiivisesti tai ei ollenkaan, selän korkeuden kasvun kanssa ruutanalla (VI). Tämän vuoksi ehdotan, että testosteroni voi säädellä somaattisen kasvun suhdetta lisääntymispanostukseen riippuen vallitsevasta predaatoriskistä. Tämän aiheen tutkimuksille on kuitenkin selvästi tarvetta myös jatkossa.

Johtopäätöksenä esitän, että kortisoli voi joko yksinään (Buchanan 2000) tai yhdessä androgeenien kanssa välittää lisääntymisen seurauksena syntyvän immuunihaitan (Braude et al. 1999) (kuva 1). Consten (2002) totesi kortisolin heikentävän koirassukupuolihormonien tuotantoa karpilla (ks. myös Haddy & Pankhurst 1999). Tämän vuoksi stressihormoneilla on todennäköisesti merkittävä osuus lisääntymisessä ja lisääntymisen aiheuttaman stressin välittäjinä. Stressi-hormonit voivat vaikuttaa myös koirassukupuolihormoniriippuvaisten seksuaali-signaalien rehellisyyteen särkikaloilla (kuva 1).

Tämän väitöskirjan osatutkimuksissa en selvittänyt kuinka testosteroni vaikuttaa spesifisten vasta-aineiden tuotantoon. Hamilton & Zuk (1982) -hypoteesin mukaan koiraat mainostavat vastustuskykyään vain kullakin hetkellä kaikkein haitallisimpia patogeeneja vastaan. Siksi on mahdollista, että testosteroni aiheuttaa immuunisuppressiota vain tietyissä spesifisissä immuunipuolustuksen osa-alueissa (Folstad & Karter 1992, Saha et al. 2004).

Alkuperäinen immuunihaittahypoteesi Folstad & Karter (1992) julkaistiin ennen seksuaalisen konfliktin teorian käsitteitä ja viimeaikaisia merkittäviä löydöksiä immunogenetiikassa ja stressimetaboliassa. Nykytiedon mukaan MHC-kudostyyppien optimointi voi olla seksuaalivalinnassa tärkeämmässä roolissa kuin pelkkä fenotyypin immunokompetenssin valinta (Siva-Jothy & Skarstein 1998). Jos naaraat aiheuttavat immuunihaitan koiraisissa käyttäen välittäjänä vain koiraille ominaista hormonia, naaraat voisivat valita geneettisesti hyvälaatuisia koiraita näiden hormoniriippuvaisten signaalien perusteella. Itse asiassa immuunihaitta-hypoteesin onkin todettu toimivan monilla nisäkkäillä ja sammakkoeläimillä, joilla testosteroni on ominainen vain koiraille (Roberts et al. 2004). Särkikalat tarjoavat tässä suhteessa mielenkiintoisen tutkimussysteemin, koska testosteroni ei ole ominainen kummallekaan sukupuolella. Tämän vuoksi vain koiraille ominaisen 11-ketotestosteronin immunologiset vaikutukset olisi syytä selvittää särkikaloilla.

Viimeaikaiset hyönteistutkimukset ovat paljastaneet, että kummallekin sukupuolelle yhteinen juveniilihormoni voi vaikuttaa niin elinikään, seksuaaliseen signaalointiin kuin immuunipuolustukseenkin (Herman & Tatar 2001, Rantala et al. 2003). Nämä tulokset tukevat immuunihaittahypoteesin ennustuksia (Folstad & Karter 1992), vaikka juveniilihormoni ei olekaan koirasspesifinen. Särkikaloilla testosteroni saattaa olla analoginen sukupuolille yhteinen hormoni, joka lisää lisääntymispanostusta, mutta joka tuottaa kustannuksia kiihdyttämällä aineenvaihduntaa, altistamalla kaloja stressaaville käyttäytymispiirteille ja tämän seurauksena, immunosuppressiivisille stressihormoneille (kuva 1).

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