Evolutionary Ecology Research, 2003, **5**: 411–419

Sexual ornamentation, androgens and papillomatosis in male roach (*Rutilus rutilus*)

Raine Kortet,* Anssi Vainikka, Markus J. Rantala, Ilmari Jokinen and Jouni Taskinen

Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, FIN-40351 Jyväskylä, Finland

ABSTRACT

According to the immunocompetence handicap hypothesis, males with attractive sexual ornamentation are more handicapped than their less ornamented rivals because of the immunosuppressive androgens required for the production of secondary sexual characters. Here we studied the predictions of the hypothesis in a wild cyprinid fish, the roach (*Rutilus rutilus*). We assayed (1) sexual ornamentation (breeding tubercles), (2) circulating androgens (11-ketotestosterone and testosterone) and (3) epidermal papilloma disease of male roach at spawning. We found that elaborated sexual ornaments were associated with high circulating concentrations of testosterone and 11-ketotestosterone. Furthermore, papillomatosis was associated with high concentrations of testosterone and elaborated sexual ornaments. The results were consistent with the predictions of the immunocompetence handicap hypothesis. It had previously been shown in the present study system that breeding tubercles of males signal resistance against a predominant parasite, as predicted by the Hamilton and Zuk hypothesis. The present results suggest that male roach with high concentrations of circulating androgens express strongly secondary sexual characters, but may be at a high risk of papillomatosis. In the present system, we propose that papilloma disease, which occurs during spawning, may contribute to the honesty of the signal of sexual ornamentation in roach.

Keywords: immunocompetence, 11-ketotestosterone, ornaments, papillomatosis, sexual selection, testosterone.

INTRODUCTION

Within sexual selection, several theories have been proposed to explain the evolution of sexual ornaments. Sexual selection is suggested to be strongest in the sex with the highest potential reproductive rate, but least investment (by means of, for example, gamete production and parental care), which, in most species, is the male (Andersson and Iwasa, 1996). The pressure of sexual selection is observed to be strong in lek-like mating systems and females have been shown to prefer males with elaborated secondary sexual characters

* Author to whom all correspondence should be addressed. e-mail: rkortet@cc.jyu.fi

(Andersson, 1994). In addition, sexual ornaments have also been suggested to work as weapons or status badges in intrasexual contests (Berglund *et al.*, 1996).

Zahavi's (1975) handicap hypothesis proposed that females prefer elaborately ornamented males, because the handicapping ornaments work as a reliable indicator of male quality to females. The Hamilton and Zuk (1982) hypothesis suggested that the quality signalled by sexual ornamentation could be a mate's genetic resistance against parasites and pathogens, which would be maintained by co-evolutionary cycles between host and parasite. Folstad and Karter (1992) presented the immunocompetence handicap hypothesis, which recognized the dual function of male androgens: they promote the development of sexual ornaments but suppress immunological defence. Therefore, male sexual ornamentation should be an honest indicator of male quality to females, since only genetically high-quality males can afford or tolerate the costs of decreased defence against parasites and pathogens.

Since the immunocompetence handicap hypothesis was proposed, several empirical and experimental studies have been conducted (Hillgarth and Wingfield, 1997). Recent results have been supportive (e.g. Skarstein and Folstad, 1996; Saino *et al.*, 1997; Kurtz and Sauer, 1999; Verhulst *et al.*, 1999; Evans *et al.*, 2000; Peters, 2000; Poiani *et al.*, 2000; Rantala *et al.*, 2002), contradictory (see Hillgarth and Wingfield, 1997; Hasselquist *et al.*, 1999) or ambiguous (Liljedal *et al.*, 1999) regarding different aspects of the hypothesis. Moreover, extensions to the hypothesis have been suggested – for example, the immune redistribution hypothesis (Braude *et al.*, 1999), the resource allocation hypothesis (Wedekind and Folstad, 1994), the sperm protection hypothesis (Folstad and Skarstein, 1997; Hillgarth *et al.*, 1997) and the hypothesis concerning different routes between genes, condition, advertising and resistance (Westneat and Birkhead, 1998). However, most studies conducted to date have used birds and have been limited to metazoan parasites.

Roach (*Rutilus rutilus*) is an iteroparous cyprinid fish species which has an intensive and short lek-like mating system (Wedekind, 1996). Male roach produce secondary sexual ornaments – breeding tubercles – which are keratin-based epidermal nodules commonly found in many fish taxa (Wiley and Collette, 1970). The breeding tubercles of teleosts are induced by several pituitary and sex hormones and they fall off soon after spawning (for a review, see Wiley and Collette, 1970). The breeding tubercles of roach have been proposed to provide females with detailed information about a male's parasite load (Wedekind, 1992) or parasite resistance (Taskinen and Kortet, 2002). There are also indications that breeding tubercles of roach may also work as a status badge in male intrasexual contests (R. Kortet, J. Taskinen, A. Vainikka and H. Ylönen, unpublished data). To our knowledge, no attempt has been made to study the three main aspects of the immunocompetence handicap hypothesis simultaneously in teleosts – that is, androgens, sexual ornamentation and the disease state of males.

Epidermal papillomatosis is a mainly virus-induced disease, which has been reported in several fish species (Bylund *et al.*, 1980; Möller and Anders, 1986; Lee and Whitfield, 1992; Mellergaard and Nielsen, 1995; Premdas *et al.*, 1995; Kortet *et al.*, 2002). Papillomata may erupt in fish due to various physiological stress factors, including anoxia and environmental pollutants (Mellergaard and Nielsen, 1995; Premdas *et al.*, 1995). The clinical picture of papillomatosis includes white, smooth, ovoid tumours on the skin and fins of fish. In severe cases, the skin may be covered totally by tumours. In a previous study (Kortet *et al.*, 2002), we found that many roach populations from the current study area had papillomatosis and that the prevalence of the disease was higher among males. The disease breaks out at the time of spawning in the spring. In general, the persistent latent infectious

agents of papilloma are suggested to be present in the population at all times, and that only part of the population may develop the disease with tumours (Lee and Whitfield, 1992). Papillomatosis has been found to cause mortality in carp (Sano *et al.*, 1991). Moreover, sex hormones were recently found to induce papillomata in whites suckers (Premdas *et al.*, 2001). Therefore, the presence of the disease may indicate a decreased defence against pathogens due to androgens.

The aim of the present study was to test the predictions of the immunocompetence handicap hypothesis using wild roach. We examined circulating concentrations of testosterone and 11-ketotestosterone, sexual ornamentation and epidermal skin disease (i.e. papillomatosis) in male roach during the breeding period. The original hypothesis predicts that high androgen concentrations in males should have a positive relationship with high sexual ornamentation (Folstad and Karter, 1992). In particular, since the sexual ornamentation of roach (i.e. breeding tubercles) is apparently produced by androgens (Wiley and Collette, 1970), we predicted high concentrations of testosterone and 11-ketotestosterone to be connected with elaborated breeding tubercle ornamentation. Moreover, the immunocompetence handicap hypothesis predicts a trade-off between androgen concentration and immune defence, which may lead, within individuals, to increased susceptibility to disease when androgen concentrations are high (Folstad and Karter, 1992). Therefore, a positive correlation between androgen concentration and disease in a population should indicate the existence of a trade-off between male sex hormones and immune defence.

MATERIALS AND METHODS

Animals

The study was conducted with the permission of the Central Finland Regional Environment Center (permission LS-16/00). Using a gill net, we collected mature roach during the spawning period (22–25 May 2000). The sampling point was near the spawning ground in Lake Jyväsjärvi, Finland (62°14′N, 25°46′E). We selected 75 mature unspawned males (length = 166.6 ± 7.2 mm; mean ± s). They were categorized into four groups according to their breeding tubercle ornamentation on their sides and the presence of papilloma tumours: (1) low-ornamented and not diseased; (2) low-ornamented and papilloma diseased; (3) high-ornamented and not diseased; (4) high-ornamented and papilloma diseased. Low-ornamented males had smooth skin with no or very few and very small breeding tubercles, while high-ornamented males had notably rough skin with numerous big tubercles. Intermediately ornamented fish were excluded. Netting and ranking was continuous and a single person conducted the ranking of all fish. To verify the repeatability of this ranking method, another group of fish ($n = 50$) was caught and different individuals conducted the ranking. Repeatability was tested using a kappa measure of agreement. The agreement between measurers was highly significant ($\kappa = 0.972$, $P < 0.001$).

Immediately after the categorization, the fish were bled from the caudal vein using a heparinized syringe and needle. After the bleeding, the length of fish was measured and the gonads were examined to ensure their maturity. All males included in the study were sexually mature and ready to spawn; they released milt.

To determine the prevalence of papillomatosis among differentially ornamented males in the sampled population, an additional group of 105 males was collected and studied

(length = 166.2 ± 16.1 mm). Categorization into high- and low-ornamented male groups was performed as described above.

Hormone analyses

Fish blood plasma was separated by centrifugation and frozen at −70°C for later determination of sex hormones. However, some of the plasma samples had deteriorated, so we could not perform 11-ketotestosterone analyses for 50 males and testosterone analyses for 61 males.

Blood testosterone concentrations were determined using a radioimmunoassay-based kit (TESTO-CTK, DiaSorin, Italy). The test tubes used in the analysis had been coated with ^{125}I-labelled testosterone antibody produced in rabbits (cross-reactivities: testosterone 100%, 5-dihydrotestosterone 6.9%, androstenedione 1.1%, 19-norethisterone 0.33% and danazol 0.15%). The sample volume (50 μl) was reduced to 30 μl on the basis of preliminary testing of roach plasma. The radioactivity of drained test tubes was measured with a gamma counter (RackGamma, 1270, LKB Wallac).

An ELISA-based kit (Biosense Laboratories, Norway) was used to determine the concentration of 11-ketotestosterone in roach plasma. The procedure was modified from that used by Cuisset *et al.* (1994). The lowest possible dilution of samples (1:20) was used to achieve high precision but still avoiding the effects of other plasma components on the determination. The ELISA plates were measured four times over 3 h after adding Ellman's reagent to the plates; the highest absorbance values (3 h time point) were used in the analyses.

Statistical analyses

The numbers of fish in the four groups for the 11-ketotestosterone assays were: low-ornamented/not diseased, $n = 20$; low-ornamented/diseased, $n = 15$; high-ornamented/not diseased, $n = 9$; and high-ornamented/diseased, $n = 6$. The numbers of fish in each group for the testosterone assays are given in Table 1. The relationship between androgens, papillomatosis and sexual ornamentation was examined using analyses of covariance, with hormone as the dependent variable, papillomatosis and ornamentation as fixed factors and fish length as a covariate. To meet the assumptions of homogeneity of the variances, we used $-1/(x+1)^3$ transformation for testosterone and $\ln(x+1)$ transformation for 11-ketotestosterone. The correlation between plasma concentrations of testosterone and 11-ketotestosterone was examined using Pearson's correlation analysis. Logistic regression was used to analyse the relationships between papillomatosis, fish length and ornamentation.

RESULTS

Hormone concentrations

There was a significant positive correlation between the circulating concentrations of testosterone and 11-ketotestosterone in males (Pearson's $r = 0.491$, $P = 0.001$, $n = 41$). Testosterone was significantly related to both breeding tubercle ornamentation and disease (ornamentation: $F_{1,59} = 10.92$, $P = 0.002$; papillomatosis: $F_{1,59} = 8.549$, $P = 0.005$; length: $F_{1,59} = 1.271$, $P = 0.264$; interaction between ornamentation and papillomatosis:

$F_{1,59} = 0.001$, $P = 0.999$). 11-Ketotestosterone was significantly related only to ornamentation (ornamentation: $F_{1,39} = 7.715$, $P = 0.009$; papillomatosis: $F_{1,39} = 0.116$, $P = 0.735$; length: $F_{1,39} = 0.851$, $P = 0.362$; interaction between ornamentation and papillomatosis: $F_{1,39} = 0.050$, $P = 0.825$). Circulating concentrations of testosterone and 11-ketotestosterone (Table 1) were more than two times higher among high-ornamented males than among low-ornamented males.

Papillomatosis prevalence

In our study population, the prevalence of papillomatosis among low-ornamented males was 35%, half that among high-ornamented males (70%). Moreover, the results of logistic regression indicated that both the length of fish and ornamentation correlated positively with the prevalence of disease (model: papillomatosis = length + ornamentation, $n = 105$: $\chi^2_2 = 19.40$. $P < 0.001$; length: $P = 0.007$; ornamentation: $P = 0.029$).

DISCUSSION

The results of the present study are consistent with the immunocompetence handicap hypothesis, in that there was a positive relationship between androgen concentrations, sexual ornamentation and papilloma skin disease during the spawning season. Male roach that were highly sexually ornamented had high concentrations of testosterone and 11-ketotestosterone as well as a higher prevalence of papillomatosis. In addition, the males with papillomatosis had higher testosterone concentrations than undiseased males. In another study, Skarstein and Folstad (1996) found that sexual ornamentation of male Arctic charr, *Salvelinus alpinus*, was positively related to parasites and negatively related to lymphocyte count.

The relationship between the expression of secondary sexual characters and testosterone has been reported among a variety of species, and testosterone is considered in most cases to be the main sex steroid needed for the production of sexual ornamentation (Hillgarth and Wingfield, 1997; Verhulst *et al.*, 1999; Evans *et al.*, 2000). In teleost fishes, 11-ketotestosterone has been suggested to act as the main androgen, but testosterone is also important (Borg, 1994). High concentrations of 11-ketotestosterone have been reported to be associated with the development of the head crest – the badge of status

Table 1. Mean and 95% confidence intervals for circulating concentrations of plasma testosterone and 11-ketotestosterone in male roach caught from Lake Jyväsjärvi during the spawning season in 2000

	Testosterone ($ng \cdot ml^{-1}$)			11-Ketotestosterone ($ng \cdot ml^{-1}$)		
Group	*n*	Mean	95% CI	*n*	Mean	95% CI
Low-ornamented, not diseased	16	0.12	0.10–0.15	20	0.57	0.38–0.76
Low-ornamented, diseased	20	0.39	0.19–0.59	15	1.08	0.23–1.90
High-ornamented, not diseased	12	0.33	0.15–0.51	9	2.13	0.63–3.62
High-ornamented, diseased	13	0.74	0.41–1.10	6	1.81	0.59–3.02

during spawning – in the fish *Salaria pavo* (Oliveira *et al.*, 2001a). Thus, the connection we found between high circulating concentrations of androgens and elaborated breeding tubercle ornamentation of male roach was as expected (see also Wiley and Collette, 1970).

Testosterone and 11-ketotestosterone concentrations were correlated in the present study, indicating that they might work in parallel in roach. Petersson *et al.* (1999) found that dominant brown trouts had higher plasma testosterone and 11-ketotestosterone concentrations than their subordinates. Elofsson *et al.* (2000) reported similar results for Arctic charr, among which testosterone and 11-ketotestosterone were intercorrelated. However, a study with Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis*, suggested that males using different reproductive strategies may express various patterns of 11-ketotestosterone and testosterone (Oliveira *et al.*, 2001b). Interestingly, the conversion of testosterone to 11-ketotestosterone is suggested to take place as part of the social and reproductive behaviour of fishes (Oliveira *et al.*, 2001a).

Under the immunocompetence handicap hypothesis (Folstad and Karter, 1992), male sexual ornamentation is a handicapping but honest signal of male quality, due to the unavoidable consequences of high concentrations of immunosuppressive androgens required to produce and maintain an effective expression of sexual ornamentation. This is a consequence of either adaptive or non-adaptive immunomodulative action of sex hormones (Hillgarth and Wingfield, 1997). In the present study, which was conducted during the spawning of the fish, high circulating concentrations of testosterone were associated with papillomatosis. This supports the results of Premdas *et al.* (2001) who found that testosterone induced papillomata in white suckers. To provide further support for our results, some previous studies have also shown that high concentrations of testosterone may be connected with an increase in parasite infections in the host (for a review, see Hillgarth and Wingfield, 1997; see also Poiani *et al.*, 2000).

Papillomatosis in fish is induced by stress (e.g. Mellergaard and Nielsen, 1995; Premdas *et al.*, 1995) and is found in roach, mainly among males, during the breeding period (Kortet *et al.*, 2002). The study by Premdas *et al.* (2001), in which testosterone implants induced papillomatosis or increased the size of existing papillomata, suggests that testosterone influences the ability of fish to control infection. In roach, this process may be reinforced by testosterone-induced formation of breeding tubercles. Fast-growing organs (breeding tubercles in this case) are particularly vulnerable to tumorigenesis during rapid growth and differentiation (Walter and Israel, 1987). However, it cannot be ruled out that testosterone also increases an individual's susceptibility to become infected. Nevertheless, the use of papillomatosis to test the predictions of the immunocompetence handicap hypothesis is well grounded, because the development of the disease is aided by the male androgen, testosterone. As suggested by many researchers (e.g. Hillgarth and Wingfield, 1997; Evans *et al.*, 2000; Poiani *et al.*, 2000), the immunosuppressive mechanism of testosterone during breeding may work together with increasing circulating concentrations of cortisol (see also Slater and Schreck, 1993). The results of a recent study with teleosts suggested a suppressive effect of cortisol on leukocyte phagocytosis, but testosterone and 11-ketotestosterone had no effect (Law *et al.*, 2001). However, testosterone was found to elicit significant *in vitro* immunosuppression of anterior kidney leukocytes from chinook salmon (Slater and Schreck, 1998).

Although there was an association between testosterone and disease, we found no relationship between 11-ketotestosterone and papillomatosis. Therefore, in roach, 11-

ketotestosterone may promote ornament development but it does not increase the risk of papillomatosis. This would provide a means of cheating: males could have an elevated 11-ketotestosterone concentration, thereby increasing sexual signalling, but would not need to bear the cost of immunosuppression. However, our results indicate that both 11-ketotestosterone and testosterone may be needed to produce breeding tubercles. Thus, we think that, in the present system, the possibility for cheating is limited. The main defence mechanisms directed against viral infections, in general, include natural killer cells, interferons alpha and beta, specific antibodies and cytotoxic T-lymphocytes (Minton and Sissons, 1992). Therefore, the above finding also may indicate different relationships of testosterone and 11-ketotestosterone with various parts of the immune system of roach.

The immunocompetence handicap hypothesis is one of the most controversial recent hypotheses of sexual selection. It has given rise to numerous new ideas and studies. The original hypothesis predicts that high concentrations of androgens in males should have a positive relationship with sexual ornamentation and a negative relationship with immune defence, leading to an increased susceptibility to diseases (Folstad and Karter, 1992). However, the variation between individuals in the amount of resources available to invest in ornamentation and immunocompetence could make those relationships difficult to observe at the population level (van Noordwijk and de Jong, 1986). Nevertheless, the observed positive correlation between testosterone and papillomatosis in the current study indicates a negative relationship between testosterone and immune defence.

Interestingly, in a previous study (Taskinen and Kortet, 2002) using the present system, we found that the breeding tubercles of wild male roach signalled a long-term resistance against the predominant, harmful trematode parasite, *Rhipidocotyle campanula*, as predicted by the Hamilton and Zuk hypothesis. Thus, we propose that, in the present system, the character that male roach signal by their ornamentation is parasite resistance, and papillomatosis is an element that contributes to the honesty of the signal. Only high-quality males can tolerate the negative effects of testosterone (cf. the immunocompetence handicap hypothesis), such as papillomatosis, which may induce secondary fungal or bacterial infections, increase predation or cause mortality of fish (Sano *et al.*, 1991).

ACKNOWLEDGEMENTS

We are grateful to J. Kupari for assistance in the field. We thank A. Peters, F. Skarstein, K. Pulkkinen, the Parasite Research Group of Jyväskylä University and two anonymous reviewers for helpful comments on the manuscript. We thank the Emil Aaltonen Foundation for financial support to R.K. The study was conducted at the Biological Interactions Graduate School of the Finnish Ministry of Education.

REFERENCES

Andersson, M.B. 1994. *Sexual Selection*. Princeton, NJ: Princeton University Press.

Andersson, M. and Iwasa, Y. 1996. Sexual selection. *Trends Ecol. Evol.*, **11**: 53–58.

Berglund, A., Bisazza, A. and Pilastro, A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol. J. Linn. Soc.*, **58**: 385–399.

Borg, B. 1994. Androgens in teleost fishes. *Comp. Biochem. Physiol. C*, **109**: 219–245.

Braude, S., Tang-Martinez, Z. and Taylor, G.T. 1999. Stress, testosterone, and the immunoredistribution hypothesis. *Behav. Ecol.*, **10**: 345–350.

Bylund, G., Valtonen, E.T. and Niemelä, E. 1980. Observations on epidermal papillomata in wild and cultured Atlantic salmon *Salmo salar* L. in Finland. *J. Fish Dis.*, **3**: 525–528.

Cuisset, B., Pradelles, P., Kime, D.E., Kuehn, E.R., Babin, P., Davail, S. and Le Menn, F. 1994. Enzyme immunoassay for 11-ketotestosterone using acetylcholinesterase as label: application to the measurement of 11-ketotestosterone in plasma of Siberian sturgeon. *Comp. Biochem. Physiol. C*, **108**: 229–241.

Elofsson, U.O.E., Mayer, I., Damsgård, B. and Wimberg, S. 2000. Intermale competition in sexually mature arctic charr: effects on brain monoamines, endocrine stress responses, sex hormone levels, and behavior. *Gen. Comp. Endocrinol.*, **118**: 450–460.

Evans, M.R., Goldsmith, A.R. and Norris, S.R.A. 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.*, **47**: 156–163.

Folstad, I. and Karter, A.J. 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.

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

Hasselquist, D., Marsh, J.A., Sherman, P.W. and Wingfield, J.C. 1999. Is avian humoral immunocompetence suppressed by testosterone? *Behav. Ecol. Sociobiol.*, **45**: 167–175.

Hillgarth, N. and Wingfield, J.C. 1997. Parasite-mediated sexual selection: endocrine aspects. In *Host–Parasite Evolution: General Principles and Avian Models* (D. Clayton and E. Moore, eds), pp. 78–104. Oxford: Oxford University Press.

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

Kortet, R., Vainikka, A. and Taskinen, J. 2002. Epizootic cutaneous papillomatosis in roach *Rutilus rutilus*: sex and size dependence, seasonal occurrence and between population differences. *Dis. Aquat. Org.*, **52**: 185–190.

Kurtz, J. and Sauer, K.P. 1999. The immunocompetence handicap hypothesis: testing the genetic predictions. *Proc. R. Soc. Lond. B*, **266**: 2515–2522.

Law, W., Chen, W., Song, Y., Dufour, S. and Chang, C. 2001. Differential *in vitro* suppressive effects of steroids on leukocyte phagocytosis in two teleosts, Tilapia and Common Carp. *Gen. Comp. Endocrinol.*, **121**: 163–172.

Lee, S. and Whitfield, P.J. 1992. Virus-associated spawning papillomatosis in smelt, *Osmerus eperlanus* L., in the River Thames. *J. Fish Biol.*, **40**: 503–510.

Liljedal, S., Folstad, I. and Skarstein, F. 1999. Secondary sex traits, parasites, immunity and ejaculate quality in the Arctic charr. *Proc. R. Soc. Lond. B*, **266**: 1893–1898.

Mellergaard, S. and Nielsen, E. 1995. Impact of oxygen deficiency on the disease status of common dab *Limanda limanda. Dis. Aquat. Org.*, **22**: 101–114.

Minton, J. and Sissons, J.G.P. 2000. Viruses, immunity to. In *Principles of Virology, Molecular Biology, Pathogenesis, and Control* (S.J. Flint, L.W. Enquist, R.M. Krug, Y.R. Racaniello and A.M. Skalka, eds), pp. 1556–1669. Washington, DC: ASM Press.

Möller, H. and Anders, K. 1986. Tumors and tumor-like lesions. In *Diseases and Parasites of Marine Fishes*, pp. 39–59. Kiel: Möller.

Oliveira, R.F., Almada, V.C., Goncalves, E.J., Forsgren, E. and Canario, A.V.M. 2001a. Androgen levels and social interactions in breeding males of the peacock blenny. *J. Fish Biol.*, **58**: 897–908.

Oliveira, R.F., Canario, A.V.M., Grober, M.S. and Santos, R.S. 2001b. Endocrine correlates of male polymorphism and alternative reproductive tactics in the Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis*. *Gen. Comp. Endocrinol.*, **121**: 278–288.

Peters, A. 2000. Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. *Proc. R. Soc. Lond. B*, **267**: 883–889.

Petersson, E., Järvi, T., Olsens, H., Mayer, I. and Hedenskog, M. 1999. Male–male competition and female choice in brown trout. *Anim. Behav.*, **57**: 777–783.

Poiani, A., Goldsmith, A.R. and Evans, M.R. 2000. Ectoparasites of house sparrows (*Passer domesticus*): an experimental test of the immunocompetence handicap hypothesis and a new model. *Behav. Ecol. Sociobiol.*, **47**: 230–242.

Premdas, P.D., Metcalfe, T.L., Bailey, M.E. and Metcalfe, C.D. 1995. The prevalence and histological appearance of lip papillomas in white suckers from two sites in Central Ontario, Canada. *J. Gt. Lakes Res.*, **21**: 207–218.

Premdas, P.D., Metcalfe, C.D. and Brown, S. 2001. The effects of 17*β*-oestradiol, testosterone and tamoxifen on the development of papillomata in *Catostomus commersoni. J. Fish Biol.*, **59**: 1056–1069.

Rantala, M.J., Jokinen, I., Kortet, R., Vainikka, A. and Suhonen, J. 2002. Do pheromones reveal male immunocompetence? *Proc. R. Soc. Lond. B*, **269**: 1681–1685.

Saino, N., Bolzern, A.M. and Møller, A.P. 1997. Immunocompetence, ornamentation, and viability of male barn swallows (*Hirundo rustica*). *Proc. Natl. Acad. Sci. USA*, **94**: 549–552.

Sano, T., Morita, N., Shima, N. and Akimoto, M. 1991. *Herpesvirus cyprini*: lethality and oncogenicity. *J. Fish Dis.*, **14**: 533–543.

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

Slater, C.H. and Schreck, C.B. 1993. Testosterone alters the immune response of chinook salmon, *Onchorhynchus tshawytcha. Gen. Comp. Endocrinol.*, **89**: 291–298.

Slater, C.H. and Schreck, C.B. 1998. Season and physiological parameters modulate salmonid leucocyte androgen receptor affinity and abundance. *Fish Shellfish Immunol.*, **8**: 379–391.

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.

van Noordwijk, A.J. and de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.*, **128**: 137–142.

Verhulst, S., Dieleman, S.J. and Parmentier, H.K. 1999. A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. *Proc. Natl. Acad. Sci. USA*, **96**: 4478–4481.

Walter, J.B. and Israel, M.S. 1987. *General Pathology*. New York: Churchill Livingstone.

Wedekind, C. 1992. Detailed information about parasites revealed by sexual ornamentation. *Proc. R. Soc. Lond. B*, **247**: 169–174.

Wedekind, C. 1996. Lek-like spawning behaviour and different female mate preferences in roach (*Rutilus rutilus*). *Behaviour*, **133**: 681–695.

Wedekind, C. and Folstad, I. 1994. Adaptive or nonadaptive immunosuppression by sex hormones? *Am. Nat.*, **143**: 936–938.

Westneat, D.F. and Birkhead, T.R. 1998. Alternative hypotheses linking the immune system and mate choice for good genes. *Proc. R. Soc. Lond. B*, **265**: 1065–1073.

Wiley, M.L. and Collette, B.B. 1970. Breeding tubercles and contact organs in fishes: their occurrence, structure, and significance. *Bull. Am. Mus. Nat. Hist.*, **143**: 145–216.

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