Sexual ornamentation and parasite infection in males of common bream (*Abramis brama*): a reflection of immunocompetence status or simple cost of reproduction?

Eva Ottová,[1] Andrea Šimková,[1]* Pavel Jurajda,[2]
Martina Dávidová,[1] Markéta Ondračková,[1,2]
Martina Pečínková[1] and Milan Gelnar[1]

[1]*Department of Zoology and Ecology, Faculty of Science, Masaryk University, Kotlářská 2,
61137 Brno and* [2]*Institute of Vertebrate Biology, Academy of Sciences of the
Czech Republic, Květná 8, 60365 Brno, Czech Republic*

ABSTRACT

Question: How does sexual ornamentation relate to parasite infection, host immune response and somatic condition status in male fish?

Hypotheses: Zahavi's (1975) handicap hypothesis proposes that producing secondary sexual traits represents a considerable handicap for males. Additionally, Hamilton and Zuk (1982) proposed that the expression of secondary sexual traits reveals a genetic resistance against parasites.

Organisms: Spawning males of common bream (*Abramis brama*) and several of its parasites (*Gyrodactylus* spp., *Diplostomum* spp., *Argulus* spp.).

Variables: Parasite abundance (for parasite infection), spleen size (for host immune response) and condition (for somatic condition status).

Results: The more tubercles on the fish, the more abundant the *Gyrodactylus* spp. The more tubercles on the fish's head, the more abundant the *Diplostomum* spp. The greater the mean length of the head tubercles, the more abundant the *Gyrodactylus* spp. and *Argulus* spp. However, we found no relationship between spleen size and either sexual ornamentation or parasite infection. Fish with larger spleens were in poorer somatic condition, but condition was not related to male ornamentation or parasite abundance.

Conclusions: Males that develop more intensive sexual ornamentation are more susceptible to metazoan ectoparasite infection, supporting the hypothesis of Hamilton and Zuk. However, our results do not support the hypothesis that immunosuppression by steroid hormones reduces immunocompetence.

Keywords: cost of reproduction, fish, immunocompetence, parasitism, sexual ornamentation.

* Author to whom all correspondence should be addressed. e-mail: simkova@sci.muni.cz

INTRODUCTION

The role of parasites in sexual selection has been highlighted by several studies predominantly using birds as a model (Read, 1988; Borgia and Collis, 1989; Møller, 1990; Poiani *et al.*, 2000). However, more recently several fish models have been applied in studies examining the pattern of sexual ornamentation as a measure of sexual showiness and parasite exposure (Wedekind, 1992; Folstad *et al.*, 1994; Skarstein and Folstad, 1996; Skarstein *et al.*, 2001; Kortet *et al.*, 2003a).

Runaway selection represents the first theory about sexual selection (Fisher, 1930) based on the female preference for a specific male character. Females choose more exaggerated male traits and this process continues until the male trait connected with female preference reduces the fitness of individuals, and the male trait becomes restricted by natural selection.

The second hypothesis explaining the function of secondary sexual traits in the evolution of sexual selection was proposed by Zahavi (1975). The development of secondary traits may represent a handicap, which on the one hand reduces male survival, but on the other hand could signify the quality of genetic resistance, which is important in sexual selection through female choice.

Hamilton and Zuk (1982) extended the handicap hypothesis and modified it as the parasite-sexual selection hypothesis based on 'good-gene' prediction. This hypothesis proposed that the secondary sexual traits reveal a genetic resistance against parasites and pathogens. Thereafter, females choose male mates according to the quality of secondary sexual traits to ensure higher resistance of the offspring – that is, females provide a choice of 'good genes' for the offspring. As suggested by Folstad *et al.* (1994), host individuals may signal their ability to resist parasites by increasing either parasite exposure or host susceptibility to parasites.

A trade-off between investment in reproduction and other somatic functions has generally been proposed (Williams, 1966). Comparing reproductive and non-reproductive individuals, if the reproductive ones increase their allocation in reproduction, then they have limited the energetic resources for other somatic costs. Therefore, an increase in male reproductive effort expressed by sexual ornamentation (for instance, intensity of coloration or development of breeding tubercles during fish breeding) may decrease individuals' energy allocation to defence against disease or parasites (Sheldon and Verhulst, 1996).

The connection between immunocompetence and the handicap hypothesis represents the immunocompetence handicap hypothesis (Folstad and Karter, 1992), which includes an endocrinological component in the parasite-sexual selection hypothesis. This hypothesis is based on the dualistic effect of steroid hormones. These stimulate the development of sexual ornamentation, but reduce immunocompetence via immunosuppression. The immunocompetence handicap hypothesis predicts a trade-off between cost of reproduction and immunocompetence. Spleen size is often used as a reliable metric of immunocompetence in birds (Saino *et al.*, 1998; Morand and Poulin, 2000) and was recently applied to fish (Skarstein *et al.*, 2001; Taskinen and Kortet, 2002; Kortet *et al.*, 2003b). Interspecifically, the trade-off was confirmed through the simple relationship between spleen size and gonad size in male birds (Morand and Poulin, 2000), but no such relationship was observed for male fish (A. Šimková *et al.*, unpublished). When considering investment in male reproduction through sexual ornamentation at the intraspecific level using fish as a model, the immunocompetence hypothesis was not confirmed in a study conducted within the breeding period (Taskinen and Kortet, 2002). However, the comparison of costs of reproduction as a result of immunosuppression by steroid hormones was observed when comparing spawning and resting fish (Skarstein *et al.*, 2001).

The present study examined the expression of breeding tubercles in one European cyprinid species, common bream (*Abramis brama* L.). The breeding tubercles of fish represent male sexual traits that play a role in male–male interactions and attempts to achieve spawning. The size of breeding tubercles is connected with male dominance behaviour and may be used in a female's choice of a high-quality mate (Kortet *et al.*, 2004).

Bream is a polygamous fish with a spawning period from May to June. Fish usually spawn on plants and stones, and the main factor determining spawning is temperature (Baruš and Oliva, 1995). This species of cyprinids was chosen because determination and measurement of breeding tubercles during the spawning period is simple. Moreover, the parasite fauna of common bream is well known (Moravec, 2001). Sexual ornamentation of this fish species is observed only in males; females do not generate breeding tubercles. Male tubercles are colourless keratin-based epidermal nodules (Schwerdtfeger and Bereiter-Hahn, 1978) that are spread all over the body, most of which are concentrated on the head and fins. After the spawning period, these structures fall off. For this reason, we used reproductive males to test the above hypotheses.

The aim of the present study was to examine the relationship between male sexual ornamentation (the presence, number and size of breeding tubercles) and parasitism (presence and abundance of metazoan parasites) in spawning fish. We wished to determine whether this relationship is connected with fish immunocompetence (measured by spleen size) and somatic condition (measured by condition factor) following the prediction of Hamilton and Zuk's (1982) hypothesis and the immunocompetence hypothesis (Folstad and Karter, 1992), or whether it represents only a simple cost of reproduction paid by male fish investing more extensively in their ornamentation.

MATERIALS AND METHODS

A total of 30 male common bream (*A. brama* L.) were collected during the breeding period – that is, the last 2 weeks of May and the first 2 weeks of June 2003 – from the Dyje River below the water reservoir Nové Mlýny (Morava river basin, Czech Republic). The fish were caught by electrofishing. Captured fish were immediately placed in a tank containing water collected from the same location and then transported to the laboratory. During storage of fish in the laboratory, the original water temperature was maintained and water was filtered using a standard aquarium filter. All fish were sacrificed within 24 h.

All individuals were measured and weighed, and the standard length (mean ± standard deviation: 40.6 ± 3.6 cm), body weight (1311.3 ± 340.5 g) and spleen size (2.61 ± 1.17 g) were recorded. As the spleen is assumed to be an important secondary lymphatic organ widely used as a simple measurable immunological variable in studies of immunocompetence in different vertebrates, we chose spleen size as the parameter with a potential role in immune response against parasites.

The breeding tubercles, as secondary sexual ornaments in fish, were counted on the head, back (the area between the head and the first hard rays of the dorsal fin), all fins and in the first scale line above and below the lateral line on the right and left sides of the body (in total, four lines of scales were counted, referred to below as 'on the body'). When we found missing tubercles on a given fish scale line, probably as a result of mechanical damage, we counted lighter traces than when all the tubercles were present. The head of each individual was fixed in 4% formalin for later measurement of the height of the tubercles on the head.

Using a calliper we measured the five highest tubercles on the head and we recorded the average height of the tubercles on the head.

The complete dissection of fish was performed using the method of Ergens and Lom (1970). Fish were examined for all metazoan parasites. Therefore, external organs (skin, fins, gills, eyes) and internal organs (intestine, hepatopancreas, spleen, protonephros, heart, swim bladder) were examined for the following groups: ectoparasites (Monogenea, Crustacea, Mollusca and Hirudinea) and endoparasites (Digenea, Nematoda, Cestoda and Acanthocephala). Parasites were collected from each individual fish and fixed: Monogenea in a mixture of ammonium picrate-glycerine, Nematoda in a mixture of glycerine-ethanol and all parasites of Digenea, Cestoda, Acanthocephala, Crustacea and Mollusca in 4% formalin. An Olympus BX 50 light microscope equipped with phase-contrast, differential interference contrast (DIC) and Digital Image Analysis (Olympus MicroImage™ for Windows 95/98/NT 4.0, Olympus Optical Co.) was used for parasite measurements and identification.

Parasite abundance (mean number of parasites per host individual) and prevalence (percentage of infected individuals) were calculated according to Bush *et al.* (1997): (1) separating counts into total ectoparasites and endoparasites; (2) considering parasite groups with meaningful abundances (i.e. Monogenea, Crustacea, Digenea and Nematoda); and (3) using different genera with sufficient numbers of parasites: *Diplozoon* sp. (Monogenea), *Dactylogyrus* spp. (Monogenea), *Gyrodactylus* spp. (Monogenea), *Ergasilus* sp. (Crustacea), *Argulus* spp. (Crustacea), *Diplostomum* spp. (Digenea), *Cotylurus* sp. (Digenea), *Contracaecum* sp. (Nematoda). Moreover, *Gyrodactylus* spp., due to its different localization on the fish, was analysed in two groups: *Gyrodactylus* parasitizing skin and *Gyrodactylus* parasitizing gills.

As a measure of male vigour and general condition status, we calculated the condition factor $K = \text{constant} \times \text{somatic weight (g)}/(\text{length (cm)})^3$. The assumption of the condition factor is that the heavier the fish is in relation to its length, the better is its condition (Bolger and Connolly, 1989).

Statistical analyses were performed using StatView 5.0 and Statistica 6.0 for Windows. All data were checked for normality using the Kolgomorov-Smirnov test. The distribution of height of the tubercles and parasite abundance did not meet the assumption of normality after log transformation of the data, and therefore non-parametric analysis was applied (Spearman correlation coefficient).

Using the Spearman correlation coefficient, strong correlations were observed between the numbers of tubercles on the back and on the fins ($R = 0.799$, $P = 0.0001$), on the back and on the body ($R = 0.767$, $P = 0.0001$), and on the fins and on the body ($R = 0.774$, $P = 0.0001$). Therefore, the total number of tubercles on the back, fins and body was used in our analyses (below referred as 'BFB'). The correlation between the numbers of tubercles on the head and those on the BFB was also significant ($R = 0.402$, $P = 0.0302$). However, as different parasites can attach to different parts of the body, which can potentially differ by their epidermis thickness, and that many abundant parasites in our study infect only the head parts (gills, eyes, skin) of the fish, we analysed the numbers of tubercles on the head and on the BFB separately. The total number of tubercles was also retained for the analyses. Multiple stepwise regression was used for analyses of the relationships between sexual ornamentation and parasite abundance. Logarithmic transformation was applied before the regression analyses.

We examined potential associations between fish size and male ornamentation/parasite

load/spleen size/condition status. When a positive relationship was observed, the variables were corrected for fish size and residual values were used for the following analyses.

RESULTS

General patterns: sexual ornamentation and parasites in male fish

The mean numbers of tubercles (± standard deviation) found on the different parts of the body were as follows: head (364.6 ± 194.5), back (71.8 ± 37.5), fins (1114.9 ± 859.6), body (206.3 ± 99.1). The mean height of tubercles observed on the head was 0.40 ± 0.50 cm. The mean number of tubercles on the BFB (1393.1 ± 971.7) and the mean total number of tubercles (1757.7 ± 1067.9) were calculated. The mean height of tubercles on the head was correlated with total number of tubercles ($R = 0.712$, $P = 0.0001$). Positive relationships were found between fish size and: total endoparasite abundance ($R^2 = 0.204$, $P = 0.0123$), total Digenea ($R^2 = 0.297$, $P = 0.0018$), *Dactylogyrus* spp. (Monogenea) ($R^2 = 0.167$, $P = 0.0252$), *Diplostomum* spp. (Digenea) ($R^2 = 0.237$, $P = 0.0063$), *Cotylurus* sp. (Digenea) ($R^2 = 0.151$, $P = 0.0341$). Moreover, fish size was positively correlated with the number of tubercles on the head ($R^2 = 0.301$, $P = 0.0017$) and total number of tubercles ($R^2 = 0.135$, $P = 0.0457$). The relationship between fish size and number of tubercles on the BFB was not significant.

All males were infected by parasites. The abundance and prevalence of parasites are given in Table 1. Parasites of Monogenea, Crustacea, Digenea and Nematoda had the highest mean abundance. *Gyrodactylus* and *Dactylogyrus* species (both belonging to Monogenea) showed the highest mean abundance and maximal prevalence.

A positive correlation was observed between different parasite groups of high abundance: *Gyrodactylus* spp. found on the gills and *Gyrodactylus spp.* found on the fins, *Dactylogyrus* spp. and *Ergasilus* sp., *Dactylogyrus* spp. and *Diplostomum* spp., *Ergasilus* sp. and *Argulus* spp., *Ergasilus* sp. and *Diplostomum* spp., *Argulus* spp. and *Gyrodactylus* spp. found on the fins, and *Argulus* spp. and *Diplostomum* spp. (Table 2).

Expression of sexual ornamentation and parasites

A positive relationship between total number of tubercles and abundance of ectoparasites was observed. The total number of tubercles revealed a significant positive association with Monogenea. The abundance of *Gyrodactylus* spp. parasitizing the fins increased with total number of tubercles (Table 3).

A significant positive relationship was found between the number of tubercles on the head and abundance of endoparasites, after correcting both variables for fish size. When analysing four parasite groups (Monogenea, Crustacea, Digenea, Nematoda), the number of tubercles on the head revealed a significant positive association with Digenea after correcting both variables for fish size. The abundance of *Diplostomum* spp. increased with number of head tubercles (Table 3). However, no significant relationship between number of tubercles on the head and abundance of parasite group or genus was found after applying Bonferroni correction.

The number of tubercles on the BFB was positively related to the abundance of ectoparasites. In the analyses calculated using four parasite groups, we found a significant relationship between the number of tubercles on the BFB and Monogenea. Within

Table 1. Mean abundance (± standard deviation) and prevalence (in %) calculated for (1) ectoparasites and endoparasites, (2) the four most frequent parasite groups and (3) the most frequent parasite genera ($N = 30$)

Ectoparasites 1303.9 ± 2331.1 100%				Endoparasites 39.7 ± 24.1 100%			
Monogenea 1298.7 ± 2331.6 100%			Crustacea 4.5 ± 9.0 63.3%		Digenea 28.4 ± 22.1 96.7%		Nematoda 10.3 ± 8.7 93.3%
Gyrodactylus spp. (gills)	*Dactylogyrus* spp.	*Gyrodactylus* spp. (fins)	*Diplozoon* sp.	*Ergasilus* sp.	*Argulus* spp.	*Diplostomum* spp.	*Cotylurus* sp.	*Contracaecum* sp.
974.1 ± 2131.6 100%	120.9 ± 96.9 100%	200.3 ± 407.0 70%	3.5 ± 3.5 76.7%	1.4 ± 2.6 43.3%	3.1 ± 8.1 50%	22.6 ± 20.5 83.3%	4.8 ± 5.6 73.3%	8.2 ± 6.9 93.3%

Table 2. Associations between parasite genera investigated in this study using the Spearman correlation coefficient

	Diplozoon sp.	*Gyrodactylus* spp. (gills)	*Dactylogyrus* spp.	*Ergasilus* sp.	*Argulus* spp.	*Gyrodactylus* spp. (fins)	*Diplostomum* spp.	*Contracaecum* sp.
Gyrodactylus spp. (gills)	N.S.							
Dactylogyrus spp.	N.S.	N.S.						
Ergasilus sp.	N.S.	N.S.	$R = 0.476$ $P = 0.0103$					
Argulus spp.	N.S.	N.S.	N.S.	$R = 0.418$ $P = 0.0245$				
Gyrodactylus spp. (fins)	N.S.	$R = 0.484$ $P = 0.0091$	N.S.	N.S.	$R = 0.493$ $P = 0.0079$			
Diplostomum spp.	N.S.	N.S.	$R = 0.425$ $P = 0.0222$	$R = 0.461$ $P = 0.0131$	$R = 0.419$ $P = 0.0239$	N.S.		
Contracaecum sp.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
Cotylurus sp.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table 3. Results of multiple regression analyses on the relationship between ornamental characters (number of tubercles on the head, number of tubercles on the BFB = back + fins + body, total number of tubercles) and different parasite groups ($N = 30$)

Ornamental characters	Parasites	b	F	R^2	P
Tubercles on the head	Endoparasites	0.355	8.604	0.235	0.0066*
	Digenea	0.823	5.11	0.154	0.0318
	Diplostomum spp.	0.912	4.537	0.139	0.0421
Tubercles on the BFB	Ectoparasites	0.893	14.4	0.34	0.0007*
	Monogenea	0.89	14.189	0.336	0.0008*
	Gyrodactylus spp. (gills)	0.842	5.185	0.156	0.0306
	Gyrodactylus spp. (fins)	2.212	24.528	0.467	0.0001*
All tubercles	Ectoparasites	0.956	8.546	0.234	0.0068*
	Monogenea	0.953	8.443	0.232	0.0071*
	Gyrodactylus spp. (fins)	2.326	12.599	0.31	0.0014*

Note: b = the slope of regression, R^2 = the regression coefficient, F-test significant at the 0.05 level. * Results significant after Bonferroni correction was applied.

Monogenea, the number of *Gyrodactylus* spp. parasitizing the gills and fins increased with the number of tubercles on the BFB (the result for *Gyrodactylus* parasitizing the gills was not significant after applying Bonferroni correction). The results are shown in Table 3.

The mean height of tubercles on the head was significantly positively related to the abundance of ectoparasites. Significant relationships between mean height of the tubercles on the head and both Monogenea and Crustacea were found. In the analyses of parasite genera, positive relationships between the height of tubercles and the abundance of both *Gyrodactylus* spp. parasitizing the fins and gills (the result for *Gyrodactylus* parasitizing the fins was not significant after applying Bonferroni correction) and *Argulus* spp. parasitizing the surface were observed (Table 4). Because of the relationship between the mean height of tubercles on the head and the total number of tubercles, the same analysis as described above was performed using the residuals from multiple regression after correcting for both fish size and total number of tubercles. Positive relationships between height of tubercles and abundance of both *Gyrodactylus* spp. parasitizing the fins and gills and *Argulus* spp. were also observed in this case. Moreover, a negative relationship between the mean height of tubercles on the head and abundance of *Contracaecum* sp. (Nematoda) ($b = -0.22$, $F = 5.234$, $R^2 = 0.157$, $P = 0.0299$) was observed.

Immune response, sexual ornamentation and parasites

A positive relationship was observed between fish body weight and spleen size ($R^2 = 0.400$, $P = 0.0002$). There was no significant relationship between spleen size and intensity of sexual ornamentation expressed either by the number or height of tubercles when correcting all variables for fish size. No significant relationship was found between spleen size and any parasite group or genera, after correcting for fish size.

Table 4. Results of multiple regression analyses on the relationships between mean height of tubercles on the head and different parasite groups ($N = 30$)

Ornamental character	Parasites	b	F	R^2	P
Mean height of tubercles on the head	Ectoparasites	0.372	20.334	0.421	0.0001*
	Monogenea	0.37	19.828	0.415	0.0001*
	Crustacea	0.255	10.052	0.264	0.0037*
	Gyrodactylus spp. (gills)	0.359	7.106	0.202	0.0126
	Gyrodactylus spp. (fins)	1.033	74.125	0.726	0.0001*
	Argulus spp.	0.241	11.093	0.284	0.0024*

Note: b = the slope of regression, R^2 = the regression coefficient, *F*-test significant at the 0.05 level. * Results significant after Bonferroni correction was applied.

Condition status, sexual ornamentation and parasites

The condition factor K (mean 1.93 ± 0.24) was calculated for each individual. No significant relationship was found between condition factor and fish size. No significant relationships were observed between condition factor and sexual ornamentation (number of tubercles or height of tubercles) or between condition factor and any parasite group. However, a significant negative relationship was observed between spleen size and condition factor ($b = -0.28$, $R^2 = 0.213$, $P = 0.0102$) after correcting spleen size for fish body size.

DISCUSSION

Spawning males and parasites

The relationship between the development of secondary sexual traits and the presence of parasites has been investigated in a limited number of fish species: salmonids (Pickering and Christie, 1980; Skarstein and Folstad, 1996; Skarstein et al., 2001), roach, *Rutilus rutilus* L. (Wedekind, 1992; Taskinen and Kortet, 2002) and three-spined stickleback, *Gasterosteus aculeatus* L. (Folstad et al., 1994). Generally, mature males have higher intensities of several ectoparasite species attached to their surface than immature males or mature females, as shown by Pickering and Christie (1980). Moreover, spawning males have higher intensities of macroparasite infection than non-spawning males (Skarstein et al., 2001).

In the present study, we examined sexual ornamentation and metaozoan parasites in males of common bream, *A. brama*, collected during the spawning period. We confirmed that the number of breeding tubercles (as a measure of sexual ornamentation in fish) is related to the presence of parasites. Thus, our results support one of the predictions of Hamilton and Zuk's (1982) hypothesis – that is, sexual ornamentation is an indicator of the degree of parasite exposure. However, whether sexual ornamentation really reflects a genetic quality – that is, the presence of 'good genes' as suggested by Hamilton and Zuk (1982) – or the relationship between sexual ornamentation and parasite infection simply represents the cost paid by males expressing more distinct sexual ornamentation during the period of higher investment in reproduction, requires further investigation.

Expression of sexual ornamentation and parasites

We found positive relationships between the expression of sexual ornamentation and the occurrence of parasite groups or parasite genera. These results are consistent with the findings of Skarstein and Folstad (1996) and Kortet *et al.* (2003a) but are in opposition to those of Wedekind (1992), who reported a negative relationship between number of tubercles on the head and body and the presence of *Diplozoon* sp. and nematodes. However, Taskinen and Kortet (2002) failed to observe a significant relationship between sexual ornamentation and intensity or prevalence for any protozoan or metazoan ecto- or endoparasites. On the other hand, they observed a positive correlation between ornamentation and the proportion of dead *Rhipidocotyle campanula*, suggesting that the proportion of dead parasites in the host may provide a measure of resistance. A study of sexual coloration and parasite infection in three-spined stickleback showed that high expression of sexual coloration is related to a higher intensity of infection of some parasite species, but a lower intensity of infection of other parasite species (Folstad *et al.*, 1994). All these findings were interpreted in the sense of Hamilton and Zuk's hypothesis, when males broadcast information about the degree of parasite exposure through secondary sexual ornamentation. However, the nature of such a relationship may be affected by non-heritable behaviour when males can develop sexual traits without the constraints imposed by parasites (Folstad *et al.*, 1994).

In the present study, we observed a positive relationship between the total number of tubercles and the presence of *Gyrodactylus* spp. Similar to this observation, Pickering and Christie (1980) found that sexually mature males of the brown trout (*Salmo trutta* L.) were more frequently infected by species of *Gyrodactylus*. The fact that sexually mature males had a significantly thicker epidermis than mature females or immature fish could explain this observation. The characteristics of the epidermis are connected with the increased susceptibility of the integument of sexually mature males during the spawning season (Pickering and Christie, 1980). We did not assess such characteristics. However, our results suggest that the number of ectoparasites could increase with the intensity of sexual ornamentation in connection with skin status. Our field observations suggest that the skin of males during spawning is distinctly damaged and disturbed at several locations on the body. Therefore, these conditions could be favourable for infestation by ectoparasites.

We also observed a positive relationship between the mean height of tubercles and the presence of two surface parasites – *Gyrodactylus* spp. and *Argulus* spp. – and a negative relationship between the mean height of tubercles and the abundance of *Contracaecum* sp. Of the studies conducted on fish parasites and sexual ornamentation, only Wedekind (1992) used height of tubercles as a measure of sexual showiness in the case of fish showing a positive relationship between height of the largest tubercles on the operculum and the presence of the gill parasite, *Diplozoon* sp. (Monogenea), and a negative relationship between height of the largest tubercles on the operculum and the presence of nematodes. However, these relationships were not significant.

We found that the number of tubercles on the head increases with increasing abundance of *Diplostomum* spp. Studying Arctic charr, *Salvelinus alpinus* L., Skarstein and Folstad (1996) found that the intensities of the eyefluke *Diplostomum* sp. correlated positively with hue as a sexual trait. Subsequently, in the same fish species, Skarstein *et al.* (2001) found that spawning males are more heavily infected by *Diplostomum* sp. than resting males. This observation could be explained by increased susceptibility of the skin to the free living larval stages ('cercariae') of *Diplostomum* spp., which attack the host. Our results are

interpreted as a cost of reproduction paid by male fish during breeding – that is, males allocate more resources to reproduction by developing more intensive sexual ornamentation and are more susceptible to metazoan parasite infection. Alternatively, it might be that more ornamented individuals have a reduced immune response in line with the immunocompetence handicap hypothesis.

Immune response, sexual ornamentation and parasites

No significant relationship was observed between spleen size and both sexual ornamentation and the presence of any parasite group in our study. Skarstein *et al.* (2001) showed that spawning males are more susceptible to parasite infection and, moreover, they have a smaller spleen than non-spawning males. Differences in spleen size have also been observed between the spawning period and pre- and post-spawning in roach (Kortet *et al.*, 2003b). This may be explained by seasonal changes in immunocompetence related to seasonal changes in investment in reproduction through the trade-off between reproduction and immune function (Kortet *et al.*, 2003b), thus supporting the immunocompetence handicap hypothesis (Folstad and Karter, 1992). However, the results of a study of fish during the spawning period suggest that spleen size does not reflect differences in sexual ornamentation (Taskinen and Kortet, 2002), as in our study. Moreover, we found no relationship between parasite intensity and spleen size in spawning fish. This finding does not confirm the immunocompetence hypothesis as presented by Folstad and Karter (1992), suggesting that spleen size is not an appropriate immunological measure for the evaluation of immunocompetence status within spawning males differing in intensity of sexual ornamentation and parasite infection. However, as steroid hormones cause a suppressive effect on the fish immune system (Hou *et al.*, 1999) and induce the creation of breeding tubercles (Kortet *et al.*, 2003a), the positive relationships between parasite intensity and the number and height of breeding tubercles suggest a potential connection with the immune response. Therefore, other measures of leukocyte and plasma IgM concentration, such as monitoring of the functional activity of leucocytes or mucus-producing components, could be applied to test the immunocompetence hypothesis. For instance, Skarstein and Folstad (1996) observed a negative correlation between lymphocytes and sexual dichromatism in Arctic charr during spawning.

Condition status, sexual ornamentation and parasites

We did not observe a significant relationship between condition factor and sexual ornamentation or parasite infection. No difference in the condition factor among males with different intensities of ornamentation was also reported by Taskinen and Kortet (2002). In the study of Wedekind (1992), only a weak relationship was observed between the number of tubercles on the head and the condition factor. Therefore, the function of males' breeding tubercles as a reflection of their vigour and condition status is not supported in the light of Hamilton and Zuk's (1982) hypothesis. Moreover, we suggest that somatic condition status is not affected by parasites in spawning males of common bream. On the other hand, the negative correlation between condition factor and the fin area of roach reported by Wedekind (1992) suggests that the fin area could be a more accurate reflection of male condition than breeding tubercles.

Trade-off between somatic condition status and immune defence

Despite the above results, we found a negative relationship between condition factor and spleen size. As condition factor represents a measure of relative body weight, this finding suggests a trade-off between different investments in life-history traits. Fish investing more in condition status have less energy for investing in immune response. This result could support the trade-off energy hypothesis, proposed by Sheldon and Verhulst (1996).

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