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Research Article

Sex and Sexual Ornamentation Associated with Survival of the Cyprinid Fish, *Rutilus rutilus*, under Disease Stress

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Abstract By conveying information of disease resistance, sexual signals may be used as cues for adaptive mate choice. Here we report observations on survival of laboratory-maintained, wild-collected, sexually mature, ready-to-spawn cyprinid fish, *Rutilus rutilus* (roach), under accidental epidemic attributed to *Flavobacterium psychrophilum*, diagnosed using species-specific PCR. The fish were maintained in a single tank. During the 27 days observation period, both the proportion of fish surviving the infection as well as the length-adjusted mean survival time of the fish that died was the highest among the high-ornamented males with large breeding tubercles, intermediary among the low-ornamented males with small or no breeding tubercles, and the lowest in females. Control fish in another tank did not show disease symptoms and experienced 100% survival. It should be noticed that this was not an experiment designed to study the survival question—the fish were in storage tanks to be used for other purposes. However, in line with earlier studies on the association between breeding tubercles and parasite resistance in *R. rutilus*, the present observations suggest that the sexual signals of roach may be indicators of survival under disease stress.

Keywords breeding tubercles; *Flavobacterium psychrophilum*; Hamilton and Zuk hypothesis; mortality; roach; spawning stress

1. Introduction

Darwin [3] proposed that elaborated male sexual ornaments of many animal taxa have evolved through sexual selection (i.e., females' preference for male displays). Since such male ornaments are often handicapping (i.e., costly in terms of survival or energetic demands) for males, their existence over evolutionary time requires stronger benefits for females than their costs for males [7,8]. Such benefits include heritable traits that enhance the fitness of offspring of females (reviewed by [1]), and are generally divided into “good-genes” benefits or “sexy-son” effects related only to the higher attractiveness of highly-ornamented males [8, 9]. One of the proposed “good genes” benefits is improved survival under parasitic attacks [4,5].

At the time of breeding, male *Rutilus rutilus* (roach) produce keratin-nodules called breeding tubercles on their head and lateral sides [28]. They have been found to signal

resistance against *Rhipidocotyle campanula* gill parasite in the current study area [19]. In addition, roach breeding tubercle ornaments have been found to signal low loads of certain parasite species [10,14,25]. If breeding tubercles could be used as indicators of heritable parasite resistance or survival under parasitic attacks, they would provide an easy tool to enhance the quality of several fishes produced artificially in hatcheries [26]. However, studies focusing on the survival of differentially ornamented males are very rare [18] and to our knowledge absent in fishes.

We aimed to study experimentally the dependence of parasite resistance upon the breeding ornamentation in roach in spring 2003. However, shortly after the collection and marking of fish during their spawning time in spring, a disease epidemic took place in the laboratory storage tank. We recorded all events in the experimental tanks before terminating the study, with fulfilling the requirements of the permission admitted by the ethical committee. Thus, as a by-product of the experiment, data on survival of fish was gathered.

2. Materials and methods

We collected roach using dip nets from a creek discharging from Lake Pirttijärvi to Lake Lahnajärvi, 35 km south-east from the City of Jyväskylä, central Finland, on May 20, 2003—right before the spawning time. Fish used this small, 950 m creek for migrating from Lake Lahnajärvi to their spawning grounds in the upper lake. Roach of this population were found to vary in their expression of the secondary sexual characters in an earlier study [19], and were therefore suitable to experiments testing the dependence of parasite resistance upon the expression of sexual signals. Prior to transportation into laboratory in buckets filled with creek water, 15 °C in temperature, fish were anesthetized using MS-222 (Sigma Chemical, St.

Louis, MO, USA) and inspected for maturity and sex by pressing gently the abdomen so that the included fish were all sexually mature and ready to spawn. Breeding tubercle ornamentation, skin roughness, was examined by palpation by hand, as described by Taskinen and Kortet [19]. The fish were categorized into three classes according to their sex and the expression of breeding tubercle ornaments on their lateral sides: high-ornamented males (very rough skin, large breeding tubercles), low-ornamented males (smooth or slightly rough skin, small or no breeding tubercles) and females. Intermediately ornamented males were excluded and killed with a sharp blow on the head. During anesthetization, the fish were marked by cutting the tip of the left ventral fin from high-ornamented males, ($n = 29$), tip of the right ventral fin from low-ornamented males ($n = 54$), and tip of the dorsal fin from females ($n = 47$). Mean \pm standard errors of the mean (SEM) length of high-ornamented males, low-ornamented males, and females were 149.2 ± 2.4 mm, 134.8 ± 1.9 mm, and 155.9 ± 2.1 mm, respectively. The 130 marked fish were kept in a single 1000 L flow-through storage tank and fed with commercial dry pellets and monitored daily. Water temperature 10.5°C (range $10.0\text{--}11.0^\circ\text{C}$) and oxygen content ≥ 7.5 mg $\text{O}_2\text{ l}^{-1}$ were measured daily (YSI 55, YSI Co., USA). Another set of roach ($n = 120$), collected from Lake Jyväsjärvi, City of Jyväskylä, Finland, by ice-fishing between April 1 and 13, 2003, were held in another tank in the same conditions as the experimental roach and used to control for the effects of laboratory conditions. Moribund fish were removed from the tanks and euthanatized by decapitation, and therefore in the present report, survival time is the time when the fish was euthanatized due to its poor condition. During the disease outbreak, monitoring on the condition of fish and removal of the moribund fish was done several times per day. Within the framework of our animal experiment permit, keeping of the fish was ended on June 16, 2003, when all the remaining fish were killed with an overdose of MS-222.

The cause of death based on the clinical signs of Flavobacteria-related disease was examined by studying the presence of *Flavobacterium psychrophilum* in fish tissues using species-specific PCR from six fish suffering skin lesions and erosion of the tail. DNA from infected tissue was extracted by lysing cells with Proteinase K and bead-beating, and the DNA was purified using phenol-chloroform extraction and NaCl-isopropanol precipitation [20]. PCR was performed using protocol designed by Toyama et al. [21]. The reaction mix contained 0.3 mM dNTPs, 0.3 μM of each primer, $1 \times$ DynaZyme buffer, and U Dyna Zyme II DNA-polymerase (Finnzymes, Espoo, Finland). The total reaction volume in each PCR tube was 40 μL with 1 μL of template DNA. *F. psychrophilum* DNA was used as a positive control and reaction mix with no template as a negative control. The PCR products were run in 0.8% agarose gel and

visualized under UV light. All fish samples were positive for *F. psychrophilum*, a bacterium that has been previously detected in wild roach by Madetoj et al. [16].

Differences between the groups of high-ornamented males, low-ornamented males, and females in the proportion of fish surviving to the end of the monitoring (27 days) were analyzed using χ^2 -test. Among the fish that died during the monitoring, the differences between the groups in their mean survival time were analyzed using one-way analysis of covariance (ANCOVA) with fish group as a factor and fish length as a covariate. Statistical analyses were performed using SPSS for Windows 11.0.1 (SPSS Inc., USA). The study was performed with the permission from the Ethical Committee for Animal Research of the University of Jyväskylä (LS-10/15.4.03).

3. Results

Lake Lahnajärvi fish started to succumb on day 20 from the beginning of the monitoring: affected fish had eroded tails and lesions in the skin. Among Lake Lahnajärvi roach, 24 out of the 130 roach (18.5%) survived until the end of the 27-days monitoring. All of the 120 Lake Jyväsjärvi roach survived till the end of the monitoring, and no disease symptoms were observed among them, suggesting that the mortality was not attributable to laboratory conditions per se. Amongst Lake Lahnajärvi roach, the highest survival was observed in high-ornamented males. Survival proportions of high-ornamented males, low-ornamented males and females in Lake Lahnajärvi fish during the monitoring were 31.0, 20.4, and 8.5%, respectively. Differences between the groups were statistically significant (χ^2 -test, $\chi = 4.842$, $df = 1$, $P = .028$).

Between days 20 and 27, the length-adjusted mean survival time of fish that died was the highest among the high-ornamented males with large breeding tubercles, intermediary among the low-ornamented males with small or no breeding tubercles, and the lowest in females (Figure 1). Results of ANCOVA suggested that there were statistically significant differences in the mean survival time between the fish groups among the fish that died during the monitoring ($F_{2,106} = 13.528$, $P < .001$). LSD post hoc tests revealed that the mean survival time in high-ornamented males was higher than in low-ornamented males ($P = .024$) or females ($P < .001$) and higher in low-ornamented males than in females ($P = .007$). In addition, the effect of the covariate, fish length, was also significant (ANCOVA, $F_{1,106} = 4.857$, $P = .030$) indicating that the mean survival time increased with fish length.

4. Discussion

In this monitoring, females had the shortest survival time while the males with the most elaborated breeding tubercles survived for the longest time. Neither the fish from

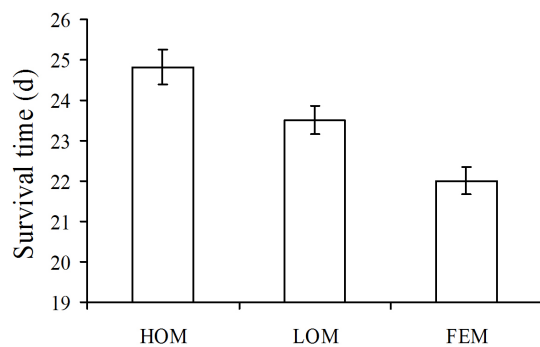


Figure 1: Mean length-adjusted survival time (\pm standard error of the mean) for roach, *Rutilus rutilus*, which died during the 27-days monitoring. HOM, LOM, and FEM stand for high-ornamented males ($n = 20$), low-ornamented males ($n = 43$), and females ($n = 43$), respectively. All differences between the three groups were statistically significant (see Section 3).

Lake Lahnajärvi nor Lake Jyväsjärvi were experimentally exposed to any pathogen, but Lake Lahnajärvi fish started to suffer from skin lesions, eroded tails, and mortality after 20 days in the laboratory. As the control fish from Lake Jyväsjärvi experienced 100% survival and showed no disease symptoms, the laboratory conditions as such were not probable to induce the mortality. Thus, the observed mortality in Lake Lahnajärvi fish was most likely caused by *F. psychrophilum* present in diseased fish individuals, or some other fish pathogen not detected in this study. Even though the fish were, in many cases, put to death before the actual die off, they were in such a poor condition that recovery could not be expected.

As all Lake Lahnajärvi fish were in one tank, it can be assumed that they were equally exposed to *F. psychrophilum*, or infection by other contributing pathogens that similarly transmit through water. Therefore, we suggest that the differences in survival of high-ornamented males, low-ornamented males, and females were related to differences in disease and stress resistance, or tolerance. When the disease broke out, the mortality increased rapidly. Mortality among the infected fish would probably have been 100% if the monitoring was prolonged. During the 7 days period when mortality took place, the difference in the mean survival time between high- and low-ornamented males was about 1.2 days. Although the difference was not bigger than that, it could have important fitness consequences in natural populations. By tolerating symptoms developing under spawning season for a longer time, fish may have a better chance to spawn successfully. Therefore, the present result of higher survival in high-ornamented males is in accordance with the hypothesis of parasite-mediated sexual selection [5] predicting that males would signal resistance by their sexual ornaments. This resistance may be heritable,

since, for example, in the whitefish, the parental breeding tubercle ornamentation has been found to indicate offspring survival [27] and offspring performance [6].

The lowest survival among females was against the prediction that male breeding ornaments would be an energetic or immunological handicap [4]. Rather, this result suggests that females, due to their higher investment into gonads in comparison to males, were less tolerant against severe stress caused by the pathogen(s). In addition, high breeding-time estradiol concentrations [22] combined with sex-specific breeding-time differences in innate immune functions could have contributed to their higher mortality of females [11].

The current results are in accordance with the previous studies [19] which indicated that roach's resistance against the digenean parasite *Rhipidocotyle campanula* is higher in high-ornamented males than in low-ornamented males. In a later study on roach, resistance against *R. campanula* was also linked to the high concentration of immunoglobulins and high activity of head kidney phagocytes, suggesting consequently that highly ornamented males in general have a more efficient immune defence than their less-ornamented rivals [24]. In addition, the elaborateness of breeding tubercle ornamentation has been found to associate with dominance over rival roach males [12]. Since high testosterone concentration was found to be positively coupled to both breeding tubercles ornamentation [13] and efficient innate immune functions [24], it is possible that the high-testosterone males, carrying the most elaborated ornaments, also have the strongest parasite resistance (see also [4]). Results by Vainikka et al. [23] showed that cortisol concentrations in roach plasma can be 10–30 times higher during spawning than outside the spawning period. However, differences in cortisol concentrations can probably not explain the higher mortality in female roach than in male roach in the present study as the cortisol levels were found by Vainikka et al. [23] to be higher in males than in females in roach during spawning.

Why the diseases broke out in Lake Lahnajärvi fish but not in Lake Jyväsjärvi fish likely relates to the different origin and handling of these fish. The Lake Lahnajärvi fish were marked by fin-cutting which can expose fish to *F. psychrophilum* [16] while Lake Jyväsjärvi fish were not. In addition, Lake Jyväsjärvi fish had had several weeks of time to acclimatize to the laboratory conditions before the newly brought Lake Lahnajärvi fish developed symptoms. Either the Lake Jyväsjärvi fish were more resistant against infections, or they were never exposed to infective agents as roach from the two lakes were kept in separate tanks. We cannot exclude other pathogens than *F. psychrophilum* as causative or contributing agents. However, the external symptoms of the disease, together with the molecular findings support our suggestion that the infection was caused by *F. psychrophilum*. *F. psychrophilum* is a difficult pathogen

in aquaculture, where epizootics include high mortality rate [17], but the bacterium has also been detected in wild fish [16] and it is evident that the host range of the pathogen is wide and not restricted to only salmonid fish [15].

All fish being in one tank meant lack of replicates in the present study. In addition, we did not perform controlled infections. Finally, the set up was not even designed to study the survival question, but we just observed events in a tank where fish were stored for another experiment. Nevertheless, the current observations describe disease-induced, rapid mortality among wild fish and capture the most critical period of life in terms of breeding success, the spawning period. Before spawning, roach may perform extensive migrations [2]. During spawning, fish may be subjected to a severe stress as indicated by their elevated plasma cortisol concentrations [23]. Resistance or tolerance of bacterial infections at the time of spawning may be particularly important for the survival and fitness of differentially ornamented males, and, as such, contribute to the evolution of sexually dimorphic ornaments. The observed sex difference in survival times could result even in biased operational sex ratios and lower reproductive capacity of the population under intensive epidemics, and could as such have important ecological consequences on fish population dynamics. Before the ultimate conclusions can be drawn, the observed patterns on the survival under stress related to spawning and disease should be confirmed by using controlled experimental infection approach.

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