Animal Behaviour 77 (2009) 823–829

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/yanbe

Male body size and breeding tubercles are both linked to intrasexual dominance and reproductive success in the minnow

Alain Jacob [a,*,1], Guillaume Evanno [a,b,c,1], Emanuela Renai [a,1], Roberto Sermier [a], Claus Wedekind [a]

[a] Department of Ecology and Evolution, University of Lausanne
[b] INRA, Ecology and Health of Ecosystems, Rennes
[c] Agrocampus Ouest, Rennes

ARTICLE INFO

Article history:
Received 15 August 2008
Initial acceptance 19 September 2008
Final acceptance 5 December 2008
Published online 30 January 2009
MS. number: 08-00538R

Keywords:
lek
male–male competition
minnow
perl organ
Phoxinus phoxinus
reproductive behaviour
secondary sexual character
sexual selection

Male dominance hierarchies are usually linked to relative body size and to weapon size, that is, to determinants of fighting ability. Secondary sexual characters that are not directly used as weapons could still be linked to dominance if they reveal determination or overall health and vigour and hence, indirectly, fighting ability. We studied the mating behaviour of the minnow, *Phoxinus phoxinus*, a cyprinid fish in which males develop breeding tubercles during the spawning season. The function of these breeding tubercles is still not clear. Using microsatellite markers, we determined male reproductive success under controlled conditions. The minnows were territorial and quickly established a dominance hierarchy at the beginning of the spawning season. Dominance was strongly and positively linked to fertilization success. Although body size and number of breeding tubercles were not significantly correlated in our sample, both large males and males with many breeding tubercles were more dominant and achieved higher fertilization success than small males or males with few tubercles. We found multimale fertilization in most clutches, suggesting that sperm competition is important in this species. Females showed behaviour that may be linked to spawning decision, that is, male dominance might not be the only determinant of male reproductive success in minnows.

Antagonistic encounters between males are often costly (Smith 1974) and dominance hierarchies may be established to reduce the intensity of such interactions (Collias 1943). Dominant males usually have larger and higher-quality territories (Foote 1990; Candolin & Voigt 2001; Andersson et al. 2002), better access to females (Fleming & Gross 1994; Quinn & Foote 1994; Creighton 2001; Wong & Candolin 2005) and they often have higher fertilization success (Wiley 1973; Dewsbury 1982; Andersson 1994; Esteve 2005).

Across various taxa, dominance is usually well indicated by body size and weight (Andersson 1994; Qvarnstrom & Forsgren 1998), but often also by secondary sexual characters that are not directly used as weapons (Clutton-Brock et al. 1980; Berglund et al. 1996; Kortet & Taskinen 2004; Kortet et al. 2004). Sometimes, male dominance seems to be linked more closely to body size than to secondary sexual characters (Zucker & Murray 1996; Hudman & Gotelli 2007), and sometimes secondary sexual characters may be the better indicators of male dominance (Kitchen et al. 2003; Kortet et al. 2004; Setchell et al. 2006; Stuart-Fox et al. 2006), especially so if secondary sexual characters indicate good health and vigour. Indeed, high resistance or tolerance to pathogens has been found in dominant males (Rantala & Kortet 2004; Ahtiainen et al. 2006) and in males with elaborate secondary sexual characters (Milinski & Bakker 1990; Wedekind 1992; Taskinen & Kortet 2002; Kortet & Taskinen 2004; Ezenwa & Jolles 2008). Secondary sexual characters can therefore be important not only in female choice but also in male–male competition (Andersson 1994).

We studied male reproductive success in regard to dominance and secondary sexual characters in the European minnow, *Phoxinus phoxinus*, a cyprinid whose mating system has so far only been qualitatively described as 'communal spawning' (Breder & Rosen 1966; Bless 1992). Mature males seem to establish a dominance hierarchy and to defend territories before females begin to spawn (Bless 1992). Males often display secondary sexual characters during the reproductive period. These characters can include conspicuous skin colours (e.g. melanin-based patterns and/or red colours: the latter are usually most pronounced around the mouth and the pectoral and pelvic fins) and breeding tubercles that are mostly located on the head. Breeding tubercles are small, colourless and horny epidermal structures that are common in many fish

* Correspondence: A. Jacob, Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland.
E-mail address: alain.jacob@unil.ch (A. Jacob).
[1] Equally contributing authors.

0003-3472/$38.00
doi:10.1016/j.anbehav.2008.12.006

species. Their functional significance is not fully understood yet (see discussion in Wiley & Collette 1970 and Wedekind et al. 2008). In the case of the minnow, breeding tubercles may simply facilitate the maintenance of body contact between the mating partners, be used as weapons during male fights, or act as signals that provide information about male genetic quality and parasitic load through visual or sensory hydrodynamic signals (Wiley & Collette 1970; Müller & Ward 1995).

We analysed the spawning behaviour of minnows in a controlled seminatural set-up. We then tested whether male body size and/or breeding tubercles are linked to dominance and to male reproductive success as confirmed by microsatellite typings of parents and offspring.

METHODS

Recording of Spawning Behaviour

Minnows were caught by electrofishing from a natural population in the catchment of the river Venoge, Vaud canton, Switzerland, in April 2007, some weeks before the spawning season. Males were anaesthetized (with Aqui-S, Aqui-S New Zealand Ltd, Lower Hutt, New Zealand; 0.04 ml/litre) and individually marked with different black and white combinations of plastic beads (diameter 2 mm) on a nylon filament that was fixed to the dorsal fin by penetrating its basal part from one side with a small needle and attaching the perforated bead on the other side. After marking, they were transferred to a basin filled with oxygenated water to recover. After 30 min, all fish had fully recovered and the anaesthesia had no apparent negative effects. The fish were then introduced into two aquaria (50×50 cm and 100 cm high, eight males with two females per aquarium) in a climate chamber. Individuals were fed twice a day with dry fish food (Tetra Min, BioActive formula), and with live zooplankton on the weekends. Four perforated metallic boxes were filled with gravel, 2–3 cm diameter, a substrate known to be preferred by minnows for spawning (Bless 1992). These filled metallic boxes fitted into four Plexiglas boxes that had been put into each aquarium before the fish were introduced. The gravel and the perforated metallic boxes allowed the spawned eggs to fall through the gravel down to a 2 cm gap between the Plexiglas and the metallic box. This way, the fish were prevented from eating eggs. In each tank, an area without gravel was left for potential use as a refuge for individuals to avoid more dominant fish.

To describe the males' dominance hierarchy and their spawning behaviour, we monitored the aquaria with eight surveillance cameras (CCD cam 1/3″ SONY Super HAD, lens angle 78°, minimum illumination 0.05 Lux, Profiline, two cameras per side and per aquarium), which were linked to a MultiCam GV-1000 System (Ecoline; see Jacob et al. 2007 for further description). We recorded all behaviour between 10 May and 1 June 2007. A seasonal change was simulated by increasing the water temperature from 7 °C to 14 °C (1 °C every 2 days) and by changing the light cycle from 8 to 13 h of light per day. Observations during the first few days indicated that fish activity depended on the light regime, with low activity in darkness and increased activity when the light was switched on in the morning. The cameras were therefore programmed to film the aquaria from 0800 hours to 2100 hours. Boxes were checked every morning for the presence of eggs. Eggs were collected and individually distributed to 24-well multiwell plates (BD Falcon San Jose, CA, U.S.A.; nontreated polystyrene, flat bottom). Each well had been filled before with 2 ml of water (14 °C) that was chemically standardized according to the OECD guidelines (OECD 1992). The isolated embryos were then incubated at 10.7 °C until hatching (no water exchange occurred in between).

On 14 June 2007, all males were anaesthetized, as described above, for biometry. We took digital photos of their foreheads so we could count the breeding tubercles later on. The diameters of individual breeding tubercles were also measured with the open-access software IMAGEJ (http://rsb.info.nih.gov/ij/). For this measurement we first sampled the four largest tubercles of the anterior part of the forehead, which are situated more or less in a rectangular pattern between the nostrils, two on the left and two on the right side of the mesial sagittal line (see Figure 1 in Frost 1943). We also measured four randomly picked forehead tubercles that were situated posterior to the eyes (the tubercles were numbered on both sides of the mesial sagittal line and then selected for measurements using a random number generator).

We described the dominance hierarchy based on the antagonistic behaviour during three different kinds of observation periods. The first covered 2 h shortly before female spawning activity started (p_{before}), the second covered 1 h from the moment female spawning activity had started (p_{during}), and the third (p_{end}) covered 30 min starting 1 h after the end of the second period. An antagonistic act was defined as an interaction between two males that ended by one male swimming away and being followed or chased by the other male. The total number of recorded antagonistic interactions were in aquarium 1: $N_1 = 445$ (60 in p_{before}; 254 in p_{during}; 131 in p_{end}); in aquarium 2: $N_2 = 978$ (428 in p_{before}; 382 in p_{during}; 168 in p_{end}). We assigned a winner and a loser for each of these interactions and calculated dominance hierarchies for each aquarium using the David's score method. This method takes the relative strength of the opponents into account (Gammell et al. 2003; De Vries et al. 2006) and results in continuous scores (instead of ranks). We calculated an overall dominance hierarchy per aquarium. We also determined dominance hierarchies for each of the three observation periods per aquarium.

To identify male territories, we used the same video sequences as for the calculations of the dominance scores. The gravel area in each aquarium was divided into 16 sections of the same size. The position of each male was recorded every 5 min but only if no female showed any spawning activity on the spawning area. Otherwise, we skipped to the next observation point 5 min later. The size of a male territory was estimated using a score s_{ij} for male i in section j, calculated as $s_{ij} = 1/n_j$ where n_j is the number of males in section j at the time of observation, that is, a male's score was weighted for the presence of other males in a given section. This procedure was followed for all observations separately to produce a sum of scores for each male in each section. We then computed a relative score for each male per section by dividing a male's score by the sum of all scores for the given section. We summed these relative scores for each male over all sections to obtain the overall territoriality per male. This way, we obtained an index that was weighted by the presence of other males in each section of the potential spawning area.

Genetic Analyses

We used microsatellite markers to genotype all adults and a random sample of offspring (that had been killed with a lethal dose of Aqui-S, 0.1 ml/litre for 30 min, at the hatchling stage). To estimate male fertilization success per clutch (c) we genotyped the following hatchling numbers: aquarium 1: $N_{c1} = 40$; $N_{c2} = 63$; $N_{c3} = 80$; $N_{c4} = 28$; aquarium 2: $N_{c5} = 32$; $N_{c6} = 33$; $N_{c7} = 30$; $N_{c8} = 6$; that is, a total of 211 individuals were analysed for aquarium 1 and 101 individuals for aquarium 2.

Genomic DNA was extracted from tissue samples using the Qiagen DNeasy Kit (Qiagen Inc., Valencia, CA, U.S.A.), following the manufacturer's protocol. We used five microsatellite loci (Ca1, Ca12, Ca3, Ca5, Ppro126) previously developed in other cyprinids