

Sperm characteristics in perch *Perca fluviatilis* L.

S. WIRTZ* AND P. STEINMANN

*Zoologisches Museum der Universität Zürich, Winterthurerstrasse 190,
8057 Zürich, Switzerland*

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Sperm densities in perch *Perca fluviatilis* males showed a positive correlation with the amount of stripped milt. Sperm flagellum length did not correlate with body mass, but a significant correlation between flagellum length and the total number of sperm produced was found.

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Sperm production is a crucial factor for the potential reproductive success of males in many species (Nakatsuru & Kramer, 1982). Especially under strong sperm competition, higher sperm numbers in the ejaculates are likely to increase a male's chance of fertilization (Parker *et al.*, 1996). But sperm production is limited by physiological constraints, *e.g.* by testis size, which often depends on the body size of a male (Schärer & Robertson, 1999). In many fish species, large males produce a multiple of the sperm number of small males (Petersen & Warner, 1998). Moreover, sperm allocation tactics can vary according to the size and status of a male or its amount of available sperm (Kazakov, 1981; Gross, 1996; Taborsky, 1998). Allometries in sperm production and gonad characteristics between small and large males have been described in several species, *e.g.* small males having relatively larger testes or higher sperm densities in the seminal fluid or differences in hormone production (Gage *et al.*, 1995; Leach & Montgomerie, 2000; Uglem *et al.*, 2002). Sperm flagellum length, motility or longevity can also depend on the size and status of a male (Gomendio & Roldan, 1991; Gage *et al.*, 1995; Stockley *et al.*, 1997; Taborsky, 1998; Scaggiante *et al.*, 1999; Mazzoldi *et al.*, 2000). Small males might at least partially compensate their physical inferiority in sperm competition.

Males of the perch *Perca fluviatilis* L. start developing their testes in August which reach maximum size in October. Testes remain at a similar level (1–2% of body mass) throughout the following months until the beginning of the spawning season (Le Cren, 1951). Testes begin to ripen early in April and show

*Author to whom correspondence should be addressed at present address: Behavioural Ecology, Zoological Institute, University of Bern, 3032 Hinterkappelen, Switzerland. Tel.: +41 31 631 9158; fax: +41 31 631 9141; email: sabine.wirtz@esh.unibe.ch

a swift drop after the beginning of the spawning season. Therefore, perch males are only equipped with a limited amount of sperm for the spawning season in April and May. Perch males perform three different mating tactics (Heeb, 1999) which depend on body size (P. Steinmann & G. Ribí, unpubl. data). Whether sperm density and sperm flagellum length in perch vary according to body mass and condition factor was examined in this study.

During the spawning seasons of 2003 and 2004, male perch were caught twice a week with a bow net in Lake Zurich, Switzerland. In 2003, a total of 93 perch were analysed for milt volume and sperm density. Sperm flagellum length were measured for a total of 23 individuals. In 2004, 34 perch were examined for milt volume and sperm density, but not for flagellum length. From each male caught, total length (L_T ; cm) and mass (M ; g) were measured and the condition factor (K) was calculated from: $K = 100 ML_T^{-3}$. The full ejaculate was obtained by applying gentle pressure to the abdomen and collected in plastic tubes. The tubes were stored at 4° C for a maximum of 60 min before being used for sperm density counting and preparation of the microscope slides. For sperm density, the stripped milt of each individual fish was stirred and diluted 1:100 in distilled water. A droplet of the diluted milt was placed on a haemocytometer (improved Neubauer counting chamber, depth 0.1 mm). For each male, at least 100 moving sperm heads were counted. The sperm density was calculated according to the following:

$$\text{sperm density (ml}^{-1}\text{)} = 1000 \times \text{number of counted sperm [area (mm}^2\text{)} \\ \times \text{chamber depth (mm)} \times \text{dilution}]^{-1}.$$

For measuring flagellum length, milt was diluted to 1:10 000 in distilled water and a droplet was placed on a microscope slide. Fixation over 10 min in 70% ethanol was followed by colouration of the samples with Colorryd solution rouge (Bioréac, Lausanne, Switzerland) and Colorryd solution bleu (Bioréac) for 5 min each. Pictures of sperm were captured with a Polaroid DMC2 digital camera (resolution 1600 × 1200 pixels) mounted on a LEITZ DMR microscope (magnification ×400, phase contrast). NIH Image software (version 1.63 f) was used to measure flagellum length of haphazardly chosen sperm from each fish, measuring from the base of the sperm head to the tip. Due to the fact that the quality of the microscope slides varied between individuals, the amount of gametes measured per male ranged between 7 and 26 (mean 13.8). For statistical analysis, the average for each fish was used as one data point.

An over six-fold variation in sperm density was found, ranging from 18.8×10^9 to 127.5×10^9 sperm ml⁻¹, with a mean of 53.6×10^9 sperm ml⁻¹. The average sperm density was higher in 2003 than in 2004 (one-way ANOVA, $F_{1,125}$, $P < 0.001$; Fig. 1). In both years, a positive correlation was found between stripped milt volume and M [Pearson correlation (two tailed), 2003: $n = 93$, $r = 0.857$, $P < 0.001$; 2004: $n = 34$, $r = 0.649$, $P < 0.001$]. Sperm densities correlated significantly with the amount of stripped milt of the examined fish in 2003 (ANCOVA, $F_{1,63}$, $P < 0.001$, $n = 93$) and in 2004 (ANCOVA, $F_{1,30}$, $P < 0.05$, $n = 34$).

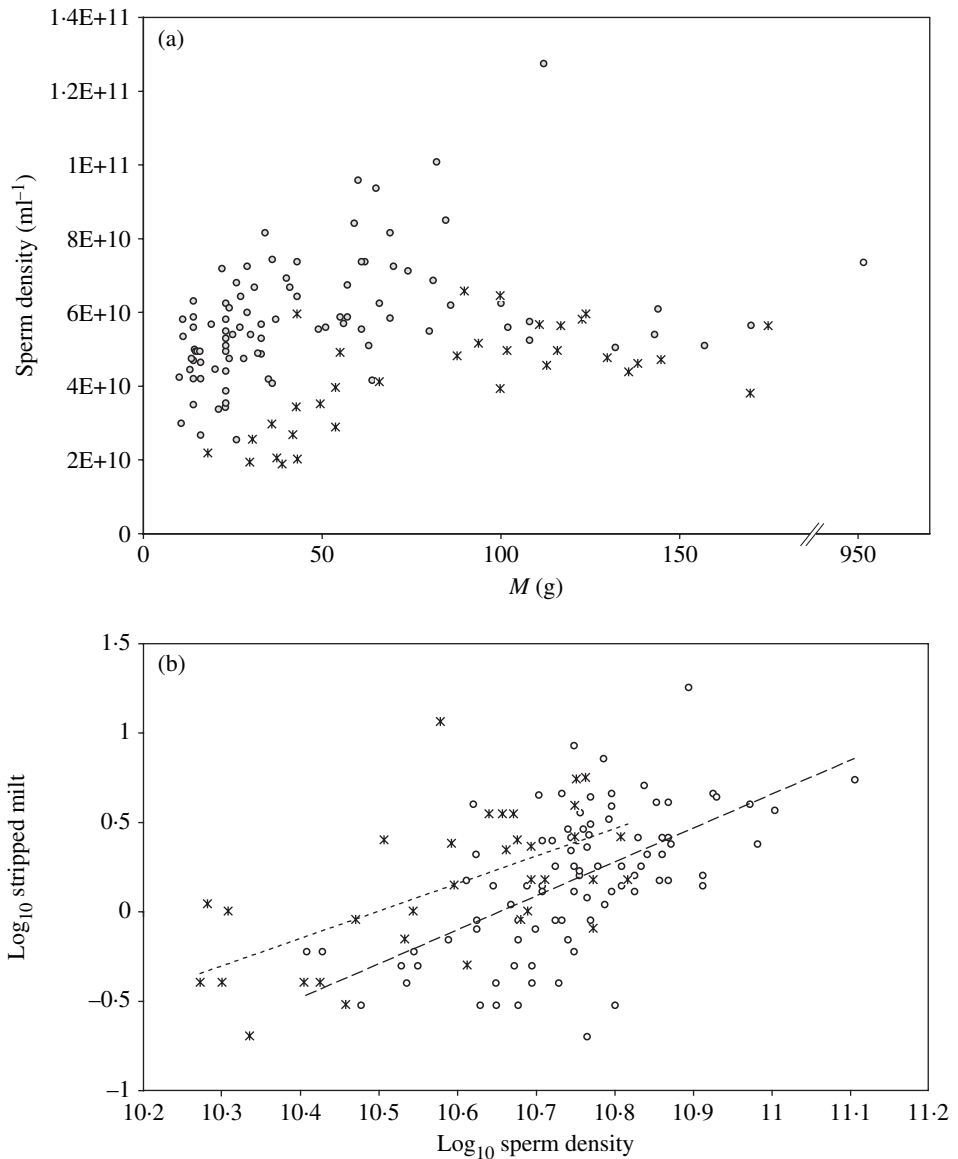


FIG. 1. Sperm density of stripped milt in relation to (a) perch body mass and (b) stripped milt volume. The curves in (b) were fitted by: 2003 $y = 1.89x - 20.202$ (—) and 2004 $y = 1.535x - 16.124$ (- -). Data were collected in 2003 ($n = 93$, o) and 2004 ($n = 34$, X).

The K of males correlated positively in both years with sperm density [Pearson correlation (two tailed), 2003: $n = 93$, $r = 0.269$, $P < 0.01$; 2004: $n = 34$, $r = 0.428$, $P < 0.05$]. Piironen & Hyvarinen (1983) found similar sperm densities in perch, ranging from 37×10^9 to 127×10^9 sperm ml⁻¹, but without specifying the M of the examined fish. As body mass to milt volume ratio is subject to annual variation (P. Steinmann & G. Ribí, pers. obs.), the observed difference in sperm density between 2003 and 2004 supports the assumption that sperm

production in perch depends both on M and on current physical state of males. Compared to other fish species (Piironen & Hyvarinen, 1983; Stockley *et al.*, 1996; Mylonas *et al.*, 2003; Rainis *et al.*, 2003; Mansour *et al.*, 2004), the average sperm density in perch is high and the variation between males is considerably large. Sperm density in externally fertilizing fish has been shown to be generally higher than in mammals or other internal fertilizers (Stockley *et al.*, 1997; Petersen & Warner, 1998; Byrne *et al.*, 2002). If high sperm densities in external fertilizers are assumed to be a result of selection, it can be concluded that perch must be subjected to a very high selection pressure for high sperm densities. According to the classification of sperm competition intensity provided by Stockley *et al.* (1996), perch males are likely to face a very high sperm competition risk during the spawning acts. The increase of sperm density with increasing body mass suggests an upper limit for sperm density, probably due to physiological constraints. When perch males grow, they first increase both sperm density and milt volume until the maximal sperm density is reached. From then on, larger milt volumes are produced while maintaining the same maximal sperm density. A similar relationship has been shown for perch females in the egg size to egg number relationship (Egloff, 1998). Sperm flagellum length of 23 perch males caught in 2003 ranged between 25.3 and 32.5 μm (mean = 27.9 μm) and did not differ much from previous results in other fish species (Stockley *et al.*, 1996; Leach & Montgomerie, 2000; Gage *et al.*, 2002). Lahnsteiner *et al.* (1995) reported the flagellum length in perch to range between 30 and 35 μm . Males used in this study ranged between 10 and 15 cm L_T and 15 and 80 g. In their study, samples of semen were fixed in an unbuffered mixture of 10% paraformaldehyde, 5% glutaraldehyde and 2% osmium tetroxide. Therefore, the difference found in this study might be due to differences in fixation procedures. For perch examined in this study, flagellum length did not correlate with M , but a positive correlation between the total number of sperm produced per male (ml stripped milt \times measured sperm density) and flagellum length was found [Pearson correlation (two tailed), $n = 23$, $r = 0.465$, $P < 0.05$; Fig. 2]. The K of males did show a positive correlation with flagellum length [Pearson correlation (two tailed), $n = 23$, $r = 0.419$, $P < 0.05$]. For internally fertilizing species it has been reported that sperm flagellum tends to be longer in species facing high sperm competition risk (Gomendio & Roldan, 1991). It is not clear, however, whether a longer flagellum can provide any advantage in sperm competition in externally fertilizing species, since sperm are brought to the eggs by water movements much more than by movements of the sperm.

Perch males show three different status-dependent mating tactics, mostly depending on body size: sneakers (small males), group spawners (midsized males) and dominant (large) males (Heeb, 1999; P. Steinmann & G. Ribi, pers. obs.). Gross (1996) provided a model stating that males should change their mating tactics according to changes in their status within a group of males (*e.g.* switching from a sneaker to a group spawner) and thereby maximize their lifetime reproductive success. The observed mating tactics in perch males agree with these predictions.

It has previously been shown for several species that sperm characteristics, such as sperm density, milt volume or flagellum length, vary according to different mating tactics of males (Leach & Montgomerie, 2000; Neff *et al.*,

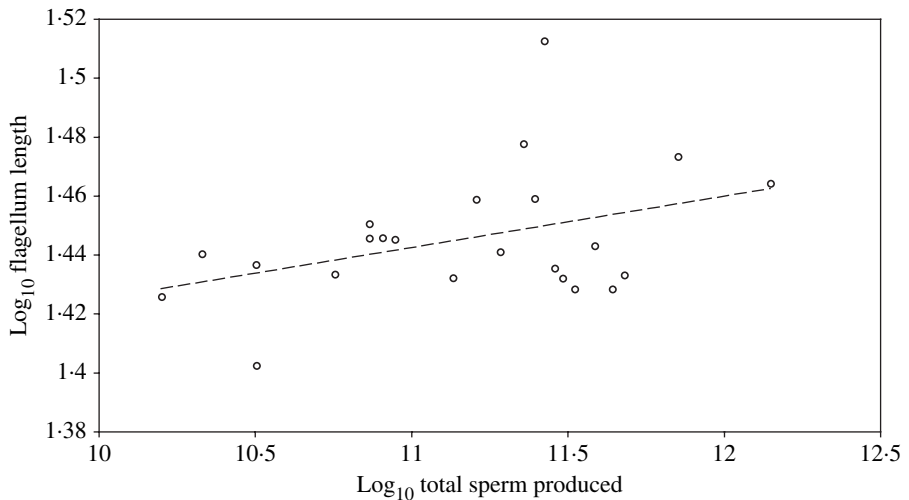


FIG. 2. The relationship between flagellum length and total number of sperm produced, where total number of sperm = [stripped milt volume (ml) \times sperm density (ml⁻¹)]. The curve was fitted by: $y = 0.0174x + 1.2507$.

2003; Aspbury & Gabor, 2004) or according to the expected sperm competition intensity (Oppliger *et al.*, 1998; Uglem *et al.*, 2002). In Atlantic salmon *Salmo salar* L., for example, no difference in flagellum length could be found between parr and anadromous males (Vladic *et al.*, 2002), but parr males showed higher sperm density, higher investment in gonads and more motile sperm 10 s after initiation than anadromous males (Vladic & Järvi, 2001). For bluegill *Lepomis macrochirus* Rafinesque, another species with alternative mating tactics, Neff *et al.* (2003) reported sperm density to be highest in satellite males. In a later study (Burness *et al.*, 2004), longer flagellum length and faster sperm (10 and 5 s after initiation) were found for sneakers. In perch, no indications for small males being able to even partially compensate for their physical inferiority by higher sperm densities, allometric sperm production or longer sperm flagellum could be found. The results of this study indicate that large and healthy males generally produce more and longer sperm than small ones. Small perch males might 'make the best of a bad situation' (Leach & Montgomerie, 2000) by exhibiting sneaker tactics or trying to participate in group spawnings and thereby allocating their small sperm supplies under unfavourable conditions and achieving much smaller fertilization success than large dominant males.

Sperm depletion in the Lake Zurich perch population has been shown to lead to a decrease in the fertilization ratio in late spawned egg strands (P. Steinmann & G. Ribí, pers. obs.). Considering the much higher reproductive potential of large males, excessive fishery efforts that preferably take out the largest fish of a population might aggravate this phenomenon.

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