Sperm velocity in an Alpine whitefish: effects of age, size, condition, fluctuating asymmetry and gonad abnormalities

D. Urbach*†, D. Bittner*‡, T. L. Lenz§, D. Bernet∥, T. Wahlι and C. Wedekind¶

*Division of Conservation Biology, University of Bern, Erlachstr 9, 3012 Bern, Switzerland and ‡Centre for Fish and Wildlife Health, University of Bern, Länggassstr. 122, P. O. Box 8466, 3001 Bern, Switzerland

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The relationship between sperm velocity and individual age, size, body condition and fluctuating asymmetry was investigated in Alpine whitefish Coregonus fatioi. The fish analysed belonged to one among several sympatric whitefish populations of Lake Thun, Switzerland, which are characterized by a high prevalence of gonad alterations. Therefore, sperm velocity data were also tested for a link between gonad deformation and sperm swimming speed. Sperm velocity was significantly lower in larger-grown individuals and in individuals of higher body condition. As expected, sperm velocity was higher in males with higher levels of fluctuating asymmetry, but it did not significantly vary with male age. Moreover, variation in sperm velocity was found to be significantly higher in individuals showing some types of gonad alterations but it did not significantly correlate with the presence of other types of alterations.

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INTRODUCTION

Sperm velocity has in recent years been recognized as a primary determinant of fertilization success in a number of species (Birkhead et al., 1999; Froman et al., 1999; Levitan, 2000; Al-Qarawi et al., 2002). The large variation in sperm velocity observed amongst males may be explained by sperm competition theory. Sperm competition occurs when several males attempt to fertilize simultaneously.

†Author to whom correspondence should be addressed at present address: Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland. Tel.: +41 21 692 42 03; fax: +41 21 692 42 65; email: davnah.urbach@unil.ch
‡Present address: Computational and Molecular Populations Genetics (CMPG), Zoological Institute, University of Bern, Baltzerstr. 6, 3012 Bern, Switzerland.
§Present address: Max-Planck-Institute for Limnology, 24302 Plön, Germany.
¶Present address: Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland.

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the same batch of eggs. The intensity of competition varies with a male’s social status: more dominant individuals are expected to experience less sperm competition than their subdominant counterparts and are hence expected to invest less into sperm production (Ball & Parker, 1996; Taborsky, 1998). Accordingly, increased sperm velocity has been found in males experiencing elevated risks of competition (Leach & Montgomerie, 2000; Vladic & Jarvi, 2001; Neff et al., 2003; Burness et al., 2004, 2005; Kortet et al., 2004; Rudolfsen et al., 2006; T. Haugland, G. Rudolfsen, L. Figenschou & I. Følstad, unpubl. data).

Dominance hierarchies which determine a male’s social status and mating role are often settled by relative body size (Gross, 1996; Qvarnstrom & Forsgren, 1998). Larger males are usually the dominant ones and occupy the favourable positions (Fleming & Gross, 1994; Quinn & Foote, 1994; Blanchfield & Ridgway, 1999). Yet, whether or not a male becomes dominant might depend on the size structure of the group of males present. This suggests dominance (Gross, 1996) and consequently investment in sperm production (Rudolfsen et al., 2006) to be plastic traits. Correlated to male size is male age, which also acts as a determinant of social status. Older males are usually higher ranked, as in bluegill Lepomis macrochirus Rafinesque (Gross, 1982), rainbow trout Oncorhynchus mykiss (Walbaum) (Liley et al., 2002) or sockeye salmon Oncorhynchus nerka (Walbaum) (Hoysak et al., 2004). Consequently, sperm velocity is expected to decrease with increasing body size and increasing age.

Dominance hierarchies might also be linked to variation among individuals in general quality (i.e. health and vigour): individuals of increased phenotypic (and genetic) quality may be particularly successful in acquiring resources (Møller & Pomiankowski, 1993) and potentially also in acquiring social dominance. Fluctuating asymmetry, $A_F$ (Witter & Swaddle, 1994; Dufour & Weatherhead, 1998a), and body condition belong to the candidate indicators of health and vigour. Fluctuating asymmetries, i.e. the phenotypic expression of developmental instabilities (Dongen, 2006), are small, random deviations from perfect symmetry which arise due to the inability of individuals to buffer themselves against genetic and environmental stress during ontogeny (Van Valen, 1962). Negative correlations have been found between $A_F$ and attractiveness (Møller & Thornhill, 1998), offspring survival (Wedekind & Müller, 2004) but also social status (Malyon & Healy, 1994; Møller et al., 1996; Dufour & Weatherhead, 1998a). These observations allow predicting a positive correlation between $A_F$ and sperm velocity. Furthermore, if dominance is indeed positively correlated to phenotypic quality and the latter is revealed in Fulton’s condition factor $K$ (Fulton, 1904), sperm velocity is also predicted to correlate negatively with $K$.

Alternatively, although existing results suggest otherwise (Rudolfsen et al., 2006), reduced sperm velocity in males of enhanced condition may also result from a trade-off between current body condition and investment into reproduction (Casselman & Montgomerie, 2004). That is, even if a male is in good condition before spawning, allocating large proportions of resources in current reproduction may occur at the cost of reduced condition at the time of spawning.

In this study, the correlations between sperm velocity and male age, male size, $K$ and levels of fluctuating asymmetry were estimated in the Alpine whitefish Coregonus fatioi Kottelat (Kottelat, 1997). The individuals used in this study
belonged to a lake in central Switzerland in which a high prevalence of gonad alterations has recently been observed (Bernet et al., 2004). Consequently, the existence of a link between variation in sperm velocity and occurrence of particular gonad alterations was also investigated.

MATERIALS AND METHODS

FISH SAMPLING, AGE DETERMINATION AND GONAD DESCRIPTION

In mid-December 2004, 66 sexually mature male Alpine whitefish were caught by bottomset gill nets on one spawning ground in Lake Thun at c. 30 m depth. After drying the area around the genital pore to avoid sample contamination, freshly killed individuals were stripped for their gametes by bilateral pressure on the abdomen towards the genital pore. Milt was collected in individual Petri dishes. Individual fish were then measured for total length (LT) to the nearest mm and weighed (M). The K values were calculated as $K = ML_T^b$. The constant $b$ is the slope of the regression of log10(M) and log10(LT) and was set at 3.0 (Bolger & Connolly, 1989). Fluctuating asymmetry was measured on pictures of the paired pectoral fins, which had been carefully cut at their bases, dried for several weeks and photographed. The pictures were taken on a dull black background under standardized light conditions. Because the third fin ray appeared to well represent the actual fin size, its length was measured and used as trait (Mazzi et al., 2002). The AF was then estimated as the absolute asymmetry of the ray length divided by the mean ray length $\left(\frac{l-r}{l+r}\right)^{-1}$, where $l = \text{left}$ and $r = \text{right}$.

Age was determined using the annulus-criteria of Berg & Grimaldi (1967). Approximately 10 scales were sampled between the adipose fin and the branch line of each fish and prepared for scalimetry, but only the scale showing the clearest annuli was used for age determinations. Age indications are given in year-classes, as sampling took place during the natural spawning period of these fish. That is, a fish with two annuli was considered to be 3 years-old at the time of sampling.

Fish were dissected and the gonads morphologically assessed. According to Bernet et al. (2004), the morphological alterations were categorized into constrictions, asymmetries, atrophy, compartmentations, adhesions or fusions to the peritoneal wall and to the lateral trunk musculature, and hermaphroditism. In a recent study, no significant differences were found in the prevalence of constrictions, asymmetries and atrophy among males sampled in several different Swiss lakes, while compartmentations and adhesions or fusions showed significantly higher prevalence in Lake Thun compared to other lakes (D. Bittner, D. Bernet, T. Wahli, H. Segner, C. Küng & C. R. Largiader, unpubl. data). Based on these results, these latter abnormalities were considered separately.

SPERM QUALITY ANALYSIS

To detect variations in sperm speed among age classes, sperm behaviour was video-recorded through a microscope and analysed with a sperm-tracking programme. Videorecording was performed using a CCD B/W video camera module (Sony, XC-ST50 CE) at 50 Hz vertical frequency, mounted on an external negative phase-contrast microscope (CH30 Olympus) with a ×10 magnification objective. Samples to be analysed consisted of pure milt from each male exposed to 4.5 µl water. Recordings were obtained as follows: for each trial, a 0.5 µl aliquot of undiluted sperm was first isolated on a glass slide, before a micropipette containing the water was dipped into it to obtain a sub-sample. The content of the micropipette, i.e. the 4.5 µl water and the sub-sample
of milt, was then immediately placed onto a cooled microscope slide (c. 5°C) with fixed cover glass (LEJA Products BV, 20 m depth). To be able to account for the time elapsed from activation, i.e. the precise moment the sperm cells get activated by contact with water, until a stable image was obtained, the video-recorder was started before sperm manipulation, and activation was identified on the video-tape by a vocal signal. The whole procedure was repeated twice for each male.

Video-recordings were analysed using the ‘HTM-CEROS sperm tracker’ software (CEROS version 12, Hamilton Thorne Research, Beverly, MA, U.S.A.), an objective tool for studying sperm motion in fishes (Kime et al., 1996, 2001; Rurangwa et al., 2004). The following set up was used: frame rate = 50 Hz; number of frames = 25; minimum contrast = 6; minimum cell size = 11 pixels. The velocity variables assessed were: average path velocity (V_{AP} = average velocity on the smoothed cell path), straight line velocity (V_{SL} = average velocity on a straight line between the start and end points of the track) and curvilinear velocity (V_{CL} = average velocity on the actual point-to-point track followed by the cell). These velocity estimates correspond to the mean velocity of all motile cells analysed. Precautions were taken to control for differences between samples in the number of cells analysed. To remove the possible effect of drift, threshold values for the two only optional settings defining static cells, i.e. V_{AP} and V_{SL}, were set at 20 μm s^{-1} for V_{AP} and 30 μm s^{-1} for V_{SL}. For statistical analyses, the average over replicated measures within each male was used for each velocity variable recorded. Sperm velocity measurements were taken 10 s after activation.

**STATISTICAL ANALYSES**

Statistical analyses were performed using the software R version 2.1.1 (R Development Core Team, 2006). Parametric analyses were used after visual investigation of the normality and the homoscedasticity of the residuals.

In this study, there were neither eggs nor ovarian fluid gradients to attract or guide the sperm cells. Consequently, cell trajectories were not expected to be linear and measurements of the actual point-to-point track followed by the cells (V_{CL}) appeared as the most pertinent indicator of sperm velocity (Urbach et al., 2005; Rudolfsen et al., 2006). Thus, only V_{CL} is shown as a speed measurement in the figures.

**RESULTS**

Among the 66 males caught, six individuals were of age 2, 38 of age 3 and 21 of age 4 years. Because of one single observation in age group 5 years, this group was discarded. Eight out of 65 males (12%) showed compartmentation and adhesion or fusion of gonads, 36 (55%) had asymmetrical, atrophied and constricted gonads, and all together, 40 (62%) males had at least one type of alteration. Mean ± s.d. L_T was 30.9 ± 1.8 cm in 2, 32.0 ± 1.6 cm in 3 and 32.7 ± 1.6 cm in 4 year-old fish.

The L_T at catch varied between age classes (two-way ANOVA, F_{2,62}, P < 0.05); after correcting for multiple comparisons with a Tukey honest significant difference (HSD) test, a significant difference in size was found between 2 and 4 year-old individuals (HSD, t_{1,25}, P < 0.05) but no significant difference was detected neither between 2 and 3 year-old fish (HSD, t_{1,42}, P > 0.05) nor between 3 and 4 year-old ones (HSD, t_{1,57}, P > 0.05). Individuals aged 2 and 3 years were consequently grouped and the correlation between sperm velocity and L_T in 4 year-old individuals was investigated separately. Sperm velocity (V_{AP}, V_{SL} and V_{CL}) significantly decreased with L_T at catch among individuals aged 2 and 3 years (Pearson’s r between −0.37 and −0.41, n = 39, P < 0.05) and among individuals aged 4 years (Pearson’s r = −0.56 (V_{AP}) and −0.65 (V_{SL}),
except for $V_{CL}$ (Pearson’s $r = -0.42$, $n = 18$, $P > 0.05$). The correlation between $L_T$ and $V_{CL}$ is shown in Fig. 1 as an example.

The value of $K$ and $A_F$ did not significantly vary between age classes (two-way ANOVA, $K$: $F_{2,62}$, $P > 0.05$; $A_F$: $F_{2,61}$, $P > 0.05$), and did not significantly correlate with each other (Pearson’s $r = 0.05$, $n = 62$, $P > 0.05$). Consequently, the correlation between $K$, $A_F$ and sperm velocity was investigated on the pooled data set, and $K$ and $A_F$ were entered simultaneously as predictor variables. Together, $K$ and $A_F$ explained a significant part of variation in $V_{CL}$ (multiple regression, $F_{2,37}$, $P < 0.05$) but not in $V_{AP}$ and $V_{SL}$ (multiple regression, $F_{2,37}$, $P > 0.05$). The negative correlation between $V_{CL}$ and $K$, and the positive correlation between $V_{CL}$ and $A_F$ are shown as an example in Fig. 2.

The prevalence of gonad alterations of either type did not differ among age classes (asymmetry, atrophy and constriction: $\chi^2$ test, d.f. = 2, $P > 0.05$; compartmentation and adhesion or fusion: $\chi^2$ test: d.f. = 2, $P > 0.05$). The link between the occurrence of gonad alterations of different types and sperm velocity could consequently be tested together with the effect of male age on velocity. No significant variation in sperm velocity was observed among age classes (MANOVA with all three velocity variables: $F_{2,56}$, $P > 0.05$, Fig. 3) and no significant difference in velocity was detected among individuals characterized or not by compartmentation, adhesion or fusion of their gonads (MANOVA: $F_{1,56}$, $P > 0.05$). Sperm velocity, however, was significantly higher in individuals characterized by asymmetry, atrophy or constriction of their gonads (MANOVA: $F_{1,56}$, $P < 0.05$). The variation in $V_{CL}$ in the presence and the absence of gonad abnormalities is shown in Fig. 4 as an example.

![Fig. 1. Correlation between sperm curvilinear velocity ($V_{CL}$) and individual total length ($L_T$) in 2 and 3 year-old individuals (○, ---, $y = 77.6 - 1.13x$) and in individuals aged 4 years (+, --, $y = 72.3 - 0.94x$).](image-url)
Finally, there was no significant difference in the variance in sperm velocity between age classes (MANOVA: $F_{2,43}, P > 0.05$) and no significant correlation between variance in sperm velocity and $L_T$ was observed in either 2 and 3 year-old fish (Pearson’s $r$ between $-0.06$ and $-0.27$, $n = 28$, $P > 0.05$) or in 4 year-old ones (Pearson’s $r$ between $-0.26$ and $0.14$, $n = 18$, $P > 0.05$).

DISCUSSION

In this study, sperm velocity decreased with size but did not vary significantly with age. A negative correlation between body size and sperm velocity is as predicted by sperm competition theory (Ball & Parker, 1996). Because large body size confers an advantage during the establishment of dominance hierarchies (A. Jacob, S. Nusslé, A. Britischg, R. Müller & C. Wedekind, unpubl. data) and because a spawning advantage for dominant males might compensate for the cost of reduced sperm quality (Taborsky, 1998), larger males are expected to show reduced sperm velocity. Alternatively, the observed decline in sperm velocity with increasing size might also suggest a trade-off between growth and sperm production. Allocation trade-offs between different life-history traits are necessary (Williams, 1966). Individuals investing in
growth may pay a cost in terms of reproductive investment and sperm production (Dewsbury, 1982; Olsson et al., 1997). Additionally, increased growth could also occur at the cost of sperm production through an immunological pathway, if increased growth results in a decrease in testosterone levels and consequently in the up-regulation of the immune response towards non-self sperm cells (Folstad & Skarstein, 1997; Kortet et al., 2004).

The absence of a significant correlation between $K$ and $A_F$ confirms existing results (Dufour & Weatherhead, 1998b; Wedekind & Müller, 2004). As previously argued, these two measurements may reveal different aspects of quality (Wedekind & Müller, 2004). The positive correlation between sperm velocity and $A_F$ strengthen the putative links between sperm velocity and social dominance, and between social dominance and $A_F$ in fishes (Dufour & Weatherhead, 1998a). The negative correlation between sperm velocity and $K$ underlines the importance of life-history trade-offs.

A number of reasons exist to expect a decrease in ejaculate performances with age, among which age-related accumulation of germ-line mutations (Crow, 1993). The relationship between age and variation in so-called ‘sperm quality’ has been investigated in fishes (Khodzher, 1981; Buyukhatipoglu & Holtz, 1984; Vuthiphandchai & Zohar, 1999; Liley et al., 2002; G. Rudolfsen, R. Müller, D. Urbach, A. Jacob & C. Wedekind, unpubl. data). Only the last work demonstrates a negative correlation between sperm velocity and male age in the whitefish *Coregonus zugensis* Nüsslin. Previous studies found no decrease either in the duration of sperm motility or in the mean motility with age.
The absence of an age effect on sperm velocity in the present study might have different origins. First, the fish used in this study may not have covered a sufficiently large number of age classes to allow the detection of an age effect on sperm characteristics. Whitefish in Swiss lakes are under strong fishing pressure (Müller et al., 2002), resulting in size and age distributions skewed towards smaller and younger individuals. Such induced selection has previously been shown to result in a decrease in age at maturity (Walsh et al., 2006), which could explain the presence of fish aged 2 to 5 years at this spawning ground. For the present study, sampling with several gillnets differing in mesh-size may have resulted in a broader range of sampled age classes. Yet, a longer life span might be necessary for effects of senescence in males to noticeably affect sperm velocity. Second, mating history might have differed between individuals, rendering an effect of senescence difficult to detect (Jones & Elgar, 2004). Several lines of evidence suggest that spermatozoa deteriorate as they get older (Vishwanath & Shannon, 1997) through damage to the DNA or to the cell membrane (Irvine et al., 2000). In fish species, ageing of sperm has also been reported and results in changes in milt quality as the spawning season progresses (Suquet et al., 1998). Consequently, a spermatozoa age effect could have confounded an effect of age per se.

Fig. 4. Sperm curvilinear velocity ($V_{CL}$) in males characterized (a) ($n = 8$) or not ($n = 57$) by compartmentations and adhesions or fusions and (b) in males characterized ($n = 36$) or not ($n = 29$) by asymmetries, atrophies and constrictions (boxplot with medians, quartiles and ranges).
Abnormalities in gonad morphology did not seem to negatively affect sperm velocity. Only very few males, however, showed fusions, adhesion and compartmentations of their gonads and the statistical power might consequently be low for drawing any strong conclusion. Additionally, the observed alterations might affect other important ejaculate characteristics not investigated in the present study. The positive correlation between sperm velocity and the occurrence of the more commonly encountered type of gonad alterations might seem slightly surprising. Constrictions, atrophies and asymmetries, however, might be seen as additional indicators of developmental instability. Thus, under the hypothesis of increased instability in lower ranked individuals discussed earlier, a positive correlation between sperm velocity and occurrence of gonad alterations is as expected.

Understanding the variation in sperm quality among individuals is particularly relevant in economically important species for which supportive breeding programmes exist, such as the whitefish ecotype used in this study. A common practice in supportive breeding is the sequential or simultaneous addition of milt from males of different age and size. Thus, if age and size correlate with sperm traits, supportive breeding might increase the variance in reproductive success among males through higher fertilization success of particular individuals (Wedekind et al., in press). This might in turns result in an undesired decrease in the effective number of breeders and consequently in reduced genetic diversity among the offspring that are released in the wild (Campton, 2004).

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