Sperm quality influences male fertilization success in walleye (*Sander vitreus*)

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Abstract: We examined how variation in sperm quality influences a male's success at fertilizing ova (male fertilization success) in a wild population of walleye (*Sander vitreus*). To do this, we conducted controlled fertilization trials using milt and eggs (ova) from wild-spawning fish and measured male fertilization success (percentage of ova fertilized) by examining eggs after 24 h of incubation. We found that both the number of sperm and sperm swimming speed (at 10 s after activation) were significantly related to fertilization success. There was, with respect to fertilization success, a relatively large return on male investment in the number of sperm, but this return diminished as the percentage of ova fertilized increased above 50%. This is in agreement with theoretical predictions based on external fertilization dynamics. When the number of sperm used in the experimental trials was kept constant, variation in sperm swimming speed (at 10 s after activation) explained approximately 90% of the variation in a male's fertilization success. These findings demonstrate that the variation in sperm quality found in wild spawning populations has the potential to dramatically influence male reproductive success.

Résumé : Nous examinons comment les variations de la qualité du sperme influencent le succès des mâles dans la fécondation des oeufs (succès de fécondation mâle) dans une population sauvage de dorés (*Sander vitreus*). Nous avons donc fait des essais contrôlés de fécondation à l'aide de laitance et d'oeufs (ova) de poissons frayant en nature et mesuré le succès de la fécondation (pourcentage d'oeufs fécondés) à l'examen des oeufs après 24 h d'incubation. Il y a une relation significative entre à la fois le nombre et la vitesse de nage des spermatozoïdes (10 s après l'activation) et le succès de la fécondation. Comptabilisé du point de vue du succès de la fécondation, l'investissement des mâles en un nombre élevé de spermatozoïdes est relativement très rentable, mais la rentabilité diminue à mesure que le pourcentage d'oeufs fécondés dépasse 50 %. Cette relation concorde avec les prédictions théoriques basées sur la dynamique de la fécondation externe. Lorsque le nombre de spermatozoïdes est maintenu constant dans les essais expérimentaux, la variation de la vitesse de nage des spermatozoïdes (10 s après l'activation) explique environ 90 % de la variation du succès de la fécondation chez les mâles. Ces résultats démontrent que la variation de la qualité du sperme observée chez les populations sauvages durant la fraye peuvent influencer de façon sérieuse le succès de la fécondation chez les mâles.

[Traduit par la Rédaction]

Introduction

A male fish's fertilization success (percentage of ova fertilized during a spawning event) under sperm competition is thought to be related to both the number of sperm released and the ability of those sperm to swim to and fertilize the ova (Ball and Parker 1996; Stockley et al. 1997). Even when a male spawns without competition from other males, sperm traits would be expected to influence fertilization success and thus overall male reproductive success. Surprisingly, however, there has been relatively little research examining how different sperm traits relate to fertilizing ability (Gage et al. 2004; Malo et al. 2005).

A male's fertilization success is predicted to increase with the number of sperm released by a male during a spawning bout, but the benefit of releasing more sperm should decrease as the total number of sperm approaches the number required to fertilize all of the available ova (Ball and Parker 1996). This relationship has been empirically examined in several externally fertilizing fishes with some support for the model. For example, the male fertilization success of bucktooth parrotfish (*Sparisoma radians*) in natural spawning events increased dramatically with the number of sperm released, reaching an asymptote close to 95% fertilization success (Marconato and Shapiro 1996). The relationship between sperm number and male fertilization success has

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also been examined using in vitro fertilizations with rainbow trout (*Oncorhynchus mykiss*) and sockeye salmon (*Oncorhynchus nerka*) (Hoysak and Liley 2001; Liley et al. 2002), but in these studies, there was much variation in the results, so a precise description of the relationship between male fertilization success and sperm number was not possible.

Male fertilization success should also increase with sperm longevity and swimming speed. The rationale for this is twofold: based on a simple physical model of random sperm movement, it is reasonable to assume that (*i*) the longer period over which sperm swim, the greater the chance of encountering an unfertilized ovum and (*ii*) faster swimming sperm encounter more ova per unit time. The influence of sperm swimming speed on a male's fertilization success is obvious when a male's sperm is competing with those of another male, but it is also important when spawning takes place in turbulent waters and sperm may remain in the vicinity of unfertilized ova for only a few seconds. Even over the 2–3 min that the spermatozoa of many fish species remain motile (e.g., Burness et al. 2004), they would swim <2 cm, at most, and thus must be deposited and remain very close to an unfertilized ovum to have any chance of fertilization.

Despite the theoretical importance of sperm swimming velocity on male fertilization success, there has been relatively little research examining this relationship. A few studies have shown that male fertilization success is positively related to sperm velocity in externally fertilizing marine invertebrates (Au et al. 2002; Kupriyanova and Havenhand 2002) and internally fertilizing Iberian red deer (*Cervus elaphus hispanicus*) (Malo et al. 2005). However, most of the evidence supporting the effect of sperm motility on male fertilization success in fish comes from measuring the proportion of forward-moving sperm and not from actual measurement of sperm swimming velocity (Geffen and Evans 2000). To our knowledge, the only study to examine the relationship between sperm velocity and male fertilization success in a fish species under somewhat natural conditions was conducted by Gage et al. (2004), who showed that the sperm velocity of Atlantic salmon (*Salmo salar*) males was the best predictor of a male's fertilization success under sperm competition. Lahnsteiner et al. (1998) also examined the influence of sperm velocity on in vitro male fertilization success in rainbow trout, but they used a specialized fertilization solution to activate the sperm, and this may not have accurately mimicked natural spawning conditions.

Longer sperm tails are expected to generate more power and enable faster swimming, thus it is predicted that male fertilization success will also increase with sperm tail length (Gomendio and Roldan 1991). Recent evidence suggests that sperm with longer tails do indeed swim faster in bluegill (*Lepomis macrochirus*) (S. Casselman and R. Montgomerie, unpublished data), and fertilization success increases with sperm tail length in the internally fertilizing snail *Viviparus ater* (Oppliger et al. 2003). The length of the end piece of the sperm tail is also related to fertilization ability in Atlantic salmon (Vladić et al. 2002). In that study, it was hypothesized that the end piece may affect the resistance of the sperm tail to viscous drag, such that sperm with longer end pieces experience reduced drag and increased fertilization success.

In this study, we investigated the effects of sperm number as well as sperm swimming speed, longevity, and morphology on male fertilization success in walleye (*Sander vitreus*). We designed our experiments to mimic some of the conditions encountered during natural spawning events — temperature, duration of sperm–ovum contact, and fertilization medium — so that our results could be used to predict the impact of ejaculate quality on reproductive success in wild populations. Walleye spawn in the spring, shortly after ice breaks up, at water temperatures 6–9 °C. Spawning takes place in rocky areas in fast-flowing water at the base of impassable falls or dams in rivers and on coarse gravel shoals in some lakes (Scott and Crossman 1973). Males congregate at the spawning grounds prior to the arrival of females, but males build no nest and are not territorial. At night, one to two females move together into shallow water with up to six males, who compete for fertilizations. Within the spawning group, males will become aggressive, with pursuit, pushing, and fin erection occurring prior to spawning (Scott and Crossman 1973). Both eggs and sperm are broadcast into the water column, where fertilization occurs; eggs then settle into crevices in the substrate where they incubate for 12–18 days before hatching.

Materials and methods

We collected mature walleye from the Bay of Quinte (44°N, 77°W), Ontario, Canada, during spawning in April 2003, using a trap net placed near the spawning grounds where fish were staging. Fish were removed from the net and held in large (200 L), insulated containers (with fresh water regularly renewed) for up to 6 h before being processed.

Fertilization experiments

All fertilizations were conducted in 500 mL clear plastic containers. For each trial, approximately 100 ova and a milt sample were placed about 5 cm apart in the bottom of a dry container. Then, 400 mL of fresh lake water was poured quickly into the container to ensure thorough and immediate mixing of ova and sperm. This also ensured that all sperm and ova came into contact with water at the same time and that all sperm were activated simultaneously. After 30 s, the water was poured off the eggs, and fresh lake water was immediately added to wash off any remaining sperm and thus standardize the amount of time available for fertilization. This final washing was performed so that we could look specifically at the effects of variation in sperm movement and morphology during the period when walleye sperm in nature are most likely to be in the vicinity of unfertilized ova.

After the fertilization trials, eggs were transported to the lab and incubated at 10–12 °C for 24 h. Then, 50 eggs from each trial were examined at 3× magnification to determine fertilization success. Only clear, undamaged eggs were counted, and eggs were categorized as being fertilized if there was segmentation of the blastodisc (Hardy 1978). We define a male's fertilization success as the percentage of ova fertilized during each trial (spawning event).

Sperm number and male fertilization success

To determine the effect of sperm numbers on male fertilization success, we first conducted fertilization trials for 10

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male–female pairs, with six replicates for each pair using different numbers of sperm in each replicate. Ten different male–female pairs were used in this experiment to provide a large sample of random male × female combinations. Each of these fertilization trials was performed immediately after ova and sperm were collected from the live adults, within 10 min of stripping the adults. For each male, we loaded a microhaematocrit (75 mm long; 1.1–1.2 mm inside diameter) capillary tube 70% full with milt and centrifuged it for 2 min at 8000 r·min^{-1} (1 r = 2π rad; 5900*g*). We then calculated spermatocrit as the ratio of packed sperm to total volume of milt (Bouck and Jacobson 1976) so that we could control for differences in sperm density among males. Using a haemocytometer, we determined the number of spermatozoa per microlitre of milt for one male, accurate to within ±5%, then used the spermatocrit for that milt sample as a standard for calculating the number of sperm per microlitre of milt for the other males from their spermatocrit values. Based on a pilot study that we had previously conducted on the fertilization dynamics of walleye, we chose six different densities of sperm to use in each trial to yield male fertilization rates ranging from 100% to 0% of ova fertilized. The volume of sperm added during these fertilization trials was calculated to produce final sperm densities of 500 000, 250 000, 100 000, 50 000, 5000, and 500 sperm·mL^{-1} water in the total volume of 400 mL of lake water used per trial. All six replicates for each male × female pair were performed virtually simultaneously to eliminate any potential order effect due to storage of milt or ova.

Sperm movement, morphology, and male fertilization success

On a different day, we conducted further fertilization trials to determine the influence of sperm traits (swimming speed, tail length) on the fertilization success of 20 different males. To minimize any effect of female identity on male fertilization success, we pooled 50 g of ova (approximately 15 000 ova) from each of five females and used this pooled batch of about 75 000 ova for all fertilizations. We used this pooled sample rather than ova from different females to minimize the effects of differential fertilization success of particular male × female combinations that might influence our results. Thus, each male was tested against the same pooled set of ova. We stored these unfertilized ova in a cooler at 4 °C after collection until we used them in a fertilization trial.

We stripped milt from each of the males immediately prior to conducting the fertilization trials to eliminate any potential change in sperm quality caused by storage. We controlled for differences in sperm density among males by measuring the spermatocrit (as above) of each milt sample used. We then calculated the appropriate volume of milt needed to ensure that the same number of sperm (5×10^4 sperm·mL^{-1}; see Results) was used from each male in the fertilization trials.

Fertilization trials were replicated five times for each male sampled and conducted in dyads (two males at a time) over a period of 8 h, using the protocol described above. It took 20–60 min to complete the trials for each dyad of males, with 60 min breaks after the fourth and sixth dyads, resulting in three groups of dyads. Because the ova were collected before these fertilization trials began, they were stored for different periods, up to 8 h, before being used in a trial.

Sperm motility measurements

We measured sperm motility for each male used in the fertilization experiments within 1 h of sperm collection. A two-step dilution process was used to ensure simultaneous activation of the sperm (Billard and Cosson 1992). To dilute the milt without activating the sperm, we added 1 µL of milt to 750 µL of an extender developed for walleye (Moore 1987), then placed 1 µL of this solution into a haemocytometer chamber. Sperm were then activated by flooding the chamber with lake water (10 °C) taken from the spawning site. To ensure that sperm motility was measured at the same temperature for all samples, the haemocytometer was maintained at 10 °C using a Peltier plate controlled with a variable DC power supply and monitored with a thermocouple. Sperm motility was recorded on videotape using a CCD video camera (Sony model XC-ST50, Hamilton–Thorne Research, Beverly, Massachusetts, USA) mounted on a negative phase contrast Olympus CH30 microscope (Hamilton–Thorne Research) at 100× magnification.

We analyzed the videotapes using a CEROS video sperm analysis system (v. 12, Hamilton-Thorne Research). The smoothed average sperm swimming velocity (VAP, µm·s^{-1}) was measured for a minimum of 25 sperm from each male at 10, 20, and 30 s after water was added to the milt sample (thereby activating the sperm). VAP is calculated using a smoothing algorithm that reduces the effect of lateral head displacement and produces a path representative of the sperm position along the sperm's track of forward movement. The length of this track is then divided by the time elapsed (0.5 s) to determine VAP. To assess the straightness of the swimming path, we also calculated a linearity index (STR) by dividing the straight-line distance (distance between the beginning and the end of the track) traveled in 0.5 s by the total distance traveled in the same period. Percent motility was measured as the proportion of sperm with both VAP > 20 µm·s^{-1} and STR > 0.20 at 10, 20, and 30 s after activation. The VAP criterion is commonly used to distinguish progressively motile fish sperm (e.g., Lahnsteiner et al. 1998), whereas the STR criterion eliminates a few sperm that are not moving forward; sperm not meeting these criteria were judged to be nonmotile.

Sperm morphology measurements

We preserved sperm for morphological analyses by diluting 5 µL of milt in 750 µL of 2% formaldehyde solution. A small drop of this solution was spread thinly across a microscope slide and air-dried. We digitized 10 haphazardly chosen sperm from each male using a microscope at 600× magnification and a CCD video camera. Using NIH Image (Version 1.62, available from http://rsb.info.nih.gov/ nih-image/), we measured the tail length of each sperm to the nearest 0.1 µm on the digitized images.

Statistical analyses

We used JMP 5.1 (SAS Institute Inc., Cary, North Carolina) for all analyses. We used repeated measures analyses of variance where appropriate when the sperm from each male was used in different fertilization trials during a single experiment and when analyzing sperm swimming speed at different periods after activation. For models with random effects (i.e., male as factor), we used the restricted maximum

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Fig. 1. Percentage of ova fertilized in relation to experimentally manipulated sperm density in walleye (*Sander vitreus*). Each data point represents an experimental trial with a single male, with sperm from the same 10 males tested at each density. Overlapping data points are jittered so that all data points are shown, except for results at 500 sperm·mL^{-1}, which are shown as triangles with sample sizes indicated for overlapping data points.

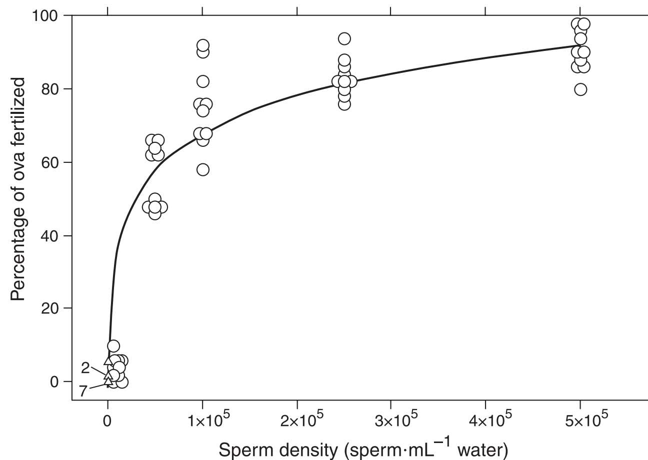
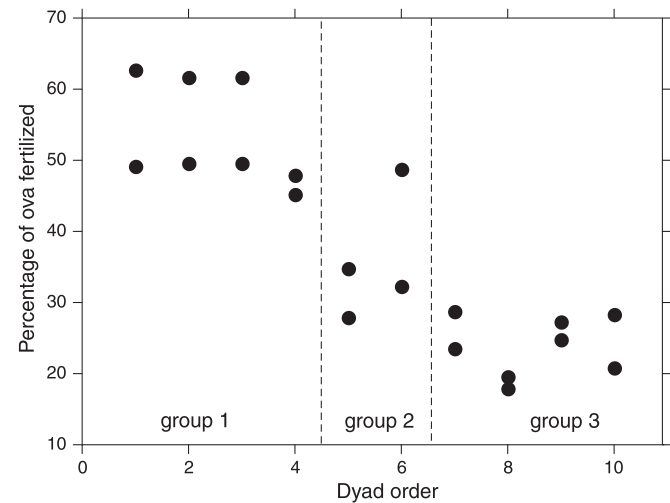


Fig. 2. Decline in mean percentage of ova fertilized with order of fertilization trials. Each data point is the mean of five trials per male, and males were tested simultaneously in dyads. Fertilization groups were delimited, as shown, for further analysis.



likelihood method for analysis. None of the analyses of variance had residuals that departed significantly from normality (Shapiro–Wilk tests, $p > 0.05$), so no transformations were needed. For analyses of covariance, interaction terms were removed if not significant (NS), indicating no significant difference in slopes among treatments.

Results

Sperm number and male fertilization success

As expected, mean male fertilization success varied significantly among trials depending upon the number of sperm used, from 1% (range 0%–6%) with a sperm density of 500 sperm·mL^{-1} water to 90.6% (range 80%–98%) with 5×10^5 sperm·mL^{-1} water (repeated measures analysis of variance, $F_{[9,45]} = 402$, $p < 0.0001$). A logarithmic equation (fertilization success = $33.8 \times \log_{10}$(sperm density) – 101.7) provided a good description of the relationship between fertilization success and the number of sperm ($r^2 = 0.89$, $p < 0.0001$; Fig. 1).

Based on this analysis, we used a density of 5×10^4 sperm·mL^{-1} water to examine the effect of sperm quality on male fertilization success in subsequent experiments. The fertilization success predicted for this sperm density (58%) was intermediate, with no replicates having close to 0% or 100% fertilization success (Fig. 1) and thus little danger of truncated results that might complicate the analysis.

Sperm movement, morphology, and male fertilization success

The order in which the fertilizations were performed had a significant effect on mean male fertilization success (Spearman's rank correlation, $r = -0.87$, $p < 0.0001$, $n = 20$ males in 10 dyads), in that mean male fertilization success decreased over the course of the whole experiment (Fig. 2). This decline in male fertilization success was not due to changes in sperm quality, as males were stripped of milt immediately prior to each dyad of trials, and thus the time between stripping milt and sperm activation was the same for each male. Moreover, sperm swimming speed (VAP at 10 s after activation) increased rather than decreased over the course of the experiment ($r = 0.48$, $p = 0.03$, $n = 20$ males in 10 dyads) for no obvious reason. We believe that this decline in male fertilization rates over the course of the experiment was likely due to reduced ovum fertility resulting from storage, possibly because of decreased oxygen supply or ovum hardening caused by accidental exposure to moisture (condensation). For further analysis, we included either dyad order or group (see Methods) in the models to control for order effects.

VAP at 10 s after activation was significantly and positively related to male fertilization success (repeated measures analysis of covariance, $F_{[1,80]} = 8.9$, $p = 0.004$, interaction term NS and removed from the model; Fig. 3), with dyad as covariate to control for order effects. In this model, VAP explained 72% of the variation in fertilization success, and the slope of the relationship between percent fertilization and VAP was 0.70. Using group as a covariate instead of dyad also revealed a significant relationship between fertilization success and VAP at 10 s after activation (repeated measures analysis of covariance, $F_{[1,80]} = 16.2$, $p = 0.0001$, interaction term NS and removed from the model), with a slope of 0.75 for the relationship between percent fertilization and VAP. Within all three groups, male fertilization success increased with VAP at 10 s after activation (Fig. 3), but this relationship was only significant for the first two groups of trials ($p \leq 0.05$, repeated measures analyses of variance within groups), with regression slopes of 0.80 (group 1) and 1.04 (group 2).

Male fertilization success was not related to VAP at 20 and 30 s after activation (repeated measures analyses of covariance, both $F_{[1,79]} < 2.57$, both $p > 0.11$, with group as a factor) nor to STR at 10, 20, and 30 s after activation (repeated measures analyses of covariance, all $F_{[1,79]} < 0.49$, all $p > 0.49$, with group as a factor). Similarly, the proportion of

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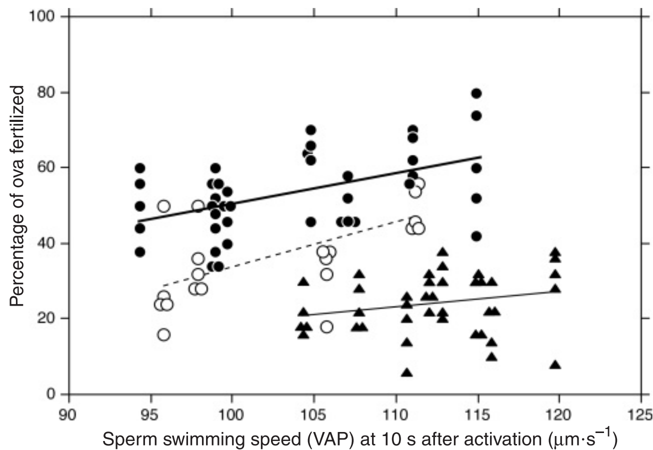
Fig. 3. Increase in fertilization success with average sperm velocity (VAP) at 10 s after activation within fertilization groups as defined in Fig. 2 (group 1 presented in solid circles, group 2 presented in open circles, and group 3 presented in solid triangles). Five trials were run with the sperm from each male; overlapping data points are jittered so that all data points are shown; regression lines from repeated measures analyses are shown.



motile sperm at 10, 20, and 30 s after activation did not influence male fertilization success (repeated measures analyses of covariance, all $F_{[1,79]} < 2.22$, all $p > 0.14$, with group as a factor).

Mean sperm tail length varied significantly among males (analysis of variance, $F_{[19,180]} = 4.62$, $p < 0.0001$, n = 10 sperm·male^{-1}). Despite this variation, there was no effect of sperm tail length on fertilization success (repeated measures analysis of covariance, $F_{[1,16]} = 0.27$, $p = 0.61$, with group as a factor). Nor was mean sperm swimming velocity (VAP) at any time after activation related to mean sperm tail length across males (at 10 s: $r = 0.16$, $p = 0.50$; at 20 s: $r = 0.30$, $p = 0.19$; at 30 s: $r = 0.03$, $p = 0.90$; $n = 20$ males).

Discussion

Our experiments demonstrated a significant effect of sperm number on male fertilization success as expected. At the highest sperm density (5 × 10^5 sperm·mL^{-1} water), male fertilization success averaged 91%. Although this is less than compete fertilization success, it is in agreement with previous studies that have found maximum male fertilization success of walleye under hatchery conditions to be 75%–86% (Moore 1987, 2003), likely due to variability in ovum quality (Moore 2003). In contrast, average male fertilization success was only 1% with sperm density of 500 sperm·mL^{-1} water.

While previous studies have found male fertilization success to be highly variable, ranging from 8% to 97% under constant experimental conditions (Hoysak and Liley 2001), we found much less variation in male fertilization success when the density of sperm was held constant. The variation in fertilization success found in other studies may have been caused by embryo mortality during incubation, as eggs were often incubated 1–3 weeks before measuring fertilization success, and mortality is often quite high during this period (S. Casselman, personal observation). In Hoysak and Liley (2001), for example, cloudy eggs were simply removed from the sample during the incubation period, apparently without assessing whether or not they contained an embryo. To minimize this potential source of variation, we incubated the eggs ≤24 h before assessing the fertilization status of the sample.

The shape of the relationship between male fertilization success and sperm number suggests that there is initially a relatively large return on male investment in ejaculates but that this return diminishes as the percentage of ova fertilized increases above 50%. For example, doubling the density of sperm from 1 × 10^5 to 2 × 10^5 sperm·mL^{-1} water increased predicted male fertilization success from 67% to only 77%. Thus, in the absence of sperm competition, the percentage of ova in a clutch that is fertilized by an individual male is directly related to the number of sperm released, and thus males should adjust ejaculate size depending upon both their prospects for further spawnings and their rate of sperm production (Shapiro and Giraldeau 1996). However, when a male competes with other males during a spawning event, other factors like sperm swimming speed and sperm–ovum interactions can influence a male's success (Gage et al. 2004).

For example, when the density of sperm in water was held constant in our fertilization trials, sperm swimming speed at 10 s after activation was significantly related to male fertilization success. Thus, in our first group, males with the fastest-swimming sperm had approximately 40% greater fertilization success than males with the slowest-swimming sperm. Across all trials, the slope of the relationship between percent fertilization success and VAP was 0.70–0.75; within the first two trial groups, this was even higher at 0.80–1.04. Thus for every increase of 1 µm·s^{-1} in sperm swimming speed (VAP), a male's fertilization success would increase by about 0.75%. Over the range of swimming speeds (VAP at 10 s after activation) observed in this study (94.3–119.7 µm·s^{-1}), this translates into an increase of 18.8% in male fertilization success. Thus, we conclude that the variation in sperm swimming speeds that we observed can have a major impact on male reproductive success.

Although our fertilization experiments were conducted using sperm from each male in isolation, our results can be extended to make predictions regarding the outcome of sperm competition involving males with ejaculates of different quality. Thus, if multiple males release the same number of sperm, the male with the fastest swimming sperm should fertilize the greatest percentage of ova and that percentage may depend upon the density of the sperm in the water, the distribution of unfertilized ova, the timing of ejaculation, and the distribution of sperm swimming speeds (R. Montgomerie and S. Casselman, unpublished data). In an experimental study of the effects of sperm traits on male fertilization success in the Atlantic salmon, Gage et al. (2004) found that sperm swimming velocity was the best predictor of male fertilization success when milt from different males competed for access to ova, even when the relative sperm densities of two different males were in a 2:1 ratio. Thus, in that study, the effect of relative sperm density was not significant, presumably swamped by the variation in sperm swimming speed. Although Gage et al. (2004) did attempt to mimic natural spawning conditions in their experiments,

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they kept mean sperm density constant so that they could examine the effects of relative swimming speeds and numbers of sperm. In nature, however, the density of sperm in water in the vicinity of unfertilized ova during spawning is likely to vary considerably because of varying turbulence in the water, distances between the sexes when gametes are released, and the relative timing of gamete release by the male(s) and female. In the present study, we showed that both factors are likely to be important determinants of male fertilization success, but more work will be needed to determine the relative importance of sperm swimming speeds and ejaculate size at sperm:ovum densities found in nature.

While there was an effect of sperm swimming velocity on male fertilization success in walleye when measured at 10 s after activation, we found no such relationship at 20 or 30 s after activation. The swimming speed of walleye sperm declines rapidly after activation, such that between 10 and 30 s after activation, swimming speed declines by about 70% (S. Casselman and R. Montgomerie, unpublished data). Previous work on the fertilization dynamics of salmon and rainbow trout found that the majority of fertilization took place within the first 20 s after activation (Hoysak and Liley 2001; Liley et al. 2002), so that speed measurements after this period are expected to be irrelevant to a male's fertilization success.

Interestingly, there was no effect of the differences among males in percent sperm motility on male fertilization success. Assuming that nonmotile sperm are incapable of fertilization (Levanduski and Cloud 1988), any decline in the proportion of motile sperm should result in decreased male fertilization success. In support of this, a positive relationship has been found between percent motility and male fertilization success in rainbow trout (Geffen and Evans 2000), but not in the serpulid polychaete *Galeolaria caespitosa* (Kupriyanova and Havenhand 2002). One possible explanation for our results is that percent motility may only be expected to affect male fertilization success when all other sperm traits are equal and when differences in percent motility dramatically influence sperm density in the water during spawning. Moreover, when the ratio of sperm to ova is very high, the fastest-swimming sperm might be expected to fertilize the majority of ova even when a high proportion of sperm are immotile, as long as the density of motile sperm is high. In our study, mean sperm motility at 10 s after activation was 79.8% (±6.4%, 95% confidence level, n = 20 males) and thus relatively high and not very variable, so an effect of motility may have been absent or merely difficult to detect.

We also found that there was no effect of sperm tail length on male fertilization success. This is initially surprising because theoretical models predict that sperm with longer tails swim faster, and there is some empirical evidence in support for this (Lahnsteiner et al. 1998; Jobling et al. 2003; Burness et al. 2004). Thus, if sperm tail length influences sperm swimming speed, then tail length should be related to fertilization success (Ball and Parker 1996). However, we found no relationship between sperm morphology and sperm swimming speed, so there was no reason to expect an effect of sperm tail length on fertilization success in this species. Similarly, Gage et al. (2002) found no relationship between sperm swimming speed and sperm flagellum length in Atlantic salmon, but again, in that species, sperm swimming speed was a major determinant of a male's fertilization success under (experimental) sperm competition (Gage et al. 2004). Undoubtedly sperm swimming speed is determined by factors other than, or maybe in addition to, sperm length, and this subject deserves more detailed study.

This study demonstrates the importance of sperm number and swimming velocity in determining male fertilization success and suggests that males able to produce large numbers of fast-swimming sperm will benefit from increased reproductive success. Further study is required to determine the impact of percent sperm motility and morphology on fertilization success when controlling other sperm traits. As this experiment was conducted in vitro, the effect of these sperm traits on fertilization success in natural spawning events should also be examined.

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