EVOLUTIONARY BIOLOGY

Alternative male morphs solve sperm performance/longevity trade-off in opposite directions

Michael Taborsky,[1]* Dolores Schütz,[1] Olivier Goffinet,[1] G. Sander van Doorn[1,2]

Males pursuing alternative reproductive tactics have been predicted to face a trade-off between maximizing either swimming performance or endurance of their sperm. However, empirical evidence for this trade-off is equivocal, which may be due to simplistic assumptions. In the shell-brooding cichlid fish *Lamprologus callipterus*, two Mendelian male morphs compete for fertilization by divergent means: Bourgeois nest males ejaculate sperm, on average, about six times farther from the unfertilized ova than do parasitic dwarf males. This asymmetry is opposite to the usual situation, in which bourgeois males typically benefit from superior fertilization opportunities, suggesting that nest males' sperm should persist longer than dwarf male sperm. The assumed trade-off between sperm swimming performance and longevity predicts that, in turn, sperm of dwarf males should outperform that of nest males in swimming efficiency. Measurement of sperm performance and endurance reveals that dwarf male spermatozoa swim straighter initially than those of nest males, but their motility declines earlier and their velocity slows down more abruptly. Nest male sperm survives longer, which relates to a larger sperm head plus midpiece, implying more mitochondria. Thus, the trade-off between sperm performance and endurance is optimized in opposite directions by alternative male morphs. We argue that the relative success of alternative sperm performance strategies can be influenced strongly by environmental factors such as the time window between gamete release and fertilization, and the position of gamete release. This is an important yet little understood aspect of gametic adaptations to sperm competition.

INTRODUCTION

Sperm competition occurs whenever sperm of two or more males compete for the fertilization of eggs (*1*–*3*). This competition is often modeled as a "fair raffle," with the probability of a given male siring offspring depending on the relative representation of his sperm number in the "fertilization set" (*4*). Males competing among themselves for fertilizations face important trade-offs. For instance, researchers have proposed that the product of sperm size and number (that is, the total ejaculate expenditure) can be traded off against some other fitness component, such as the expenditure in obtaining further matings (*5*, *6*). Other trade-offs may exist at the level of gametes. Studies have shown the risk of sperm competition to influence sperm morphology and performance (*7*, *8*). In externally fertilizing species, it is difficult for males to monopolize partners or fertilizable eggs, and several males may release sperm at a time, causing scramble competition among sperm (*9*), where the number of scrambling males is the prime determinant of the intensity of sperm competition (*10*). Therefore, in external fertilizers, theory predicts that with increased sperm competition, selection on sperm traits should increase and faster sperm should evolve (*8*, *11*, *12*). Sperm velocity is a key component of the success of spermatozoa in sperm competition (*13*). The situation can be more complex, and predictions may be less straightforward; however, if males pursuing different fertilization tactics compete with each other and the raffle is hence "loaded" (*14*).

A trade-off between sperm motility and longevity results from the fact that both criteria of sperm performance, swimming speed and endurance, depend on the amount of available energy (*15*), hence on sperm size (*2*). For example, Yamamoto *et al.* showed sperm with high swimming velocity in Dolly Varden char *Salvelinus malma* to be shorter-lived (*16*). The size of sperm as a trait is the sum of the length of the flagellum, which serves forward propulsion, and the sperm head plus midpiece containing what is required for the fertilization of eggs, in particular the mitochondria and energy reserves responsible for flagellar movement. The ratio between the lengths of the flagellum and sperm head apparently determines the relationship between sperm morphology and swimming performance (*17*).

If selection maximizes sperm velocity in situations of intense sperm competition, then the flagellum length should increase with the level of sperm competition risk. This has been confirmed by comparative studies, for example, among Lake Tanganyika cichlids (*18*) and in myobatrachid frogs (*19*). However, the high swimming speed attained by a long flagellum inevitably drains the energy reserves of sperm more quickly. All else being equal, sperm longevity should therefore be inversely related to relative flagellum length. Comparative studies of sperm length have supported this inverse relationship in freshwater fishes (*12*), in sea urchins *Heliocidaris erythrogramma* (*20*), and in three-spined sticklebacks *Gasterosteus aculeatus* (*21*). In other cases, the trade-off between sperm velocity and longevity, and in particular its relationship to sperm competition, is less clear (*2*, *22*). We argue that, to clarify the influence of sperm competition on sperm characteristics, it is necessary not only to relate sperm velocity and longevity to sperm morphology (especially length) but also to consider the particular conditions under which different ejaculates compete for fertilization (*23*).

Males performing alternative reproductive tactics (ARTs) (*24*) in externally fertilizing species are typically exposed to asymmetrical sperm competition [a "loaded raffle" (*1*, *4*)]. In general, large bourgeois males (*25*) defending females or territories show elaborate secondary sexual characters and invest relatively little in sperm production (*4*, *9*, *14*). Selection allows parasitic males to overcome the monopolization of females by bourgeois males and to exploit the latter's reproductive effort (*9*, *26*). Parasitic males have less elaborate secondary sexual characters but invest relatively more in their gonads and in sperm production

[1]Department of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland. [2]Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 11103, 9700 CC Groningen, Netherlands.
*Corresponding author. Email: michael.taborsky@iee.unibe.ch

than bourgeois males (*26*–*28*). At the gametic level, the evidence for consistent differences in sperm morphology and performance between males adopting divergent reproductive tactics within a species is equivocal (*9*). Some studies found parasitic male sperm to contain more adenosine 5′-triphosphate (ATP), to be faster or more motile, but to live shorter than bourgeois male sperm (*15*, *29*–*32*). Other studies did not find this pattern or revealed the opposite, sometimes even within the same species (*30*, *33*–*36*). Contrary to predictions of the hypothesis that sperm velocity is traded off against longevity, in a few cases, parasitic males had both faster swimming and longer-lived sperm than bourgeois males [*Salmo salar* (*27*, *32*), *Gobius niger* (*30*), and *Telmatochromis vittatus* (*37*); but see the study of Reinhardt and Otti (*22*) for methodological concerns].

In view of these contradicting results, we argue that research has not considered some crucial factors determining the fertilization success of males pursuing divergent ARTs (*9*, *28*, *29*). In their review, Reinhardt *et al.* (*23*) determined that sperm shows extreme phenotypic plasticity and that the role of natural selection acting either on sperm function or on male and female microenvironments enabling optimal plastic performance of sperm is still unclear. They concluded that ignoring environmental effects on sperm characteristics reduces fitness predictability under sperm competition (*23*). For example, most studies of the influence of sperm competition on the evolution of sperm characteristics do not account for differences in the time window between sperm activation and fertilization that exist between different tactics, and sperm competition is often assumed to be a fair raffle. However, bourgeois males are usually much closer to the eggs than parasitic males and proximity to the female during spawning can be critical for fertilization success (*38*). Sperm of parasitic males will typically reach the eggs later than sperm of bourgeois males. Therefore, sperm of parasitic males may be selected for the endurance needed to survive the longer time interval it takes until the egg is reached; typically, local water movement rather than sperm swimming activity largely determines this time interval. If a trade-off exists between sperm speed and longevity, then this may result in the evolution of slower swimming speed of parasitic male sperm than that of bourgeois males.

We think that the obscure patterns of how sperm competition and fertilization context shape sperm morphology and behavior can be elucidated by studying systems where the relationships between male roles in spawning are reversed from the standard situation. In the shell-brooding cichlid fish *Lamprologus callipterus*, two genetically determined alternative male morphs compete for the fertilization of eggs. These males are highly divergent with regard to growth and body size, reproductive behavior, and gamete investment (*39*–*42*) due to the effect of disruptive selection on genetic alternative reproductive morphs (*43*). In contrast to the usual situation in which parasitic males ("sneakers") are disadvantaged in sperm competition due to their inferior opportunities when attempting to fertilize eggs, here, males of the parasitic morph are better off than their bourgeois competitors (*42*).

Bourgeois males of *L. callipterus* (here referred to as "nest males") defend a territory and collect empty shells of the snail *Neothauma tanganicense*, which they defend as spawning substrate (*44*). Females ready to spawn enter a shell in a male's territory and deposit their eggs inside. Genetically distinct dwarf males attempt to wriggle past a spawning female into the tip of the shell, from which they try to fertilize the eggs (*39*, *41*). If they manage to pass the female, then they fertilize on average about three-fourth of the clutch (*42*). Nest and dwarf males release sperm more or less simultaneously, that is, without a fixed spawning sequence but at very different distances. The fertilization asymmetry between male types regarding their position and communication potential with the female runs contrary to most cases hitherto known, because during sperm release the parasitic dwarf males are much closer to freshly deposited eggs than to nest males (*42*). Nest males fertilize the eggs from outside the snail shell without visual and bodily contact with the female (*45*). By contrast, dwarf males fertilize the eggs from inside the snail shell and hence spawn in direct contact with the female [see Fig. 1 in the study of Taborsky (*3*)] (*39*). Other things being equal, because of this distance asymmetry, nest male sperm will take longer to reach the egg than dwarf male sperm. Therefore, given that, for ejaculates, the long distance between the nest male genital pore and the freshly deposited egg is largely covered through water currents caused by female pectoral fin movements (*45*), nest male sperm should benefit from increased longevity to a greater extent than from high sperm performance, which is contrary to the conditions of dwarf males. Hence, in *L. callipterus*, the specific spatial relations at spawning predict that sperm of nest and parasitic males should specialize in opposite directions. In contrast to dwarf males, nest male sperm should be selected to live longer and thus have longer heads to contain sufficient energy reserves in the midpiece than dwarf male sperm, which differs from most other aquatic systems with ARTs.

There is intense sperm competition between nest males and dwarf males; on average, the latter fertilize the majority of eggs when they manage to enter a shell during spawning (*42*). Here, we aim to examine the trade-off between sperm performance and longevity and the specialization in one direction or the other of males pursuing tactics that diverge diametrically from the usual fertilization opportunities of ARTs. We measured the swimming performance and longevity of sperm in *L. callipterus* and the decline of sperm performance with time. In addition, we measured sperm sizes of both male types, separating the sperm head plus midpiece from the flagellum. We compared these variables between the two male morphs and tested for correlations between sperm activity parameters and sperm morphology.

RESULTS

Sperm activity parameters

Sperm performance measures

The swimming performance of spermatozoa differed significantly between nest and dwarf males. The beat cross frequency (Fig. 1A), the straightness (in %; Fig. 1B), the linearity (in %; Fig. 1C), and the mean angular head displacement (MAD) (Fig. 1D) of sperm fitted well to a complementary cumulative gamma distribution (table S1, nonlinear model fits), which provides an appropriate model of the time to failure of a system (in this case, sperm performance; for a graphical explanation of the different performance measures, see fig. S1). Dwarf male sperm performance declined faster than nest male sperm performance, as indicated by significant differences in the scale parameter of the fitted exponential or gamma distributions (table S1, A and B, parameter β) for beat cross frequency, straightness, linearity, and MAD. The shape parameter of the fitted gamma distributions (table S1A, parameter α) differed significantly between the two male types in beat cross frequency, linearity, and MAD, implying that the quality of dwarf male sperm decreased not only quicker but also more abruptly for these measures of performance. The straightness of dwarf male spermatozoa significantly exceeded that of nest male spermatozoa shortly after activation (parameter *k*: 3% higher in dwarf males than in nest males between 30 and 120 s after sperm release), but this relationship was subsequently reversed (that is, between 120 and 240 s after

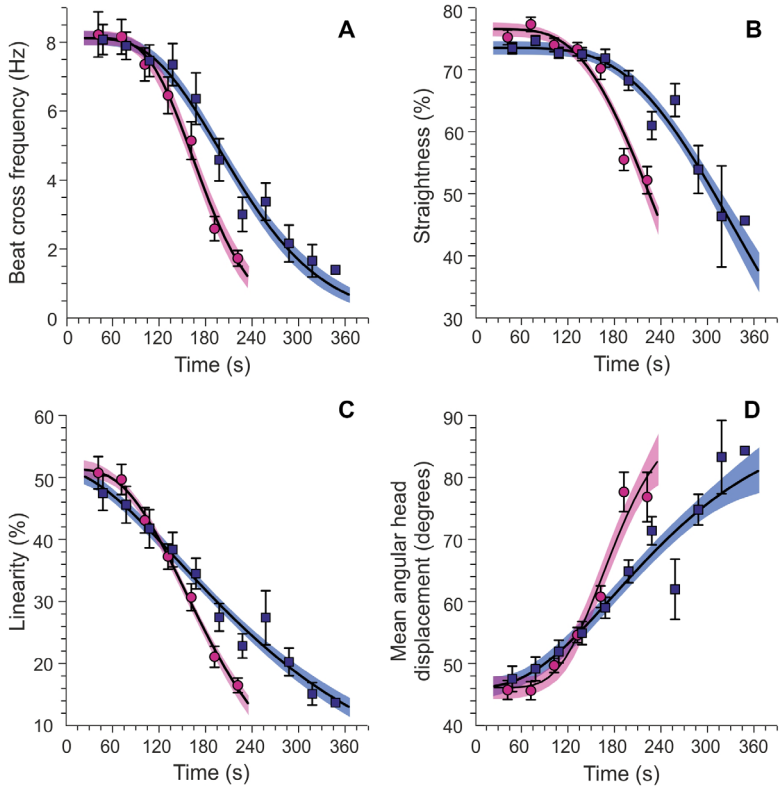

Fig. 1. Sperm performance measures. Means and 95% confidence intervals at different times after sperm release (time = 0) for territorial males (blue squares) and dwarf males (pink circles) and fitted curves (black lines) with 95% confidence intervals (blue area, nest males; pink area, dwarf males) are given. (**A**) Beat cross frequency (Hz). (**B**) Straightness (VSL/VAP). (**C**) Linearity (VSL/sampled path). (**D**) MAD (degrees). Note that the measures at the measurement points on the abscissa (60, 90, …, 360 s) always represent values obtained during a 30-s period (for example, the first point denotes results of the swimming performance of sperm measured between 30 and 60 s). The number of individuals using each tactic varied between the different intervals after sperm release: up to 180 s, n = 14 nest males + 13 dwarf males; 210 s, n = 14 nest males + 10 dwarf males; 240 s, n = 12 nest males + 8 dwarf males; 270 s, n = 9 nest males; 300 s, n = 8 nest males; 330 s, n = 5 nest males; 360 s, n = 1 nest male.

sperm release; Fig. 1B). Two minutes is also the average time window between laying subsequent eggs (*45*).

Sperm motility measures

An exponential distribution best represented the percentage of motile sperm (Fig. 2A), average path velocity (VAP) (Fig. 2B), curvilinear velocity (VCL) (Fig. 2C), and straight line velocity (VSL) (Fig. 2D) (table S1B). All four parameters declined quicker in dwarf than in nest males (see significant differences in table S1B).

Sperm longevity

Sperm of nest males survived significantly longer than sperm of dwarf males [Fig. 3; analysis of variance (ANOVA): $F_{13,10} = 14.284$, $P < 0.001$].

Sperm length

Spermatozoa of nest males were about 10% longer than those of dwarf males (mean ± SD: 35.73 ± 2.99 µm versus 32.43 ± 4.19 µm; two-sample t test: $T_{13,10} = 2.2$, $P = 0.039$; Fig. 4). This was mainly due to the different lengths of sperm heads plus midpiece (2.96 ± 0.61 µm versus 2.44 ± 0.36 µm; two-sample t test: $T_{13,10} = 2.42$, $P = 0.025$; Fig. 4), whereas flagellum length did not differ significantly between sperm of different male morphs (32.77 ± 2.63 µm versus 30.00 ± 4.38 µm; two-sample t test: $T_{13,10} = 1.88$, $P = 0.073$; Fig. 4).

Relationship between sperm morphology and performance

The sperm head plus midpiece length of individual males correlated significantly positively with sperm swimming linearity, beat cross frequency, VAP, and VSL, whereas there was a negative but nonsignificant relationship with MAD, which is a measure of nonlinearity of sperm movement due to depleting energy reserves (Table 1).

Sperm longevity correlated positively with total sperm length of individual males [Pearson correlation analysis (r) = 0.424, N = 23, P = 0.043]. Mean sperm head plus midpiece length and mean flagellum length of different males (independent variables) related significantly to sperm longevity (dependent variable; multiple regression analysis: $F = 5.581$, df = 2, $r = 0.598$, $P = 0.012$), which was due to differences in sperm head plus midpiece length ($T = 2.672$, df = 1, $P = 0.015$; Fig. 5) but not in flagellum length ($T = 1.331$, df = 1, $P = 0.198$). The residuals of the regression of sperm longevity and sperm head plus midpiece length were, on average, positive for nest males (mean ± SD, 15.33 ± 38.7) and negative for dwarf males (−19.92 ± 31.7), reflecting a significant difference (two-sample t test comparing residuals: $T_{13,10} = 2.34$, $P = 0.029$).

DISCUSSION

Our data confirm the predicted trade-off between sperm performance and endurance (*2*) in a species with alternative male mating tactics, where nest and parasitic dwarf males adopt divergent solutions to sperm competition according to their particular roles during spawning. In *L. callipterus*, dwarf male sperm start to swim straighter and more linearly right from the start than nest male sperm, but this performance

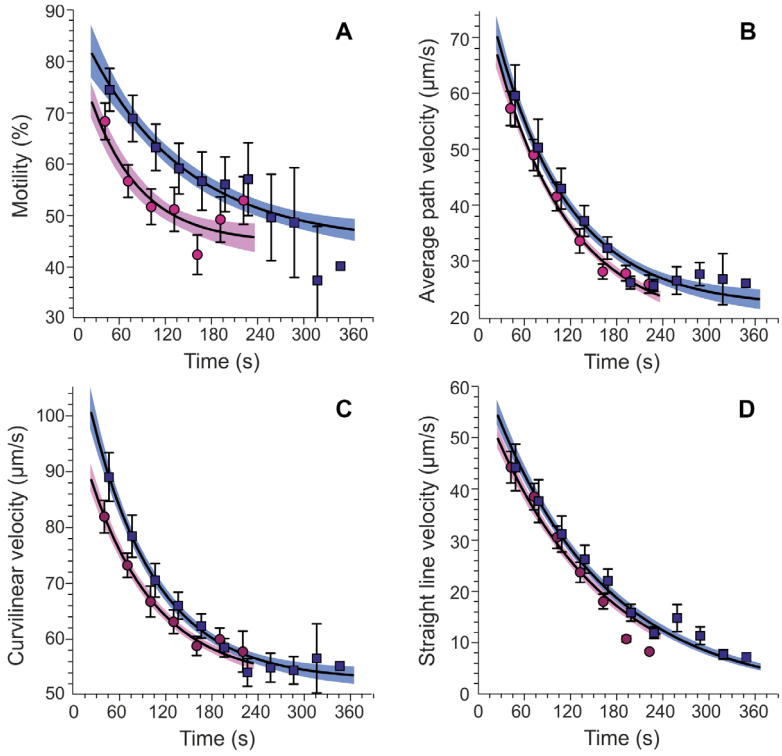

Fig. 2. Sperm motility measures. Means and 95% confidence intervals at different times after sperm release for territorial males (blue squares) and dwarf males (pink circles) and fitted curves (black lines) with 95% confidence intervals (blue area, nest males; pink area, dwarf males) are given. (**A**) Percent motile cells. (**B**) VAP (µm/s). (**C**) VCL (µm/s). (**D**) VSL (µm/s). Sample sizes as in Fig. 1.

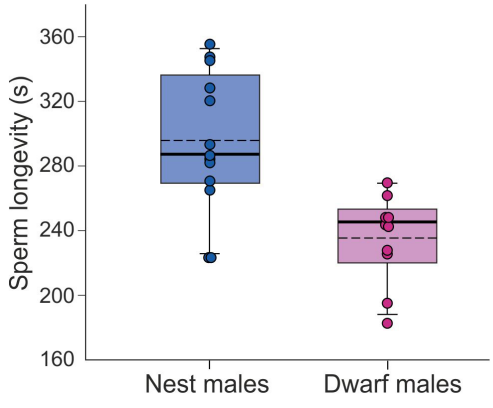

Fig. 3. Longevity of spermatozoa (VSL > 10 µm/s). Circles show data points of nest males (n = 13; blue) and dwarf males (n = 10; pink). Boxes denote interquartile ranges, medians (bold line), and arithmetic means (dashed line). Whiskers show the 10th and 90th percentiles.

declines faster in dwarf than in nest male sperm. Also, the beat cross frequency declines more quickly in dwarf males than in nest males. In contrast, nest male sperm swim less straightly at the beginning but live longer than dwarf male sperm, and generally, nest male ejaculates show a higher percentage of motile sperm. Because dwarf male ejaculates are released much closer to the fertilization site than those of nest males, it is more advantageous for dwarf males to produce sperm with good swimming performance than with long endurance. Nest male sperm have to survive much longer until the distance between the sperm release site and the fertilizable egg is covered, which happens mainly through water current produced by female pectoral fin movements (*45*). Therefore, nest males benefit more from producing sperm with high sperm longevity.

Male body size within each male type related neither to any of the measured sperm activity parameters nor to sperm length (Table 2). This is similar to the eastern mosquitofish *Gambusia holbrooki*, where male body size is the key factor determining their pre-copulatory mating success, but Locatello *et al.* found no correlation between male body size and sperm traits (*46*).

Consistent with the existence of a trade-off between sperm performance and longevity of sperm, our results confirm the predicted specializations of nest and dwarf male sperm in opposite directions. Furthermore, in *L. callipterus*, selection for swimming performance and longevity of sperm acts in opposite directions to the usual situation in species with ARTs, resulting from divergent conditions regarding male proximity at spawning. As opposed to nest males, dwarf male sperm are released at a very close distance to the egg, reaching it immediately. Hence, given the limited energy reserves of spermatozoa, dwarf males should evolve to maximize sperm performance at the cost of endurance. By contrast, sperm of nest males should specialize in longevity, because their sperm must remain active for a relatively long period before reaching the egg from the point at which the ejaculate is released.

All sperm performance measures except the MAD decreased with time, which reflects the depletion of ATP reserves during sperm activity (*33*). In contrast, MAD typically increases with time after sperm activation, because ATP depletion causes sperm to swim in a more irregular or "S-shaped" path (*33*). The decline in performance occurred at tactic-specific rates, with faster rates in dwarf than in nest males. Dwarf male sperm swam more linearly and straighter than nest male sperm until about 2 min after sperm activation. Subsequently, this pattern was

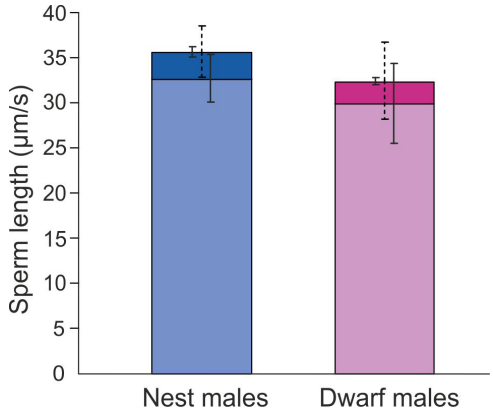

Fig. 4. Sperm length. Total sperm length (total column), sperm head plus midpiece length (dark areas), and length of flagellum (light areas) of nest males (n = 13) and dwarf males (n = 10). Arithmetic means ± SDs (dashed lines for total sperm length) are shown.

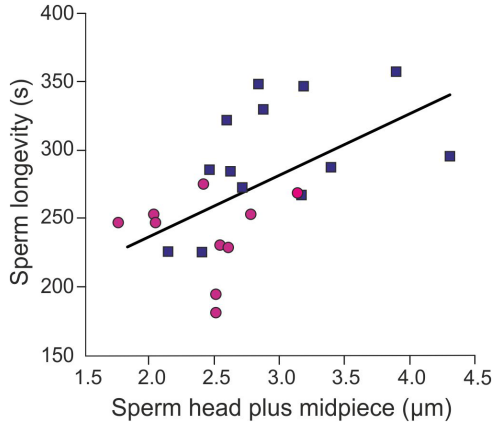

Fig. 5. Sperm length and longevity. Relationship between mean sperm head plus midpiece length of 13 nest males (blue squares) and 10 dwarf males (pink circles) and the longevity of their sperm. Fitted regression line: $y = 44.98x + 147.05$ (N = 23).

reversed, which probably reflects the quicker depletion of energy reserves in the faster-performing sperm of dwarf males. Research has shown that the fertilization ability of sperm declines over time in fish as well as in other organisms (*11*, *34*, *47*). This is due to various reasons, not least of which is a depletion of available energy [reviewed by Reinhardt (*47*)].

The swimming performance of sperm primarily relates to sperm morphology, especially to the absolute and relative sizes of different main spermatozoa components. In *L. callipterus*, the total length of spermatozoa differs between nest and dwarf males, with nest male sperm being approximately 10% longer than dwarf male sperm. This is mainly due to the different lengths of the sperm head including the midpiece, which contains the mitochondria and energy reserves required for flagellar movement (*48*). Therefore, spermatozoa with larger heads have more energy at their disposal and can perform longer (*36*). Overall, in *L. callipterus*, longer sperm heads improved both swimming performance (Table 1) and longevity (Fig. 4) of spermatozoa. However, the residuals of the regression between sperm head length and longevity were positive in nest males and negative in dwarf males (Fig. 4), suggesting that benefits of sperm performance and longevity might select for increased energy reserves in sperm of nest males but not of dwarf males. Nest male sperm might increase swimming performance without compromising longevity when jointly increasing propulsion (flagellum length) and energy reserves (midpiece size), whereas dwarf male sperm seem to be selected for high initial velocity at the expense of reduced sperm endurance, which is a trait that is probably of minor importance for dwarf male sperm.

Selection may also act on other trade-offs, such as sperm number versus sperm quality (*2*), which might override effects of selection on the trade-off between sperm performance and endurance. For example, in bluegill sunfish, where three male tactics compete for fertilizations, spermatozoa of sneaker males showed faster initial swimming speed but shorter periods of motility than the sperm of bourgeois males (*15*), whereas the latter seemed to outlive sperm of parasitic males (*31*) [but see the studies of Burness *et al.* (*33*) and Leach and Montgomerie (*35*)]. However, in this species in the field, the distance between males and females at spawning was much shorter in bourgeois males than in sneakers but much longer in bourgeois males than in satellites (*38*); the latter typically position themselves between the bourgeois male and the female during spawning (*49*). Nevertheless, the sperm longevity measures did not differ between satellite males and sneakers (*31*), despite their radically different positions and differences of timing at spawning. Based on this discrepancy, Burness *et al.* have concluded that sperm performance measures in bluegill sunfish may not relate straightforwardly to differences in sperm competition risk between male tactics (*33*), because relative sperm numbers released at spawning may be of overriding importance in this species (*50*).

Seminal plasma (*51*) and ovarian fluid (*52*) can also influence sperm performance and thus the outcome of sperm competition. For example, in the ocellated wrasse *Symphodus ocellatus*, where nest males produce faster but less sperm than sneaker males, the presence of ovarian fluid reduces the advantage of having more sperm while increasing the relative importance of sperm velocity in nest males. Nevertheless, ovarian fluid did not affect sperm from alternative male morphs differently (*53*). In salmonids, ovarian fluid can influence sperm performance in various ways (*54*), and subtle effects manifested in internally fertilizing guppies where sperm velocity was higher in ovarian fluids of unrelated females in comparison to sisters, thus favoring paternity of unrelated males (*55*). In *L. callipterus*, however, sperm of nest males have to pass a great distance before reaching the egg, which is laid far inside a snail shell. Hence, for most of the time between sperm release and encounter with the egg, sperm have to perform and survive in fresh water, without the potential influence of ovarian fluid. Thus, ovarian fluid is unlikely to strongly affect sperm performance and the outcome of sperm competition between the two male morphs in this species.

Sperm parameters were shown to adjust flexibly in other species with male ARTs [for example, see the study of Smith and Ryan (*56*)]. In *L. callipterus*, however, as nest and dwarf males represent distinct genetic morphs (*42*), keeping their divergent fertilization tactics throughout life (*39*, *41*), it seems likely that the observed tactic-specific differences in sperm morphology and performance remain rather constant and are at least partly based on genetic divergence.

Here, we have focused on the different trade-off gametes may face if males pursue alternative fertilization tactics. However, one should keep in mind that males pursuing ARTs may also face a number of other trade-offs, in particular at the levels of ejaculate expenditure and mating behavior. For instance, expenditure in ejaculates may be traded off against the effort required to obtaining additional matings (*5*, *6*). In different wrasse species, for example, bourgeois males seem to divert energy from ejaculate expenditure to activities that increase their fitness in alternative ways, such as mate acquisition and guarding (*57*, *58*). In

Table 1. Sperm size and swimming performance. Pearson correlations of mean sperm head plus midpiece length per male and swimming performance of sperm at 180 s after sperm release (all $N = 23$). Significant correlations are highlighted in bold.

	Linearity	Beat cross frequency	Mean head displacement	VAP	VSL
r	0.551	0.649	−0.292	0.533	0.605
P	**0.006**	**0.001**	0.177	**0.006**	**0.002**

Table 2. Body size and sperm characteristics. Pearson correlations of male body size (SL) with sperm activity parameters at 60 s after activation and sperm lengths in nest males ($n = 13$) and dwarf males ($n = 10$). There were no significant relationships.

	Nest males		Dwarf males	
	r	*P*	*r*	*P*
Beat cross frequency	0.016	0.957	0.424	0.222
Straightness	0.142	0.644	0.361	0.306
Linearity	0.017	0.955	0.139	0.701
MAD	−0.088	0.776	−0.1	0.782
Percent motile sperm	−0.403	0.172	−0.433	0.211
VAP	0.041	0.895	−0.059	0.871
VCL	0.165	0.591	0.216	0.548
VSL	0.049	0.873	0.120	0.741
Longevity	−0.108	0.725	−0.039	0.916
Total sperm length	0.082	0.789	−0.427	0.218
Sperm head length	0.138	0.653	0.395	0.258
Sperm tail length	0.062	0.84	−0.441	0.202

L. callipterus, nest males may be at such a severe disadvantage relative to the dwarf males during spawning that it might pay them to increase almost all the fitness characteristics of individual sperm (Figs. 2 to 4). In contrast, nest males save investment in ejaculate production when competing for fertilizations with dwarf males (*59*).

In conclusion, our data show that nest and dwarf males of *L. callipterus* solve the trade-off between sperm performance and endurance in opposite directions. This specialization of sperm activity seems to reflect the divergent conditions during fertilization encountered by alternative male morphs. Characteristics of nest male sperm correspond to the large time window between sperm activation and fertilization in these males, whereas sperm competition and the privileged position during spawning might be mainly responsible for sperm characteristics of dwarf males. This highlights the importance of the ecological context when considering sperm trait variations (*23*, *60*). We argue that the spatial and temporal dynamics of sperm in competition during the fertilization process merits consideration when studying functional properties of spermatozoa. In particular, when there is sperm competition between males pursuing different reproductive behaviors, the tactic-specific time window between sperm release and fertilization is apparently of crucial importance for the optimization of sperm traits.

MATERIALS AND METHODS

Study species

L. callipterus is a polygynous shell-brooding cichlid endemic to Lake Tanganyika, showing an extreme inter- and intrasexual size dimorphism. Nest males (mean body weight ± SD, 36.1 ± 7.4 g) are 7.4 to 12.1 times heavier than females (2.7 ± 0.96 g) (*44*, *61*, *62*) and 42 to 83 times heavier than parasitic dwarf males (0.9 ± 0.3 g) (*39*, *41*, *63*, *64*). Spawning of a whole clutch in the field takes between 6 and 8 hours, where females deposit their eggs one by one, with intervals between subsequent depositions exceeding 2 min (*45*). Separate ejaculates are released for the fertilization of each egg, which involves a tight coordination process between female and male during spawning (*45*). More than 20 females may simultaneously breed in a nest (*65*), and nest owners may spawn with up to four females at a time (*59*). After spawning, females care for the eggs and hatched larvae inside the shell for a period of 10 to 14 days (*40*). Medium-sized males that will develop into nest males once they have passed a certain threshold size occasionally behave as sneakers by dashing into a nest to release sperm over the shell entrance in which a female is spawning (*39*, *41*, *61*). These opportunistic sneaker males belong to the nest male genotype (*41*, *62*).

Experimental setup and stimulation of the males

For the experiments, we used fish from a population maintained in the laboratory in Bern, which were derived from wild caught males and females in Mpulungu, Zambia (F_1 and F_2 generations). The temperature in the tanks was kept constant at 26°C (±0.5°C). To induce reproductive motivation and sperm production of males, they were provided with access to gravid females. Tanks with a size of 420 liters were partitioned asymmetrically (3:1) with plastic mesh (hole diameter, ≤1.5 cm), only allowing dwarf males and females to pass, but not nest males. The larger compartment contained five shells of *N. tanganicense* serving as territory for the nest male.

In the morning of day 1 of each of 15 replicate trials, a nest male, three dwarf males, and three females were placed into the larger compartment of the tank. Females that had spawned were replaced by new females. In each tank, one dwarf male haphazardly chosen as focal test male was fin-clipped. The fish were kept in this setup for 4 days. On days 5 and 7, we stripped ejaculates from the nest male and the fin-clipped dwarf male.

Of all test males, standard lengths were determined to the nearest 0.1 cm. Nest males were, on average, 10.1 ± 0.86 cm long [standard length (SL); mean ± SD; range, 9.0 to 11.0 cm], and dwarf males were 3.6 ± 0.52 cm long (range, 3.0 to 4.0 cm). One nest male and two dwarf males could not be successfully stripped; therefore, sperm characteristics were determined from 14 nest males and 13 dwarf males.

Sperm activity parameters

In nature, *L. callipterus* dwarf males fertilized ca. 77% of eggs when in direct competition with nest males (*42*). Because it is yet unknown which of the potential performance differences between nest and dwarf male sperm influence this asymmetric success, here we report all sperm performance measures separately. Sperm was collected by stripping (see section S1). Two different types of sperm activity measures were collected at room temperature (22° ± 1°C): (i) measures representing sperm performance and (ii) measures representing sperm motility and

endurance. Sperm activity was captured with a charge coupled device camera mounted on a dark filter contrast Leica microscope at ×200 magnification. The following sperm parameters were recorded in real time (see fig. S1).

Sperm performance measures

Beat cross frequency (Hz: number of beats/s) is the number of times the centroid of the sperm head crosses the computer-generated mean trajectory of that cell. This parameter provides an estimate of flagellar beat frequency based on movement of the sperm head (*66*). It influences both sperm performance and motility and determines sperm swimming straightness and linearity. Straightness (%) is calculated by dividing the straight line distance between the first and last point on the smoothed path by the distance along the smoothed path (VSL/VAP; see motility measures below) and is expressed in percent. Linearity (%) is calculated by the straight line distance between the start and end points, divided by the travel distance along the actual path taken (VSL/VCL), and is expressed in percent. MAD [degrees (°)] is equal to the average change in direction of the head from frame to frame, displayed as an average value for each analyzed track.

Sperm motility measures

Percent of motile cells (%) corresponds to the number of motile spermatozoa divided by the sum of motile plus immotile sperm within the field of view. During our analysis, we manually recorded the number of immotile, that is, not moving, cells in the field of view. For practical reasons (time required for the preparation of sample for microscopic analysis), sperm motility measurement started 30 s from activation. VAP (μm/s) gives the velocity of the sperm along a smoothed path based on a moving average over a number of sampled points. VCL (μm/s) closely approximates the velocity of the sperm along its actual path, calculated as the sum of the incremental distances covered between subsequent frames divided by the total time of the track. VSL (μm/s) is calculated by dividing the straight line distance between the start and end points of the track by the total time of the track.

Sperm longevity

As an index of the duration of sperm motility, we used the time in seconds until the mean VSL of all measured spermatozoa during a 30-s measurement period was ≤10 μm/s (*67*). Sperm swimming slower than 10 μm/s were considered "dead" and no longer capable of fertilization (*67*).

Sperm length

The preparation of sperm for morphological measurements is described in section S2. To measure sperm length, we used a light microscope at ×400 magnification with an H5 phase-contrast filter. Head length, including the midpiece, was measured from the intersection of the flagellum across the midline of the sperm head to its forward apex (*68*); total length was measured from the apex of the head to the end of the terminal filament. Because of a technical failure, we lost the sperm size data of one nest male and two dwarf males. In the case of one additional dwarf male, neither of the two prepared slides had measurable spermatozoa because of insufficient coloration. Therefore, our sample of sperm size measures included 20 spermatozoa each from 13 nest males and 10 dwarf males.

Relationship between size and swimming performance of spermatozoa

We hypothesized that sperm performance should be determined mainly by the energy reserves contained in the midpiece of the sperm head. Therefore, we analyzed the relationship between mean total sperm length, flagellum length, and sperm head plus midpiece length of a male and the swimming performance of his sperm. We tested sperm at an interval after sperm release at which energy limitations should be expected to influence performance already, but at which spermatozoa of all tested males were still alive, that is, at 180 s after sperm release (see Figs. 1 and 2). For this analysis, we used the ejaculates of 13 nest males and 10 dwarf males.

Data analysis

To compare the performance and motility measures of sperm between nest and dwarf males, we fitted the data to a nonlinear model using the NonlinearModelFit routine implemented in Mathematica 7. This routine estimates the model parameters using nonlinear least-squares fitting and calculates SEs and 95% confidence intervals for the parameter estimates by locally approximating the model with a linear function in the parameters (*69*). Using this information, we performed tests of parameter significance, based on a t test statistic, to evaluate whether the best-fit models differed significantly between nest and dwarf males. We then reduced the model stepwise by systematically eliminating parameter differences between nest and dwarf males that were found to be nonsignificant, starting with the parameter difference with the highest (most nonsignificant) P value.

The data for the sperm performance measures beat cross frequency, straightness, linearity, and MAD showed an accelerating or sigmoidal decrease in performance with time and were well described by a complementary cumulative gamma distribution (see section S3). This distribution is frequently used to model the time to failure of a system. The motility measures (motility, VAP, VCL, and VSL) declined approximately exponentially and were well described by a fit to decaying exponential functions.

With a one-way ANOVA, we checked for differences in sperm longevity between male types. We used Pearson correlation analyses to check for a relationship between male body size and sperm length within each morph and independent samples t tests to test for differences in sperm length between male types. Pearson correlation analyses and multiple regression analyses were used to check for relationships between sperm length and performance. Because neither the sperm activity parameters measured at 60 s after activation (corresponding to half the average time interval between deposition of subsequent eggs) nor sperm length correlated with male body size within each morph (see Table 2), we did not include male body size in our analyses. The analyses were done using SPSS version 22.0. All tests were two-tailed.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/4/5/eaap8563/DC1

Supplementary Materials and Methods

section S1. Ejaculate collection and preparation

section S2. Sperm length measurement

section S3. Gamma distribution

fig. S1. Representation of the different tracking parameters of sperm performance.

table S1. Model fitting results of sperm performance and motility parameters.

table S1A. Sperm performance parameters fitted to a complementary cumulative gamma distribution.

table S1B. Motility measures fitted to an exponential function.

References (*70*–*72*)

REFERENCES AND NOTES

1. G. A. Parker, Sperm competition and the evolution of ejaculates: Towards a theory base, in *Sperm Competition and Sexual Selection*, T. Birkhead, A. P. Møller, Eds. (Academic Press, 1998), pp. 3–54.

2. R. R. Snook, Sperm in competition: Not playing by the numbers. *Trends Ecol. Evol.* **20**, 46–53 (2005).
3. M. Taborsky, Sperm competition in fish: 'Bourgeois' males and parasitic spawning. *Trends Ecol. Evol.* **13**, 222–227 (1998).
4. G. A. Parker, Sperm competition games: Raffles and roles. *Proc. R. Soc. Lond. B* **242**, 120–126 (1990).
5. M. A. Ball, G. A. Parker, Sperm competition games: External fertilization and "adaptive" infertility. *J. Theor. Biol.* **180**, 141–150 (1996).
6. M. A. Ball, G. A. Parker, Sperm competition games: Inter- and intra-species results of a continuous external fertilization model. *J. Theor. Biol.* **186**, 459–466 (1997).
7. C. Boschetto, C. Gasparini, A. Pilastro, Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.* **65**, 813–821 (2011).
8. C. C. Smith, Opposing effects of sperm viability and velocity on the outcome of sperm competition. *Behav. Ecol.* **23**, 820–826 (2012).
9. M. Taborsky, Alternative reproductive tactics in fish, in *Alternative Reproductive Tactics: An Integrative Approach*, R. F. Oliveira, M. Taborsky, H. J. Brockmann, Eds. (Cambridge Univ. Press, 2008), pp. 251–299.
10. G. A. Parker, M. A. Ball, P. Stockley, M. J. G. Gage, Sperm competition games: Individual assessment of sperm competition intensity by group spawners. *Proc. R. Soc. Lond. B* **263**, 1291–1297 (1996).
11. D. R. Levitan, Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. *Proc. R. Soc. Lond. B* **267**, 531–534 (2000).
12. P. Stockley, M. J. G. Gage, G. A. Parker, A. P. Møller, Sperm competition in fishes: The evolution of testis size and ejaculate characteristics. *Am. Nat.* **149**, 933–954 (1997).
13. M. J. G. Gage, C. P. Macfarlane, S. Yeates, R. G. Ward, J. B. Searle, G. A. Parker, Spermatozoal traits and sperm competition in Atlantic salmon: Relative sperm velocity is the primary determinant of fertilization success. *Curr. Biol.* **14**, 44–47 (2004).
14. G. A. Parker, Sperm competition games: Sneaks and extra-pair copulations. *Proc. R. Soc. B* **242**, 127–133 (1990).
15. G. P. Burness, S. J. Casselman, A. I. Schulte-Hostedde, C. D. Moyes, R. Montgomerie, Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). *Behav. Ecol. Sociobiol.* **56**, 65–70 (2004).
16. T. Yamamoto, N. Hirohashi, E. Fujiwara, T. Suzuki, H. Maruta, H. Omiya, S. Kitanishi, Relationships between body size and secondary sexual characters, and sperm characters in male Dolly Varden char (*Salvelinus malma*). *Ecol. Freshw. Fish* **26**, 397–402 (2017).
17. S. Humphries, J. P. Evans, L. W. Simmons, Sperm competition: Linking form to function. *BMC Evol. Biol.* **8**, 319 (2008).
18. S. Balshine, B. J. Leach, F. Neat, N. Y. Werner, R. Montgomerie, Sperm size of African cichlids in relation to sperm competition. *Behav. Ecol.* **12**, 726–731 (2001).
19. P. G. Byrne, L. W. Simmons, J. D. Roberts, Sperm competition and the evolution of gamete morphology in frogs. *Proc. R. Soc. Lond. B* **270**, 2079–2086 (2003).
20. J. L. Fitzpatrick, F. Garcia-Gonzalez, J. P. Evans, Linking sperm length and velocity: The importance of intramale variation. *Biol. Lett.* **6**, 797–799 (2010).
21. T. C. M. Bakker, M. Hollmann, M. Mehlis, M. Zbinden, Functional variation of sperm morphology in sticklebacks. *Behav. Ecol. Sociobiol.* **68**, 617–627 (2014).
22. K. Reinhardt, O. Otti, Comparing sperm swimming speed. *Evol. Ecol. Res.* **14**, 1039–1056 (2012).
23. K. Reinhardt, R. Dobler, J. Abbott, An ecology of sperm: Sperm diversification by natural selection. *Annu. Rev. Ecol. Evol. Syst.* **46**, 435–459 (2015).
24. R. F. Oliveira, M. Taborsky, H. J. Brockmann, *Alternative Reproductive Tactics: An Integrative Approach* (Cambridge Univ. Press, 2008), pp. 1–507.
25. M. Taborsky, Bourgeois and parasitic tactics: Do we need collective, functional terms for alternative reproductive behaviours? *Behav. Ecol. Sociobiol.* **41**, 361–362 (1997).
26. M. Taborsky, Sneakers, satellites, and helpers: Parasitic and cooperative behavior in fish reproduction, in *Advances in the Study of Behavior*, P. J. B. Slater, J. S. Rosenblatt, C. T. Snowdon, M. Milinski, Eds. (Academic Press, 1994), vol. 23, pp. 1–100.
27. M. J. G. Gage, P. Stockley, G. A. Parker, Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): Theoretical and empirical investigations. *Philos. Trans. R. Soc. Lond. B* **350**, 391–399 (1995).
28. M. Taborsky, F. Neat, Fertilization mode, sperm competition, and cryptic female choice shape primary sexual characters in fish, in *The Evolution of Primary Sexual Characters in Animals*, J. Leonard, A. Cordoba-Aguilar, Eds. (Oxford Univ. Press, 2010), pp. 379–408.
29. T. Haugland, G. Rudolfsen, L. Figenschou, I. Folstad, Sperm velocity and its relation to social status in Arctic charr (*Salvelinus alpinus*). *Anim. Reprod. Sci.* **115**, 231–237 (2009).
30. L. Locatello, A. Pilastro, R. Deana, A. Zarpellon, M. B. Rasotto, Variation pattern of sperm quality traits in two gobies with alternative mating tactics. *Funct. Ecol.* **21**, 975–981 (2007).
31. B. D. Neff, P. Fu, M. R. Gross, Sperm investment and alternative mating tactics in bluegill sunfish (*Lepomis macrochirus*). *Behav. Ecol.* **14**, 634–641 (2003).
32. T. V. Vladić, T. Jarvi, Sperm quality in the alternative reproductive tactics of Atlantic salmon: The importance of the loaded raffle mechanism. *Proc. R. Soc. Lond. B* **268**, 2375–2381 (2001).
33. G. P. Burness, C. D. Moyes, R. Montgomerie, Motility, ATP levels and metabolic enzyme activity of sperm from bluegill (*Lepomis macrochirus*). *Comp. Biochem. Physiol. A* **140**, 11–17 (2005).
34. D. J. Hoysak, N. R. Liley, Fertilization dynamics in sockeye salmon and a comparison of sperm from alternative male phenotypes. *J. Fish Biol.* **58**, 1286–1300 (2001).
35. B. Leach, R. Montgomerie, Sperm characteristics associated with different male reproductive tactics in bluegills (*Lepomis macrochirus*). *Behav. Ecol. Sociobiol.* **49**, 31–37 (2000).
36. C. C. Smith, M. J. Ryan, Evolution of sperm quality but not quantity in the internally fertilized fish *Xiphophorus nigrensis*. *J. Evol. Biol.* **23**, 1759–1771 (2010).
37. K. Ota, D. Heg, M. Kohda, Sperm phenotypic plasticity in a cichlid: A territorial male's counterstrategy to spawning takeover. *Behav. Ecol.* **21**, 1293–1300 (2010).
38. J. A. Stoltz, B. D. Neff, Male size and mating tactic influence proximity to females during sperm competition in bluegill sunfish. *Behav. Ecol. Sociobiol.* **59**, 811–818 (2006).
39. T. Sato, M. Hirose, M. Taborsky, S. Kimura, Size-dependent male alternative reproductive tactics in the shell-brooding cichlid fish *Lamprologus callipterus* in Lake Tanganyika. *Ethology* **110**, 49–62 (2004).
40. D. Schütz, G. Pachler, E. Ripmeester, O. Goffinet, M. Taborsky, Reproductive investment of giants and dwarfs: Specialized tactics in a cichlid fish with alternative male morphs. *Funct. Ecol.* **24**, 131–140 (2010).
41. M. Taborsky, The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *J. Hered.* **92**, 100–110 (2001).
42. S. Wirtz Ocana, P. Meidl, D. Bonfils, M. Taborsky, Y-linked Mendelian inheritance of giant and dwarf male morphs in shell-brooding cichlids. *Proc. R. Soc. Lond. B* **281**, 20140253 (2014).
43. L. Engqvist, M. Taborsky, The evolution of genetic and conditional alternative reproductive tactics. *Proc. R. Soc. Lond. B* **283**, 20152945 (2016).
44. D. Schütz, M. Taborsky, Giant males or dwarf females: What determines the extreme sexual size dimorphism in *Lamprologus callipterus*? *J. Fish Biol.* **57**, 1254–1265 (2000).
45. D. Schütz, Z. Heg-Bachar, M. Taborsky, D. Heg, Spawning coordination of mates in a shell brooding cichlid. *Int. J. Evol. Biol.* **2012**, 517849 (2012).
46. L. Locatello, M. B. Rasotto, B. Adriaenssens, A. Pilastro, Ejaculate traits in relation to male body size in the eastern mosquitofish *Gambusia holbrooki*. *J. Fish Biol.* **73**, 1600–1611 (2008).
47. K. Reinhardt, Evolutionary consequences of sperm cell aging. *Q. Rev. Biol.* **82**, 375–393 (2007).
48. F. Lahnsteiner, R. A. Patzner, Sperm morphology and ultrastructure in fish, in *Fish Spermatology*, S. M. H. Alavi, J. Cosson, K. Coward, G. Raffiee, Eds. (Alpha Science International, 2008), pp. 1–51.
49. P. Fu, B. D. Neff, M. R. Gross, Tactic–specific success in sperm competition. *Proc. R. Soc. Lond. B* **268**, 1105–1112 (2001).
50. J. A. Stoltz, B. D. Neff, Sperm competition in a fish with external fertilization: The contribution of sperm number, speed and length. *J. Evol. Biol.* **19**, 1873–1881 (2006).
51. J. A. Lewis, T. E. Pitcher, The effects of rival seminal plasma on sperm velocity in the alternative reproductive tactics of Chinook salmon. *Theriogenology* **92**, 24–29 (2017).
52. R. C. Firman, C. Gasparini, M. K. Manier, T. Pizzari, Postmating female control: 20 years of cryptic female choice. *Trends Ecol. Evol.* **32**, 368–382 (2017).
53. S. H. Alonzo, K. A. Stiver, S. E. Marsh-Rollo, Ovarian fluid allows directional cryptic female choice despite external fertilization. *Nat. Commun.* **7**, 12452 (2016).
54. S. E. Yeates, S. E. Diamond, S. Einum, B. C. Emerson, W. V. Holt, M. J. G. Gage, Cryptic choice of conspecific sperm controlled by the impact of ovarian fluid on sperm swimming behavior. *Evolution* **67**, 3523–3536 (2013).
55. C. Gasparini, A. Pilastro, Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy. *Proc. R. Soc. Lond. B* **278**, 2495–2501 (2011).
56. C. C. Smith, M. J. Ryan, Tactic-dependent plasticity in ejaculate traits in the swordtail *Xiphophorus nigrensis*. *Biol. Lett.* **7**, 733–735 (2011).
57. R. R. Warner, D. Y. Shapiro, A. Marcanato, C. W. Petersen, Sexual conflict: Males with highest mating success convey the lowest fertilization benefits to females. *Proc. R. Soc. Lond. B* **262**, 135–139 (1995).
58. S. H. Alonzo, R. R. Warner, Allocation to mate guarding or increased sperm production in a Mediterranean wrasse. *Am. Nat.* **156**, 266–275 (2000).
59. D. Schütz, L. Tschirren, G. Pachler, P. Grubbauer, M. Taborsky, Sperm-limited males save ejaculates for future matings when competing with superior rivals. *Anim. Behav.* **125**, 3–12 (2017).
60. L. H. Apostólico, J. E. A. R. Marian, Dimorphic ejaculates and sperm release strategies associated with alternative mating behaviors in the squid. *J. Morphol.* **2017**, 1–16 (2017).
61. D. Schütz, M. Taborsky, The influence of sexual selection and ecological constraints on an extreme sexual size dimorphism in a cichlid. *Anim. Behav.* **70**, 539–549 (2005).
62. D. Schütz, G. A. Parker, M. Taborsky, T. Sato, An optimality approach to male and female body sizes in an extremely size dimorphic cichlid fish. *Evol. Ecol. Res.* **8**, 1393–1408 (2006).
63. M. Taborsky, Conflict or cooperation: What determines optimal solutions to competition in fish reproduction? in *Behaviour and Conservation of Littoral Fishes*, R. Oliveira, V. Almada, E. J. Goncalves, Eds. (ISPA, 1999), pp. 301–349.

64. S. Wirtz Ocana, D. Schuetz, G. Pachler, M. Taborsky, Paternal inheritance of growth in fish pursuing alternative reproductive tactics. *Ecol. Evol.* **3**, 1614–1625 (2013).
65. T. Sato, M. M. Gashagaza, Shell-brooding cichlid fishes of Lake Tanganyika: Their habitats and mating systems, in *Fish Communities in Lake Tanganyika*, M. Hori, M. Nagoshi, Y. Yanagisawa, Eds. (Kyoto Univ. Press, 1997), pp. 219–240.
66. S. O. Mack, D. P. Wolf, J. S. Tash, Quantitation of specific parameters of motility in large numbers of human sperm by digital image processing. *Biol. Reprod.* **38**, 270–281 (1988).
67. F. Lahnsteiner, B. Berger, T. Weismann, R. A. Patzner, Determination of semen quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. *Aquaculture* **163**, 163–181 (1998).
68. M. J. G. Gage, C. P. Mac Farlane, S. Yeates, R. Shackleton, G. A. Parker, Relationships between sperm morphometry and sperm motility in the Atlantic salmon. *J. Fish Biol.* **61**, 1528–1539 (2002).
69. Wolfram Mathematica Inc. Documentation Center, *Statistical Model Analysis* (2012); http://reference.wolfram.com/mathematica/tutorial/StatisticalModelAnalysis.html.
70. Y. Hirano, H. Shibahara, H. Obara, T. Suzuki, S. Takamizawa, C. Yamaguchi, H. Tsunoda, I. Sato, Relationships between sperm motility characteristics assessed by the computer-aided sperm analysis (CASA) and fertilization rates in vitro. *J. Assist. Reprod. Genet.* **18**, 213–218 (2001).
71. L. Robertson, D. P. Wolf, J. S. Tash, Temporal changes in motility parameters related to acrosomal status: Identification and characterization of populations of hyperactivated human sperm. *Biol. Reprod.* **39**, 797–805 (1988).
72. R. V. Hogg, A. T. Craig, *Introduction to Mathematical Statistics* (Macmillan, ed. 4, 1978).

Acknowledgments: We thank S. Wirtz Ocana for help with handling the fish and for her general support, M. Häsler for sharing his expertise of obtaining sperm, Z. Heg-Bachar and C. Lovy for their help with stripping the fish, and D. Heg for statistical advice. All experimental protocols were approved by the Veterinary office of the Kanton Bern. The experiments were carried out in accordance with relevant guidelines and regulations. **Funding:** M.T. was supported by the Swiss National Science Foundation (SNSF grants 31003A-122511 and 310030B-138660). G.S.v.D. is grateful for the support of the European Research Council (ERC Starting Grant 30955) and the Netherlands Organization for Scientific Research (NWO VIDI grant 864.11.012). **Author contributions:** M.T. and O.G. designed the study. O.G. collected the samples and analyzed the sperm. D.S. and G.S.v.D. analyzed the data and prepared figures. M.T., D.S., and G.S.v.D. wrote the manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 2 September 2017
Accepted 17 April 2018
Published 23 May 2018
10.1126/sciadv.aap8563

Citation: M. Taborsky, D. Schütz, O. Goffinet, G. S. van Doorn, Alternative male morphs solve sperm performance/longevity trade-off in opposite directions. *Sci. Adv.* **4**, eaap8563 (2018).