1

2 **Do the fastest sperm within an ejaculate swim faster in**

3 **subordinate than in dominant males of Arctic charr?**

4

5

6

7

8 Vaz, J.*, Folstad, F., Rudolfsen, G. and Figenschou, L.

9 Department of Evolution and Ecology

10 Institute of Biology

11 University of Tromsø

12 N-9037 Tromsø, Norway

13

14 *Corresponding author Vaz, J. (e-mail: jonvaz@stud.ibg.uit.no)

15

Do the fastest sperm within an ejaculate swim faster in subordinate than in dominant males of Arctic charr?

Vaz, J.*, Folstad, F., Rudolfsen, G. and Figenschou, L.

ABSTRACT

Theoretical models predict that subordinate males should have higher sperm velocity to compensate for their disadvantage mating role and because they experience sperm competition more frequently than dominant males. Differences in mean velocity between sperm of dominant and subordinates in the predicted direction are also documented for a few species – including the Arctic charr (*Salvelinus alpinus*). Yet, this difference in mean velocity does not imply that the fastest sperm within an ejaculate, which are those most likely to fertilize the eggs, swim faster in subordinates than in dominants. We studied the 5 and 10 % fastest sperm cells in ejaculates of dominant and subordinate Arctic charr. Before individuals attained their status, there were no differences in velocity between the fastest sperm of males that later became dominant or subordinate. Yet, after establishment of social position, subordinates showed significantly higher sperm swimming speed of the fastest cells in the first 30 seconds after activation (i.e., at 15, 20 and 30 s post activation). Males that became subordinates showed no change in sperm speed of the fast cells, compared to pre-trial levels, whereas males that became dominant reduced the speed of their sperm (15 seconds post activation) compared to pre-trial levels. Our results suggest that males that attain social dominance are unable to maintain high sperm velocity, not even among the small fraction of the fastest cells.

KEY WORDS:

Arctic charr, evolution, hierarchical status, sperm velocity, sperm competition.

INTRODUCTION

Dominance hierarchies are common among males living in groups during the mating season. Within these hierarchies individuals adopt reproductive strategies according to their within-group social position (Taborsky 1998). A common tactic held by dominant males is guarding females while they are receptive, so that other males get less access to their mature eggs (Alonzo and Warner 2000). Subordinate males, on the other hand, may adopt sneaky mating tactics by clustering around the dominant, and sometimes more attractive males, to steal fertilizations (Beehler and Foster 1988). Such subordinate males always experience sperm competition whereas dominant males may avoid sperm competition when successful in guarding the females. Thus, dominant and subordinate males may experience different risk and intensity of sperm competition, which may lead to a higher investment in sperm among subordinates (Parker 1998).

In species with internal fertilization, sperm competition occurs in the female's own reproductive system, where females may have the opportunity to manipulate the outcome of the fertilization process through cryptic female choice (Eberhard 1998). Although cryptic female choice may also occur in species with external fertilization (Urbach et al. 2005), these species leave fewer avenues for female influence over which sperm will fertilize their eggs. Thus, fertilization of eggs in species with external fertilization should, to a large extent, be determined by a male's ejaculate characteristics and the ejaculate characteristics of the competing males (Ball and Parker 1996), rather than by cryptic female choice (Parker 1998). Accordingly, individuals from several external fertilizing species have been shown to produce ejaculates corresponding to that expected for their reproductive role. That is, ejaculates from subordinate males have higher sperm density

(Gage et al. 1995; Leach and Montgomerie 2000; Liljedal and Folstad 2003; Liljedal et al. 1999; Pilastro and Bisazza 1999) and higher mean sperm velocity than ejaculates from dominant males (Burness et al. 2004; Rudolfsen et al. 2006). The latter may be important as sperm velocity has been documented to have a positive effect on fertilization rate (Gage et al. 1995; Kupriyanova and Havenhand 2002; Levitan 2000; Liljedal et al. 2005).

When eggs are fertilized externally, faster sperm should be more competitive, holding everything else equal, because they could reach the egg more quickly than slower sperm (Snook 2005). However, previous studies on differences in sperm velocity between mating tactics have all used mean sperm velocity values calculated from all motile sperm cells evaluated (Gage et al. 2004; Kupriyanova and Havenhand 2002; Lahnsteiner et al. 1998; Levitan 2000; Liljedal et al. 2005). The appropriateness of this measure relies on the assumption that all sperm cells are of importance for fertilization, an assumption that need not be valid. As only a small, fast fraction of sperm cells within an ejaculate is likely to be the ones fertilizing the eggs, sperm velocity of the fastest cells, rather than the average velocity of all cells, may be a more adequate measure of fertilizing potential – at least for external fertilizers.

The teleost Arctic charr (*Salvelinus alpinus*) has external fertilizations. Males aggregate annually at spawning grounds where they compete intensely before and during the arrival of sexually mature females. When mature females arrive, one or several large males closely guards them, while smaller, presumably subordinate individuals, circle around (Fabricius 1953; Sigurjonsdottir and Gunnarsson 1989). Dominant males spawn in synchrony with the female close to the released eggs, whereas subordinate males “sneak” and usually spawn a few seconds after the female has released her eggs (own

1 observations). Compared to dominant male charr, subordinates show higher average sperm
2 velocity (Rudolfsen et al. 2006). Yet, whether they produce the fastest swimming sperm
3 cells is still unclear.

4 The aim of this study is to examine if sperm cells from subordinate Arctic charr are
5 faster than sperm from dominant males when only measuring the sperm velocity of the
6 fastest fraction of the sperm cells in the ejaculate. That is, rather than testing whether
7 average sperm velocity among dominant and subordinate male charr differ, which has been
8 done elsewhere (Rudolfsen et al. 2006), we specifically question whether the mean sperm
9 velocity of the 5 and 10 % fastest sperm cells in ejaculates differ between dominant and
10 subordinate males.

11

12 METHODS

13 Sampling and handling

14 On four consecutive nights, in mid September 2003, 48 male Arctic charr were
15 captured at one spawning ground in lake Fjellfrøsvatn, Northern Norway (see Rudolfsen et
16 al. (2006) for method descriptions). The fish never stayed in the gill nets for more than 15
17 minutes, and were later stored in collecting cages until next morning. Then, the fishes were
18 anaesthetized (10-12 ml of benzocain per 10 l of water), fork length (nose to caudal cleft)
19 was measured to the nearest mm and each individual was tagged at the dorsal fin with a
20 white plastic tag attached with Floy`s elastic vinyl filament after methods described by
21 Rikardsen (2000). Thereafter, each individual was stripped for all available sperm (see

below). The fish were then allowed to recover in separate water tanks before they were placed with another individual of similar size in a chicken-wire cage (40 x 60 x 90 cm). Maximum length difference within pairs was 5 mm. The cages were placed in the lake at about 1.5 m depth, 2-3 m apart and they were then left for approximately 24 hours before the observations started (see below). After 3 days of observations, the fishes were brought to the laboratory, where they were killed with a blow to their head. Thereafter, a second sperm sample was collected from each individual.

Observations

The social rank, i.e., whether the fish was dominant or subordinate, was determined in each of the 24 pairs by counting the number of aggressive acts from each individual during 5-minute periods. Aggressive acts were defined as a nip, a bite or an initiation of a chase. Different observers watched each cage 2 times a day (midday and evening) for 3 days (30 minutes observation-period altogether) using water binoculars. The data from the observers was pooled since the correlation between observers is known to be high (see Liljedal and Folstad (2003)). The male performing most aggressive acts within a pair was considered dominant (only 3 of the 24 subordinate individuals performed aggressive acts at all during our observation period).

Sperm sampling

As available mature sperm was sampled before the experiment started, the milt collected at the second stripping was produced during the experimental period. When sampling milt, the area around the fish's genital pore was dried to avoid activation of the sperm by water. One person stripped all fish by applying pressure to the abdominal cavity towards the genital pore. The sperm drops were collected in a Petri dish and then sampled

1 with a syringe and stored in Eppendorf tubes at approximate lake water temperature (c.a.
2 10º C). Within two hours after sampling, video-recordings of the sperm movements were
3 made from each individual's ejaculate.

4 **Velocity measurements**

5 The sperm was activated by adding 4.5 µl of water to an aliquot of less than 0.12 µl
6 of sperm placed on a standard counting chamber (Leja products). Video recordings of
7 sperm were made using a Sony CCD black and white video camera (XC-ST50CE PAL) at
8 50 Hz vertical frequency, mounted on an external negative phase-contrast microscope
9 (Olympus CH30) with a 10-x objective. The recordings were stored on DV tapes.
10 Computer assisted sperm analysis (CASA) has been shown to be an objective tool for
11 examining sperm motility in fish (Elofsson et al. 2003; Kime et al. 1996; Kime et al. 2001)
12 and the video recordings were later analyzed using a HTM-CEROS sperm tracker (CEROS
13 version 12, Hamilton Thorne Research, Beverly, MA, USA, see Rudolfsen et al. (2006) for
14 further details). We obtained ejaculates for 46 males, and the sperm velocity was measured
15 for a period of 0.5 s at 15, 20, 30 and 40 s after activation. We used velocity of the average
16 point-to-point track followed by the cell (VCL), because sperm cells did not have any
17 ovarian fluid gradient or a target (i.e., an egg) to ease orientation.

18 **Statistics**

19 The average sperm velocity values from this experiment have been extensively
20 analyzed by Rudolfsen and coworkers (2006). We examined the mean velocity of both the
21 10 and 5% fastest sperm cells within the evaluated sperm samples for each male. Non-
22 parametric tests were used because the data did not follow a normal distribution. A
23 Wilcoxon signed rank test was used to compare differences in sperm speed before and after

1 the experiment (i.e., the caging). T-tests and Mann-Whitney U tests were used to compare
2 the sperm velocity between dominant and subordinate males, both before and after caging.

3 In order to present the frequency distribution of sperm speed, 20 seconds after
4 activation, we selected the same number of sperm cells from each male of every pair. This
5 was done by randomly deleting sperm cells from the recordings of the male with the
6 highest number of sperm cells evaluated until it equaled the number of recordings from the
7 male with the lowest number of sperm cells in the pair. Finally, 1367 sperm cells for each
8 dominant (n = 23) and subordinate (n = 23) males were selected.

9 The mean number (± *s.d.*) of sperm cells represented for each male for the 10%
10 fastest sperm cells is 10 (± 5.7) cells from before males were caged, and 9 (± 5.7) cells
11 from after males were caged. For the 5 % fastest sperm cells the corresponding numbers
12 are 5 (± 2.8) cells, from before caging, and 5 (± 2.9) cells, for after caging. We used
13 StatView, version 5.0.1 (SAS Institute Inc.) for the statistical analyses.

14

15 **RESULTS**

16 There was no significant pre-caging difference in sperm velocity of the 5 and 10 %
17 fastest sperm cells between males that later became dominant or subordinate (Table 1).
18 Yet, after four days with social interactions in the cages, sperm velocity of the 5 and 10%
19 fastest sperm cells were significantly higher in subordinate than in dominant males at 15,
20 20 and 30 seconds after activation. No significant difference was observed 40 seconds after

1 activation (Table 1). The cumulative frequency distributions of the measurements of sperm
2 velocity after caging in dominant and subordinate males are given in Figure 1.

3 When comparing sperm velocity before and after caging, within the status groups,
4 sperm velocity among subordinate males did not change significantly, neither for the 5 nor
5 for the 10 % fastest sperm cells, at any time after activation (Table 2). Dominant males, on
6 the other hand, have significantly lower sperm velocity after caging compared to pre-
7 caging levels at 15 s after activation. Yet, no significant difference was found in dominants
8 20, 30 and 40 s post activation. Moreover, sperm velocity of the 10 and 5 % fastest sperm
9 cells decreased significantly with the time elapsed since activation in both types of males
10 (Figure 2 and 3).

11

12 **DISCUSSION**

13 Before each individual was caged with another individual of similar size, there
14 were no differences in sperm velocity of the fastest sperm cells between those who later
15 turned out to be either dominant or subordinate. Yet, after hierarchical status was
16 established, i.e., after 4 days of caging, individuals that turned out to be subordinates had
17 higher velocity of the fastest sperm cells than individuals that became dominants. This
18 difference was a result of a reduction in sperm velocity in dominant males, with
19 subordinate males maintaining their pre-trial sperm velocity.

20 Subordinate males in our study have the highest velocity of the fastest sperm in the
21 first 30 s after activation. Our finding is in accordance with sperm competition theory (Ball
22 and Parker 1996), and similar results have been found in other studies (Burness et al. 2004;

1 Gage et al. 1995; Neff et al. 2003; Uglem et al. 2001; Vladic and Jarvi 2001). For
2 example, Rudolfsen and coworkers (2006) showed that subordinate males of Arctic charr
3 had higher mean sperm velocity than dominant males, 15 and 20 seconds after activation,
4 and in bluegills (*Lepomis macrochirus*), sneakers had significantly higher velocity than
5 dominant males, 5 and 10 seconds after activation ((Burness et al. 2004), however see
6 (Burness et al. 2005)). Yet, the present study is the first to examine the fraction of cells
7 most likely to fertilize the eggs, and our results suggest that the above findings also hold
8 for the fastest sperm cells. Sperm velocity is partly dependent on the amount of resources
9 available for the sperm cell (Jeulin and Soufir 1992) and is also positively related to sperm
10 length (Gomendio and Roldan 1991). In bluegills, sneakers (subordinates) have about 1.5
11 times more ATP before sperm activation than parentals (dominants), and also slightly
12 longer flagella (Burness et al. 2004). Sneakers also have higher average swimming speed,
13 5 to10 seconds after activation. A high initial sperm velocity may be adaptive in Arctic
14 charr as swimming speed of charr sperm seem to be closely related to fertilization success
15 under sperm competition (Liljedal et al. 2005). Additionally, high sperm velocity should be
16 particularly adaptive for subordinates of external fertilizers because they reproduce in an
17 unfavorable role, later and further away from eggs than the dominant males.

18 Surprisingly, dominant males seem to down-regulate the velocity of their fastest
19 sperm cells whereas subordinate males have no change in sperm swimming speed of their
20 fastest sperm cells during the experiment. This may indicate that fish included in the
21 experiment were too small to attain dominance in their natural spawning environment,
22 where individuals approximately 10-15 cm longer normally dominate (own observations).
23 Thus, many of our pre-trail milt samples may be from originally subordinate individuals

and adjustments of sperm velocity during the trail should consequently only be evident in individuals changing their pre-trail status, that is, in the individuals becoming dominant. These results indicate that males potentially able to mate guard females may have marginal fitness benefits by maintaining an investment in sperm production comparable to that of non-guarding males.

Our results suggests that subordinate males not only compensate for their disfavored role by producing higher sperm density (Liljedal and Folstad 2003) or by having higher mean sperm velocity (Burness et al. 2004; Rudolfsen et al. 2006), but also by holding the fastest portion of sperm cells in the ejaculate, the one which are most likely to fertilize the eggs.

ACKNOWLEDGMENTS

Thanks to Audun Stien for his help with the statistics. We are very grateful to Marie Figenschou, Davnah Urbach and Jacob Lohm for their indispensable assistance in the field.

1 **REFERENCES**

2 Alonzo, S.H. and Warner, R.R. 2000. Allocation to mate guarding or increased sperm
3 production in a Mediterranean wrasse. Am. Nat. **156**(3): 266-275.
4 Ball, M.A. and Parker, G.A. 1996. Sperm competition games: external fertilization and
5 "adaptative" infertility. J. Theo. Biol. **180**(2): 141-150.
6 Beehler, B.M. and Foster, M.S. 1988. Hotshots, hotspots and female preference in the
7 organization of lek mating systems. Am. Nat. **131**(2): 203-219.
8 Burness, G., Casselman, S., Schulte-Hostedde, A., Moyes, C. and Montgomerie, R. 2004.
9 Sperm swimming speed and energetics vary with sperm competition risk in bluegill
10 (*Lepomis macrochirus*). Behav. Ecol. Soc. **56**(1): 65-70.
11 Burness, G., Moyes, C.D. and Montgomerie, R. 2005. Motility, ATP levels and metabolic
12 enzyme activity of sperm from bluegill (*Lepomis macrochirus*). Comp. Biochem.
13 Physio. Mol. Inte. Physio. **140**(1): 11-17.
14 Eberhard, W.G., 1998. Female roles in sperm competition. *In* Sperm competition and
15 sexual selection, *Editied by* T.R. Birkhead and A.P. Møller. Academic press,
16 London. pp. 91-116.
17 Elofsson, H., McAllister, B.G., Kime, D.E., Mayer, I. and Borg, B. 2003. Long lasting
18 stickleback sperm; is ovarian fluid a key to success in fresh water? J. Fish Biol.
19 **63**(1): 240-253.
20 Fabricius, E. 1953. Aquarium observations on the spawning behaviour of the char, *Salmo*
21 *alpinus*. Institute of Freshwater Research Report.

1 Gage, M.J., Macfarlane, C.P., Yeates, S., Ward, R.G., Searle, J.B. and Parker, G.A. 2004.
2 Spermatozoal traits and sperm competition in Atlantic Salmon: Relative sperm
3 velocity is the primary determinant of fertilization success. Cur. Bio. **14**(1): 44-47.
4 Gage, M.J.G., Stockley, P. and Parker, G.A. 1995. Effects of alternative male mating
5 strategies on characteristics of sperm production in the Atlantic salmon (*Salmo*
6 *salar*): Theoretical and empirical investigations. Philos. Trans. R. Soc. Lond. B
7 Biol. Sci. **350**(1334): 391-399.
8 Gomendio, M. and Roldan, E.R.S. 1991. Sperm size influences sperm competition in
9 mammals. Proc. R. Soc. Lond. B. Biol. Sci. **243**(1308): 181-185.
10 Jeulin, C. and Soufir, J.C. 1992. Reversible intracellular ATP changes in intact rat
11 spermatozoa and effects on flagellar sperm movement. Cell Motil Cytos. **21**(3):
12 210-222.
13 Kime, D.E., Ebrahimi, M., Nysten, K., Roelants, I., Rurangwa, E., Moore, H.D.M. and
14 Ollevier, F. 1996. Use of computer assisted sperm analysis (CASA) for monitoring
15 the effects of pollution on sperm quality of fish; Application to the effects of heavy
16 metals. Aqua. Tox. **36**(3-4): 223-237.
17 Kime, D.E., Van Look, K.J.W., McAllister, B.G., Huyskens, G., Rurangwa, E. and
18 Ollevier, F. 2001. Computer-assisted sperm analysis (CASA) as a tool for
19 monitoring sperm quality in fish. Com. Biochem. Physiol. Tox. Pharm. **130**(4):
20 425-433.
21 Kupriyanova, E. and Havenhand, J.N. 2002. Variation in sperm swimming behaviour and
22 its effect on fertilization success in the serpulid polychaete *Galeolaria caespitosa*.
23 Inver. Rep. Dev. **41**(1-3): 21-26.

1 Lahnsteiner, F., Berger, B., Weismann, T. and Patzner, R.A. 1998. Determination of semen
2 quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal
3 plasma parameters, and spermatozoal metabolism. Aquaculture **163**(1-2): 163-181.
4 Leach, B. and Montgomerie, R. 2000. Sperm characteristics associated with different male
5 reproductive tactics in bluegills (*Lepomis macrochirus*). Behav. Ecol. Soc. **49**(1):
6 31-37.
7 Levitan, D.R. 2000. Sperm velocity and longevity trade off each other and influence
8 fertilization in the sea urchin *Lytenchinus variegatus*. Proc. R. Soc. Lond. B. Biol.
9 Sci. **267**(1443): 531-534.
10 Liljedal, S. and Folstad, I. 2003. Milt quality, parasites, and immune function in dominant
11 and subordinate Arctic charr. Can. J. Zool. **81**(2): 221-227.
12 Liljedal, S., Folstad, I. and Skarstein, F. 1999. Secondary sex traits, parasites, immunity
13 and ejaculate quality in the Arctic charr. Proc. R. Soc. Lond. B. Biol. Sci.
14 **266**(1431): 1893-1898.
15 Liljedal, S., Rudolfsen, G. and Folstad, I. 2005. The relative importance of sperm quality
16 and cryptic female choice in predicting male fertilization success in sperm
17 competition. In: Liljedal 2004 Factors influencing sperm production, sperm
18 competition and male fertilization success in the Arctic charr, *Salvelinus alpinus.*
19 University of Tromsø. Tromsø.
20 Neff, D., Fu, P. and Gross, M. 2003. Sperm investment and alterative mating tactics in
21 bluegill sunfish (*Lepomis macrochirus*). Behav. Ecol. **14**(5): 634-641.
22 Parker, G.A., 1998. Sperm competition and the evolution of ejaculates: towards a theory
23 base. *In* Sperm competition and sexual selection, *Editied by* T.R. Birkhead and A.P.
24 Møller. Academic Press, London. pp. 826.

1 Pilastro, A. and Bisazza, A. 1999. Insemination efficiency of two alternative male mating
2 tactics in the guppy (*Poecilia reticulata*). Proc. R. Soc. Lond. B. Biol. Sci.
3 **266**(1431): 1887-1891.
4 Rikardsen, A. 2000. Effects of Floy and soft VIalpha Tags on growth and survival of
5 juvenile Arctic charr. Am. J. Fish. Man. **20**(3): 720-729.
6 Rudolfsen, G., Figenschou, L., Folstad, I. and Figenschou, M. 2006. Rapid adjustments of
7 sperm characteristics in relation to social status. Proc. R. Soc. Lond. B. Biol. Sci.
8 **273**: 325-332.
9 Sigurjonsdottir, H. and Gunnarsson, K. 1989 Alternative Mating Tactics of Arctic Charr,
10 *Salvelinus-Alpinus*, in Thingvallavatn, Iceland. Environ. Biol. Fishes **26**: 159-176.
11 Snook, R.R. 2005. Sperm in competition: not playing by the numbers. Trends Ecol. Evol.
12 **20**(1): 46-53.
13 Taborsky, M. 1998. Sperm competition in fish: 'bourgeois' males and parasitic spawning.
14 Trends Ecol. Evol. **13**(6): 222-227.
15 Uglem, I., Galloway, T.F., Rosenqvist, G. and Folstad, I. 2001. Male dimorphism, sperm
16 traits and immunology in the corkwing wrasse (*Symphodus melops L.*). Behav.
17 Ecol. Soc. **50**(6): 511-518.
18 Urbach, D., Folstad, I. and Rudolfsen, G. 2005. Effects of ovarian fluid on sperm velocity
19 in Arctic charr (*Salvelinus alpinus*). Behav. Ecol. Soc. **57**(5): 438-444.
20 Vladic, T.V. and Jarvi, T. 2001. Sperm quality in the alternative reproductive tactics of
21 Atlantic salmon: the importance of the loaded raffle mechanism. Proc. R. Soc.
22 Lond. B. Biol. Sci. **268**(1483): 2375-2381.
23

1 **TABLES**

2 **Table 1.** Descriptive data of the 10 % and 5 % fastest sperm cells before and after caging

3 in dominant and subordinate individuals. Time is seconds since activation and mean sperm

4 velocity (VCL) is measured in μm/s for both dominant and subdominant. Effect size is the

5 *t-value* from an unpaired t-test.

6
7
8 **A.** 10% fastest. (*n*= 46)
9

	Time	Velocity of dominants	Velocity of subordinates	Effect sizes	*p-value*
Before	15	148.8	157.1	-0.75	0.46
	20	127.8	125.4	0.36	0.72
	30	87.7	81.8	1.32	0.19
	40	84.6	83.5	0.23	0.82
After	15	131.2	144.5	-2.75*	<0.01
	20	122.3	133.2	-2.57	0.01
	30	81.8	90.4	-2.19*	0.03
	40	79.5	85.9	-1.11	0.27

10
11
12 **B.** 5 % fastest. (*n*= 46)
13

	Time	Velocity of dominants	Velocity of subordinates	Effect sizes	*p-value*
Before	15	155.1	165.4	-0.81	0.42
	20	134.8	130.8	0.57	0.58
	30	90.2	85.1	1.14	0.26
	40	93.2	94.1	-0.14	0.89
After	15	134.4	151.4	-3.16*	<0.01
	20	129.3	141.0	-2.29	0.03
	30	88.2	93.1	-2.07	0.04
	40	86.6	96.8	-1.39	0.17

14 ** Z-values from a Mann-Whitney U test.*

1 **Table 2.** Wilcoxon signed rank tests for the 10 % (A) and 5 % (B) fastest sperm
2 cells in dominant and subordinate males after and before caging. Time is seconds since
3 activation and mean sperm velocity (VCL) is measured in μm/s for both dominant and
4 subdominant.

5

6
7 **A.** 10 % fastest. (*n*= 23)
8
9

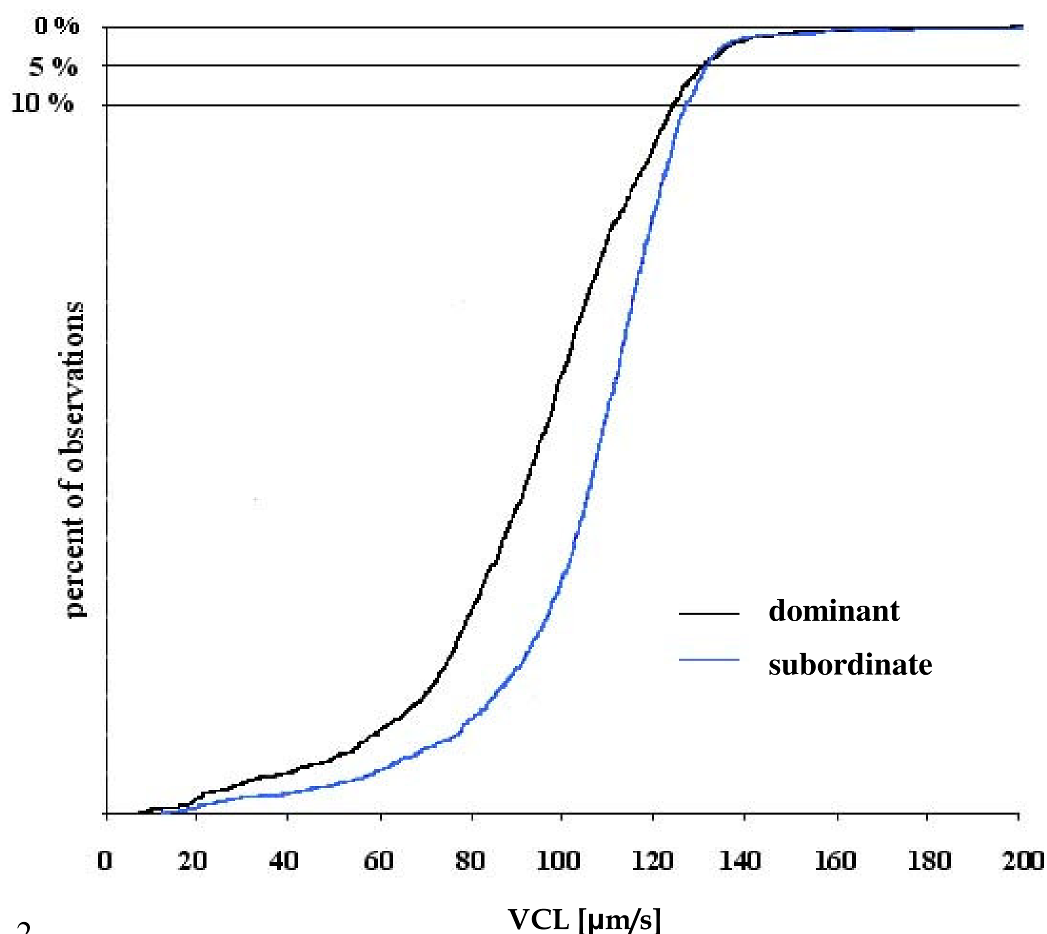
	Time	Before	After	*z-value*	*p-value*
Velocity of dominants	15	148.8	131.2	-3.11	<0.01
	20	127.8	122.3	-1.34	0.18
	30	87.7	81.8	-1.10	0.27
	40	84.6	79.5	-1.20	0.23
Velocity of subordinates	15	157.1	144.5	-1.00	0.32
	20	125.4	133.2	-0.67	0.50
	30	81.8	90.4	-1.52	0.13
	40	83.5	85.9	-0.31	0.76

10
11
12 **B.** 5 % fastest. (*n*= 23)
13

	Time	Before	After	*z-value*	*p-value*
Velocity of dominants	15	155.1	134.4	-3.35	<0.01
	20	134.8	129.3	-0.89	0.38
	30	90.2	88.2	-1.30	0.19
	40	93.2	86.6	-1.16	0.24
Velocity of subordinates	15	165.4	151.4	-1.10	0.27
	20	130.8	141.0	-0.94	0.35
	30	85.1	93.1	-1.40	0.16
	40	94.1	96.8	-0.12	0.90

14
15

1 **FIGURES**



2
3

4 **Figure 1**. Cumulative frequency distribution graph of sperm velocity (VCL [µm/s])

5 of dominant and subordinate males after caging represented by the 5 and 10% fastest

6 sperm cells. Individuals that become dominant after caging have a significant lower mean

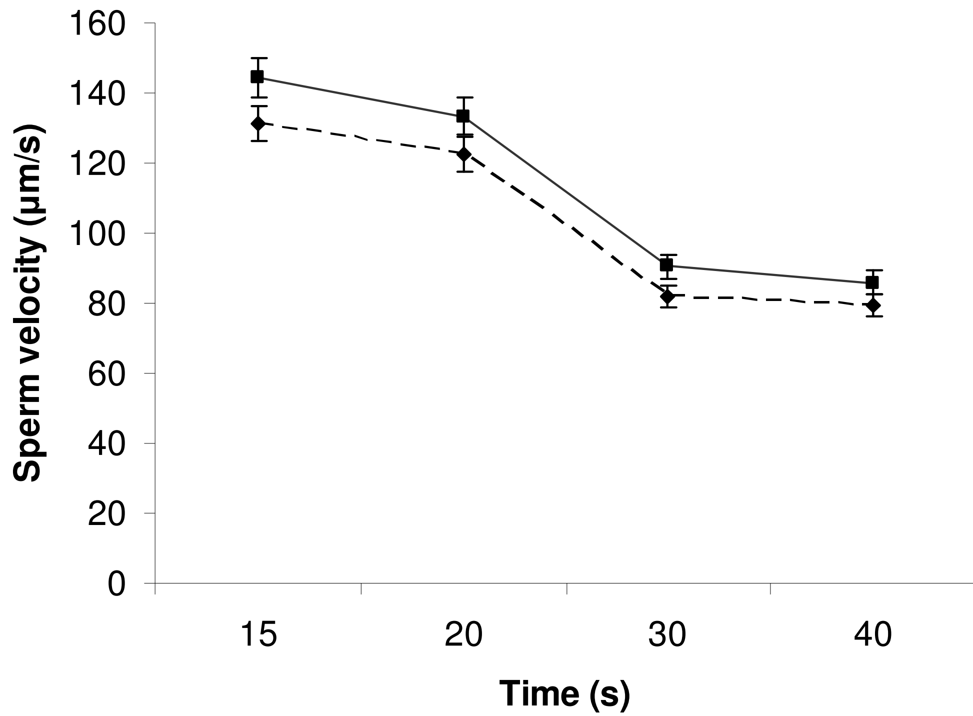
7 sperm velocity than individuals that became subordinate, 20 seconds after activation

8 (subordinates have a larger right skewness than dominants). The mean (±*s.d.*) sperm speed

9 of dominant individuals is 93.9 (±27.3) and 105.3 (±23.6) for subordinates. Significant

10 differences were observed between the two cumulative graphs (*t*= -11.9; *p*<0.001; *n*=

11 1367). The skewness values are –0.5 for dominant males and –1 for subordinate males.



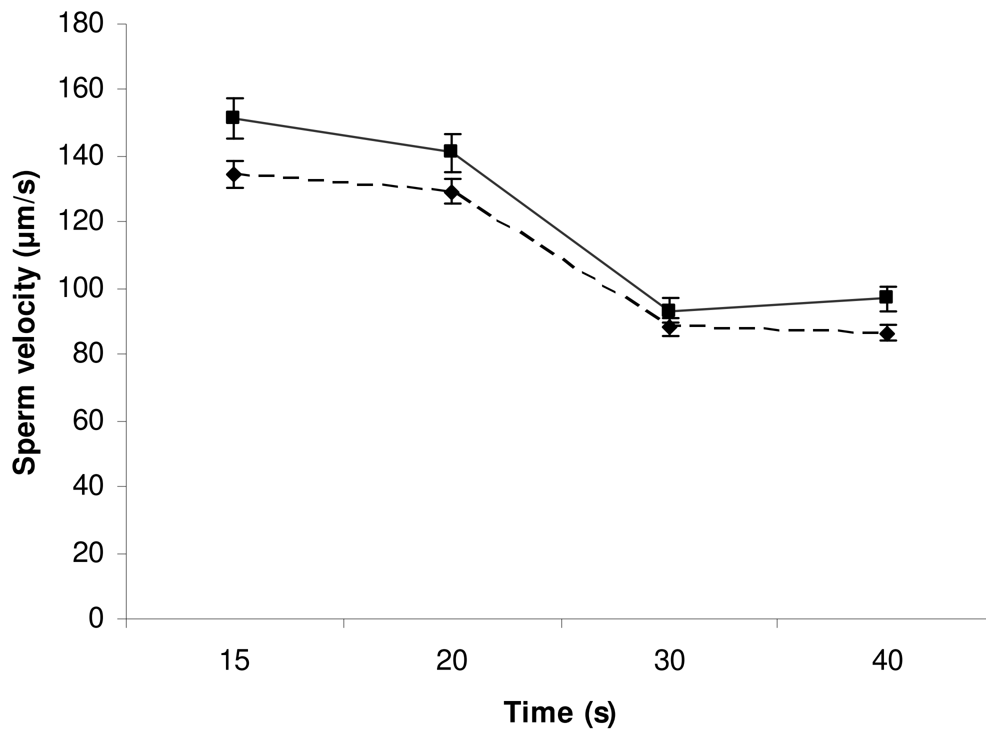
1

2 **Figure 2**. Line graph illustrating differences in sperm velocity (mean and 95 % confidence

3 interval) of the 10 % fastest sperm cells for dominants (♦) and subordinate (■) males,

4 during 15, 20, 30 and 40 seconds after activation.

5



1

2 **Figure 3.** Line graph illustrating differences in sperm velocity (mean and 95 % confidence

3 interval) of the 5 % fastest sperm cells for dominants (♦) and subordinate (■) males, during

4 15, 20, 30 and 40 seconds after activation.

5

6

7

8

9

10

11

12

13