BIO – 3910

Master thesis in evolutionary and behavioural ecology

Examining the phenotypic plasticity in ejaculates of the Arctic charr by experimentally inducing successive changes in social status.

Bjørnar Strøm

May 2007

Faculty of Science

Department of Biology

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Incipit

"*Sexual selection…depends, not on a struggle for existence, but on a struggle between males for possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring*"

Charles Darwin 1859

"*Sexual selection results from competition for female gametes, not for females*"

William G. Eberhard 1996

Prologue

This thesis was conceived in the time between September 2005 and May 2007. I was given the opportunity to examine sperm competition in the Arctic charr by Professor Ivar Folstad. By steady guiding in the field by some of Norway and Spains most dedicated and hard working Arctic charr researchers, I gathered data through three weeks of scientific excellence. Aiding this inexperienced naturalist with both theoretical and practical superiority was Geir Rudolfsen, Lars Figenschou, Thomas Haugland and Jonathan Vaz Serrano. The thesis has in itself been projecting phenotypic plasticity, it seemed a necessity in order to evolve. As May approached, my somewhat high flying thoughts and ideas behaved in a gregarious way, and soon the thesis presented itself.

I believe that science is best described by the words of Claude Bernard: *"If an idea presents itself to us, we must not reject it simply because it does not agree with the logical deductions of a reigning theory"*.

Tromsø, May 2007

Bjørnar Strøm

Abstract

According to theory, a male's reproductive role should predict investment in ejaculate quality, i.e. subordinate males should invest in ejaculate quality to compensate for mating in unfavourable roles, and vice versa for dominant males. The Arctic charr is known to possess fluctuating characteristics of its sperm triggered by rapid changes in social status. The relationship between social status and phenotypic plasticity in Arctic charr ejaculates was examined through a caging experiment. Changes in status of size matched males were experimentally induced through two successive social encounters. Results show that males inhabiting subordinate roles throughout the experiment were able to maintain sperm swimming speed, as did males going from a dominant to a subordinate state. Further, males attaining and defending dominant roles did not decrease investment in their ejaculates as one would expect. Surprisingly, subordinate males attaining dominance showed increased sperm swimming speed in early post activation. The investments in short term fitness benefits become evident in this experiment, partly, as males becoming subordinate will not increase initial sperm swimming speed because it is possibly at an optimum already, but in particular, as males attaining dominance increase initial sperm swimming speed. As the rapidly altered competitive ability of Arctic charr ejaculates may increase individual fitness, individual positioning in a social hierarchy is an important aspect of sperm competition.

Keywords: Arctic charr; sperm competition; ejaculate characteristics; phenotypic plasticity; social status

Introduction

The evolution of anisogamy has led to females becoming the choosier sex (Stearns and Hoekstra 2005). In species without parental care, male fitness is limited by the number of opportunities to mate, whereas female fitness is limited by the number of eggs that she can produce. In such species we find that males compete for access to females and their eggs (Bateman 1948). The component of natural selection associated with mating success is termed sexual selection (Darwin 1871; Andersson 1994). Sexual selection makes way for both pre- and post-copulatory adaptations to mate choice, where pre-copulatory adaptations entail processes regarding evaluation of potential partners and benefits associated with choice of partner (Andersson 1994). Post-copulatory sexual selection, on the other hand, leads to adaptations for sperm selection in females, and adaptations in male ejaculates influencing the outcome of copulation (Birkhead and Møller 1998; Snook 2005). Adaptations in ejaculates are driven by sperm competition, and occur when sperm from two or more males compete for the fertilization of a given set of ova (Parker 1970). Sperm competition is found amongst both internal and external fertilizing species in a variety of taxa (Birkhead and Møller 1998).

When males compete for the opportunity to mate, a social hierarchy forms, and individuals adopt different reproductive tactics according to their level of dominance (Gross 1996; Taborsky 1998). Dominance can be settled by aggressive behaviour (Farentinos 1972; Sigurjónsdóttir and Gunnarsson 1989; Sneddon et al. 2006), and it is positively correlated with the frequency of copulations (Farentinos 1972; Johnsen et al. 2001). Moreover, studies on reproductive behaviour in external fertilizers have shown that socially dominant males have a higher probability of fertilization than subordinate males (DeWoody and Avise 2001). Ejaculates from disfavoured males always encounter ejaculates of other males, thus males mating in disfavoured roles experience a higher level of sperm competition compared to males mating in favoured roles (Parker 1990a; Parker 1990b). Accordingly, subordinate males should invest more into the competitive ability of their ejaculate, e.g. by having a higher number of sperm released in ejaculates (Parker 1990a; Parker 1990b; Parker 1993; Gage et al. 1995; Ball and Parker 1996), or by increasing sperm swimming speed (Ball and Parker 1996). Furthermore, the intensity of sperm competition in external fertilizers is positively correlated with a number of ejaculate characteristics, such as gonadosomatic index (Gage et al. 1995; Stockely et al. 1997; Uglem et al. 2001), sperm number (Gage et al. 1995; Stockely et al 1997; Alonzo and Warner 2000; Liljedal and Folstad 2003), sperm density (Leach and

Montgomerie 2000; Liljedal and Folstad 2003; Burness et al. 2005; Rudolfsen et al. 2006) and sperm swimming speed (Burness et al. 2004; Rudolfsen et al. 2006; see however Burness et al. 2005; Stoltz and Neff 2006). Additionally, the positive correlation between sperm swimming speed and fertilization success has been observed through empirical studies both in internal (Birkhead et al. 1999; Froman et al. 1999) and external fertilizing species (Gage et al. 2004; Casselman et al. 2006).

Cryptic female choice, which is the female's preferential use of sperm from one male over sperm from another (Thornhill 1983; Eberhard 1996), may also influence male reproductive success. Although there are indications of such mechanisms in external fertilizers (Urbach et al. 2004), the ways in which cryptic female choice can operate are fewer in external fertilizing species than in internal fertilizing species. That is, in external fertilizers sperm manipulating mechanisms like sperm storage, sperm displacement and sperm removal inside the female reproductive tract are not present (Eberhard 1996; Birkhead and Møller 1998). Accordingly, ejaculate characteristics are predicted to be an important aspect of external fertilization dynamics (Ball and Parker 1996).

The Arctic charr (*Salvelinus alpinus*) is an external fertilizing teleost where males aggregate and interact aggressively on spawning grounds, both before and after females arrive (Figenschou et al. 2004). In this lek-like context sperm competition becomes evident, as males adopt different reproductive tactics, i.e. mate guarding or sneaking, according to their individual level of social dominance. Furthermore, no parental care is exerted, and both males and females may encounter successive opportunities to mate during the spawning period (Fabricius and Gustafson 1954; Sigurjónsdóttir and Gunnarsson 1989). Rapid changes in ejaculate characteristics such as sperm density (Liljedal and Folstad 2003; Rudolfsen et al. 2006) and sperm swimming speed (Rudolfsen et al. 2006) has been observed, as well as differential investment in ejaculates between males inhabiting different social roles (Liljedal and Folstad 2003; Figenschou 2005; Rudolfsen et al. 2006; Haugland 2006; Serrano et al. 2006; Figenschou et al. 2007). In addition, sperm swimming speed seems to be an important factor influencing the ejaculate`s competitive ability, as it is positively correlated with fertilization success (Liljedal 2004).

This study aims to examine how the phenotypic plasticity of Arctic charr ejaculates is related to changes in individual social status. That is, who makes adjustments in their ejaculate when social status changes: the dominant or the subordinate male? In a caging experiment with pairs of size matched male Arctic charr, changes in individual status were experimentally induced through two successive social encounters. Each male's social status

from the first round of pairing was used to pair males with the same status in a second encounter. This means that two dominant males was paired together, and likewise two subordinate males, thus provoking one of the two males in each pair to change their status. In previous empirical work fish has been observed through a single round of pairing, and initial status has been unknown (Rudolfsen et al. 2006). By observing fish through two successive encounters, changes in individual social status can be related to the corresponding adjustments in primary sexual characters. Previous empirical work has shown that males attaining social dominance seem to reduce ejaculate investment (Rudolfsen et al. 2006; Serrano et al. 2006). Thus, lowered ejaculate quality is expected in males attaining dominance.

Methods

a) Fish sampling

During the course of 13 days in mid September 2005, 64 male Arctic charr were caught in Lake Fjellfrøsvatn in northern Norway (69°N). The fish were all reproductively active, and they were caught with gill nets at one spawning ground. The fish never stayed in the net longer than 15 minutes, and they were carefully cut out of the net with scissors. Fish with signs of injury was excluded from the experiment. At shore the fish was put into cages over night, before handling the following day.

b) Fish handling, caging and observation

Before handling, the fish was anaesthetized with benzocaine (approximately 10 ml per 10 L water). Then the length of the fish was measured, from nose to caudal cleft, to the nearest 0.1 cm (25.21 cm ± 1.22 SD). The fish was tagged with a specific number, and one of four different forms of white tags (square, circle, rectangle or triangle), for individual recognition. The tag was sowed into the midst of the dorsal fin using an elastic vinyl filament. By applying a gentle bilateral abdominal pressure the fish was stripped of its ejaculate, so that ejaculate samples collected during the experiment would contain sperm produced during the experimental period. Four fish of equal size (maximum 3 % deviation in length) was used to complete one quartet, consisting of two pairs. The fish was paired in chicken wire cages (90 x 60 x 40 cm), and placed at 2 m depth, approximately 2 m apart. Observation began the following day.

In this first round of observation (R1), each cage was observed for 5 minutes, two times a day, for three days. The fish's relative social rank (dominant or subordinate) was determined by the number of aggressive acts (a nip, a bite or a chase) by each individual (Liljedal and Folstad 2003). The presence of an observer has no significant effect on fish aggressiveness, nor does it alter hierarchical position (Liljedal and Folstad 2003). The fish with most aggressive acts was considered to be dominant.

On day four the fish was anaesthetized with benzocaine and an ejaculate sample was collected. Before collecting the ejaculate, the fish's abdomen was gently dried with paper, from the ventral fins down to the area around the genital pore, to avoid activation or contamination of the sperm. Examination of Arctic charr ejaculates in the present study is of those attained through manually stripped ejaculates under experimental conditions. After the

sample was collected, the fish was paired again, this time with a fish from the corresponding cage in the quartet. The two dominants were paired in one cage, and the two subordinates in another (figure 1), and observation commenced the following day. The cages, now consisting of two dominant males in one, and two subordinate males in another, were observed in the same manner as before. After this second observational period (R2), social position was assigned. Four groups termed DD-males, DS-males, SD-males and SS-males (capital letters refer to status in R1 and R2 respectively) was used to describe the status of each fish through the experiment, e.g. fish that was dominant in both rounds was termed DD-males.

On the eight day the fish was killed in a random order with a swift blow to the head, and a new ejaculate sample was collected in the same manner as before.

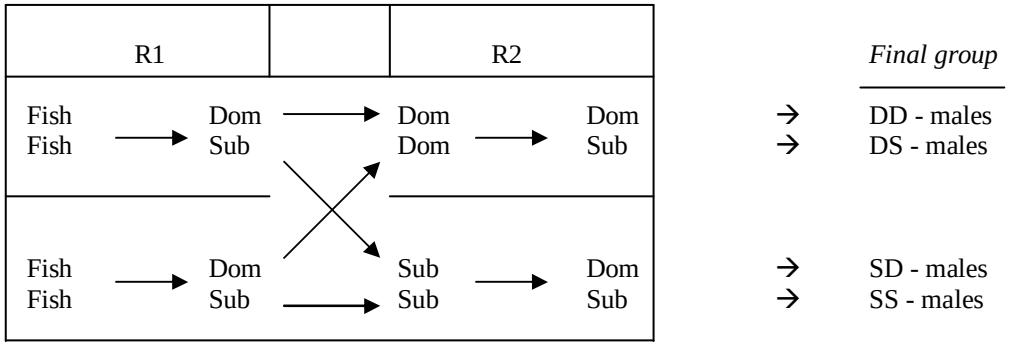

Figure 1: Four male Arctic charr of equal size are put into two cages, with two fish in each cage. After three days of observation (R1), their status is determined (Dominant = Dom, Subordinate = Sub). An ejaculate sample is collected, and the male is paired with a male from the corresponding cage that is occupying the same social role. This means that the dominant males from the two cages are put together in one cage, and likewise with the subordinate males. Then there is another three days of observation (R2), now observing two dominants in one cage and two subordinates in another. After R2 their new status is determined. An ejaculate sample is collected and the fish is killed. The final groups describe the male's status through the experiment. This procedure was followed for 64 fish, giving 16 initial quartets.

c) Ejaculate characteristics and Computer Assisted Sperm Analysis

Ejaculate samples were taken from each fish after three days in cage (day 4), and at the end of the caging period (day 8). Ejaculate volume was estimated in syringes to the nearest 0.01 ml and the sample was then stored in closed Eppendorf tubes at 4°C. The following measurements were all done within one hour after the ejaculate was collected. Within three hours, sperm storage-time does not influence sperm swimming speed (Haugland 2006).

Spermatocrit, which is the percentage of a given volume of sperm that is occupied by sperm cells, was measured by centrifuging a homogenized proportion of ejaculate in a

capillary tube for 195 s at 11500 rpm, using a Compur mini-centrifuge. This gives us a reliable measurement of sperm density (Aas et al. 1991).

Different parameters of sperm swimming behaviour was measured using Computer Assisted Sperm Analysis (CASA) (HTM- CEROS sperm tracker, CEROS version 12, Hamilton Thorne Research, Beverly, MA, USA) which is an objective tool for measuring sperm motility in fish (Kime et al. 1996; Kime et al. 2001). CASA quantifies different measures of sperm characteristics using video recordings of swimming sperm. A Sony CCD black and white video camera (XC- ST50CE PAL) was mounted on a negative phase-contrast microscope (Olympus CH30) with a 10 x objective. 0.12µl of sperm was activated by 4.5µl of water on standard counting chambers (Leja Products BV) of stable temperature (4 - 9ºC). Sperm cell movement was recorded for approximately 1 minute. Measurements included in this study were sperm motility (percent motile sperm cells) and sperm velocity (sperm swimming speed). The velocity parameters were: mean average path velocity (VAP), mean straight line velocity (VSL), and mean curvilinear velocity (VCL), all measured in µm/s. The analysis was set up with frame rate 50 Hz, minimum contrast 16 and minimum cell size 5 pixels. Parameters were quantified from the video recordings at every 10 seconds from 10 – 50 seconds post activation. The quantification was a `snap-shot` of sperm cell movement, lasting 0.5 seconds and containing 25 frames. To avoid assessment on sperm cells showing non-flagellar movement due to drift, cells with VAP < 20 µm/s and VSL < 10 µm/s were excluded. To avoid assessment on cells that moved in and out of frame (Burness et al. 2004; Burness et al. 2005; Haugland 2006), cells that appeared in less than 15 successive frames were also excluded from the analyses. Sperm cells included in CASA were not expected to have a straight line trajectory, since there were no eggs or ovarian fluid to guide them. Thus, VCL was chosen as the parameter of sperm swimming speed to be assessed in further analyses, since this record the point-to-point movement of the cell. A sperm index was created; sperm index (ml) = (spermatocrit x (sperm volume / 100)), a variable describing total sperm cell volume in the sampled ejaculate.

Sperm swimming speed showed a significant decrease with time post activation, both in R1 and R2 (repeated measures ANOVA, $F_{(4,244)} = 244.6$, $p < 0.0001$ and $F_{(4,184)} = 210.8$, $p < 0.0001$, respectively), as did the measure for motility in both rounds (repeated measures ANOVA, $F_{(4,244)} = 204.3$, $p < 0.0001$ and $F_{(4,184)} = 115.9$, $p < 0.0001$, respectively). However, there was no significant status specific effect on neither sperm swimming speed-decline in R1 or R2 (repeated measures ANOVA, $F_{(4,240)} = 0.10$, $p = 0.98$ and $F_{(4,160)} = 1.11$, $p = 0.35$, respectively), nor motility-decline in R1 or R2 (repeated measure ANOVA, $F_{(4,240)} = 0.08$, $p =$

0.99 and $F_{(4,160)} = 1.71$, $p = 0.15$, respectively). Since there were no status specific differences in decline of sperm swimming speed or motility between 10 – 50 s, the measurements at 10 s post activation were used when analysing differences between males of different status groups. However, when analysing repeated measures from the same fish, measurements from 10 – 50 s post activation was included.

d) Statistical analysis

Statistical analyses were done using R 2.1.1 (R Development Core Team 2005) and StatView for Windows version 5.0.1. Data distributions of sperm trait variables were checked for normality and heteroscedasticity using model checking plots in R. Transformations of data were done where assumptions for parametric testing were not met. In order to test if ejaculate characteristics such as velocity, motility, density, volume and sperm index differed between R1 and R2, a students paired t-test was used. A non-parametric wilcoxon signed rank test was used if transformations could not be done. Additionally, to test if there were differences in sperm characteristics between individuals of different social status, a one way ANOVA was used with attained status as predictor variable. Sperm swimming speed at 10 s post activation was positively correlated with sampling date, both in R1 ($r = 0.28$, $p = 0.027$) and R2 ($r = 0.293$, $p = 0.046$), as was motility at 10 s post activation in R2 ($r = 0.559$, $p < 0.0001$), thus sampling date was included as a covariate when analysing these measures. P-values reported are from two-tailed tests. No adjustments for multiple comparisons are made (Nakagawa 2004). Sample size varies between analyses, as not all measurements were obtained from all individuals. Reported means and SD are from original untransformed frequency distributions.

Results

A one way ANOVA with status as predictor variable and sampling date as a covariate, showed no significant difference in sperm swimming speed at 10 s post activation in R1 between dominant (120 ± 34, n = 32) and subordinate (120 ± 38, n = 30) males ($F_{(1,58)} = 0.38$, p = 0.54). Further, there was no significant difference in sperm swimming speed at 10 s in R2 between dominant (128 ± 29, n = 22) and subordinate (118 ± 30, n = 20) males ($F_{(1,38)} = 0.11$, p = 0.74). There was no significant difference in motility at 10 s in R1 between dominant (84 ± 19, n = 32) and subordinate (83 ± 19, n = 30) males ($F_{(1,60)} = 0.08$, p = 0.78). Further, there was no significant difference in motility at 10 s in R2 between dominant (84 ± 16, n = 22) and subordinate (78 ± 22, n = 20) males ($F_{(1,38)} = 0.162$, p = 0.69). However, there was a significant difference in spermatocrit between status in both R1 ($F_{(1,61)} = 21.0$, p < 0.0001, figure 2) and R2 ($_{dom}$; 9.3 ± 3.9, n = 24, $_{sub}$; 21.6 ± 14.2, n = 19, $F_{(1,41)} = 16.9$, p = 0.0002). In addition, there was a borderline significant difference in ejaculate volume between dominant (0.282 ± 0.145, n = 32) and subordinate (0.206 ± 0.161, n = 32) males in R1 ($F_{(1,61)} = 3.9$, p = 0.052). Further, there was a significant difference in ejaculate volume between dominant (0.190 ± 0.156, n = 27) and subordinate (0.059 ± 0.071, n = 27) males in R2 ($F_{(1,52)} = 15.66$, p = 0.0002). The sperm index was borderline significantly different in R1 between dominant (0.019 ± 0.011, n = 32) and subordinate (0.030 ± 0.029, n = 32) males ($F_{(1,62)} = 3.98$, p = 0.050), but not in R2 between dominant (0.016 ± 0.015, n = 27) and subordinate (0.01 ± 0.014, n = 27) males ($F_{(1,52)} = 2.76$, p = 0.102).

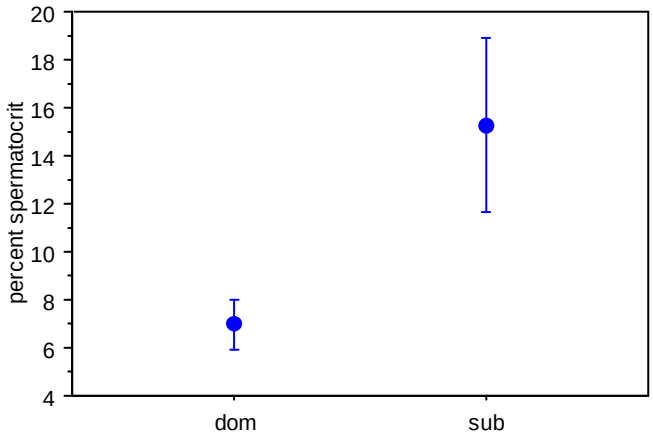

Figure 2: Mean spermatocrit values (%) in ejaculates from dominant (n = 32) and subordinate (n = 31) males in R1. Vertical bars denote 95 % confidence intervals.

Males inhabiting a dominant social role throughout the experiment (DD-males) showed a significant increase in sperm swimming speed at 30, 40 and 50 s post activation from R1 to R2. There was a significant increase in spermatocrit, and ejaculate volume showed a decrease approaching significance. Means, test statistics and p-values are given in table 1.

Males that lost their dominant role from R1, and became subordinate in R2 (DS-males) showed no change in sperm swimming speed at 10 s post activation between R1 and R2. However, at 20 and 30 s post activation there were significant increases. The spermatocrit values showed a significant increase (figure 3), and ejaculate volume showed significant decrease. Means, test statistics and p-values are given in table 2.

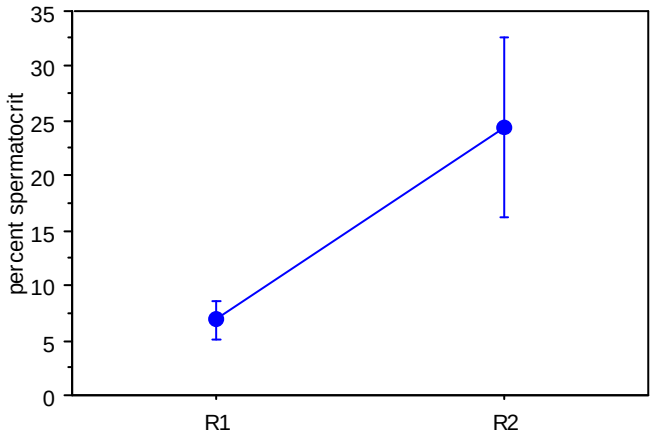

Figure 3: Mean spermatocrit values (%) in ejaculates from males that were dominant in R1 (n = 16), and then became subordinate in R2 (n = 13). Vertical bars denote 95% confidence intervals.

Males that were subordinate throughout the experiment (SS-males) showed a significant decrease in ejaculate volume, and a significant decrease in sperm index, from R1 to R2. Means, test statistics and p-values are given in table 3.

Males that were subordinate after the first bout and then attained dominance in the second bout (SD-males), showed an increase in sperm swimming speed at 10 s post activation approaching significance (figure 4), from R1 to R2, and this increase was significant at 20 s post activation. For the motility measurement at 10 s post activation there was an increase between R1 and R2 approaching significance. Means, test statistics and p-values are given in table 4.

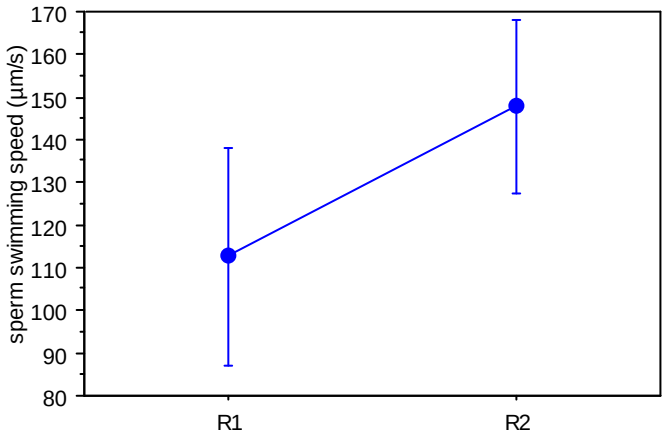

Figure 4: Mean sperm swimming speed (VCL in µm/s) at 10 seconds post activation of sperm cells in ejaculates from males that were subordinate in R1 (n = 10), and then became dominant in R2 (n = 6). Vertical bars denote 95% confidence intervals.

Discussion

In addition to differences in ejaculate traits between males of different social status, rapid adjustments in ejaculate characteristics were observed. Results show that males inhabiting subordinate roles throughout the experiment, as well as males going from a dominant to a subordinate state, did not increase investment in sperm between rounds, but rather maintained initial sperm swimming speed. Further, males attaining and defending dominant roles did not decrease investment in their ejaculates as one would expect. Surprisingly, subordinate males attaining dominance showed both increased sperm swimming speed and increased motility in early post activation. Additionally, this group was the only group that was able to maintain production of ejaculate volume.

Differential investment in ejaculates

As previous studies have shown there are phenotypical differences in primary sexual characters between males of different social status (Leach and Montgomerie 2000; Rudolfsen et al. 2006; Liljedal and Folstad 2003; Figenschou et al. 2007). Concurring with these studies in respect to sperm density, this study shows that dominant males have a lower sperm density than subordinates both in R1 and R2. As for sperm swimming speed, there were no differences between dominant and subordinate males. This is in accordance with empirical work on male fowl (*Gallus gallus domesticus*) (Pizzari et al. 2007), and bluegills (*Lepomis macrochirus*) (Burness et al. 2005). However, other studies have shown significant differences in sperm swimming speed between individuals of different status, with subordinates having faster sperm than dominants (Froman et al. 2002; Burness et al. 2004; Rudolfsen et al. 2006). Moreover, Leach and Montgomerie (2000) observed that stripped ejaculates from sneaker male bluegill contained significantly fewer sperm cells than stripped ejaculates from dominant males. However, subordinate males in the present study tended to hold a larger volume of sperm cells in their ejaculates than dominant males in R1. In R2, on the other hand, there was no difference. Furthermore, dominant males had a larger ejaculate volume in both rounds than subordinate males, which is not in accordance with previous results from Arctic charr (Liljedal and Folstad 2003; Rudolfsen et al. 2006), but in concurrence with results from bluegill (Leach and Mongomerie 2000). A meta-analysis of studies on sperm swimming speed in Arctic charr, with data from the present study included, show that there is an overall difference between dominant and subordinate males (Haugland

2006). As the present study was not able to detect this difference, it is suggested that sample sizes used are not large enough to yield unambiguously significant results.

DD - males

There was no difference in initial sperm swimming speed between R1 and R2, but increases were observed at 30, 40 and 50 s post activation. Spermatocrit levels showed a slight increase, along with a slight drop in sperm volume. There was no difference in motility or sperm index. As for sperm swimming speed, the results do not concur with those of Pizzari and co-workers (2007). They observed that sperm mobility (a measure of sperm velocity) dropped in male fowl that remained dominant. The underlying causality for the different sperm allocation patterns observed in internal and external fertilizing species may be that the male fowl, an internal fertilizer, can monopolize the female to a greater extent than the male Arctic charr, an external fertilizer, can. Thus, the selection pressure for reduced sperm swimming speed when mating in a favourable role may be stronger in internal fertilizers. Furthermore, males included in this study will most likely spawn in disfavoured roles on the spawning grounds, as favoured males are approximately 10 – 15 cm longer (Serrano et al. 2006; personal observation). This may explain why DD-males do not decrease ejaculate investments. How ejaculate traits differ between pre-trial levels and R1 is not known, but there is a possibility that DD-males have already decreased investments in their ejaculate in R1 (Rudolfsen et al. 2006; Serrano et al. 2006). As time from activation increase, externally fertilizing males mating in favoured roles fertilize more eggs than subordinates do (Schulte-Hostedde and Burness 2005), and this may explain why DD-males show increased sperm swimming speed in late post activation.In sum, males attaining and defending dominant roles did not decrease investments in ejaculate quality, as one should expect.

DS - males

There was increased sperm swimming speed at 20 and 30 s post activation from R1 to R2, and also increased spermatocrit parallel to decreasing ejaculate volume. There was no difference in motility or sperm index between rounds. As opposed to what should be expected from sperm competition theory, DS-males did not increase investment in sperm swimming speed 10 s post activation, nor did they increase the sperm index in R2. Male fowl is observed to reduce sperm mobility when going from a dominant to a subordinate role (Pizzari et al. 2007), which is not in accordance with results from the present study. A proximate reason as to why increased sperm swimming speed is observed at 20 and 30 s post activation, but not at 10 s,

can be that the decrease in ejaculate volume from R1 to R2 may influence sperm swimming speed negatively (Engqvist and Reinhold 2007). Also, bio-chemical changes in seminal fluid between rounds, or other mechanisms in relation to the extra-gonadal environment may be in play (Miura and Miura 2003). Even though DS-males show significantly higher spermatocrit values in R2 than in R1, they do not have a higher sperm index. Thus maturation of sperm seems to be constant. In sum, increased investment in ejaculate quality was observed, although this did not entail the measurement of initial sperm swimming speed.

SS - males

There was a decrease in sperm volume from R1 to R2, as well as a decrease in sperm index, i.e. males had fewer sperm cells in their ejaculates in R2. There was no difference in either spermatocrit, sperm swimming speed or motility between rounds. Results regarding sperm swimming speed are in concurrence with Pizzari and co-workers (2007), who observed that SS-male fowl was able to maintain sperm mobility after a second social encounter. Moreover, these results concur with a study on the fastest sperm cells within ejaculates of Arctic charr, where it is indicative that males becoming subordinate does not show any change in sperm swimming speed compared to pre-trial levels (Serrano et al. 2006). Results are also in concurrence with a study on Arctic charr ejaculates involving all sperm cells (Rudolfsen et al. 2006). As opposed to the present study, Rudolfsen and co-workers (2006) observed that males becoming subordinate increased their spermatocrit, but they did not observe the dramatic decrease in sperm volume. The comparison of studies is not truly applicable, as Rudolfsen and co-workers (2006) did not know fish` pre-trial status, but indicate assumptions of fish being subordinate before the social encounter. The lack of increased investment in sperm swimming speed by SS-males may be explained by their small sperm reserves in R2 (Engqvist and Reinhold 2007). As individual stress response may influence social position (Gilmour et al. 2005), and further, influence how plasma androgen levels fluctuate (Castranova et al. 2005), a potential for producing sperm of high quality may be present, but energetic demands may supersede the conceptualization of this potential. In sum, although the effects of being under dominance are apparent in this group, and fewer sperm cells are matured in R2, SS-males are still able to maintain sperm swimming speed.

SD - males

An increase in sperm swimming speed at 20 s post activation was observed from R1 to R2. In addition, increases in both sperm swimming speed and motility at 10 s post activation was

approaching significance. There were no differences in either spermatocrit or sperm index between rounds, and this group was the only group that was able to maintain production of ejaculate volume. Results are conclusive with results from male fowl (Pizzari et al. 2007), who observed increased mobility in sperm from SD-males shortly after a social challenge. However, it has previously been documented that male charr attaining dominance reduce initial sperm swimming speed (Rudolfsen et al. 2006; Serrano et al. 2006), which is not in accordance with results from the present study (note remarks regarding comparison of studies above). Considering that the closure of the egg-micropyle happens when the egg comes in contact with water (Billard 1988), and that fertilization occurs within seconds after gamete release (Liley et al. 2002), there should be selection for high initial sperm swimming speed in external fertilizing fish species. The response in ejaculate investments by males attaining dominance, at least in experimental caging, may be induced by a decrease in stress level from R1 to R2. When stress is reduced in R2, because fish are no longer under dominance, males react with investment in initial sperm swimming speed, thus increasing the short term fitness benefits associated with having competitive sperm at the next spawning event. In sum, against predictions this group was observed to increase rather than decrease ejaculate investment.

Conclusion

The rapid changes in primary sexual characters of Arctic charr suggest highly responsive phenotypic plasticity. The environmental factor triggering these changes seems to be the individual mating role attained as males interact with other males. Status seems regulated by level of stress, and the individual response to stress may be genotypically determined (Pottinger and Carrick 1999). Thus, the individual response to stress seems to be an important mechanism influencing the outcome of sperm competition. A pivotal underlying assumption when discussing the observed changes in ejaculate characteristics is that fish included in this study will most likely spawn in disfavoured roles on the spawning grounds (Serrano et al. 2006; personal observation). Males mating in disfavoured roles will have a decreased probability of fertilization if they do not increase investment in ejaculate quality, compared to males mating in favoured roles. However, males mating in favoured roles can encounter successive opportunities to copulate, and may consequently adjust the ejaculated sperm volume in each spawning event, to the possibility of obtaining future copulations. Thus, males inhabiting different mating roles may differ in short term versus long term fitness benefits as a result of ejaculate allocations. Males attaining and defending favourable mating roles did not decrease ejaculate quality as could be expected. Both SS- and DS-males was able to maintain

investments in their ejaculates competitive ability. Against predictions, subordinate males attaining dominance did not decrease ejaculate investments, but rather increased investments. It seems that both males attaining dominance and males becoming subordinate will experience changes in their ejaculate characteristics. They will have ejaculate`s of optimal competitive ability as a result of their immediate social status. The investments in short term fitness benefits become evident in this experiment, partly, as males becoming subordinate will not increase initial sperm swimming speed because it is possibly at an optimum already, but in particular, as males attaining dominance increase initial sperm swimming speed. Assuming fish inhabit subordinate mating roles on the spawning ground, it is not adaptive to decrease ejaculate investment when a shift in social role can reprogram sperm to be more competitive, in this case increase the short term fitness benefits of an individual male.

Tables

Tables 1 – 4: Changes in sperm trait variables between R1 and R2 in relation to a change in social status; VCL (µm/s), motility (%), spermatocrit (%), volume (ml) and sperm index (ml). Significance denoted with *, ** and *** for alpha- levels < 0.1, < 0.05 and < 0.01, respectively. Test statistics are from students paired t-test, except for sperm volume in SS-males where a wilcoxon signed rank test was used.

Table 1: DD-males (Dominant in R1 - dominant in R2)

Sperm trait	mean (SD)		df	t-value	p	
	R1	R2				
VCL 10 s	119(31)	120(29)	15	-0.1002	0.92	
VCL 20 s	87(21)	93(14)	15	-0.9681	0.35	
VCL 30 s	57(12)	65(7)	15	-2.4765	0.0257	**
VCL 40 s	46(6)	50(4)	15	-2.3953	0.0301	**
VCL 50 s	45(4)	50(5)	15	-3.3782	0.0041	***
Motility 10 s	86(10)	80(18)	15	0.7254	0.5	
Motility 20 s	87(13)	80(16)	15	1.0416	0.3141	
Motility 30 s	82(13)	80(16)	15	0.0747	0.9414	
Motility 40 s	54(24)	61(15)	15	-1.0661	0.3032	
Motility 50 s	32(15)	37(11)	15	-1.4293	0.1734	
Spermatocrit	7.1(2.6)	9.7(3.9)	15	-2.4898	0.03	**
Volume	0.32(.13)	0.24(.16)	15	1.9365	0.07	*
Sperm index	0.022(.011)	0.022(.017)	15	0.0149	0.9883	

Table 2: DS-males (Dominant in R1 - subordinate in R2)

Sperm trait	mean (SD)		df	t-value	p	
	R1	R2				
VCL 10 s	121(37)	126(27)	12	-0.6282	0.5416	
VCL 20 s	87(23)	100(14)	13	-3.2262	0.0066	***
VCL 30 s	58(16)	69(6)	13	-3.6591	0.0029	***
VCL 40 s	46(6)	49(5)	13	-1.5574	0.1434	
VCL 50 s	44(5)	47(4)	13	1.2772	0.2234	
Motility 10 s	82(25)	82(15)	12	0.8566	0.4085	
Motility 20 s	81(23)	81(14)	13	0.2256	0.825	
Motility 30 s	77(20)	79(16)	13	-0.3099	0.7616	
Motility 40 s	56(26)	66(18)	13	-1.1457	0.2726	
Motility 50 s	30(16)	35(12)	13	-1.6489	0.1231	
Spermatocrit	6.9(3.3)	24.4(13.5)	12	-5.6872	0.0001	***
Volume	0.24(.15)	0.06(.07)	15	6.5703	0.00001	***
Sperm index	0.017(.011)	0.013(.014)	15	0.8789	0.3933	

Table 3: SS-males (Subordinate in R1 - subordinate in R2)

Sperm trait	mean (SD)		df	t-value	p		
	R1	R2					
VCL 10 s	120(43)	103(30)	6	0.5107	0.6278		
VCL 20 s	91(29)	87(17)	6	1.1214	0.305		
VCL 30 s	63(17)	66(14)	6	-0.9987	0.3565		
VCL 40 s	49(9)	55(12)	6	-1.2732	0.25		
VCL 50 s	45(9)	52(7)	6	-0.0077	0.994		
Motility 10 s	81(27)	70(30)	6	0.1964	0.8508		
Motility 20 s	83(25)	77(21)	6	0.0451	0.9655		
Motility 30 s	77(29)	79(17)	6	-0.972	0.3686		
Motility 40 s	60(26)	65(25)	6	-1.3222	0.2343		
Motility 50 s	38(19)	47(25)	6	-0.9469	0.3802		
Spermatocrit	18.8(12.7)	15.6(14.7)	5	1.8129	0.1296		
Volume	0.19(.07)	0.05(.08)	11	z=-2.667	0.0076	***	(Wilcox s.r.)
Sperm index	0.036(.031)	0.006(.007)	10	3.6752	0.00428	***	

Table 4: SD-males (Subordinate in R1 - dominant in R2)

Sperm trait	mean (SD)		df	t-value	p	
	R1	R2				
VCL 10 s	113(36)	148(19)	5	-2.1189	0.0876	*
VCL 20 s	85(23)	109(10)	5	-2.9982	0.0302	**
VCL 30 s	59(15)	67(8)	5	-1.1378	0.3068	
VCL 40 s	48(6)	46(3)	5	0.5418	0.6112	
VCL 50 s	45(5)	48(5)	5	-1.583	0.1743	
Motility 10 s	80(14)	93(6)	5	-2.225	0.0767	*
Motility 20 s	80(15)	92(8)	5	-1.9623	0.1070	
Motility 30 s	78(16)	91(10)	5	0.5396	0.6126	
Motility 40 s	54(27)	61(19)	5	-0.9846	0.37	
Motility 50 s	29(18)	31(13)	5	-0.2355	0.8232	
Spermatocrit	11.9(5.8)	8.4(3.9)	7	0.7275	0.4905	
Volume	0.11(.08)	0.12(.13)	10	-0.3228	0.7535	
Sperm index	0.11(.008)	0.09(.009)	10	0.6864	0.508	

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