

Spawning behavior of Arctic charr (*Salvelinus alpinus*): spawning synchrony, vibrational communication and mate guarding.

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*BIO-3950 Master thesis in Biology, Northern populations and Ecosystems
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Front page picture:
*Torvald Egeland at lake Fjellfrøsvatn
observing spawning Arctic charr
during the 2016 spawning season.*

Photo: *Magnus Beyer Brattli*

Acknowledgement

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Magnus Beyer Brattli

Tromsø, May 2017

Abstract

A mismatch between male and female gamete release can result in reduced or failed fertilization in external fertilizers, sperm competition and reduced paternity. Here, I video monitored spawning behavior of free living Arctic charr (*Salvelinus alpinus*), and analyzed details of their reproductive biology. In total, 157 spawnings were recorded. Females clearly preferred spawning with the dominant male, and even under sperm competition a high level of synchrony in timing of gametes released was observed. Although the average sneaker released his milt 0.6 seconds after the spawning female, sneakers spawned with higher synchrony than the dominant male in single male spawning event. Approximately 50% of the recorded spawning events occurred under sperm competition, where each event included an average of 2.7 males. Sneakers were more exposed to sperm competition than dominant males. An influx of males in close proximity to the female occurs during the behavioral sequences leading up to egg release, but this influx seems not dependent on egg release (i.e., in situations of “near” spawnings). This suggests that there is something other than gonadal product that attract males to the spawning female. I suggest that vibrational communication between the spawning couple reveals time of gamete release to surrounding males. This might explain the relative high level of synchrony observed in both male tactics, yet, vibrational communication comes with the cost of higher detectability from surrounding males. Throughout this study, mate guarding seems to be the prevailing factor for paternity in Arctic charr. Mate guarding effect accessibility to females, sperm competition, synchrony of gamete release and subsequent egg predation.

Keywords: *Arctic charr, Salvelinus alpinus, reproductive behavior, spawning synchrony, sperm competition, female choice, mate guarding, quivering, vibrational communication, signal, acoustic communication*

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1 Introduction

In a blink of an eye hundreds of eggs and millions of sperm are released in open water when external fertilizers pass on their genes to the next generation. For external fertilizing species, large eggs are often fertilized by small sperm entering the eggs small single opening (the micropyle) (Kobayashi and Yamamoto, 1981; Yanagimachi et al., 1992). In some salmonids, for example, in trout (*Salmo trutta L*), the micropyle stays open for approximately 40 seconds before osmotic swelling blocks the micropyle and prevents sperm from fertilizing the egg (Ginsburg, 1963; Billard, 1992; Hoysak and Liley, 2001). Unlike mammalian egg cells, the first sperm cell to reach the egg (i.e., that enter the micropyle), fertilizes the egg (Kobayashi and Yamamoto, 1981; Yanagimachi et al., 1992; Hoysak and Liley, 2001). Given these constraints, a mismatch between male and female gamete release can result in reduced or failed fertilization. Additionally, given sperm competition, the blocking of the micropyle by foreign sperm might result in reduced paternity (Kobayashi and Yamamoto, 1981). Synchrony in gamete release is therefore particularly important for external fertilizing species with eggs including a micropyle (Mjølnerød et al., 1998; Yeates et al., 2007).

Annually, breeding Arctic charr (*Salvelinus alpinus*) gathers on specific spawning grounds to reproduce by releasing their gonadal products in the external environment. Here, on shallow water, females ready to release their eggs seem to attract males to their desired spawning site. The spawning males often adopt different mating tactics, either dominant or subordinate, according to their hierarchical status (Sigurjónsdóttir and Gunnarsson, 1989; Figenschou et al., 2007). Their differing status and tactics is easily distinguished by recognizable behavioral and morphological traits (Sigurjónsdóttir and Gunnarsson, 1989). The male spawning tactic may be conditional (Liljedal and Folstad, 2003; Rudolfsen et al., 2006) and body size seems to be an important factor in the choice of spawning tactic (Sigurjónsdóttir and Gunnarsson, 1989). Bigger dominant males often acquire a guarding tactic, protecting and defending the spawning female against other surrounding males by aggressive behavioral traits like biting and chasing (Sigurjónsdóttir and Gunnarsson, 1989). In the presence of a dominant male, smaller subordinate males often adopt a sneaking spawning behavior. Here, sneakers circulate the spawning female and occasionally try to court the female in an inadvertent moment of the protective dominant male. The subordinate sneakers may also try to fertilize the

female gametes by rushing into the spawning site and releasing their milt shortly after the dominant male and the female have spawned (Sigurjónsdóttir and Gunnarsson, 1989). The males spawning tactics seem to be highly plastic as they can shift between dominant and subordinate behavior depending on interactive males (Rudolfson et al., 2006).

Conflicts between males trying to fertilize the eggs is common (Sørum et al., 2011, personal observations). Bigger dominant males have the advantage of spawning close to and in synchrony with the spawning female. Smaller subordinate males, on the other hand, are forced by the aggressive bigger male to spawn out of synchrony and further away from the released gonadal products of the female (Sørum et al., 2011; Egeland et al., 2016). This may leave fewer unfertilized eggs available for the subordinate male and the eggs to be more dispersed and difficult to fertilize. As a consequence of these behavioral characteristics, sperm competition occurs with subordinate males trying to fertilize a limited number of dispersed, unfertilized eggs (Birkhead and Møller, 1998; Egeland et al., 2016).

In species where the males shows alternative reproductive tactics, reproductive behavior is of particular interest (Taborsky, 1998; Hoysak and Liley, 2001). These behaviors are tailored to increase a males chance of fertilizing the eggs, and physiological adaptations to each tactic would involve adjustments of reproductive organs, spermatozoa and other seminal products (Parker, 1984; Taborsky, 1998). Increasing the chance of fertilization by expressing one trait may also reduce the investment in an alternative trait, therefore a trade-off between different traits might be expected (Taborsky, 1998). For spawning Arctic charr, subordinate fish is disfavored during their “delayed gamete release” and increased distance to the already dispersed eggs from the spawning female. Subordinates seem to compensate for these disadvantages by producing more sperm that also swim faster in water than sperm from dominant males (Rudolfson et al., 2006). Yet, sperm from subordinates swims slow in water diluted ovarian fluid, compared with sperm from dominants, suggesting that sperm cells of dominants are tailored to swim in a different environment than sperm from subordinates (Egeland et al., 2016). Thus, sperm competition in charr seem to be a “loaded raffle” (Parker, 1990).

High synchrony in gamete release relies on good communication for a coordinated release. Many species of fish are supposed to use vibrational signals to synchronize spawning (Satou et al., 1991), and for the landlocked red salmon (*Oncorhynchus nerka*) the vibrational signals made by trunk muscle activity during courtship between male and female are detected and processed by the lateral line system to elicit the synchronized spawning behavior (Satou et al., 1994a). These vibrations act as timing cues to enabling synchronicity of the gamete release.

As shown by Sørnum and coworkers (2011), dominant and subordinate charr may differ in how synchronous they manage to ejaculate with the spawning female, both in situations with and without sperm competition. Additionally, the average time delay in gamete release under sperm competition between the dominant male and the first sneaker was 0.68 seconds (Sørnum et al., 2011). Females also initiated spawning with dominant males in 73.3% of all observed events, and 55.6% of the spawning events occurred under sperm competition. Yet, only 45 spawning were included in their study. In order to increase the knowledge about spawning behavior among free-living charr, more and improved data is needed to be able to conduct an experiment that closely mimics the actual spawning situation (see Egeland et al., 2015 for a first attempt). I therefore conducted further observations on spawning individuals of the same population using underwater cameras aimed at stationary females. Although replicating previous observations are relevant (Ioannidis, 2005; Van Bavel et al., 2016), I also had the ability to observe whether quivering from spawning individuals could be detected, as this might explain the influx of males in the proximity to the female right before egg release (see Sørnum et al., 2011).

2 Methods

Some of the data presented in this study have previously been analyzed and described in Sørum and coworkers (2011). In this former study, conducted in 2006-2007, spawning behavior was recorded for 69 hours and 40 minutes, showing 45 spawning events. To increase the sample for the present study, recording of spawning behavior was conducted for 284 hours and 28 minutes during the 2016 spawning season using the same approach as Sørum and coworkers (2011), but with improved camera quality enabling a more detailed evaluation of charr behavior. 110 hours and 42 minutes of the 2016 recordings were analyzed. Her 112 new spawning events were observed and data from 2006-2007 and 2016 were combined and analyzed together. This summed up 180 hours and 22 minutes of analyzed video resulting in 157 spawning events.

The quivering from the courtship behavior of a spawning couple, made a distinguish sound which was recorded by the recording camera. 32% of the 2016 videos were analyzed by only using the sound file to identify a spawning. This resulted in identification of 33 spawning events. The remaining 68% was analyzed by watching the video, resulting in identification of 79 additional spawning events. Controlling the accuracy of only using sound files to localize a spawning, was conducted by first identifying spawnings from the video and then letting a different person identify spawnings from the sound file only. The match between the two separate ways of identifying spawning events was 100%.

2.1 Study site and video recordings

The study was carried out during the spawning period from mid-September to early October in lake Fjellfrøsvatnet, Troms, Norway (N 7669870, 672665). Video monitoring of spawning Arctic charr on their lek sites was conducted on known spawning locations in and around spawning site 3 (see Figenschou et al., 2004). Low density of fish at other spawning grounds prevented the use of these other locations.

Cameras used in the survey varied in technical specifications but all were “action sport cameras” equipped with watertight housing and a wide-angle lens. With 8 cameras in total, all belonging to the GoPro family with models from GoPro hero 2, 3 and 4 (edition: plus, silver and black). Chosen settings for video quality was 1080p with 60fps.

The camera recorded both image and sound, and since there were minor technical differences in camera design, and housing, sound and video quality differed slightly.

On the spawning sites, cameras mounted on tripods were aimed towards stationary females that appeared to be preparing to spawn. The distance from the camera to the spawning female differed from approximately 0.3 to 1 meters. The recording unfolded as long as battery capacity allowed (approx. 90 to 270 minutes), and memory was rarely the limiting factor. The recording cameras were left undisturbed on the spawning site for minimal human interference until replaced by new cameras. The procedure often resulted in an exchange of cameras morning, forenoon and afternoon. Cameras brought in from the spawning site were immediately emptied for videos and recharged.

The spawning event took place in shallow water (0.2 - 2 meters deep) often near land or on banks close to land. The preferred spawning habitats consist of small to intermediate size rocks covered in algae interspersed with areas of gravel. Females ready to release their gametes hover a few centimeters above their chosen spawning site while being guarded by a dominant male. Females seem to get more stationary the closer she is to spawning and this increases the chance of recording the actual spawning event. All recordings were carried out under daylight conditions, yet night and sunset hours might be the periods with the most spawning activity (Bolgan et al., 2017).

2.2 The spawning event and its definitions

In accordance to Sørum and coworkers (2011), spawning was defined when the following 4 different types of spawning behaviors (adapted from Fabricius, 1953; Fabricius, 1954; Sigurjónsdóttir and Gunnarsson, 1989; Sato, 1991; Fleming, 1996) had taken place:

1. The female lay stationary close to the bottom substrate with an erected anal fin and with the upper body slightly pointing upwards.
2. The male (both dominant and sneaker) courted the female as he approaches the female from the back, slowly initiating quivering in the moment his head touches the female's tail. The male's quivering increases as he glides further forward close up to the female's body. The female often responds by quivering shortly after the quivering male touches her body.

3. Quivering increases in strength until both male and female gape. The female often gape first. Gamete release occurs at maximal mouth opening. Males milt can be visible as a cloud in the water and eggs can also be seen both soaring in the water or lying on the bottom substrate. Male and female propels slight upwards and forwards with an open mouth and lifted head.
4. The male and the female separates and returns to where the spawning started. The female often seeks right back to the place where the eggs were released. The male also returns to chase away other males from the spawning location.

In cases of reproductive competition, the sneaker would either dart into the spawning site and release their milt in sperm competition with the dominant male, or single sneakers may court the female to spawn without sperm competition.

2.3 Guarding and sneaking tactics

Stationary females tend to be more aggressive against smaller subordinate males, than against bigger dominant males employing the guarding tactic (Bolgan et al., 2016). Additionally, the dominant guarding male is recognized by his behavioral traits as often consist of: laying above the female, swimming slowly nearby the female or attacking other males (Sigurjónsdóttir and Gunnarsson, 1989). The sneaker, on the other hand, is typically characterized by approaching and swimming slowly near the female (Sigurjónsdóttir and Gunnarsson, 1989). Identifying the type of mating tactic of a male in proximity to the female in a pre-spawning behavior is therefore easy. In the 157 recorded spawnings, every female was protected by one dominant male guarding her from the surrounding subordinate males. Competing males would spawn by either stimulating the female to release her eggs in the absence of a dominant male, or by releasing their milt over the females eggs after the dominant male have stimulated the female to spawn with him.

2.4 Spawning synchrony

The *Avidemux 2.6 video processing* program (version 2.6.18), enabled analysis of spawning synchrony and time of maximal mouth opening defined gamete release (Fleming, 1996; Fitzpatrick and Liley, 2008). Not all the spawning females was appropriately recorded, and in 16 of the total 157 recorded spawnings females spawned

with their head pointed away from the camera or other individuals masked the gaping fish, impeding the exact measurements needed. These spawnings were excluded when estimating spawning synchrony.

2.5 Spawning located by “sound”

The high amplitude quivering of the courtship behavior of a female and a male Arctic charr could be recorded and identified as a distinct sound curve (see figure 1). By placing the camera close to the spawning female, cameras would record vibration as sound from spawning individuals as far as 5 to 6 meters away. All cameras used to record spawning behavior were placed so that vibration could be recorded. Vibrations made by a quivering fish makes a soundwave that is easy distinguishable from other sounds not related to the actual spawning event. Yet, occasionally the recording camera registered soundwaves from spawning individuals located in a blind angle of the camera. Video was therefore used to verify the observed soundwave, and used to locate spawning events. By using the *wave pad sound editor* software (version 6.59) to visualize and analyze the extracted sound files from a recorded spawning video, it was possible to pinpoint the exact time of a spawning. Compared to watching videos in search for spawning events, observing the sound tracks minimizes the time used to discover spawning events from the videos.

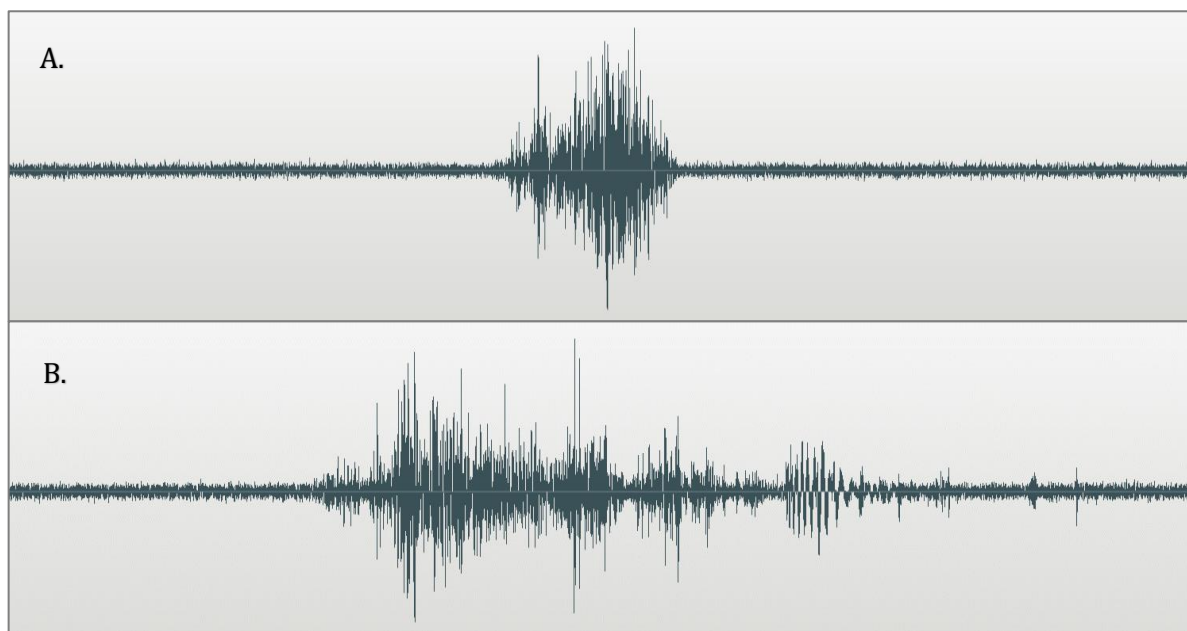


Figure 1: Soundwave extracted from recorded sound fil of spawning Arctic charr: **A:** Soundwave of a spawning without sperm competition. **B:** Soundwave of a spawning with sperm competition including, four spawning males.

2.6 Male density, sperm competition and gamete release

In accordance with Sørum and coworkers (2011), male density was defined as the number of surrounding males within a radius of a fish length distance (approximately 25 cm, see figure 2) from the spawning female. The density was recorded at specific points in time from four seconds before to four seconds after female gamete release. Sperm competition was defined to occur when more than one male released milt at the same spawning event. Asynchrony in gamete release was estimated by noting time of milt release relative to time of egg release at a precision of 16.6 milliseconds (60 frames per second).



Figure 2: Male density was estimated as number of males in proximity to the spawning female (one fish length, ca. 25 cm, illustrated on the picture).

2.7 “Near” spawning: Male density and vibrational communication

Density of neighboring males at “near” spawning events (i.e., where neither the dominant male or the female released their gametes, but apart from this had all other pre-spawning behaviors present) was examined in a similar way as male density in spawning events (see above). Egg release, which did not happen in these cases, was estimated to “occur” after a quivering period comparable to that recorded from actual spawnings. That is, I used average length of the quivering period leading up to real spawnings to estimate the likely spawning time at “near” spawnings. “Near” spawning

events was carefully chosen to fulfill the spawning criteria's, and were therefore not randomly selected. 20 females were observed and one sample per female was included.

2.8 Statistical analysis

In the examined population, individuals were not tagged to avoid influencing the natural spawning behavior. Thus, the possibility of pseudoreplication is therefore present and also likely. Yet, the observations spreads across three years of recording during the approximately one- month long spawning period. Additionally, the 2006-2007 video recordings were carried out on several different spawning sites (Sørum et al., 2011). I therefore assumed the frequency of pseudoreplication was low, and treated each spawning event as an independent data point. I used R software (version 3.2.2)(R Core Team, 2015) and IBM SPSS statistics 24 (version 24.0.0.0)(SPSS, 2017) for the statistical analysis. P-values are reported two-tailed.

3 Results

3.1 Female preference

Females spawned when courted by the guarding male in 125 of 157 spawning events. In three of the 125 cases both dominant and subordinate male courted the female simultaneously. Females indicated a clear preference for dominant male (Binomial test, $n = 154$, $p < 0.0001$).

3.2 Gamete synchrony, sperm competition and different male tactics

The dominant male ejaculated in average 0.13 seconds (SD \pm 0.18, $n = 97$) after the spawning female (One sample t -test, $t_{96} = 7.2$, $p < 0.0001$). The first sneaker, on the other hand, ejaculated on average 0.41 seconds (SD \pm 0.47, $n = 75$) after the spawning female (One sample t -test, $t_{74} = 7.6$, $p < 0.0001$). By pooling all the values of spawning sneakers, the average sneaker spawned with a delay of 0.6 seconds ($n = 106$) after the female. The dominant male released milt first in 73 of the 85 spawning events with sperm competition (89.1%). Here, there was a significant difference in synchrony in milt release between the guarding male, the first sneaker and second sneaker (Brown-Forsythe & Welch F test ANOVA, $n = 57, 60, 22$, respectively, $F_2 = 45.9$, $p < 0.0001$, see figure 3).

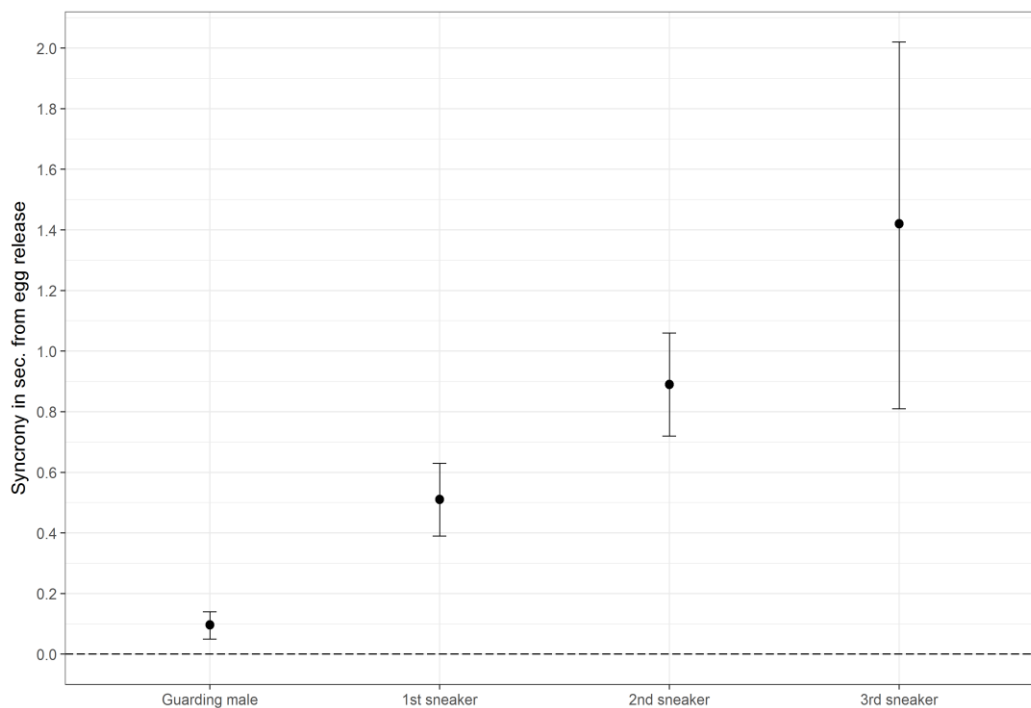


Figure 3: Synchrony (mean and \pm 95% CI) between male milt release and female egg release under sperm competition ($N=85$). Sample size differs among male spawning tactics. Dotted horizontal line indicates time of female egg release.

In single spawning events, the female spawn with the sneaker in 17 of the 72 events (Binomial test, $n = 72$, $p < 0.0001$). Moreover, in these single male spawning events, sneaker milt was released more in synchrony with the female egg release, compared to milt release of the guarding males (Mann-Whitney U-test, $U = 114$, $p < 0.0001$; guarding 0.17 ± 0.18 (mean \pm SD, $n = 41$) and sneaker 0.01 ± 0.1 (mean \pm SD, $n = 15$), see figure 4). Determining time of gamete release was not possible in 16 of the single spawning events. In 72.8 % of the spawning events, the female was the first to release gametes.

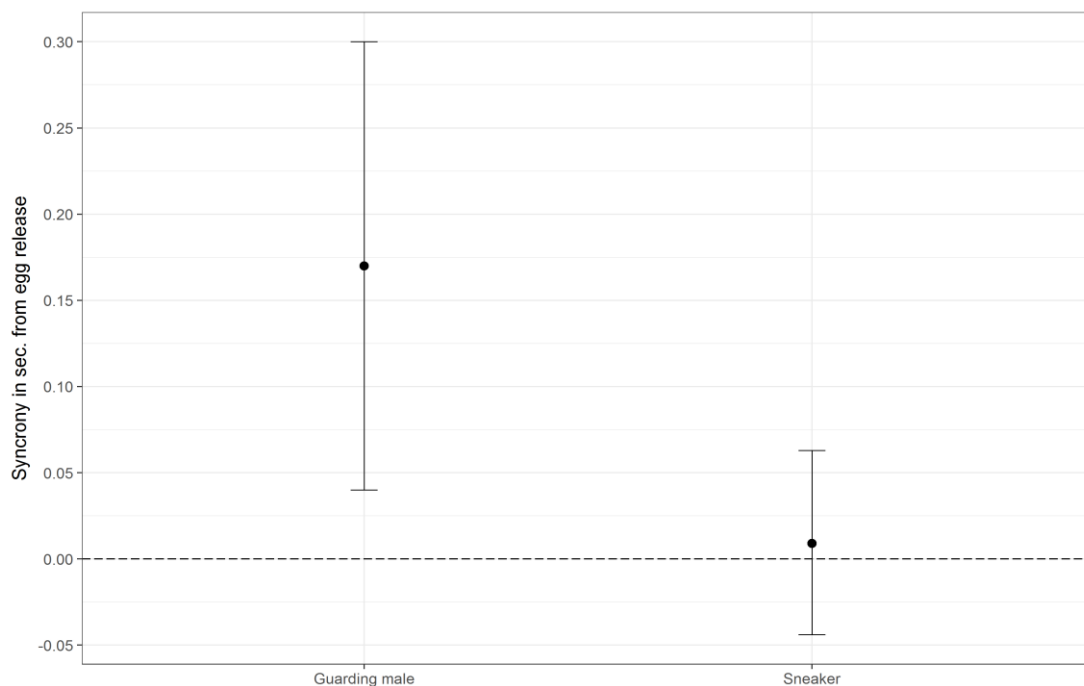


Figure 4: Synchrony (mean and \pm 95% CI) between guarding ($n = 41$) and sneaker ($n = 15$) male milt release in single male spawning events, relative to female egg release. Dotted horizontal line indicates time of female egg release.

3.3 Intensity and risk of sperm competition

Sperm competition can be expressed as intensity (number of males releasing milt during a spawning) or risk (probability of experiencing sperm competition) of sperm competition. I found that there was sperm competition in 85 (54.1%) of the 157 analyzed spawning events. Thus, spawning with more than one male was not significantly more frequent than single male spawning events (Binomial test, $p = 0.34$, $n = 157$). In spawnings with sperm competition, the mean number of males releasing milt was 2.72 ± 0.76 (mean \pm SD, range 2-6, $n = 85$). When including the single spawning events (all 157 spawning events), the mean number of male releasing milt during egg

release decreased to 1.92 ± 1.09 (mean \pm SD, range 1-6, $n = 157$). In total, I registered 301 male milt releases through the 157 recorded spawning events. 229 milt releases were in sperm competition (76.1%), compared to 72 in single male spawning events (23.9%). Thus, more ejaculates were released in sperm competition than in single spawning events (Binomial test, $p < 0.0001$, $n = 301$). 152 of the 171 (88.9%) sneaker ejaculates experience sperm competition whereas 79 of the 130 dominant ejaculates experienced sperm competition (60.8%). That is, guarding and sneaker males did significantly differ in risk of sperm competition (Pearson's Chi-squared test, $\chi_1 = 32.72$, $p < 0.0001$).

3.4 Male density at female spawning

Male density in proximity to the spawning female started to increase between -1.0 seconds and -0.75 seconds before female egg release. That is, density of males which ranges from 0 - 9 during the spawning event, reached its maximum 1.50 seconds after egg release (mean 3.92 males per female, SD \pm 1.73, see figure 5). At the time of egg release, the mean number of surrounding males was 2.16 (SD \pm 1.17, range 1-8). Males released milt from 0.7 seconds before egg release to 2.5 seconds after egg release. During this time window, there was a mean increase of 1.9 males (120%) in the proximity of the female. There was also a mean increase of 0.5 (31%) males in the period from first male released its milt to female egg release.

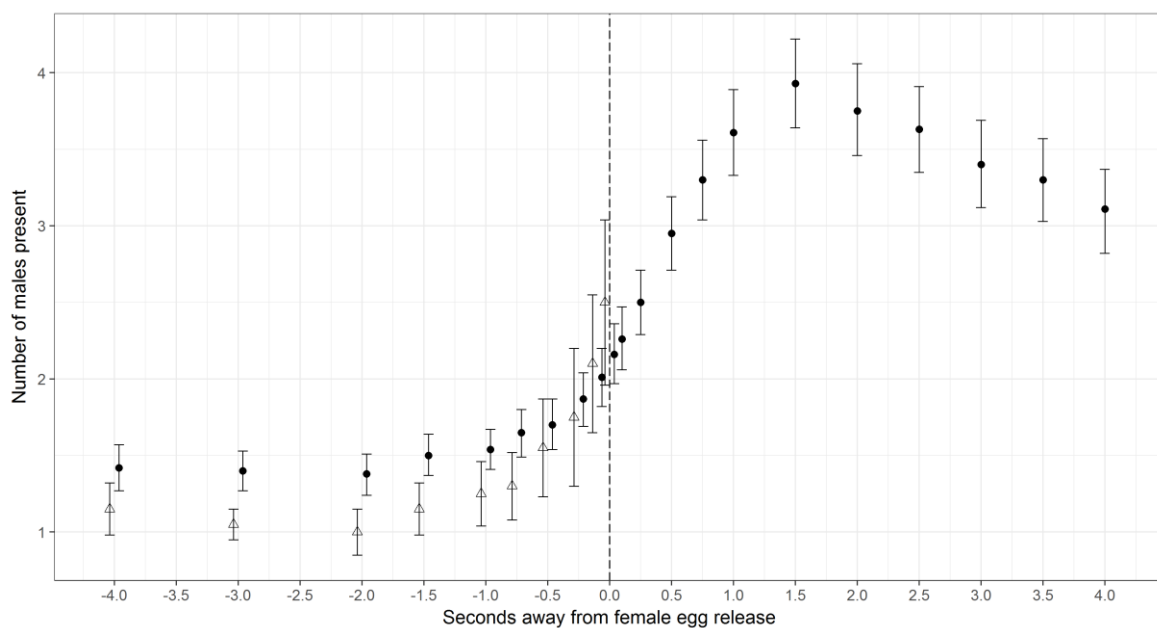


Figure 5: Number (mean and \pm 95% CI) of males in proximity to the spawning female in spawnings (solid circle, $n = 157$) and in "near" spawnings (open triangle, $n = 20$). Dotted vertical line indicates time of female egg release.

3.5 “Near” spawning density

In “near” spawning event there was also a significant increase in density of males in the four seconds preceding estimated female “gamete release” (Pearson correlation test, $r = 0.374$, $p < 0.0001$, $n = 220$, see figure 5). However, compared to spawning event, “near” spawning events had on average fewer males present in the timespan from 2 to 0.75 seconds before “female gamete release” (T-test: $1.58 < t < 2.05$, $0.04 < p < 0.11$, $n = 141$). From 0.5 seconds to female gamete release “near” and actual spawning events were more similar (T-test: $-1.2 < t < 0.67$, $0.231 < p < 0.73$, $n = 141$). At estimated time of “egg release” the mean number of males in proximity to the female was higher in “near” than in actual spawning events (Mean \pm SD, “near” spawning: 2.5 ± 1.15 , real spawning: 2.16 ± 1.75). There was however no correlation between the length of the quivering period and (i) number of males releasing milt (Pearson correlation test, $r = 0.127$, $p = 0.289$, $n = 71$), (ii) density of males in proximity to the female at egg release (Pearson correlation test, $r = -0.003$, $p = 0.982$, $n = 71$), or (iii) with the relative increase of males in the vibrational timespan (Pearson correlation test, $r = 0.149$, $p = 0.214$, $n = 71$). Quivering length of the courting male was measured in 71 spawnings.

4 Discussion

Spawning females were found to prefer spawning with the dominant male. Additionally, like Sørum and coworkers (2011), I found that the spawning female experienced a high level of synchrony in gamete release with the courting male. The females which most often released gametes first were shortly followed by the dominant or subordinate(s) ejaculation. The majority of ejaculates were released under sperm competition and the guarding and sneaker males differed in the risk of sperm competition with a higher intensity of sperm competition among sneaker males. However, in single male spawning events, the sneaker, ejaculated more in synchrony with the female than the dominant male. Additionally, as density of males in proximity to the female right before “egg release” increased also in “near” spawning events, there must be other forms of communication involved in a spawning that than represented by the released gonadal products.

4.1 Female preference

Female preference for dominant males has not previously been documented in charr. Yet, in the present study the majority of females spawned when courted by the guarding male (in 125 out of 157 events). Size is a well-known mate choice criteria in Salmonids (Bolgan et al., 2016), and females have in the presence of small males been shown to delay their spawning, allowing larger males to displace them (Blanchfield and Ridgaway, 1999; Gaudemar et al., 2000). Male size is also known to be an important factor for eliciting the behavior leading to spawning. A study of Atlantic salmon (*Salmo salar*), also indicated that relative mate size seemed to be important for female mate choice, and in the absence of courtship behavior, male size alone increased the spawning behavior of the female (Gaudemar et al., 2000). Yet, I observed that females occasionally also spawned with subordinate males. It seems unlikely that the female, in these spawnings, did not perceive subordinate males as smaller than the dominant male, suggesting that spawning by females were not mistaken. Benefits by females in these cases may arise from exposing eggs to sperm from several males, resulting in higher genetic variation among offspring (Jennions and Petrie, 2000; Reichard et al., 2007). It could be argued that female charr may actually not be performing a direct mate choice, rather a passive choice. Given female choice of spawning ground and nest site, females may express passive mate choice. In case, mate guarding and social dominance among males becomes

paramount. Thus, although females may sometimes exert preference for subordinate individuals, they may mostly be subjected to spawning with the guarding male. In sum, I believe that size-dependent dominance among males is the prime driver in what may appear to be female mate choice in charr.

Salmonid males do not provide parental care, but larger males are better nest defenders, and females might derive direct benefits from spawning with large males through higher egg survival (Bisazza and Marconato, 1988; Berejikian et al., 2000; Kvarnemo and Forsgren, 2000). In charr, eggs are exposed to predation and in this study even females were observed foraging on own eggs (personal observations, appendix figure. A). This has not been documented in other studies. However, analysis of stomach contents have shown that charr are eating eggs during the spawning period (Malmquist et al., 1992). Although some authors have suggested that the dominant male never forage on eggs after spawning (Sigurjónsdóttir and Gunnarsson, 1989), observations of dominant males foraging on eggs they recently may have fertilized occurred (personal observations, appendix figure. A). To conclude whether individuals foraged or guarded their own fertilized gametes, their behavior was monitored for 4-5 minutes, controlling whether eggs were dropped or not. I did not observe this protecting behavior, and seemingly they are foraging on own fertilized eggs. This behavior is potentially caused by failed camouflaging of the eggs in the bottom substrate, and possibly due to high predation pressure in terms of male density surrounding the spawning site, causing male and female to forage on eggs which apparently will not evade predation anyway. Additionally, females spawning with larger males are shown to experience less egg foraging by surrounding males, indicating an indirect benefit of spawning with large males.

4.2 Synchrony

In sperm competition events, females experienced higher synchrony when spawning with the guarding male than when spawning with the subordinate male. By synchronizing the ejaculation with female egg release, the courting male reduces the effect of sperm competition. In Atlantic Salmon, a 2 seconds delay in sperm release reduced paternity by approximately 40% in spawning events under sperm competition (Yeates et al., 2007). Even though the average charr sneaker ejaculate their milt only 0.47 seconds after the dominant male, the effect of sperm competition is necessarily not

comparable in the two species. That is, unlike charr, which spawn in still water, salmon spawn in flowing water, rendering the physical properties of the two fertilization environments quite different. Close imitations of natural sperm competition on charr shows that when sneaker males released ejaculates after the guarding male there was no difference in fertilization success (Egeland et al., 2015). The initial higher sperm velocity and higher sperm numbers may partly compensate for the sneakers lack of synchrony. Yet, this benefit might be outweighed by sneakers lower sperm velocity in water-diluted ovarian fluid compared to dominants (Egeland et al., 2016). In single male spawning events on the other hand, the sneaker males released their gametes with significantly higher synchrony than dominant males. By releasing milt in high synchrony with the female, eggs are forced to pass through a cloud of milt in the water (Fitzpatrick and Liley, 2008). The high synchrony exhibit by sneakers suggest that sneakers lack of synchrony under sperm competition is caused by the dominant male mate guarding, rather than sneakers lack of ability to synchronize gamete release (Sørnum et al., 2011). Thus, mate guarding seems to have an effect on subordinate's ability to synchronize their ejaculation with the egg release.

4.3 Sperm competition

Even though the female was protected by one dominant male in every spawning situation, the bigger male could not prevent sperm competition. Approximately 50% of the observed spawnings occurred under sperm competition and in these cases around 3 males participated. Yet, compared to guarding males, sneakers experience a higher intensity of sperm competition, suggesting that there is an effect of guarding on the likelihood of experiencing sperm competition. Although females also show aggressive behavior towards sneaker males (personal observation), females might have benefits from sperm competition. Females getting their eggs fertilized under sperm competition, are observed to achieve a higher fertilization success and a higher offspring survival relative to females with eggs fertilized by a single male (Shapiro et al., 1994; Keil and Sachser, 1998; Liljedal et al., 1999). Exposing eggs to sperm from several males might result in higher genetic variation in offspring (Jennions and Petrie, 2000; Reichard et al., 2007). Yet, approximately 50% of the observed spawning were single male spawning events. These events may have occurred either when density of surrounding males was low, or when the surrounding males were occupied in intersexual interactions resulting

in a late arrival to the spawning female. Thus, aggressive behaviors from both the dominant male and the female may reduce the intensity of sperm competition, but my estimated number of interacting males in all spawnings (close to 2) hints to a situation where ejaculates investments should be at the highest (Parker et al., 1996). Thus, it is not surprising that it is in this particulate species that tailoring of sperm production to the different fertilizing environments (i.e., one dominant by ovarian fluid and one by water) exist, producing a loaded raffle (Egeland et al., 2016).

4.4 Male density

There was a clear increase of males in proximity to the spawning female seconds before female egg release. A similar increase is observed in “near” spawning events where there is no release of either male or female gametes. This indicates that there is some other factor than gonadal products attracting males to the spawning couple. Signals between the spawning pair are thought to be received visually or tactually, since olfactory signals might be too slow to convey such urgent messages (Uematsu and Yamamori, 1982). Also, it is unlikely that the attractor for sneaker males are visual cue only. That is, individuals headed away from the pre-spawning pair are sometimes observed to turn and head for the spawning pair simultaneously as the courtship quivering begins and before the actual spawning occur (personal observations). Moreover, the spawning individuals in a pair are always positioned such that neither of them would be able to see gamete release from the partner (i.e., it occurs in a dead angle of his/her vision). Thus, communication signals related to spawning synchrony are thought to be detectable vibrations. In captive experiments of spawning behavior of landlocked red salmon, visual patterns are not alone essential for eliciting the male spawning behavior. Yet, the vibrational and visual cues had to spatially coincide with each other to elicit the male spawning behavior (Satou et al., 1994b). So, the observed pre-spawning density increase, could be caused by surrounding males picking up the vibrational signal used between the spawning pair. Vibrational signals could be informing the sneakers about time and space of gamete release, possible explaining the relatively short delay in sneakers milt release and the observed influx of males close to egg release. If noticeable vibrations attract males to the courting couple, it might be argued that a long vibration should attract more males than a shorter vibration. Yet, no correlation was found between length of vibration and the number of males related to

the spawning event. Thus, rather than length of vibration, frequency might be the important component of vibrational communication. This concurs with findings in landlocked red salmon where the male behavior was clearly influenced by the vibrational frequency of the model female (Satou et al., 1994b). Consequently, the frequency of vibrational signals could be the main signal to how the spawning pair synchronize their gametes release and surrounding males may eavesdrop on these signals for synchronizing their spawning.

Throughout this study, mate guarding seems to be the prevailing factor for paternity in Arctic charr. Mate guarding effect accessibility to females, sperm competition, synchrony of gamete release and subsequent egg predation. Thus, mate guarding influences the outcome of the spawning situation, effecting fertilization and paternity. By obstructing competition, advantageous positioning, tailoring of sperm production and synchronized milt release, dominant male's sperm have increased chances of reaching the micropyle. Yet, a synchronized gamete release requires good communication, and charr seem to have developed signals to synchronize gamete release with the cost of increased detectability by surrounding males. The latter, result in more intense sperm competition. Applying vibrations decreases the apparent benefit of being dominant as it also enables subordinates to engage in sperm competition and synchronize their ejaculation. Conceivable, this could be the reason to why sneaker behaviors and sperm competition among charr evolved in the race against the micropyle.

5 Further recommendations

In order to determine the influence of vibrational communication on male density surrounding the female, experiments of vibrational communication through “sounds” would be of interest. Reproducing the “sound” of courting individual on the spawning ground, while monitoring the behavior of the fish in proximity to the sound source would help us understand how males are able to anticipate time of female gamete release. Additionally, considering egg foraging, it could be possible to conduct an experiment to test whether this behavior is due to failed camouflaging of the eggs in the bottom substrate, or due to high predation pressure in terms of male density surrounding the spawning site. By placing a glass plate between the female and the bottom substrate, and then remove and render all individuals except the spawning couple (by fine masked nets), it would be possible to test the explanation of egg foraging. The glass plate would make the eggs visible and exposed, and the yarn would prevent the spawning couple from experiencing egg foraging by other individuals. Additionally, it would be of interest to test whether this phenomenon of foraging occurs more frequently under single spawning events as under sperm competition events.

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7 Appendix

A.

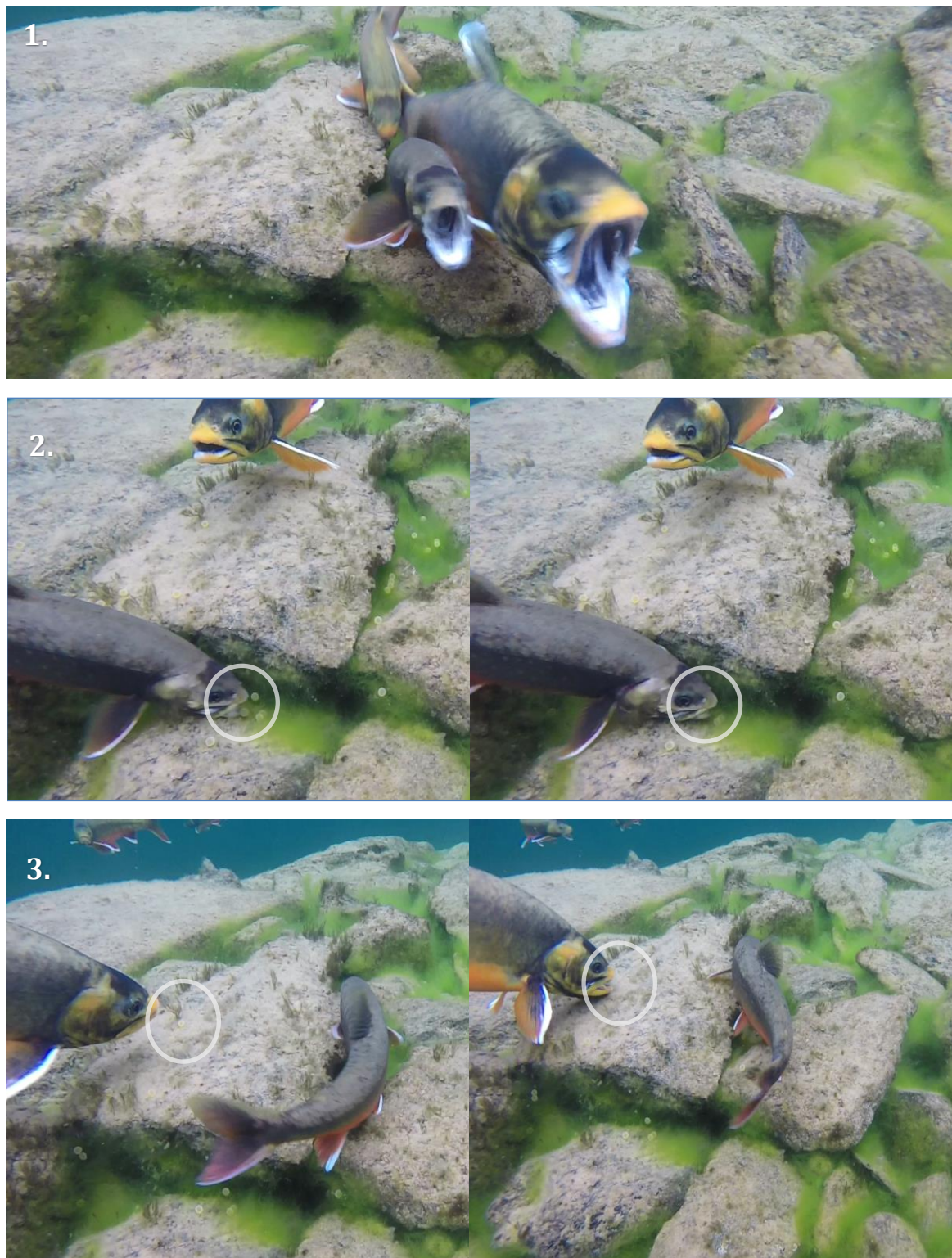


Figure A: 1: Gamete release under sperm competition. 2: Female forage on own eggs. 3: The spawning dominant male forage on eggs he may have fertilized. Individuals foraging on eggs are the spawning couple shown in in picture 1.

