Hatchery selection may depress the number of motile sperm but intensify selection for their swimming velocity in the Arctic charr

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RUNNING HEADLINE: Hatchery selection on sperm traits

Abstract The ability of captive breeding programs to maintain genetic diversity and fitness has often been questioned. Recent studies suggest that fitness loss can be extremely rapid in various traits, but it is poorly known how captive breeding affects sperm quality and thus male fertility. We studied the potential effects of hatchery-induced selection on traits indicative of semen quality, in four generations of captive bred Arctic charr *Salvelinus alpinus* L. We found that the number of motile sperm cells decreased, but that the swimming velocity of the sperm increased over generations. The independent effects of inbreeding and hatchery selection on semen traits could not be separated, but since in small captive broodstocks both of them often act together, the present results should indicate real changes of semen traits in such situations. Taken together, the present data suggest that the fitness loss in some semen traits (number of motile sperm) can be extremely rapid, but selection on other, closely-related traits (swimming velocity) may delay or counteract the overall deterioration of male fertilizing ability during captivity.

Keywords: captive breeding, fitness, hatchery selection, inbreeding, sperm quality

Introduction

Many endangered fish populations are routinely maintained in hatcheries, using artificial fertilization (Utter and Epifanio 2002; Wedekind et al. 2007). Captive breeding practices often produce variation in male fertilization success, which increases the risk of inbreeding and may cause the loss of genetic diversity. Inbreeding has severe effects on individual fitness (e.g. Hedrick and Kalinowski 2000; Drayton et al. 2007) and traits that are closely related to reproduction (Zajitschek et al. 2009). In addition to the loss of genetic diversity, captive breeding may also cause selective changes in various phenotypic traits (e.g. Fleming et al. 2002; Ford 2002; Frankham 2008). Captive environments are often radically different from the natural habitats, and this selection pressure may favour phenotypes that are maladaptive in the wild (Lynch and O'Hely 2001; Wedekind 2002; Heath et al. 2003; Saikkonen et al. 2011). Although selective changes in morphological and behavioural traits during captive breeding have been well demonstrated (e.g. Hard 1995; Håkansson and Jensen 2005), it is poorly known how captivity selection affects male fertility.

Theoretically unusually high sperm volumes (large sperm: egg ratio) commonly used in *in vitro* fertilizations (Rurangwa *et al.* 2004) may lead to relaxed selection on some semen quality traits in captivity, which could in turn lead reduction in sperm quality. On the other hand the fact that captive breeding practices increases the risk of inbreeding suggest that potential decline in sperm quality may be directly related to inbreeding depression. Supporting this view the detrimental effects of inbreeding on semen traits have been well demonstrated: It reduces the ejaculate volume, number of motile sperm and/or number of normal sperm (Roldan et al. 1998; Gomendio et al. 2000; van Eldik et al. 2006; Gage et al. 2006; Fitzpatrick and Evans 2009; Zajitschek et al. 2009). However, selection and inbreeding often have opposite effects on fitness-related traits, and it has been demonstrated that selection can delay or hinder the detrimental effects of inbreeding (Connor and Bellucci 1979; Wade et al. 1996). Furthermore, genetic changes in one sperm trait can generate evolutionary responses in other traits (Simmons and Moore 2009). Thus, relaxed selection and/or inbreeding may lead to reduced quality in some semen trait(s), but simultaneously intensify the selection for improved semen quality with respect of some other, closely related trait(s).

We studied the effects of hatchery selection on semen quality in both wild and hatchery reared salmonid fish, Arctic charr *Salvelinus alpinus*. Our primary aim was to investigate whether the differential hatchery history of our study populations and potential effect of inbreeding could have affected ejaculate quality of the males. As semen traits are expected to be under strong directional selection (Konior et al. 2005; see also Moore et al. 2004) and because such traits should strongly suffer from inbreeding depression (Charlesworth and Charlesworth 1999), we expected that measurable effects on these traits should occur, even within a few generations.

Material and Methods

Experimental males

Mature male Arctic charr individuals were haphazardly sampled from the wild (n = 8 individuals), 1st (n = 5) and 4th hatchery generation (n = 4) as well as from a mixed group of 2nd and 3rd generation fish (2nd/3rd generation; n = 4) in October 2007. The

2nd/3rd generation was established by mixing the fish from different generations (22 % 2nd generation and 78 % 3rd generation fish). All the fish originated from the Lake Inarinjärvi (69° 0’ N, 27° 43’ E). In September 2007, the wild fish were gill-netted from their natural spawning areas in the lake. The hatchery fish were obtained from two aquaculture stations of the Finnish Game and Fisheries Research Institute; from Sarmijärvi, Inari (1st and 2nd/3rd generation) and from Ohtaoja, Taivalkoski (4th generation). The initial number of founders (i.e. wild origin fish) was > 30 males and > 30 females in the 1st and 2nd/3rd hatchery generation, but only 2 males and 6 females in the 4th generation. All hatchery generations have been maintained without any additional gene flow from the wild and eggs have been fertilized with paired fertilizations (1 female x 1 male). Due to lowest number of founder individuals and the longest breeding history in hatchery, the detrimental effects of inbreeding were expected to be most evident in the 4th generation. In all generations, selected males were stripped for all available milt for seven to 12 days prior to the experiment and kept isolated from the rest of the population. After the isolation period all the males were stripped again to obtain milt for sperm analyses.

Sperm measurements

Males were anesthetized with tricaine methanesulfonate (MS-222, Sigma Chemical Co., St. Louis, MO, USA) and carefully stripped for all available milt. Then approximately 0.1 µl of sperm were activated with 4.5 µl of 2:1 ovarian fluid: water mixture (Urbach et al. 2005; Janhunen et al. 2009). The ovarian fluids for all males were obtained from three females from the 1st hatchery generation. The sperm of the all males were activated with the ovarian fluid of all three females (full-factorial design). Sperm quality differences between hatchery generations were measured by using computer-assisted sperm analysis (CEROS v.12, Hamilton Thorne Research, Beverly, MA, USA) (see Rudolfsen et al. 2006 and Kekäläinen et al. 2010 for details). The parameters measured included: average path velocity (VAP), straight line velocity (VSL) and curvilinear velocity (VCL) and percentage of motile sperm cells (Rurangwa et al. 2004). Sperm velocity and the percentage of motile sperm cells were measured 20 s after activation. To control for the effect of sperm storage time on motility parameters all the video recordings were performed within 24 hours from the sperm stripping. For statistical analyses, the average value of replicated measures within each male was used. As the three velocity parameters were highly correlated (Pearson, $r > 0.95$ in all cases), only VSL was used in statistical analyses.

Statistical analyses

Main effects of male and female and their interaction (random factors) as well as the main effect of generation (fixed factor) on sperm traits were obtained with linear mixed-effects (lmer) package lme4 in R (version 2.9.0, R Core Development Team 2007). Statistical significance of fixed and random factors and the interaction between male and female were tested using log-likelihood ratio statistics (LLR $\lambda 2$). We followed Baayen et al. (2008) and fitted the models with and without the explanatory variable and compared the quality of the fits between models. Restricted maximum likelihood (REML) method was used for parameter estimation. According to Baayen et al. (2008) significance at the 5% level in a two-tailed test for the fixed effects coefficients were gauged by checking whether the absolute value of the t-statistic exceeds 2. The model fit was verified using visual examination of normal probability plots and residual plots. Percentages of the motile cells were arcsine square root transformed to improve data normality. Ordered-heterogeneity tests (OH tests: Rice and Gaines 1994; Wedekind et al. 2001) were used to analyze the effects of generation on male sperm traits. In OH tests variation among populations (generations in our case) has both a heterogeneity component (*P*-value from a variance heterogeneity test) and an ordering component (measured by Spearman's rank correlation). All presented *P*-values are from two-tailed tests with $\alpha = 0.05$.

Results

The mean total length of the studied males did not differ between generations (ANOVA, $F_{3,20} = 2.313$, $P = 0.112$): 43.2 cm ± 3.9 SE (wild), 47.7 cm ± 1.0 SE (1st generation), 53.6 cm ± 2.4 SE (2nd/3rd generation) and 49.1 cm ± 0.9 SE (4th generation). The sperm velocity and the proportion of motile sperm differed between males, (χ^2 = 75.27 and 4.78, $df = 1$, $P < 0.001$ and $P = 0.029$), which accounted for 72.4% and 67.4% of the total variation in sperm velocity and motile sperm percentages, respectively. In addition, the three females explained a small (2.8%) but significant part of the variation in sperm velocity ($\chi^2 = 8.22$, $df = 1$, $P = 0.004$). No female effect was found for proportion of motile sperm ($\chi^2 < 0.01$, $df = 1$, $P = 0.998$). Male-female interactions were insignificant for both sperm quality measures (both $\chi^2 < 0.01$, $df = 1$ and $P = 0.990$). The effect of generation was statistically significant (χ^2 = 10.76 and 21.95, $df = 3$, $P = 0.013$ and $P < 0.001$ for sperm velocity and motile sperm proportions, respectively) (Figure 1). Motile sperm percentage decreased over generations (OH test, r_sP_c = - 0.999, $P < 0.001$), but the average velocity of the sperm increased (OH test, r_sP_c = 0.790, $P < 0.05$).

Discussion

Our results suggest that during four generations of hatchery breeding the detrimental effects of inbreeding and/or relaxed selection on sperm motility may reduce the number of motile sperm of the Arctic charr. On the other hand, our results also indicate that the observed reduction in motile sperm numbers may intensify selection for faster swimming sperm. We are unaware, whether the observed inter-generation differences in semen traits reflect genetic changes or just phenotypic plasticity. However, since both of them can drive microevolutionary changes within a species, also the phenotypic differences attributable to plasticity may be indicative of ongoing selection (West-Eberhard 1989; Losos et al. 2000).

Although most captive breeding programs aim to maintain genetic diversity and fitness over several generations, even the most carefully designed programs can lead to substantial fitness losses within one or a few generations (Kostow 2004; Araki et al. 2007, 2008; Fraser 2008). However, the evolutionary mechanism causing this fitness decline is unknown (Araki et al. 2007). Semen traits are expected to be one of the first phenotypic traits responding to selection and due to the complexity of spermatogenesis and the highly specialized function of spermatozoa they may be particularly sensitive indicators revealing inbreeding depression (Gage et al. 2006; see also Fitzpatrick et al. 2009). This suggests that the rapid decline in reproductive success of captive bred animals in the wild could be partly related to inbreeding depression at least when the broodstock sizes are small. However, due to large sperm: egg ratios commonly used in *in vitro* fertilizations (Rurangwa *et al.* 2004) it is also possible that high sperm density (unnaturally high number of motile sperm) may lead to relaxed selection on motile sperm.

In the present study 4th generation fish were reared in another hatchery than the 1st and 2/3nd generation fish. Thus, we cannot completely rule out the possibility that differential breeding conditions could have affected our results. Since these hatcheries are located in different water systems, differences in water temperature and certain chemical and physical parameters of the environment could not be controlled. On the other hand, we activated all the sperm in the similar temperature and used the highly concentrated ovarian fluid: water solutions, which were obtained from the same three females, which suggest that the hatchery-specific variation in these factors should not severely bias our results. In addition, the fish in both hatcheries were maintained in similar rearing densities and fed with the same commercial fish food (Rehuraisio, Emo-Vital, astaxanthin content 80 mg kg^{-1}), suggesting that hatchery-specific sperm quality differences were not related to nutritional or stress related differences between hatcheries. Furthermore, the omission of the 4th generation males may not dramatically change our main conclusions: The parallel trend in motile sperm numbers is still present and the sperm velocity tends to be higher in generations 1 and 2/3 than in wild fish, although the positive trend disappears (see Figure 1). Moreover, the mean size of the fish did not differ between generations, suggesting that size-related differences did not bias our results.

In the present study, it was not possible to experimentally manipulate the level of inbreeding and thus study the effects of hatchery selection independently from the effects of inbreeding. Therefore, we cannot make a clear distinction between these two underlying causes. However, as in many captive breeding programs the sizes of the broodstocks are often small, the detrimental effects of inbreeding often cannot be avoided (Fraser 2008). Thus, in many cases inbreeding and hatchery selection unavoidability act together, which suggest that present results could indicate true selective changes of sperm traits during hatchery rearing.

In conclusion, our results suggest that the interaction of hatchery-induced selection and inbreeding can reduce motile sperm numbers in males even within a few hatchery generations, but that the selection for increased sperm swimming velocity may reduce or hinder the fitness loss of the males. Although idea of captivity-induced changes in semen traits has received some theoretical support, to our knowledge, this is the first indication that such changes may really take place in practice. Even if differential breeding conditions may not seriously bias our results, further studies controlling for this potentially biasing effect are needed to determine the generalizability of our findings.

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Figure 1. Mean (± SE) sperm velocities (VSL, μm s^{-1}, a) and mean proportion of motile cells of males (b) in different hatchery generations.