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ARTICLE

Sperm Quality of Hatchery-Reared Lake Trout Throughout the Spawning Season

Katelynn Johnson and Ian A. E. Butts
Department of Biological Sciences, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B 3P4, Canada

Chris C. Wilson
Ontario Ministry of Natural Resources, Aquatic Research and Development Section, Trent University, 2140 East Bank Drive, Peterborough, Ontario K9J 7B8, Canada

Trevor E. Pitcher*
Department of Biological Sciences, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B 3P4, Canada

Abstract

The objective of this study was to investigate variation in sperm quality metrics (motility, velocity, linearity, longevity, and density) of hatchery-reared Lake Trout *Salvelinus namaycush* throughout the spawning season. Seasonal variation in sperm quality was investigated using both a regression and repeated-measures approach. Sperm was collected from the same 16 individuals over four sampling periods, separated by 3-week intervals. Regression analyses showed that 7–27% of the variation in sperm traits could be explained by seasonal variation, indicating that seasonality can have a significant impact on the quality of sperm. Significant positive linear relationships were found for percent motility and linearity at 5 s postactivation. Significant negative quadratic relationships were found for velocity at 5 s postactivation, longevity, and density, whereas a positive quadratic relationship was found for linearity at 10 s postactivation. Repeated measures ANOVAs showed a significant effect of season for percent motility and linearity at 5 and 10 s postactivation, velocity at 10 s postactivation, and longevity. Our findings are important for optimizing fertilization protocols for hatchery production and can also be used to understand reproductive biology and ecology of wild Lake Trout stocks.

Lake Trout *Salvelinus namaycush* have historically been a significant commercial and economic species important to North American inland lakes and the Great Lakes. Over the past half-century, declines in a number of Lake Trout populations, largely owing to Sea Lamprey *Petromyzon marinus* predation and overfishing (Jensen 1994; Walters et al. 1980), have resulted in the establishment of restocking and rehabilitation programs (Piller et al. 2005). Although captive breeding of this species has occurred for several decades, only remnant populations remain in some of the Great Lakes, including Lake Huron and Lake Superior (Guinand et al. 2003). Today, hatchery populations are used primarily to stock lakes in need of rehabilitation (Evans and Willox 1991). As restoration efforts have become increasingly relevant, investigating gamete quality under a captive environment is important for the successful propagation of this species.

Sperm quality, which includes measures of sperm motility, velocity, longevity, and density, are considered the primary determinants of fertilization success (Casselman et al. 2006; Tuset et al. 2008). Essentially, these sperm quality indices can be used to optimize fertilization protocols, which become important for producing large numbers of progeny from a limited number

*Corresponding author: tpitcher@uwindsor.ca
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of eggs. Sperm quality has been found to be highly dependent on season (Babiak et al. 2006; Billard 1986; Munkittrick and Moccia 1987). For example, in Atlantic Halibut *Hippoglossus hippoglossus*, sperm density, percentage of motile cells, velocity, and linearity were found to vary significantly throughout the course of the spawning season (Babiak et al. 2006). The most common pattern of seasonal change in sperm quality across species is linear or quadratic. Linear patterns generally increase or decrease steadily throughout the spawning season (e.g., Butts et al. 2010), whereas quadratic patterns generally represent bell-shaped curves in which sperm quality increases at the start of the season, peaks during the middle, and decreases towards the end of the season (e.g., Papadaki et al. 2008). It is therefore important to optimize reproductive protocols to reflect the natural biology of propagated species.

An understanding of seasonal changes in sperm quality can be used to optimize fertilization success for hatchery production (Suquet et al. 1998), improve gamete storage techniques (Rideout et al. 2004), increase the efficiency of selective breeding programs (Butts et al. 2010), and assess the impacts of sperm aging (Alavi et al. 2008). In this study, we assessed variation in sperm quality of hatchery-reared Lake Trout over a 2-month interval that spanned the natural spawning season.

METHODS

Broodstock and sperm sampling.—Variations in sperm quality traits were assessed for 7-year old hatchery-reared Lake Trout from the Ontario Ministry of Natural Resources Codrington Fisheries Research Facility, Codrington, Ontario. Fish originated from wild spawn collections in 1983 (two generations earlier), from native Lake Trout populations in two interconnected lakes in Haliburton, Ontario (Clean and Macdonald lakes: lat 45.2501, long −78.5329). All fish were housed in two fiberglass raceway tanks (6.1 × 1.5 m working depth of 0.6 m) fed by untreated water from a local stream source and were kept under a natural photoperiod and temperature regime. Fish were fed AquaBrood feed (7.5-mm pellet, 45% protein, 20% lipid; Corey Nutrition Company, Fredericton, New Brunswick) at 0.5% body weight per day. At 3-week intervals in 2010 (October 28, November 16, December 7, and December 27) milt was collected from the same 16 individuals in the context of a repeated measures experimental design. The mean ± SE total length and weight of the broodstock at the onset of spawning were 582.94 ± 6.73 mm and 1,894.12 ± 79.22 g. Fish were anaesthetized using 40–50 mg/L solution of MS-222 to minimize stress during handling and stripping (Syndel International, Vancouver, British Columbia). Milt samples were collected, using slight pressure to the abdomen and massaging towards the urogenital pore, in 532-mL Whirl-Pak plastic bags (Nasco, Newmarket, Ontario) and stored in a cooler. Extra care was taken to ensure that urine, feces, blood or water did not contaminate the milt sample. The initial male ejaculate was discarded in a standardized manner. Approximately 2 mL of ejaculate was then collected using sterilized Pasteur pipettes.

Sperm activity.—Sperm were video-recorded using a digital black and white video camera (XC-ST50, Sony, Japan) module at 50 Hz vertical frequency, mounted on an external-phase contrast microscope (CX41 Olympus, Melville, New York) with a 10 × negative-phase magnification objective (Pitcher et al. 2009). Sperm metrics (motility, velocity, longevity, and linearity) were assessed by activating an aliquot (<0.2 μL) of milt into a chamber of a 2X-CEL glass slide 110 (Hamilton Thorne, Massachusetts) covered with a coverslip (22 × 22 mm) with 10 μL of stream water. A bionomic controller (model BC-110) and heat exchanger (model HEC-400, 20/20 Technology Inc., Wilmington, North Carolina) were used to maintain water temperature at 8.6 ± 0.1°C (the temperature of the activation water during the first sampling date). Once recordings were taken, sperm traits were analyzed using the HTM-CEROS sperm analysis system (version 12, CEROS, Hamilton Thorne Biosciences, Beverly, Massachusetts) set at the following: number of frames = 60, minimum contrast = 11, photometer = 55–65, minimum cell size = 3 pixels. Sperm motility, velocity, and linearity were analyzed at 5 and 10 s postactivation. Sperm motility was calculated as the percentage of motile cells divided by the total number of cells. Sperm velocity was measured as the average velocity measured over the actual point to track followed by the cell. Sperm-path linearity was measured as the departure of the cell track from a straight line. Linearity is the straightness with which a sperm cell moves per unit of distance traveled. Straighter swimming sperm will have a larger linearity value. Longevity was estimated as the time for approximately 95% of the sperm cells to become immotile (Gage et al. 2004). For each male, the mean value of all sperm cells per each activation was used for statistical analysis.

Sperm density.—Sperm density was estimated by adding 1.5 μL of milt to 500 μL of Cortland's saline solution (7.25 g/L NaCl; 0.38 g/L KCl; 0.47 g/L $MgSO_4$: $7H_2O$; 0.4 g/L Na_2HPO_4: H_2O; 1.0 g/L $NaHCO_3$; 0.22 g/L $MgCl_2$; 1.0 g/L $C_6H_{12}0_6$) to prevent activation. The sperm suspension was then gently mixed using a wide-bore transfer pipette, and 10 μL was loaded onto a Neubauer-improved haemocytometer. Sperm cells were counted in 5 of the 25 squares (1 mm^2) on the haemocytometer (4 corner squares and 1 middle square). Sperm density was then estimated by taking the mean number of sperm cells in the 5 squares, multiplying by 25 and then by 10 (the depth of each chamber in the haemocytometer). This number was then multiplied by the initial volume of the sample to obtain the total number of sperm cells in 1 mL of milt (Pitcher et al. 2009).

Statistical analyses.—To examine sperm quality of Lake Trout throughout the spawning season, we used two statistical approaches. In the first approach, we examined seasonal variation in sperm motility, velocity, linearity, longevity, and density by fitting either linear or quadratic equations to the data (PROC REG; SAS Institute 2003). This allowed us to create predictive models to explore the shape (positive or negative) of

seasonal variation. Linear and quadratic equations were chosen a-priori to fit the data based on the available literature (e.g., Lahnsteiner et al. 1996, 1998; Butts et al. 2010). Final equation selection (linear or quadratic) was based on an F-statistic: $df_j(r^2_j - r^2_i)/(1 - r^2_j)$, where: r^2_i = the r^2 for the ith order, r^2_j = the r^2 for the next higher order, df_j = the degrees of freedom for the higher-order equation with j degrees of freedom in the numerator and $df_j = n - j - 1$ degrees of freedom in the denominator (McDonald 2009).

In the second approach we analyzed the data using a series of repeated measures mixed-model ANOVAs (PROC MIXED; SAS Institute 2003). By using this approach we were able to determine how the four sampling periods differed throughout the spawning season (i.e., October 28 versus December 7). Repeated measures mixed-model ANOVAs, for sperm-related variables, were run at each postactivation time. Akaike's (AIC) and Bayesian (BIC) information criteria were used to assess which covariance structure (compound symmetry, autoregressive order, or unstructured) was most appropriate (Littell et al. 1996). Sampling date was considered fixed, whereas male identity was considered random and included as the subject in the repeated statement. Tukey posthoc analyses were used to compare least-squares means between treatments.

All data were analyzed using SAS statistical software (version 9.1; SAS Institute Inc., Cary, North Carolina). Residuals were tested for normality using the Shapiro–Wilk test, and homogeneity of variances were tested using plot of residuals versus fit values. Sperm velocity, density, and longevity were $\log_{10}$ transformed, while sperm motility and linearity were arcsine square-root-transformed when data deviated from normality and homoscedasticity (Zar 1996).

RESULTS

Motility

There was a significant positive linear relationship between sampling date and sperm motility at 5 s postactivation ($r^2 = 0.18$; $F_{1, 63} = 14.0$, $P < 0.001$, $y = 8.34x + 39.28$); however, there was no significant relationship at 10 s postactivation ($r^2 = 0.07$; $F_{2, 63} = 2.5$, $P = 0.09$, $y = 21.51x + 3.41x^2 + 17.32$; Figure 1a). Sampling date had a significant effect on sperm motility at 5 s ($F_{3, 35.1} = 6.43$, $P = 0.0014$) and 10 s postactivation ($F_{3, 33.6} = 7.82$, $P = 0.0004$; Figure 1b).

Velocity

There was a significant negative quadratic relationship between sampling date and sperm velocity at 5 s postactivation ($r^2 = 0.10$; $F_{2, 63} = 3.23$, $P = 0.046$, $y = 40.93x - 8.27x^2 + 70.0$) and a marginally significant negative quadratic relationship at 10 s postactivation ($r^2 = 0.09$; $F_{2, 63} = 3.05$, $P = 0.05$, $y = 23.64x - 4.88x^2 + 49.73$; Figure 1c). Sampling date had a significant effect on sperm velocity at 10 s postactivation ($F_{3, 32.9} = 3.81$, $P = 0.02$) but not at 5 s postactivation ($F_{3, 44.9} = 2.04$, $P = 0.12$; Figure 1d).

Linearity

There was a significant positive linear relationship between sampling date and sperm path linearity at 5 s postactivation ($r^2 = 0.27$; $F_{1, 63} = 22.99$, $P < 0.0001$, $y = 6.77x + 44.56$), while a significant positive quadratic relationship was found at 10 s postactivation ($r^2 = 0.21$; $F_{2, 63} = 8.31$, $P < 0.001$, $y = -18.75x + 4.55x^2 + 79.92$; Figure 1e). Sampling date had a significant effect on sperm path linearity at 5 s ($F_{3, 34.1} = 11.2$, $P < 0.0001$) and 10 s ($F_{3, 33.4} = 9.2$, $P = 0.001$) postactivation (Figure 1f).

Longevity

A significant negative quadratic relationship was found between sampling date and sperm longevity ($r^2 = 0.13$; $F_{2, 63} = 4.40$, $P = 0.02$, $y = 7.30x - 1.69x^2 + 17.66$; Figure 2a). Running the analysis of variance (ANOVA) showed that sampling date had a significant effect on sperm longevity ($F_{3, 35} = 3.2$, $P = 0.036$; Figure 2b).

Density

There was a significant negative quadratic relationship between sampling date and sperm density ($r^2 = 0.12$; $F_{2, 63} = 4.10$, $P = 0.02$, $y = 1.00x - 0.18x^2 + 0.13$; Figure 2c). Sampling date had a marginally significant effect on sperm density ($F_{3, 35.7} = 2.8$, $P = 0.05$; Figure 2d).

DISCUSSION

Quantifying sperm quality throughout the spawning season is important for estimating a stock's reproductive potential (Trippel 1999, 2003), timing of optimal fertilization for hatchery production (Rana 1995), and improvement of short-term and long-term storage (cryopreservation) techniques for many captivity-bred and endangered species (Rideout et al. 2004). Variation in sperm quality across the spawning season has been previously reported for a number of freshwater and marine fishes (e.g., Billard 1986; Munkittrick and Moccia 1987; Beirão et al. 2011). Results have shown that within and across species, seasonal changes in sperm quality can differ. For example, one study on Turbot *Scophthalmus maximus* (Suquet et al. 1998) demonstrated that motility decreased linearly as the spawning season progressed, while a significant linear increase in sperm motility was shown for Red Porgy *Pagrus pagrus* (Mylonas et al. 2003). For sperm velocity, negative quadratic relationships were found in Barbel *Barbus barbus* (Alavi et al. 2008) and Atlantic Cod *Gadus morhua* (Rouxel et al. 2008). Other studies have shown that velocity gradually decreased throughout the spawning season, such as in Eurasian Perch *Perca fluviatilis* (Alavi et al. 2010), while a linear increase in velocity was found in Atlantic Cod (Butts et al. 2010). In terms of longevity, studies on Rainbow Trout *Oncorhynchus mykiss* (Büyükhatipoylu and Holt 1984) and Puntazzo *Diplodus puntazzo* (Papadaki et al. 2008) found negative quadratic

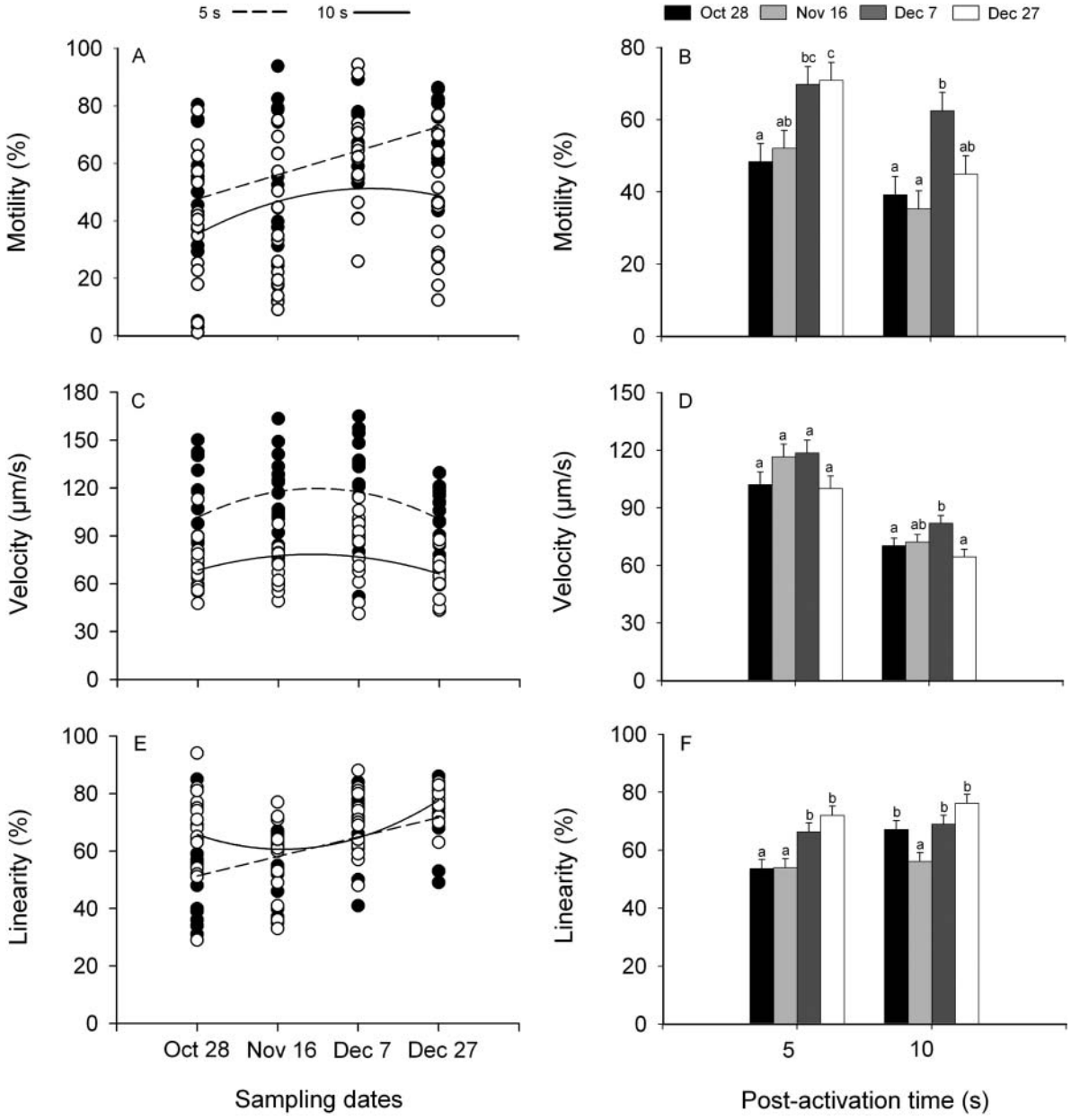

FIGURE 1. Sperm quality metrics measured for Lake Trout. Panels (**A, B**) show motility, (**C, D**) velocity, and (**E, F**) linearity. Filled circles in panels on left (A, C, E) represent mean individual values (± 1 SE) for each of the 16 male Lake Trout at 5 s postactivation; open circles represent mean individual values for 10 s postactivation. Dashed lines in these panels represent sperm at 5 s postactivation; solid lines represent sperm at 10 s postactivation. In panels on right (B, D, F), means (±1 SE) with shared letters did not differ significantly from one another based on Tukey posthoc analyses.

relationships. In contrast, a study on Brazilian Flounder *Paralichthys orbignyanus* (Lanes et al. 2010), found that sperm longevity increased as the spawning season progressed.

For Lake Trout we found significant linear increases for sperm motility and a significant negative quadratic relationship for sperm velocity at 5 s postactivation, as well as a significant negative quadratic relationship for longevity. These results show that even though sperm velocity decreases at the end of the spawning season, sperm still have the ability for movement, although they have a shorter duration of motility. Additionally, sperm quality was generally lower at the onset of the spawning season as they may not have gained the capacity for forward movement (Billard 1986; Mylonas et al. 2003). Essentially, this may be linked to the seasonal variation in progestin, 17α,20ß-dihydroxy-4-pregnen-3-one, which induces sperm maturation in salmonids (Baynes and Scott 1985; Nagahama 1994).

Understanding how sperm density changes throughout the season is necessary for determination of optimal sperm-to-egg

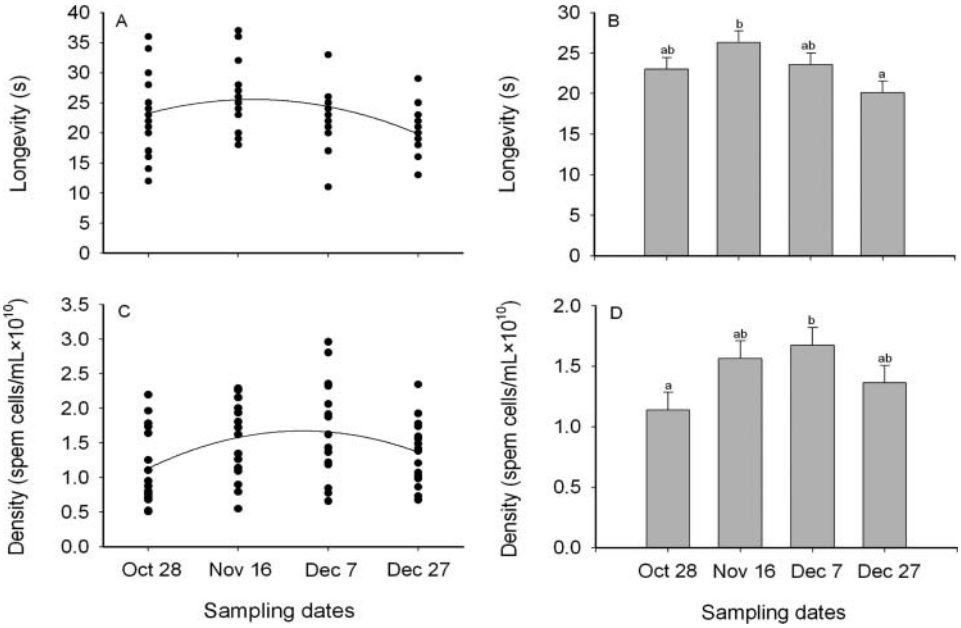

FIGURE 2. The relationship and effect of sampling date as a plot and bar graph on longevity (**A, B**) and density (**C, D**). In panels on right (B and D) means (±1 SE) with shared letters did not differ significantly from one another based on Tukey posthoc analyses.

ratios to essentially maximize fertilization success (Butts et al. 2009; Casselman et al. 2006). Quantifying density can also be useful for estimating the biochemical properties of a known volume of milt (i.e., ATP, protein concentration; Boryshpolets et al. 2009). Here, we found a negative quadratic relationship for sperm density, the densest sperm samples occurring on December 7 (or middle of the season). Studies on Atlantic Cod (Rouxel et al. 2008) and Yamú *Brycon amazonicus* (Cruz-Casallas et al. 2007) found similar relationships. Increases in sperm density throughout the spawning season have been found in Atlantic Salmon *Salmo salar* (Piironen 1985), and Atlantic Cod (Butts et al. 2010; Rakitin et al. 1999). In contrast, studies on Rainbow Trout (Büyükhatipoylu and Holt 1984), Snow Trout *Schizothorax richardsonii* (Agarwal and Raghuvanshi 2009), Brown Trout *Salmo trutta* (Hajirezaee et al. 2010) and Atlantic Salmon (Aas et al. 1991) found that sperm density decreased throughout the season.

In salmonids, gametogensis is a discontinuous process where sperm is released from the sperm ducts over several months, aging throughout the spawning period (Billard 1986). During this timeframe, declines in ATP levels (Dreanno et al. 1999), hormonal activity of the sperm duct (Koldras et al. 1996; Shangguan and Crim 1999), and seminal plasma contents (i.e., ions, proteins, antioxidants; Ciereszko et al. 1996; Hajirezaee et al. 2010) have been reported. All of these biochemical and physiological milt indices have been linked to sperm activity (Lahnsteiner et al. 1996, 1998). Therefore, further work should be undertaken to explore how these indices are affecting specific sperm quality in Lake Trout. In addition, researchers should investigate how diet, stage of maturation, environmental conditions (e.g., temperature, photoperiod), and broodstock stress can affect sperm quality in captivity.

In conclusion, we found that 7–27% of the variation in sperm traits can be explained by seasonal variation, indicating that seasonality can have a significant impact on the quality of sperm motility, velocity, linearity, longevity, and density. Understanding this seasonal variation in sperm quality is important for quantifying paternal effects on fertilization success. For example, based on the available literature for another salmonid (Tuset et al. 2008), seasonal differences in sperm velocity that we have shown would result in about 10% difference in fertilization success. Overall, these findings have relevance for the aquaculture industry and salmonid rehabilitation programs, as well as for estimation of a male's reproductive potential in the wild.

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