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Swimming kinematics and temperature effects on spermatozoa from wild and captive shortnose sturgeon (*Acipenser brevirostrum*)

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ABSTRACT

Computer-assisted semen analysis (CASA) and cluster analysis were used to compare spermatozoa swimming kinematics and milt quality between wild and captive shortnose sturgeon (*Acipenser brevirostrum*). Milt samples from 27 shortnose sturgeon were collected in May 2016 and June 2017. Of these, 19 were wild caught in the Saint John River, New Brunswick, Canada, and eight were from a captive population at the Mactaquac Biodiversity facility. The following kinematic variables were measured immediately following sperm activation (~5 s), at 30, 60, and 180 s post-activation; average path velocity (VAP); straight-line velocity (VSL); curvilinear velocity (VCL); amplitude of lateral head displacement (ALH); beat cross frequency (BCF); straightness (STR); linearity (LIN); wobble (WOB); percent motility (MOT). Analyses were conducted at 7, 10, and 14 °C to determine potential effects of temperature on kinematics. Principal components analysis (PCA) of original kinematic variables yielded two main components, a speed/wobble component along with a movement pattern component. Hierarchical cluster analysis (HCPC) indicated there were distinct subpopulations, with composition of clusters the result of fish source (wild-caught or captive). Wild-caught fish had greater sperm densities ($P = 0.0064$) and sperm swimming speeds compared to captive fish ($P < 0.05$). Temperature had a significant effect only on captive spermatozoa, and this result was not consistent between time periods. There was no effect of hormonal manipulation on spermatozoa motility kinematics. Results indicate there are significant differences in measures of milt quality between wild and captive shortnose sturgeon, indicating an effect of rearing condition on reproductive potential, which may affect fertilization success.

1. Introduction

In terms of reproduction, it is widely viewed that sperm are an infinite resource while eggs are in limited supply (Mcbride et al., 2015; Schültz et al., 2017). In many fish species, milt quantity is limited, however, and ejaculate volumes are hormonally regulated for fish that spawn repeatedly throughout the season (Rakitin et al., 1999; Alavi et al., 2004). Additionally, because in most fish there is external fertilization, with the release of gametes into potentially rapid water flow environments, it is unlikely that unlimited spermatozoa are available to fertilize eggs deposited by females in such conditions (Trippel, 2003; Butts et al., 2012a). There is variation in sperm density and motility variables among species (Alavi et al., 2004), and it is likely that these differences affect not only fertilization success, but also reproductive strategies, and behavior (Cattelan et al., 2016; Lehnert et al., 2018; Poli et al., 2018)

Sturgeon and paddlefish are among the most endangered fish in the world, with all 27 species listed on the International Union for

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Conservation of Nature and Natural Resources (IUCN) Red List (International Union for Conservation of Nature and Natural Resources, 2016). There is a downward population trend for many sturgeon species due to blocked spawning migration passage, overfishing, climate change, environmental degradation, and pollution (Billard and Lecointre, 2001; Bronzi et al., 2011; Alavi et al., 2012a). With the global decrease of important fish stocks, attention has been focused on expanding aquaculture and fish farming practices for many species, including sturgeon, and it is expected that this trend will continue (Kime et al., 2001; Alavi and Cosson, 2005; Dzyuba et al., 2017).

Sturgeon spermatozoa are unique in morphology, physiology, and biochemistry (Linhart and Kudo, 1997; Alavi et al., 2004; Psenicka et al., 2007; Alavi et al., 2009), and have extended periods of motility compared to sperm of other freshwater fish (Linhart et al., 1995; Alavi et al., 2004; Psenicka et al., 2008; Alavi et al., 2012b). The average motility period after activation of salmonid sperm is ≤ 60 s (Percheca et al., 1993; Kime et al., 2001), while sturgeon sperm motility can continue for several minutes or hours (Alavi et al., 2004; Cosson et al., 2008; Psenicka et al., 2010; Alavi et al., 2012b).

Studies of reproduction for both wild and captive fish often requires the use of hormonal stimulants to acquire gametes (Zohar and Mylonas, 2001; Mylonas et al., 2009). The effects of hormone type, dose, and time of collection on sperm production have been studied, and it is generally accepted that use of hormones to stimulate spawning increases spermatozoa motility and osmolality, and affects ionic concentrations of sperm (Ohta et al., 1997; Clearwater and Crim, 1998; Izquierdo et al., 2001; Ingermann, 2008). Although much research has been conducted on milt quality, reports on differences in quality between captive and wild stocks are lacking. It is obvious that environmental conditions differ between captive and wild individuals and it is logical that such differences could affect spermatogenesis, milt quality, and quantity. Understanding the effects of captivity on the reproductive capacity of fish is important not only for conservation efforts but also for aquaculture, as it is becoming the predominant source of fish production globally (Naylor et al., 2000; Subasinghe et al., 2009; Olsen and Hasan, 2012; FAO Fisheries and Aquaculture Department, 2013).

Additionally, examining sperm performance over a range of temperatures can provide insight on potential effects of climate change. For aquatic environments, there are expected changes in thermal regimens due to climate change, and temperature is a key factor affecting gamete quality and development in fish (Pankhurst and Munday, 2011; Dorts et al., 2012; Butts et al., 2014). Climate change also results in the potential for an uncoupling of photoperiod and temperature cues that could alter the reproductive phenology of fish due to asynchronous timing of critical life cycle cues (Hovel et al., 2017; Hillebrand et al., 2018).

Unfortunately, little is known about the reproductive strategies of many sturgeon species (Doroshov et al., 1997; Williot et al., 2005; Secor, 2008). Research on spermatozoa functions and characteristics can provide insight to the species reproductive strategies, evolutionary histories, as well as the effect of domestication on reproductive performance. The objectives of the present study are to 1) examine the effect of temperature and time post-activation on spermatozoa swimming kinematics and 2) determine if there are differences in sperm quality between captive and wild shortnose sturgeon *Acipenser brevirostrum* Lesueur 1818. It is anticipated that this study will provide insight on shortnose sturgeon reproductive biology in the wild, as well as the effects of their domestication on sperm quality, and information that can improve artificial reproduction procedures in captivity.

2. Materials and methods

2.1. Experimental specimens

A. brevirostrum were caught using 5″ gillnets between 11 May and 1 June 2016, and 10 May and 26 May 2017, in the Saint John River, New Brunswick. Nets were deployed for a soak time not exceeding 4 h. Upon capture, individuals were transported to a riverside flow-through holding tank (Sæplast Americas Inc., Saint John, NB, Canada). Individuals were assessed for the capacity to express milt by placing fish in a trough and applying pressure along the abdomen from anterior to posterior. If milt was expressed the individual was placed in a second flow-through tank separate from non-spawning males and females.

2.2. Sperm collection

Males with the capacity for spermiation were placed in a trough with their ventral surface exposed. Pressure was applied from anterior to posterior to express milt. The initial ejaculate was discarded and the area surrounding the urogenital opening wiped dry to avoid contamination with water and fecal matter. A length of Tygon tubing (˜7.5 cm) attached to a 10 mL syringe was inserted into the urogenital opening. Pressure was continually applied to the abdomen while the syringe plunger was withdrawn. When ˜5 mL of milt was collected, the tube was removed, and the remainder of the syringe filled with air and sealed. This ensured enough oxygen remained in the syringe for sperm respiration. A minimum of two 5 mL samples were collected per fish. Milt was placed on a towel over ice in an insulated cooler and transported to the Mactaquac Fish Biodiversity Facility's laboratory where it was then transferred to a refrigerator (˜4 °C). Nineteen wild male *A. brevirostrum* were used for milt analyses. Of these 19 males, 10 readily expressed milt upon capture. An additional nine males not readily expressing milt were selected for hormonal stimulation of spermiation based on ultrasonic imaging of the gonads. These males were treated with luteinized hormone-releasing hormone analogue (LHRHa, Syndel, Nanaimo, BC, Canada) using procedures previously described with sturgeon (Conte et al., 1988). This was to ensure any differences observed between captive and wild fish were not an effect of hormonal manipulation. The same hormonal treatment procedure was conducted with 36 captive fish from the population at the Mactaquac Fish Biodiversity Facility, with successful milt collection from eight individuals. Milt was collected ˜24 h following hormonal treatment. Sperm collection methods for hormonally-treated individuals were performed as previously described in this manuscript.

2.3. Spermatozoa density

Density of spermatozoa was determined using an improved Neubauer haemocytometer and compound microscope (Olympus CX-41) at 10x magnification. Cells were first diluted 500-fold with seminal plasma as this prevents activation. Two counts of four squares (1 mm^2) were conducted and the mean of each square calculated.

2.4. Spermatozoa activity

Three 2.0 mL Eppendorf vials were filled with ˜1.0 mL of milt and placed in one of three temperature baths (7, 10, and 14 °C) representing approximately 3 °C less, similar to, and 4 °C greater than the river water temperature at the onset of spawning. Milt samples were acclimated for ˜5 min before analyses. A vial of activating medium (AM) consisting of river water and 1% bovine serum albumin (BSA by wt/volume) was placed in each temperature bath. The BSA was added to the activating medium to prevent spermatozoa sticking to the slide (Rouxel et al., 2008). Spermatozoa activity was recorded at 0, 30, 60, 180 s post activation using an Olympus CX-41 compound microscope equipped with a 10x negative phase contrast objective and digital video camera (JAI CM-040GE, JAI Inc. Miyazaki, Japan). The 0 s experiments were conducted to determine values for sperm motility variables immediately following activation and were taken within 5 s of activation. The camera was attached to a Dell Latitude E5540 laptop via an Ethernet cable. Images were analyzed in real-time using the CEROS II Hamilton-Thorne computer assisted semen analysis (CASA) software. The system recorded images at 60 frames/s. Prior to analyses, the appropriate dilution of activating medium to milt was determined for each male. The goal is to dilute milt to limit the number to 40–80 spermatozoa per field of view when examined using the microscope. Assessment of this number of spermatozoa allows for greater efficiency when using the CEROS II system. After this ratio was determined the appropriate volumes of milt and AM were pipetted into an Eppendorf tube and shaken for 5 s (final volume ˜1.0 mL). This vial was placed into the appropriate temperature bath for the duration of analyses. Milt/AM (5 μL) was pipetted under a coverslip (35 x 25 mm) into one chamber of a Hamilton-Thorne two-chambered 20 μm slide (2X-CEL, Hamilton-Thorne, Massachusetts, United States of America). Slide temperature was maintained using a Linkam PE120 temperature regulated stage (Linkam Scientific Instruments, Surrey, United Kingdom). To record the 5 and 30 s time intervals, a pipette tip was immersed in a vial of undiluted milt and blotted onto one chamber of a Hamilton-Thorne two-chambered 20 μm slide and covered with a coverslip. Activating medium (5 μL) was placed under the coverslip and as a result of capillary action was drawn into the chamber. It took < 5 s for capillary action to cease and at that point the 5 s time period videos were recorded. The same method was used for the 30 s time period. The order in which temperature treatments were analyzed was randomly generated. The following spermatozoa activity variables were analyzed: VCL-curvilinear velocity (μm/s), sum of the incremental distances moved in each frame along the sampled path divided by the time taken for the sperm to cover the track; VSL-straight line velocity (μm/s), straight line distance between the start and end points of the track divided by the time taken for the sperm to cover the track; VAP- average path velocity (μm/s), smoothed sperm head positions in a running average along the path travelled; ALH- amplitude of lateral head displacement (μm), distance sperm head moves from average path; BCF- beat cross frequency, number of times sperm head crosses the average path trajectory; STR- straightness (%), ratio of VSL/VAP; WOB- wobble (%), ratio of VAP/VCL; LIN- linearity (%) the straight line distance between the start and end points of the track divided by the sum of the incremental distances along the actual path (= VSL/VCL* 100); MOT- motility (%), percentage of motile sperm, those moving a distance greater than their head length.

2.5. Statistical analyses

All data were analyzed using RStudio (R Core Team, 2015), using MASS (Venables and Ripley, 2002), HH (Heiberger, 2018), and FactoMineR (Le et al., 2008) packages. Significance was considered at $P \leq 0.05$. Assumptions of normality and homogeneity of variance were assessed using Shapiro Wilk's and Levene's tests, respectively. Interactions were considered significant at $P \leq 0.05$.

2.5.1. Sperm density

An ANOVA was conducted to determine if there were differences between captive, hormone-treated, and non-treated wild-caught fish. Because there were no differences between the two wild sources, these data were pooled. A Welch's two-sample *t*-test was conducted to determine if there is a difference between males that were captive and wild-caught with regard to sperm density.

2.5.2. Spermatozoa kinematics

Kinematic variables describing spermatozoa are, by definition, correlated and will inflate the chance of committing a Type I error if statistical analysis is conducted for each variable separately. To avoid this potential error, principle components analysis (PCA) was used to generate independent orthogonal axes. The independent axes generated are linear combinations of the initial variables and are no longer correlated. PCA also serves as a data reduction method that has been used when working with CASA data (Núñez-Martínez et al., 2006; Martínez-Pastor et al., 2008). Because a correlation matrix was used for the PCA, there was only interpretation of the components with an eigenvalue > 1, based on the Kaiser selection criterion. Mixed model ANOVAs were performed on each of the components across treatments. Independent variables were source (captive and wild, between subjects factor) and temperature (7, 10, and 14 °C, within subjects factor). Any significant effects were analyzed *post-hoc* using the Tukey's HSD test.

Motility data were analyzed separately from kinematic variables. An ANOVA was performed to determine the effect of source, temperature, and time on motility. Any significant effects were analyzed *post-hoc* using Tukey's HSD test.

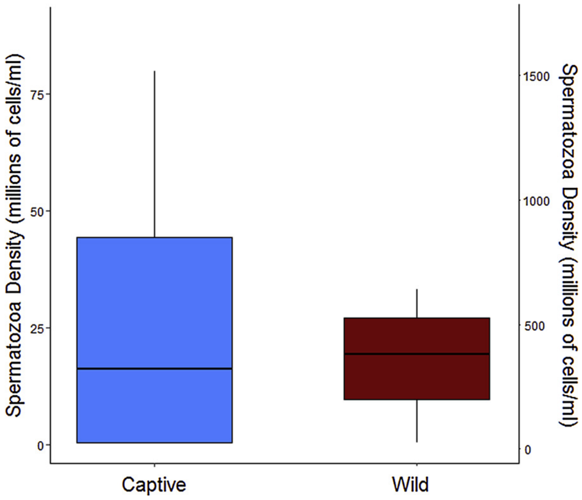

Fig. 1. Spermatozoa density of captive and wild-caught *A. brevirostrum*; Wild-caught fish had greater sperm densities than captive fish (means ± S.E.M, *n* = 8 captive fish, 19 wild-caught fish).

2.5.3. Cluster analyses

Hierarchical clustering of retained principal components was performed on data from each time subset using the HCPC function from the FactoMineR package. This method is used in conjunction with PCA, using the PCA results to identify groups (i.e. clusters) of similar objects, while retaining the original variables to later define clusters. Each spermatozoon was assigned to a cluster such that kinematic measures were similar to other sperm in the same cluster, but significantly different to the kinematic measures of sperm belonging to other clusters. The number of clusters was determined using Ward's Criterion based on Euclidean distances that have previously been reported (Martinez et al., 2013; Ibănescu et al., 2018).

3. Results

3.1. Spermatozoa density

There was no difference in sperm density between hormone-treated and non-treated wild fish (ANOVA, df = 24, $P = 0.466$), so these data were pooled. There was an effect of source on spermatozoa density (Welch's *t*-test, df = 18.67, $P = 0.0002$), with wild fish having a greater density than captive fish (Fig. 1).

3.2. Effect of source and temperature on spermatozoa kinematics

The PCA analysis of all kinematic data resulted in two principal components (PCs) with eigenvalues > 1. These two components explained 89% of the variation. PC1 was heavily loaded with descriptors of swimming speed and wobble and is, therefore, the "speed/wobble" component. PC2 is heavily loaded with beat cross frequency and straightness and is the "movement pattern" component (Table 1).

Although mixed model ANOVA results indicated there were main effects, there was an interaction between source, temperature, and time (df = 717, $P = 0.016$), so data were split by time. This was done to further explore potential effects of source and

Table 1
Contribution of original kinematic variables to initial principal components.

Variable Variance Explained (%)	PC1 (Speed/Wobble) 63	PC2 (Movement Patterns) 27
VAP (μm/s)	0.962166	−0.19999
VSL (μm/s)	0.96749	−0.04475
VCL (μm/s)	0.950612	−0.22379
ALH (μm)	0.66314	−0.54843
BCF	−0.35271	0.805856
STR (%)	0.475191	0.83754
LIN (%)	0.782927	0.55983
WOB (%)	0.923055	0.281759

VAP = average path velocity; VSL = straight line velocity; VCL = curvilinear velocity; ALH = amplitude of lateral head displacement; BCF = beat cross frequency; STR = straightness of track; LIN = linearity of track; WOB = wobble.

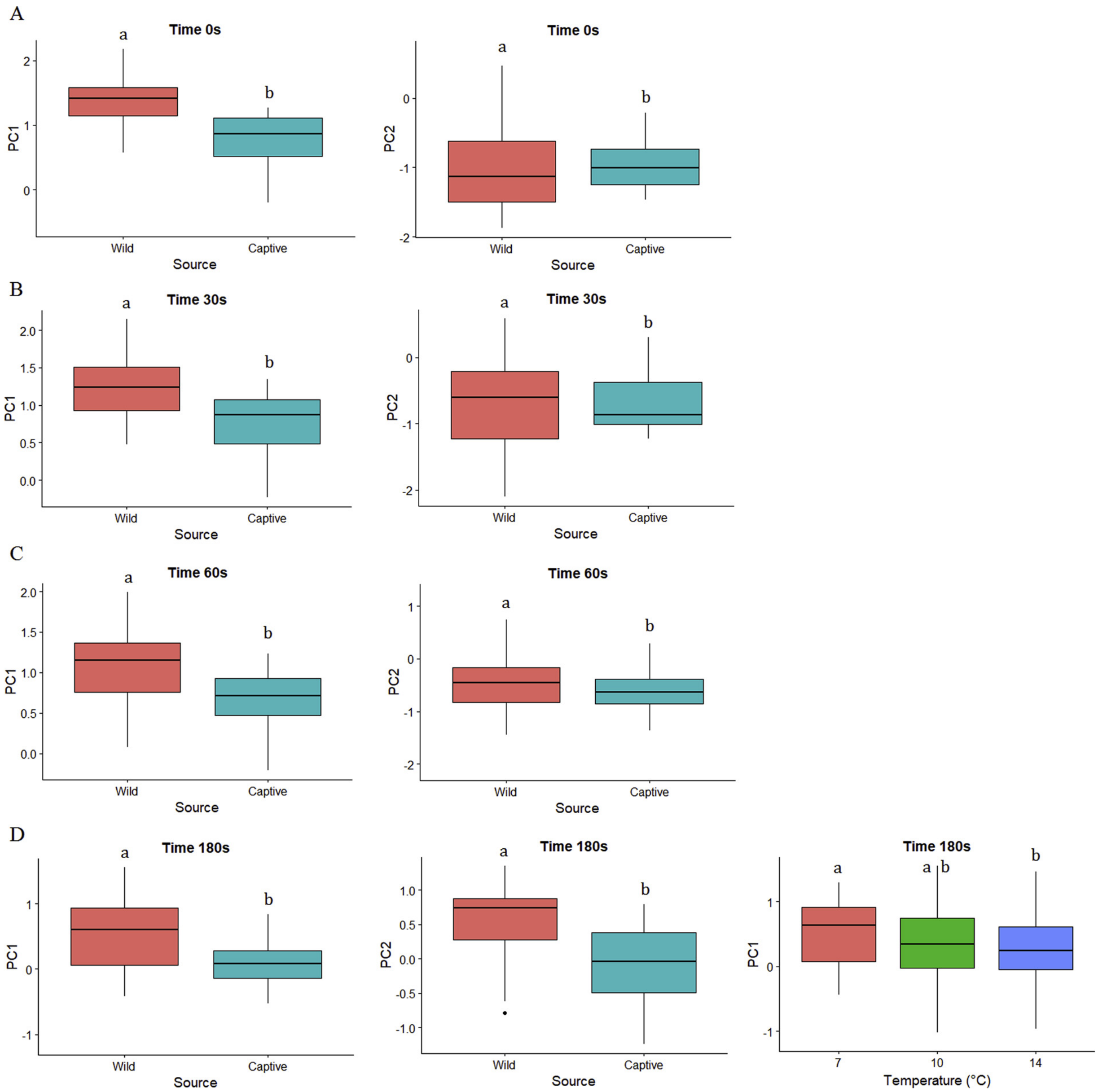

Fig. 2. Plots of significant PCA results for 0, 30, 60, 180 s post-activation (mean ± S.E.M, n = 8 captive fish, 19 wild-caught fish, for each time period); Note temperature was only significant at 180 s post-activation. PC1 = speed/wobble component, PC2 = movement pattern component; Different lower-case letters indicate differences between groups.

temperature on sperm motility kinematics. Fig. 2 depicts only PCA scores with effects for each time period.

The two-way ANOVA results of each time period are subsequently described.

3.2.1. *Time 0s*

There was an effect of source (captive or wild-caught) on both the speed/wobble (ANOVA, df = 24, P = 0.001) and movement pattern (ANOVA, df = 24, P = 0.019) components. There was no effect of temperature on the speed/wobble (ANOVA, df = 46, P = 0.127) or movement pattern (ANOVA, df = 46, P = 0.117) components.

3.2.2. *Time 30s*

There was an effect of source on both the speed/wobble (ANOVA, df = 24, P = 0.015) and movement pattern (ANOVA, df = 24, P = 0.033) component. There was no effect of temperature on the speed/wobble component (ANOVA, df = 46, P = 0.476). There was an effect of temperature (ANOVA, df = 46, P = 0.019) on the movement pattern component.

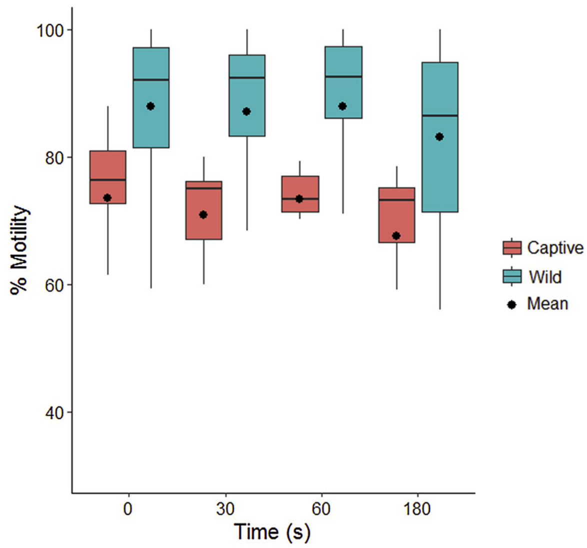

Fig. 3. Percent motility of sperm from captive and wild-caught *A. brevirostrum* over time (mean ± S.E.M, n = 8 captive fish, 19 wild fish); Solid black circles represent the mean of each group.

3.2.3. Time 60s

There was an effect of source on both the speed/wobble (ANOVA, df = 24, P = 0.033) and movement pattern (ANOVA, df = 24, P = 0.002) components. There was no effect of temperature on both the speed/wobble (ANOVA, df = 46, P = 0.276) or movement pattern (ANOVA, df = 46, P = 0.249) components.

3.2.4. Time 180s

There was an effect source on both the speed/wobble (ANOVA, df = 24, P = 0.045) and movement pattern (ANOVA, df = 24, $P < 0.001$) components. There was also an effect of temperature on only the speed/wobble component (ANOVA, df = 46, P = 0.009).

Results of the ANOVA examining the effects of source, temperature, and time on motility indicated there were effects of source and time ($P < 0.001$), and no effect of temperature (P = 0.150). There was, however, an interaction between source and time (df = 717, $P < 0.001$), so data were split by time. There was an effect of source on motility at all time periods ($P \leq 0.05$), with spermatozoa from wild-caught males having greater motility than those from captive males (Fig. 3).

3.3. Cluster analyses of spermatozoa kinematics

Results from cluster analyses provided evidence for grouping sperm within each time period into unique populations based on values of original kinematic variables. At each time period, there was an effect of source determining cluster formation (x^2, df = 2, $P < 0.001$). Data in Table 2 indicates the composition of each cluster based on values of original kinematic variables, as well as the percentage of sperm from each source within clusters for all time periods.

Cluster plots are depicted in Fig. 4. Results of the cluster analysis of each time subset allowed the following data assessments.

3.3.1. Time 0s

Spermatozoa were grouped into three clusters. In the first cluster were sperm that had less than average values for kinematic measures, with 84.6% of sperm coming from captive fish. In the second cluster were sperm with values less than the average velocity measures, increased beat frequency, and straight trajectories. This cluster is composed of 63.6% spermatozoa from wild-caught fish. The third cluster contains sperm with a greater than average velocity, path linearity, and lesser beat frequency. Of this cluster, 97.1% of the sperm were from wild-caught fish.

3.3.2. Time 30s

Spermatozoa were grouped into four clusters. The first cluster comprised sperm with less than average values for velocity variables and greater beat frequency. Of these sperm, 88.9% were from captive fish. The second cluster consisted of sperm with a greater than average beat frequency, but less than average velocity. Of these, 76.0% of the sperm were from wild-caught fish. Sperm from the third cluster had a greater than average amplitude of head movement, and less than average beat frequency and linearity. There were 55.5% of spermatozoa in the third cluster from wild-caught fish. The fourth cluster contained sperm with greater than average velocity and path linearity. Of these sperm, 92.9% were from wild-caught fish.

Table 2
Cluster analysis results for each time period along with proportion of sperm from each source per cluster.

Time	Cluster	Breakdown	Variable	V-Test	*P* Value
			BCF	−2.522	1.17E-02
			ALH	−3.298	9.73E-04
		Captive: 84.6%	VAP	−5.19311	2.07E-07
	1		VSL	−5.23178	1.68E-07
			VCL	−5.38836	7.11E-08
		Wild-Caught: 15.4%	STR	−5.95454	2.61E-09
			LIN	−6.05116	1.44E-09
			WOB	−6.71647	1.86E-11
			BCF	4.161566	3.16E-05
		Captive: 36.4%	STR	2.091725	3.65E-02
0 s			VSL	−2.34783	1.89E-02
	2		VCL	−2.98912	2.80E-03
		Wild-Caught: 63.6%	VAP	−3.05693	2.24E-03
			ALH	−3.08405	2.04E-03
			VCL	6.957616	3.46E-12
			VAP	6.880192	5.98E-12
		Captive: 2.9%	VSL	6.205499	5.45E-10
	3		WOB	5.706422	1.15E-08
			ALH	5.502898	3.74E-08
		Wild-Caught: 97.1%	LIN	3.797439	1.46E-04
			STR	2.337495	1.94E-02
			BCF	−2.25869	2.39E-02
			ALH	−2.76307	5.73E-03
			BCF	−3.22006	1.28E-03
			VAP	−3.56881	3.59E-04
	1	Captive: 88.9%	VSL	−3.63998	2.73E-04
		Wild-Caught: 11.1%	VCL	−3.89132	9.97E-05
			LIN	−5.12721	2.94E-07
			WOB	−5.89372	3.78E-09
			STR	−6.16383	7.10E-10
			BCF	6.177091	6.53E-10
			STR	2.208518	2.72E-02
	2	Captive: 24%	ALH	−3.95971	7.50E-05
		Wild-Caught: 76%	VSL	−4.06218	4.86E-05
30 s			VCL	−4.23087	2.33E-05
			VAP	−4.45694	8.31E-06
			ALH	5.148444	2.63E-07
	3	Captive: 44.5%	LIN	−2.68568	7.24E-03
		Wild-Caught: 55.5%	BCF	−2.85328	4.33E-03
			STR	−3.02213	2.51E-03
			VSL	6.91662	4.63E-12
			VAP	6.22419	4.84E-10
			LIN	6.160977	7.23E-10
	4	Captive: 7.1%	WOB	5.961945	2.49E-09
		Wild-Caught: 92.9%	VCL	5.744748	9.21E-09
			STR	4.090038	4.31E-05
			ALH	2.020676	4.33E-02
			BCF	−2.04007	4.13E-02
			BCF	5.324516	1.01E-07
			ALH	−3.37659	7.34E-04
		Captive: 16.7%	LIN	−4.36541	1.27E-05
	1	Wild: 83.3%	VCL	−5.60151	2.12E-08
			VSL	−5.71509	1.10E-08
			WOB	−5.77492	7.70E-09
			VAP	−6.04158	1.53E-09
			VSL	−2.05588	3.98E-02
		Captive: 70%	WOB	−3.56775	3.60E-04
	2	Wild: 30%	BCF	−4.26795	1.97E-05
60 s			LIN	−4.72489	2.30E-06
			STR	−6.19844	5.70E-10
		Captive: 23.5%	LIN	4.326793	1.51E-05
	3	Wild: 76.5%	WOB	4.000521	6.32E-05
			STR	3.737844	1.86E-04
			VSL	6.363969	1.97E-10
			VCL	6.207559	5.38E-10
		Captive: 0%	VAP	6.174874	6.62E-10
	4	Wild: 100%	WOB	4.159279	3.19E-05
			LIN	3.74025	1.84E-04

(*continued on next page*)

Table 2 (*continued*)

Time	Cluster	Breakdown	Variable	V-Test	*P* Value
180 s	1	Captive: 77.8% Wild: 22.2%	ALH	3.249015	1.16E-03
			STR	2.431363	1.50E-02
			VAP	−3.03817	2.38E-03
			VCL	−3.31875	9.04E-04
			VSL	−3.55368	3.80E-04
			WOB	−5.80762	6.34E-09
			LIN	−6.0146	1.80E-09
			BCF	−6.51208	7.41E-11
			STR	−7.4709	7.96E-14
	2	Captive: 18.2% Wild: 81.8%	BCF	5.051331	4.39E-07
			ALH	−3.26796	1.08E-03
			VCL	−4.01844	5.86E-05
			VSL	−4.04933	5.14E-05
			VAP	−4.39115	1.13E-05
	3	Captive: 13.3% Wild: 86.7%	VSL	7.179585	6.99E-13
			VAP	7.083581	1.40E-12
			VCL	6.945902	3.76E-12
			WOB	6.68707	2.28E-11
			LIN	6.553936	5.60E-11
			STR	4.610234	4.02E-06
			ALH	3.846127	1.20E-04

VAP = average path velocity; VSL = straight line velocity; VCL = curvilinear velocity; ALH = amplitude of lateral head displacement; BCF = beat cross frequency; STR = straightness of track; LIN = linearity of track; WOB = wobble.

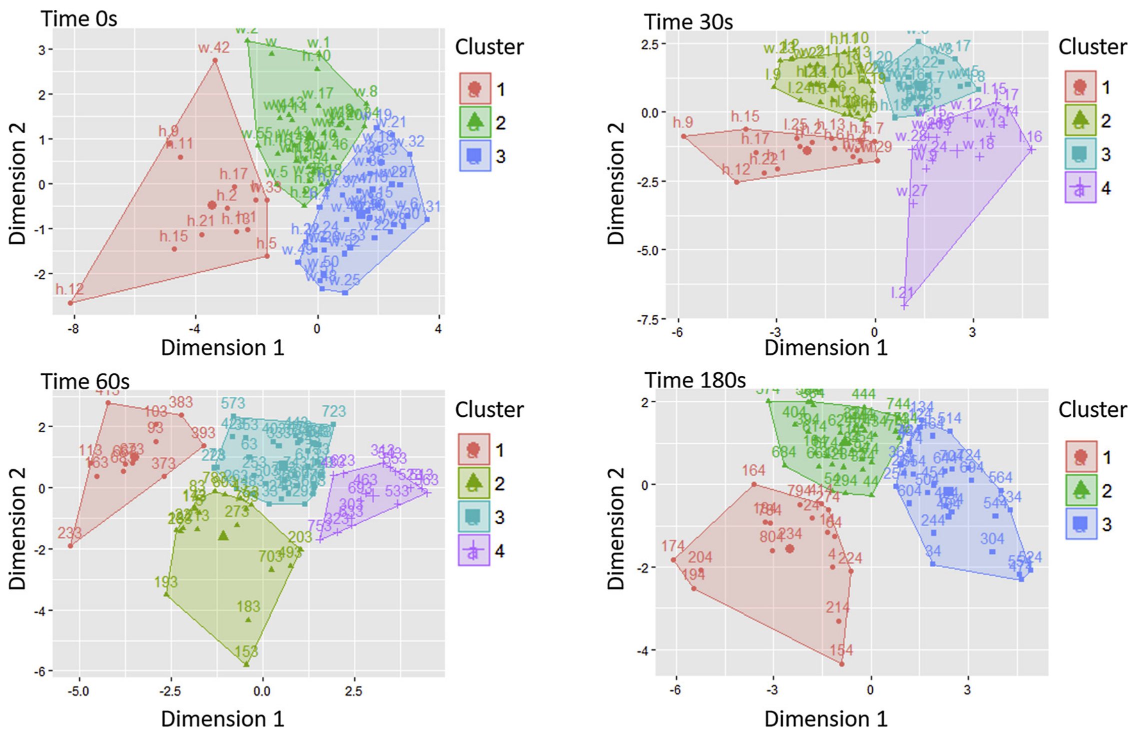

Fig. 4. Cluster plots for each time period; Source was a predictor of how individuals were grouped at all time periods; Dimensions 1 and 2 represent the speed/wobble and movement pattern components, respectively.

3.3.3. Time 60s

Spermatozoa were grouped into four clusters. The first cluster contained sperm with less than average velocity measures and greater beat frequency. There were 83.3% of the sperm in the first cluster from wild-caught fish. The second cluster contained sperm that had less than average velocity, beat frequency, and linearity. Of the sperm in the second cluster, 70.0% were from captive fish. The third cluster was comprised of spermatozoa that had greater than average path linearity and straightness. There were 76.5% of these sperm from wild-caught fish. The fourth cluster comprised sperm with greater than average velocity and path linearity. Of these, 100% were sperm from wild-caught fish.

3.3.4. Time 180s

Sperm were grouped into three clusters. The sperm of the first cluster had less than average velocity, beat frequency, and linearity. Of the sperm within this cluster, 77.8% were from captive fish. The second cluster contained sperm with greater than average beat frequency and less than average velocity. There were 81.8% of the sperm in this group from wild-caught fish. The third cluster was sperm with greater than average velocity and path linearity. Of these, 86.7% were from wild-caught fish.

4. Discussion

The results of the present study provide strong evidence that spermatozoa density, motility, and swimming kinematics are markedly different between captive and wild-caught *A. brevirostrum* (see supplementary material). Sperm from captive individuals had lesser motility compared to sperm from wild-caught fish. To ensure this was not an effect of hormonal treatment in captive fish, wild-caught males not readily expressing milt were also treated with hormone to stimulate expression of milt. There was no significant difference between wild males receiving hormonal treatment and their non-treated wild counterparts for sperm density as well as values of all kinematic variables. These results indicate that observed differences in spermatozoa density and motility kinematics are likely attributed to rearing conditions. The effects of rearing condition on sperm density and quality has been examined for multiple species (Leung-Trujillo and Lawrence, 1987; Izquierdo et al., 2001; Rideout et al., 2004; Rurangwa et al., 2004; Mylonas et al., 2009); however, these previous studies typically focused on differences only within a captive population and did not include samples from wild-sourced individuals. Studies using hormonal treatments to stimulate spawning in captive fish often indicate there is increased sperm motility and decreased density because hormonal treatments often result in increases in seminal plasma volume (Dabrowski et al., 1994; Clearwater and Crim, 1998). In the present study, wild-caught *A. brevirostrum* had greater sperm densities than captive fish. This difference was not observed between hormone-treated and non-treated wild-caught males, indicating that differences in density are unlikely an effect of hormonal treatment. Such variations in sperm density between captive and wild-caught individuals have been observed previously in other species (Ceballos-Vazquez et al., 2003; Skjaersaan et al., 2009); however, Lehnert reported there were greater sperm densities in farmed chinook salmon (*Oncorhynchus tshawytscha*) compared to wild-caught counterparts (Lehnert et al., 2012). In addition to lesser sperm densities, in the present study there was also lesser sperm motility in captive than wild-caught fish. This has been observed in other fish species, such as beluga (*Huso huso*), Atlantic bluefin tuna (*Thunnus thynnus*), and lebranche mullet (*Mugil liza*), and has been attributed to temperature, nutrition, water quality, and rearing environment (Zupa et al., 2013; Madadi and Khara, 2016; Magnotti et al., 2018).

Results from the principal components analysis (PCA) indicated there were two main components that explained 89% of the overall variation in the data. The first component (speed/wobble) was heavily weighted with measures of velocity and path linearity, while the second component (movement pattern) was weighted with head movement variables. Over all time periods there was an effect of source on both components, with captive fish having lower PC scores compared to wild-caught fish. There was an effect of temperature on sperm from captive fish 180 s after motility initiation on the speed/wobble component. Because the effect of temperature was only observed in sperm from captive fish, it could be that these individuals have a narrower range of temperatures at which their sperm have optimum motility compared to wild-caught fish. This could be related to differences in thermal regimens of a river habitat compared with a captive habitat. Because inflow water of the hatchery comes from deep in a head pond, there is little variation in water temperature. Wild-caught fish have the potential to encounter a wider range of temperatures compared to captive fish due to fluctuations in the river environment from depth, shading, and flow, and perhaps this enables for adaptations that result in sperm with greater motility over a wider range of temperatures. There are few reports on the effect of broodstock rearing temperature on sperm quality, but results of studies that have been conducted indicate species-specific responses. Optimal sperm quality in river lamprey (Cejko et al., 2016) and Siberian sturgeon (Williot et al., 2000) occurred when broodstock were maintained at below average rearing temperatures, while European eel (Gallego et al., 2012) sperm had the greatest motility when individuals were maintained at greater than average rearing temperatures. Understanding optimal thermal conditions for rearing and successful reproduction is essential, not only for captive programs but also for species conservation. Climate change is expected to affect river habitats, and this can affect the development and behavior of fish. Additionally, habitat alteration and damming affect water temperature and flow patterns, resulting in potentially large thermal changes within a short time period (Lassalle et al., 2010; Dripps and Granger, 2013; Maheu et al., 2016). This suggests that further research needs to be conducted to determine optimal thermal conditions for rearing and successful reproduction of *A. brevirostrum* in the laboratory and field.

Cluster analyses results were similar at each time subset. At all times, the cluster containing sperm with above average velocities was dominated by sperm from wild-caught fish. Source was the variable that characterized the clusters at all time periods. Differences in sperm quality between captive and wild-caught stocks have been observed in other species (Izquierdo et al., 2001; Rurangwa et al., 2004; Mylonas et al., 2009); however, the present study is, to our knowledge, the first examination of how captivity affects sperm quality in *A. brevirostrum*. Lesser spermatozoa motility and velocity observed in captive males indicates the likelihood of decreased fertilization potential, as these are commonly used as a proxy for fertilization success (Gage et al., 2004; Casselman et al., 2006; Skjaersaan et al., 2009; Gasparini et al., 2010).

There have been many studies on the possible causes for the variation in sperm quality observed in fish (Bromage et al., 2001; Alavi and Cosson, 2005; Mousa and Mousa, 2006; Chemineau et al., 2008; Butts et al., 2012b) The results from these studies indicate water temperature, salinity, stocking density, sex ratio, and diet as possible factors affecting spermatogenesis and sperm quality. It would be beneficial to determine which of these factors, along with possible others, affect sperm quality in *A. brevirostrum*. Understanding the conditions that result in production of high quality sperm is of great importance not only for aquaculture but also conservation of various species.

In conclusion, results of this study provide strong evidence that there are differences in sperm quality using CASA, as well as differences in spermatozoa density, between captive and wild-caught *A. brevirostrum*. Wild-caught fish had consistently greater quality sperm, using swimming kinematics as a proxy for quality. Wild-caught fish also had milt with greater spermatozoa densities. These findings indicate increased fertilization potential for sperm from wild-caught *A. brevirostrum* compared to those in captivity. These findings will contribute to knowledge not only on sturgeon reproduction and sperm characteristics, but also to fish reproduction in general. Results from the present study also support the need to conduct species-specific research as generalizations between species, at least in terms of sperm quality, are often not possible and impractical.

Declaration of interest

Authors declare no conflict of interest involved in this study.

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Appendix A. Supplementary data

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