Animal Reproduction Science 202 (2019) 65–72

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Sperm motility of the Nile tilapia (*Oreochromis niloticus*): Effects of temperature on the swimming characteristics

Borys Dzyuba[a,*], Marc Legendre[b], Jean François Baroiller[b,c], Jacky Cosson[a]

[a] University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic
[b] ISEM, Université de Montpellier, CNRS, IRD, EPHE, Montpellier, France
[c] CIRAD, UMR ISEM, F-34398 Montpellier, France

ARTICLE INFO

Keywords:
Sperm velocity
Temperature effect
Sperm motility
Tilapia

ABSTRACT

Results of previous studies with different fish species, mostly from temperate- or cold-water habitats, indicate a species-specific diversity regarding the relationship between environmental temperature and values for sperm motility variables. In the current study, there was appraisal of environmental temperature effects on sperm motility of tilapia *Oreochromis niloticus,* a tropical fish species selected because of its aquaculture importance and capacity to reproduce in a broad range of water temperatures. Effects of environmental temperature on the spermatozoa motility characteristics were studied by temperature-controlled video-microscopy and CASA analysis at temperature range from 5 to 50 °C. It appeared that the Nile tilapia spermatozoa exhibit an unexpected capacity to express very different velocity characteristics over this temperature range. In the lower temperature range (5–10 °C), the percentage of motile cells was markedly variable among males. An abrupt increase in the linearity index was observed between 15 and 20 °C suggesting a physiological threshold in sperm movement at about 20 °C which is the minimum temperature for reproduction in the Nile tilapia. With faster spermatozoa velocity, there was a reduction of the motility duration at the greater temperatures. Initially, there is an increase in sperm velocity as the temperature increased until the maximal velocity occurred at 40 to 50 °C which is a temperature beyond that which occurs in natural spawning conditions. Results of the present study clearly indicate the importance of considering ambient temperature when charactering sperm motility and in determining optimal temperature conditions for fertilization in fish.

1. Introduction

Present knowledge related to sperm motility in fish (Morisawa, 1985, 1994; Cosson, 2008) can be summarized as follows: 1) spermatozoa motility is a prerequisite for fertilization; 2) motility initiation appears as an extremely rapid response of the spermatozoon flagellar apparatus to environmental signals (Prokopchuk et al., 2015), primarily osmolality or ionic composition (Morisawa and Suzuki, 1980); 3) depending on the species, sperm motility activation as a result of environmental signals can be "osmotic" or "ionic" (Morisawa and Suzuki, 1980); 4) spermatozoa motility results from a complex series of biochemical reactions involving signal sensing and transduction through the spermatozoon plasma membrane (Dzyuba and Cosson, 2014) with regulation of the signal being energy-dependent at the flagellar dyneins and for activation of the flagellar axoneme (Inaba et al., 1998).

* Corresponding author at: Zátiší 728/II, 389 25 Vodňany, Czech Republic.
E-mail address: bdzyuba@frov.jcu.cz (B. Dzyuba).

https://doi.org/10.1016/j.anireprosci.2019.01.010
Received 25 September 2018; Received in revised form 14 January 2019; Accepted 28 January 2019
Available online 29 January 2019
0378-4320/

Results of previous studies on different fish species, mostly from temperate or cold water, indicate there is an inverse relationship between environmental temperature and duration of sperm motility which could be the result of expenditure of limited energy stores available for motility (Alavi and Cosson, 2005; Dadras et al., 2017). Experimental evidence is insufficient for a general understanding of the underlying physiological mechanisms by which temperature affects fish sperm functionality. Studies of the effect of relatively greater temperatures on sperm velocity yielded results that were ambiguous and inconsistent with results being particularly scarce for tropical fish.

The group of tilapia species (*Cichlidae*) is ranked second after carps in terms of world aquaculture production. Among these, the Nile tilapia (*Oreochromis niloticus*) represents a major fish species for aquaculture worldwide. Its total production amounted to about 3.9 million tons in 2015 (Fisheries and aquaculture software. FishStatJ - software for fishery statistical time series, 2017). Its reproduction occurs over a wide range of habitats and thermal conditions, from 20 to 40 °C (Philippart and Ruwet, 1982; Trewavas, 1983; Baroiller and Toguyeni, 2004; Bezault et al., 2007). In a previous study (Legendre et al., 2016), with another tilapia species (*Sarotherodon melanotheron heudelotii)* there was elucidation of a specific environmental ion-dependent mechanism of sperm motility activation that allows sperm motility to occur in ambient water salinities from 0 to greater than 70. In the present study, there is a focus on the characterization of phenotypic plasticity of sperm activation and motility characteristics of *O. niloticus* sperm in response to changes in ambient temperature when there is constant osmotic and ionic conditions. Other than a contribution to basic knowledge on fish sperm thermal tolerance and physiology, consideration of the results of this study should lead to subsequent improvements in assisted reproductive aquaculture technologies.

2. Materials and methods

2.1. Fish and rearing conditions

The fish were the descendants of specimens of the Manzala (Egypt) Lake aquaculture domestic strain maintained for more than 18 years at the ISE-M experimental facility in Montpellier (France). Males become sexually mature at 5–6 months of age. Sperm characteristics were analyzed when the fish were 12–36 months old. A total of 22 males (80–400 g) was examined in the present study, of which 14 could be manually stripped to collect sperm.

All broodstock of *O. niloticus* with a photoperiod being imposed of 12 h light/12 h dark, at a stocking density of 10–30 fish/m^3 and a sex-ratio of 1:3 (male:female), in 500- to 2000-L indoor tanks connected to a water recirculation system; equipped with mechanical and biological filters to maintain good water quality. Water temperature was maintained at 26–29 °C using thermostatically (Biotherm 2000) controlled 300-W submersed heaters and oxygen was maintained near saturation with use of air stone bubbling technologies. All fish were fed commercial pelleted diets *ad libitum* (32% and 41% crude protein, for adults and juveniles, respectively).

2.2. Sperm collection and characteristics

The fish were anaesthetized (Eugenol, 0.05 mL/L), placed on a bench and the urinary bladder was emptied by applying gentle hand pressure at the posterior part of the abdomen. The surface of the abdomen was carefully dried with absorbent paper and the males were then stripped for sperm collection with the drops of sperm being directly collected into a micropipette at the opening of the genital papilla. The aspirated sperm (50–400 µL depending upon individuals) was transferred in 1.5-mL Eppendorf tubes (FlipTube, Gemü GmbH, Switzerland) and immediately stored on crushed ice until subsequent motility determinations were made.

2.3. Sperm motility assessment

Sperm motility was observed using a negative phase contrast microscope (UB 200i, PROISER, Valencia, Spain). Motility was recorded at 25 fps with an ISAS 782 M digital camera (PROISER, Valencia, Spain) on the bottom surface of the activation medium droplet that was placed on a glass slide. Video records were analyzed using the Integrated Semen Analysis System software (ISASv1, PROISER, Valencia, Spain). Analysis was performed at 1-s intervals to estimate the values for the following variables: percent motile cells, VCL (curvilinear velocity over the actual path, µm/s), VSL (average velocity of the sperm head through the straight line connecting the first and last position of the track, µm/s), LIN (linearity of track, VSL/VCL). The following settings of ISASv1 were applied for making the assessments: 1) threshold for sperm motility: VCL = 20 µm/s, 2) species: 'fish', 3) calibration: pre-adjusted using PROISER for use of the objective lens.

In all motility assessment trials, the same swimming medium (SM) was used for sperm activation with composition being as follows: NaCl, 40 mM; Tris − HCl, 10 mM; pH 8.2; 0.25%, pluronic acid; and osmolality, 97 mOsm/kg.

The sperm motility characteristics were studied in a range of temperatures from 5 to 50 °C. For temperature control during sperm motility assessment, a temperature-controlled stage (SEMIC® BIOELEKTRONIKA, Krakow, Poland) was fitted in place of the regular stage of the microscope. The actual temperature in the assay drop on the stage was controlled by using a copper-constantan type T thermocouple with a diameter 0.1 mm (Model L-0044 T, Omega, Stamford, USA), connected to a thermocouple data logger (OM-CP-QuadTemp2000, Omega, Stamford, USA). For rapid temperature equilibration and prevention of condensation, a thin coverslip was used instead of a regular glass slide. A drop of 30 µL of SM was smeared on the coverslip pre-adjusted on the temperature-controlled stage and 0.3 to 0.5 µL of milt was rapidly mixed in the drop of SM. An initial video-recording was started at 30 s after mixing the milt and SM followed by conducting a second recording at 120 s.

The total duration of sperm motility at temperatures of 15, 25, 35 or 45 °C was determined in larger volumes, after mixing in a 500-μL plastic tube 10 μL of sperm of an individual fish and 200 μL of SM adjusted to the experimental temperature for 1 min before adding sperm. During the motility assessment phase, the tube containing the sperm activated in SM was maintained in a temperature-controlled block (PCR machine, MJ Research Inc., PTC-100) used to adjust the temperature to the selected experimental condition. Sperm activity was estimated in 20 μL aliquots of the sperm suspension and examined using the microscope at 30, 60, 180, 360, 720 and 900 s after sperm activation. Drops of sperm suspension were pipetted from the tube and smeared on the coverslip pre-adjusted on the temperature-controlled stage and sperm motility was recorded using the approach that was previously described in this manuscript.

2.4. Statistical methods

To obtain data on post-activation dynamics for sperm motility variables during the entire motility assessment period, sperm samples from four males were used. Data for sperm motility at 30 and 120 s post-activation at 5 to 50 °C were obtained from sperm samples of six males. Each experiment was performed in triplicate.

Data for percentage motility change during the entire motility assessment period are presented using linear regression. Values related to linear regression such as slope, intercept, coefficient of determination R^2 and *P*-values were calculated using the GraphPad Prism version 6 for Windows software (La Jolla, CA, USA). Differences between values of slopes and intercepts were determined using the *t*-test with Bonferroni corrections being calculated using a "difference test calculator" of the Statistica V 12.0 package (Statsoft Inc., Tulsa, OK, USA).

The values for VCL, VSL and LIN variables obtained from the measurement of the motility of more than 24,000 spermatozoa were analyzed. For statistical analysis, values for motility variables of each spermatozoon were averaged considering the male number and experimental factors (post activation time and temperature). These averaged values were assessed for normality and homogeneity of variances using the Shapiro–Wilks test and Levene's test, respectively. As *P*-values for these tests were greater than 0.05 in all groups, a two-way repeated measures parametric ANOVA was applied with temperature of SM and post-activation time as main effects and "temperature x time" as the interaction term. When there was a significant interaction between effects detected, one-way parametric ANOVA models were applied to determine the effect of temperature for each post-activation time and effect of post activation time for each temperature using the multiple comparisons procedure of the Tukey *post-hoc* test.

Values for percentage motility at 5–50 °C and 30–120 s post motility activation intervals were not normally distributed (Shapiro–Wilks test, $P < 0.05$), therefore, the nonparametric Mann-Whitney U test was applied to detect differences at 30 and 120 post activation "points of interest" only. In figures, data are depicted as mean with standard error of mean (SEM, whiskers) while values for results obtained using the Tukey *post-hoc* tests are presented as separate tables in the supplementary materials 1 and 2. Null hypotheses were rejected at $P < 0.05$ for all performed tests. All statistical analyses and plotting were conducted using Dell Statistica (data analysis software system), version 13 (Dell Inc., Tulsa, OK, USA).

3. Results

3.1. Spontaneous motility

Spermatozoa movement was not observed either in milt (for samples devoid of contamination by urine) or after dilution in seminal fluid. By contrast, motility was fully activated (> 90% motile cells) after sperm placement in the experimental SM at the temperature of fish maintenance (28 °C).

Most sperm collected in this study (with one exception, a sample with poor motility and not used in subsequent analyses) had maintenance of the initial potential for motility activation for periods of 2 to 3 h when stored undiluted in the individual's seminal fluid on crushed ice. Taking into consideration these results, the analysis of sperm motility occurred within 2 h from stripping of semen from males for all samples in the present study.

3.2. Sperm motility at different times post motility induction when there was imposing of different temperatures

The percentage of motile cells during the period of motility assessment was markedly influenced by temperature and post-activation time (Fig. 1, Table 1). The total period during which motility was sustained was about 8 min at 45 °C and was progressively longer when the temperature of SM was less being about 11, 13 and 15 min at 35, 25 and 15 °C, respectively. The slopes of the linear regression lines for percent motility and time elapsed from sperm activation were greater when temperatures were greater with significant differences in slope among the four temperatures at which assessments occurred (from 15 to 45 °C at 10 °C increments).

The values for the two velocity variables (VCL and VSL; Fig. 2A and B, respectively) were highly dependent on temperature during the early part of the motility assessment period (first minute of motility assessment) but were similar and of lesser values after 3 min of assessment. During the motility assessment period there were the greatest values for VCL and VSL at 45 °C at 30 s post-activation, whereas there were no differences between 30 and 60 s post-activation at 25 °C and 35 °C and at all post-activation times at 15 °C. There were differences in values of the VCL between 15 and 35 °C, as well as at 15 and 45 °C at 30 s post-activation and between 15 and 45 °C at 60 s post-activation. The VSL, value was less at 15 °C than at 25, 35 and 45 °C at 30 s post-activation, whereas at 60 s post-activation there was a difference only between 15 and 45 °C.The linearity of sperm tracks (LIN) was less at 15 °C than when there were assessments at all other temperatures at 30 s post-activation and this pattern of LIN was sustained at the other motility

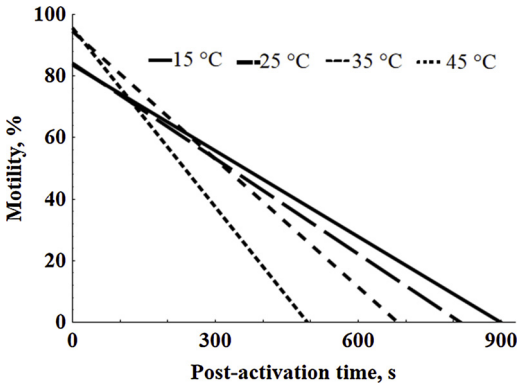

Fig. 1. Linear regression lines of sperm motility percentage as a function of time elapsed since activation at different temperatures of the swimming medium; Descriptive statistics are presented in Table 1; Data from sperm samples from three males in three repetitions were used.

Table 1
Descriptive statistics of linear regression lines of sperm motility percentage as a function of time elapsed since activation at different temperatures of the swimming medium.

Parameter	Temperature, ˚C			
	15	25	35	45
Slope, mean ± SD	–0.093 ± 0.008[a]	–0.103 ± 0.009[b]	–0.138 ± 0.010[c]	–0.194 ± 0.020[d]
Y-intercept, mean ± SD	84 ± 4[a]	85 ± 4[a]	95 ± 5[b]	96 ± 5[b]
R^2	0.85	0.88	0.91	0.93
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Within a row, values sharing one superscript in common do not differ at $P < 0.05$.

assessment times except for the decrease in LIN at the latest times of assessment at 15, 35 and 45 ˚C (Fig. 2C). Detailed description of statistical significance is presented in Supplementary material 1.

3.3. Initial values for sperm motility variables at different temperatures

Sperm motility was observed at a wide range of temperatures when sperm were placed in the SM (5–50 ˚C). At 5 ˚C, there was only motility of sperm of three males (averaged values: VSL). By contrast, there was a large percentage of motile cells at 50 ˚C (> 90%) but the duration of motility was less, never exceeding 1 min. For these reasons, data for sperm motility at 5 and 50 ˚C were not included for statistical analysis.

Over a temperature range from10 to 45 ˚C, there was a large amount of variation for curvilinear velocity (VCL, mean values range 31–76 μm/s), and linear velocity (VSL, mean values range 12–53 μm/s) both at 30 s and 2 min post-activation (Fig. 3A, B). At 20 and 25 ˚C, both VSL and VCL were markedly greater than at 10 ˚C and there was a further increase in values for both variables when there were increases in temperature. There were no differences in values for VCL or VSL at 40 and 45 ˚C. There was an abrupt increase in the linearity index (VSL/VCL) between 15 and 20 ˚C (Fig. 3C). At the lesser temperatures (5–10 ˚C) when sperm were placed in SM, the sperm were partially activated but there was a great amount of variability in response among males in the motility percentage at 30 s after sperm activation. The motility percentage at 10 ˚C increased between 30- and 120-s post-activation, indicating there was a delay in the initiation of spermatozoa motility after placement of sperm in SM (Fig. 3D). By contrast, there was a large percentage of motile cells (> 90%) when the temperatures were between 15 and 45 ˚C after placement in SM. At the 15 and 45 ˚C temperatures, there were no further increases in motility percentage after the initial 2 min subsequent to placement of sperm in SM.

4. Discussion

Results of the present study indicate environmental temperatures greatly affect values for all sperm swimming variables of the Nile tilapia. In this species, spermatozoa have a marked change in values for sperm velocity and linearity at the 5–50 ˚C temperature range, however, with the increase in sperm motility as temperature increases there is a lesser duration of motility post-activation at the greater temperatures.

Results depicted in Figure and Table 1 for percentage of swimming cells at various times subsequent to motility initiation allow for a prediction of both sperm motility duration and initial motility percentage values that cannot be obtained experimentally because of technical reasons. The relatively lesser motility percentage at the two lesser temperatures (15 and 25 ˚C) possibly indicates that there were more spermatozoa for which motility was induced early post-activation when the temperatures were greater (35 and 45 ˚C). Considering that sperm motility activation is controlled through a cell signaling network of biochemical reactions (Dzyuba

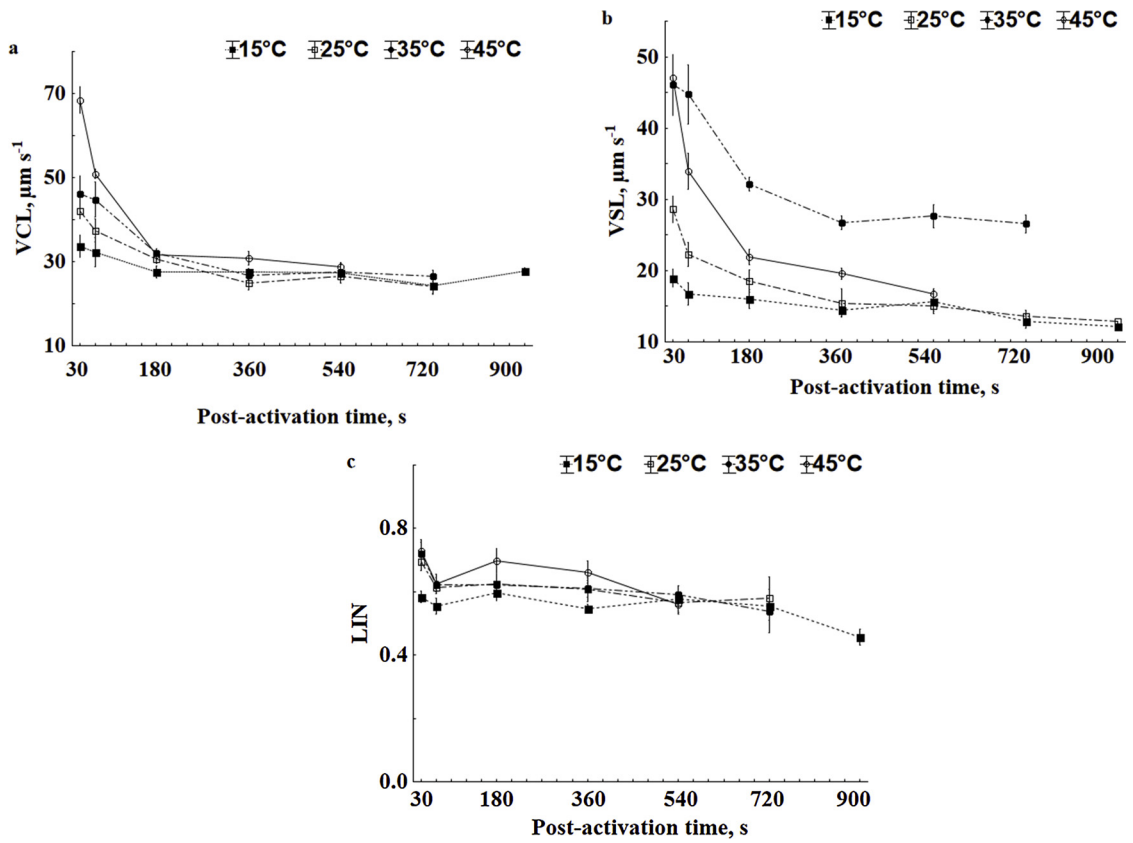

Fig. 2. Values for spermatozoa motility variables at different post-activation times as affected by temperature of the swimming medium; A – curvilinear velocity (VCL), B – average velocity of the sperm head through the straight line (VSL), C – linearity (LIN); Mean (markers) with SEM (whiskers) are depicted; For significance of differences see tables with Tukey test in attachment 1.

and Cosson, 2014), the phenomenon of gradual increase of fraction of motile cells at the initial period after motility induction in the present study is likely associated with inhibition of the reactions involved in signaling for sperm motility. The intersection of the regression lines with the X axis also provides indications about duration of the sperm motility period indicating that the duration of the motility period varies from 900 s at 15 °C to 500 s at 45 °C. This observation leads to the conclusion that the greater the temperature surrounding the sperm, the shorter the duration of motility subsequent to motility activation. As a consequence, a decrease of the values for velocity combined with an increase of the motility duration leads to the prediction that the total average distance covered by the sperm cells in a population should remain quite constant, thus minimally impairing chances of sperm-egg contact. In general for fish sperm, when there is a decrease of the temperature of the SM, there is an associated increase of sperm motility duration (Alavi and Cosson, 2005). This association is well established for a large variety of fish species possessing a wide temperature ranges of natural spawning. The cold water spawning burbot (*Lota lota*; Lahnsteiner and Mansour, 2012) and warm water spawning cascudo-preto *(Rhinelepis aspera*; Bombardelli et al., 2013) are a few representatives for which there have been evaluations of such associations. There are more examples of these associations as a result of the research of Lahnsteiner (2011); Billard and Cosson (1992); Dadras et al. (2016); Mehlis and Bakker (2014) and as reviewed by Alavi and Cosson (2005).

As previously reported for sperm of many other fish species, during the motility period, the decrease of motility percentage is occurring in concert with a decrease of the values for the swimming variables for spermatozoa (Cosson, 2008). This is depicted in Fig. 2A and B where there is a marked decrease of VSL and VCL values especially during the initial portions of the motility period (first 3 min post-activation). The linear decrease of sperm velocity allows for extrapolation from these curves the value of the velocity at the initiation time point (interception with the Y axis in Fig. 2A) and prediction of a maximum VCL value of 75 to 80 μm/s at 45 °C and in Fig. 2B a maximum VSL value of 47 to 50 μm/s at 45 °C. These values are about twice that for maximum VCL and VSL respectively, as depicted in Fig. 2A and B, but at the least temperature (15 °C). In both cases (VCL and VSL), the velocity values plateau when there are longer periods of time for the motility phase but there is motility of a lesser fraction of the sperm population as depicted in Fig. 1.

From the results depicted in Fig. 2A and B, it was decided for the present study to concentrate on the variations of the initial sperm velocity only (30 s and 120 s post-activation) but at a broader range of temperatures (5–50 °C). As depicted in Fig. 3A, the initial curvilinear velocity (VCL) was about two times greater as compared with the values at the two extreme temperatures (5 compared with 50 °C) while in the same range of temperatures, the straight-line velocity (VSL) value was almost three times regardless of time

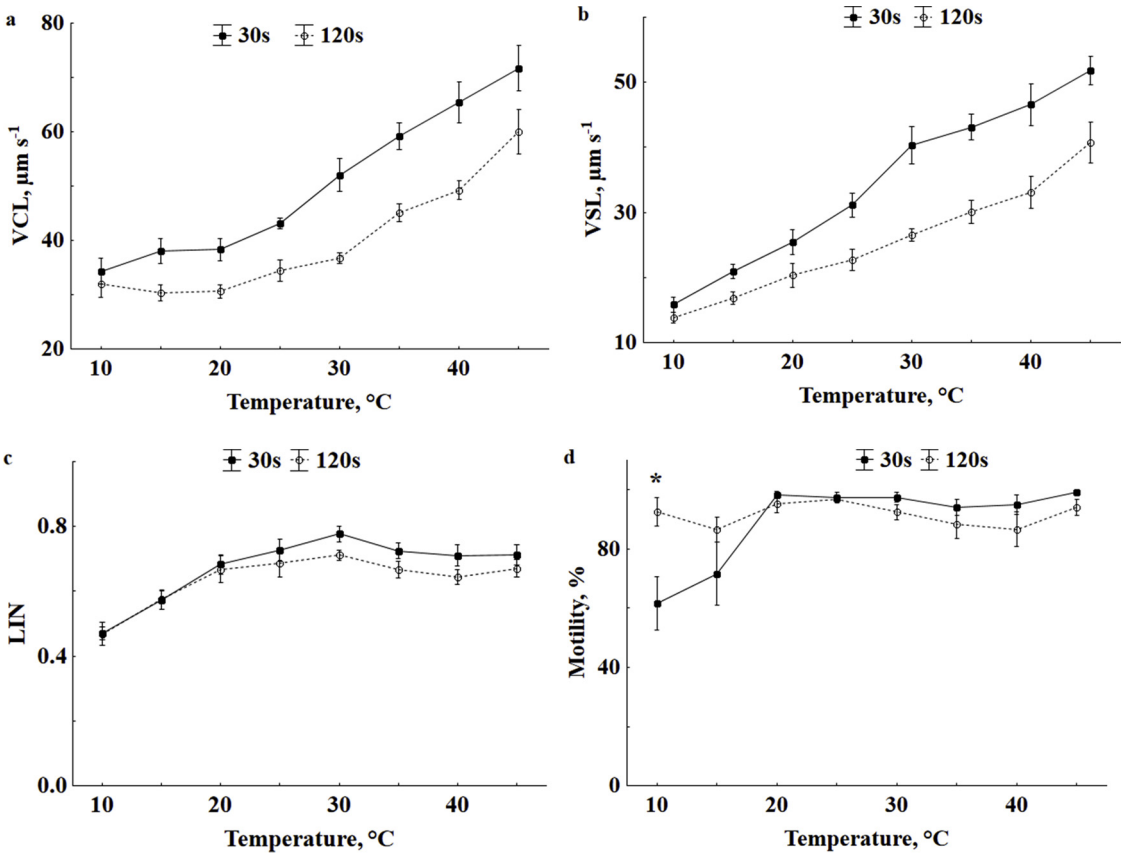

Fig. 3. Values for spermatozoa motility variables at 30 s and 120 s post-activation (P-A) time in relation to temperature of the swimming medium; A – curvilinear velocity (VCL), B – average velocity of the sperm head through the straight line (VSL), C – linearity (LIN), D – motility percentage (Motility); In A, B, C: Mean (markers) with SE (whiskers) are depicted; For significance of differences see tables with Tukey test in attachment 2; In D: values marked by asterisk are different at 10 °C (Mann-Whitney U test, $P < 0.007$).

point (30 s or 120 s post-activation) when assessments occurred.

For the linearity index (LIN), the values gradually increased when the temperature was in the range of 10–20 °C and were maximal at 30 °C. The value for LIN when temperatures were in the upper range of those imposed in the present study was almost twice that as the value at 10 °C, which indicates that there is greater circular movement of sperm when temperatures are less and that sperm movement is straighter when the surrounding conditions become warmer. The greater circular movement for the sperm tracks has been frequently associated with the concentration of intracellular Ca^{2+} ions (Brokaw, 1991). In the case of tilapia spermatozoa, the changes in pattern of the sperm swimming track at different temperatures may occur as a result of intracellular Ca^{2+} signaling (Morita et al., 2006).

There are few reports about the effects of temperature on fish sperm motility. In rainbow trout (*Oncorhynchus mykiss*) spermatozoa, Cosson et al. (1985) and Billard and Cosson (1992) assessed the beat frequency of flagella and values varied from 32 Hz at 5 °C to 55 Hz at 21 to 26 °C with the latter value corresponding to a sperm velocity of about 250 μm/s. Billard and Cosson (1986) observed a marked increase of the flagellar beat frequency of demembranated/reactivated sperm flagella (from 23 to 82 Hz) when there was an increase in the temperature from 5 to 25 °C. This flagellar beat frequency would be associated with an increasing ATP utilization rate with increasing temperatures. The resulting increase of sperm velocity is because of the temperature effect, primarily on the axoneme of the sperm tail. The duration of motility after motility induction of trout sperm varied from 5 min at 5 °C to 30 s at 25 °C. The effect of temperature on the motility duration is marked greater in trout than in tilapia sperm, a feature that could be related to the relatively lesser and narrow range of temperatures at which trout generally reproduce (6–8 °C). The effect of temperature on the motility duration was studied by Lahnsteiner and Mansour (2012) in three other cold-water fish species (brown trout, *Salmo trutta*; burbot, *L. lota;* and grayling, *Thymallus thymallus*), leading to the conclusion that a low temperature is beneficial for increasing the duration of sperm motility subsequent to sperm activation. There was a species-specific initial velocity of spermatozoa (measured at 10 s post-activation). Velocity of spermatozoa was less when the temperature of the activation medium was increased from 4 to 8 °C in **brown** trout and burbot while velocity was not different in the temperature range of 4 to 16 °C in grayling (Lahnsteiner and Mansour, 2012). There was an absence of the effect of temperature on sperm velocity in a temperate water spawning fish, the European perch (*Perca fluviatilis*) (Lahnsteiner, 2011) and gilthead seabream (*Sparus aurata*) (Lahnsteiner and Caberlotto, 2012). In contrast, there was an increase in swimming velocity with increasing temperatures in common carp (*Cyprinus carpio*; Dadras et al.,

2016), Senegalese sole (*Solea senegalensis*; Diogo, 2010) and the three-spined stickleback (*Gasterosteus aculeatus*; Mehlis and Bakker, 2014). Results of the present study provide additional information about possible responses of spermatozoa to ambient temperatures. These responses include the increase of sperm velocity without a decrease in percentage of motile sperm in an extremely wide temperature range. Additionally, the temperatures in this experimental range are not consistent with those during natural spawning. With hake (*Merlucius merlucius*) spermatozoa, flagellar beat frequency increases from 13 Hz at 5 °C to 60 Hz at 30 °C (Cosson et al., 2010). Results in a comparative study of freshwater (rainbow trout) and marine (hake) fish indicate there is a temperature dependency of beat frequency on rate of ATP store utilization by the flagellar dynein. In the present study, results from the study of Nile tilapia, a warm water species, indicate that the initial sperm velocity after motility induction consistently increases as temperature increases until there is a temperature well beyond that prevailing during natural spawning conditions that approaches being harmful as a result of denaturation of some proteins.

The effect of high temperatures on fish sperm velocity is largely species specific, with there being either a negative, nil or an optimal effect of temperature on sperm motility (Mehlis and Baker, 2014). The results of the present study supplement those from this previous study because of the positive effect of temperature on spermatozoa until there is a temperature at which sperm lose the capacity for motility.

5. Conclusion

The results of the present study clearly indicate the importance of ambient temperature on values for sperm motility variables and optimal fertilization conditions in fish. The response of Nile tilapia spermatozoa to ambient temperature is an increase of velocity without a decrease of the percentage motility at an extremely wide range of temperatures (5–50 °C). Additionally, the temperatures affecting sperm movement is of a much wider range than that which prevails during natural reproduction in this species. The mechanisms through which temperature affects sperm motility in this tropical fish species remain to be studied in more detail both at the membrane as well as at the axoneme.

Declarations of interest

None.

Funding

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project “CENAKVA” No. CZ.1.05/2.1.00/01.0024 and Project “CENAKVA II” No. LO1205 under the NPU I program), CZ.02.1.01./0.0/0.0/16_025/0007370 Reproductive and genetic procedures for preserving fish biodiversity and aquaculture, and by ANR-CLIMSEX project (N°15-CE02-0012) “Water warming: lessons from tilapia’s adaptation”.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 652831 (AQUAEXCEL2020). This output reflects only the author’s view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

Acknowledgements

The authors greatly appreciate comments and valuable writing assistance from Professor W.L. Shelton during manuscript preparation.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anireprosci.2019.01.010.

References

Alavi, S.M.H., Cosson, J., 2005. Sperm motility in fishes. I. Effects of temperature and pH: a review. Cell Biol. Int. 29, 101–110.

Baroiller, J.F., Toguyeni, A., 2004. The Tilapiini tribe: environmental, and social aspects of reproduction and growth. In: Safran, P. (Ed.), Fisheries and Aquaculture, Encyclopedia of Life Support Systems (EOLSS), Developed Under the Auspices of the UNESCO. Eolss Publishers Co. Ltd., Oxford, pp. 230–256.

Bezault, E., Clota, F., Derivaz, M., Chevassus, B., Baroiller, J.-F., 2007. Sex determination and temperature-induced sex differentiation in three natural populations of Nile tilapia (*Oreochromis niloticus*) adapted to extreme temperature conditions. Aquaculture 272 (Supplement 1), S3–S16.

Billard, R., Cosson, M.P., 1986. Sperm motility in rainbow trout, *Parasalmo mykiss*; effect of pH and temperature. In: Zohar, Y., Breton, B. (Eds.), Reproduction in Fish: Basic and Applied Aspects in Endocrinology and Genetics. INRA Press, Paris, pp. 161–167.

Billard, R., Cosson, M.P., 1992. Some problems related to the assessment of sperm in freshwater fish. J. Exp. Zool. 261, 122–131.

Bombardelli, R.A., Sanches, E.A., Baggio, D.M., Sykora, R.M., Souza, B.Ed., Tessaro, L., Piana, P.A., 2013. Effects of the spermatozoa: oocyte ratio, water volume and water temperature on artificial fertilization and sperm activation of cascudo-preto. Rev. Bras. Zootec. 42, 1–6.

Brokaw, C.J., 1991. Calcium sensors in sea urchin sperm flagella. Cell Motil. Cytoskel. 18, 123–130.

Cosson, J., 2008. The motility apparatus of fish spermatozoa. In: Alavi, S.M.H., Cosson, J.J., Coward, K., Rafiee, G. (Eds.), Fish Spermatology. Alpha Science International Ltd, Oxford, pp. 281–316.

Cosson, M.-P., Billard, R., Gatti, J.L., Christen, R., 1985. Rapid and quantitative assessment of trout spermatozoa motility using stroboscopy. Aquaculture 46, 71–75.

Cosson, J., Groison, A.L., Fauvel, C., Suquet, M., 2010. Description of hake (Merlucius merlucius) spermatozoa: flagellar wave characteristics and motility parameters in various situations. J. Appl. Ichthyol. 26, 644–652.
Dadras, H., Dzyuba, V., Cosson, J., Golpour, A., Dzyuba, B., 2016. The in vitro effect of temperature on motility and antioxidant response of common carp Cyprinus carpio spermatozoa. J. Therm. Biol. 59, 64–68.
Dadras, H., Dzyuba, B., Cosson, J., Golpour, A., Siddique, M.A.M., Linhart, O., 2017. Effect of water temperature on the physiology of fish spermatozoon function: a brief review. J. Aquac. Res. Dev. 48, 729–740.
Diogo, P.A.C., 2010. Sperm Motility in *Solea senegalensis*: Effect of Temperature, Salinity, pH and Ovarian Fluid, Faculty of Science and Technology, University of Algarve. Faro, pp. p. 73.
Dzyuba, V., Cosson, J., 2014. Motility of fish spermatozoa: from external signaling to flagella response. Reprod. Biol. 14, 165–175.
Fisheries and aquaculture software, 2017. FishStatJ - Software for Fishery Statistical Time Series. FAO Fisheries and Aquaculture Department. Rome. http://www.fao.org/fishery/statistics/software/fishstatj/en. Accessed 7 February 2018.
Inaba, K., Morisawa, S., Morisawa, M., 1998. Proteasomes regulate the motility of salmonid fish sperm through modulation of cAMP-dependent phosphorylation of an outer arm dynein light chain. J. Cell. Sci. 111, 1105–1115.
Lahnsteiner, F., 2011. Spermatozoa of the teleost fish *Perca fluviatilis* (perch) have the ability to swim for more than 2 hours in saline solutions. Aquaculture 314, 221–224.
Lahnsteiner, F., Caberlotto, S., 2012. Motility of gilthead seabream *Sparus aurata* spermatozoa and its relation to temperature, energy metabolism and oxidative stress. Aquaculture 370-371, 76–83.
Lahnsteiner, F., Mansour, N., 2012. The effect of temperature on sperm motility and enzymatic activity in brown trout *Salmo trutta*, burbot *Lota lota* and grayling *Thymallus thymallus*. J. Fish Biol. 81, 197–209.
Legendre, M., Alavi, S.M.H., Dzyuba, B., Linhart, O., Prokopchuk, G., Cochet, C., Dugué, R., Cosson, J., 2016. Adaptations of semen characteristics and sperm motility to harsh salinity: Extreme situations encountered by the euryhaline tilapia Sarotherodon melanotheron heudelotii (Dumeril, 1859). Theriogenology 86, 1251–1267.
Mehlis, M., Bakker, T.C.M., 2014. The influence of ambient water temperature on sperm performance and fertilization success in three-spined sticklebacks (*Gasterosteus aculeatus*). Evol. Ecol. 28, 655–667.
Morisawa, M., 1985. Initiation mechanism of sperm motility at spawning in teleosts. Zool. Sci. 2, 605–615.
Morisawa, M., 1994. Cell signaling mechanisms for sperm motility. Zool. Sci. 11, 647–662.
Morisawa, M., Suzuki, K., 1980. Osmolality and potassium ion: their roles in initiation of sperm motility in teleosts. Science 210, 1145–1147.
Morita, M., Takemura, A., Nakajima, A., Okuno, M., 2006. Microtubule sliding movement in tilapia sperm flagella axoneme is regulated by Ca^{2+}/calmodulin-dependent protein phosphorylation. Cell Motil. Cytoskel. 63, 459–470.
Philippart, J.C., Ruwet, J.C., 1982. Ecology and distribution of tilapias. In: Pullin, R.S.V., Lowe-McConel, R.H. (Eds.), The Biology and Culture of Tilapias. ICLARM, Manila, pp. 15–60.
Prokopchuk, G., Dzyuba, B., Bondarenko, O., Rodina, M., Cosson, J., 2015. Motility initiation of sterlet sturgeon (*Acipenser ruthenus*) spermatozoa: Describing the propagation of the first flagellar waves. Theriogenology 84, 51–61.
Trewavas, E., 1983. Tilapiine Fishes of the Genera *Sarotherodon*, *Oreochromis*, and *Danakilia*. British Museum (Natural History), London.