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Semen evaluation in *Chirostoma jordani* (Woolman, 1894) and *Chirostoma humboldtianum* (Valenciennes, 1835), Mexican native species (Atheriniformes: Atherinopsidae)

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Abstract: The knowledge of the reproductive physiology of fish of commercial interest is fundamental to optimize reproduction in wildlife or in captivity, so this research aims to determine and compare the seminal quality of *Chirostoma jordani* and *Chirostoma humboldtianum*. A sample of 20 specimens of each species from the state of Tlaxcala and Mexico City were obtained, respectively, from which the semen was obtained by slightly pressing the abdominal region in the operculum-caudal direction, collected with a micropipette of 100 µL and placed in microcentrifuge tubes. According to the results, the mean volume of semen was 2.73 ± 1.0 µL for *C. jordani* and 2.47 ± 1.8 µL in *C. humboldtianum*, while the mean sperm concentration was 9.24 ± 4.32 x 10^6 spermatozoa mL^{-1} and 15.58 ± 13.81 x 10^6 spermatozoa μL^{-1} respectively, with 95.10 ± 4.90 % and 92.10 ± 1.89 % of live cells respectively, and average motility of 437.85 ± 90.37 s and 549.40 ± 31.80 s respectively. Finding significant differences in the motility time between both species. Information that contributes to the knowledge of the reproductive biology of *C. jordani* and *C. humboldtianum* to improve their reproduction in captivity or wildlife.

Keywords: Seminal volume; sperm concentration; sperm viability; sperm motility; atherinopsidae

Introduction

The white and charal fishes are considered endemic species and they are distributed naturally although most of them were introduced in 16 States of the Mexican Republic: Aguascalientes, Coahuila, Chihuahua, Durango, Guanajuato, Hidalgo, Jalisco, México, Michoacán, Morelos, Nuevo León, Puebla, San Luis Potosí, Sinaloa, Tamaulipas and Veracruz (Espinosa *et al*., 1993).

Based on the studies conducted by Miller *et al*. (2005), they were reallocated within the *Menidia* genus, although it is customary to allocate them within the previous genus: *Chirostoma* (Scharpf, 2007).

These genus includes 19 species and some of them possess high commercial relevance, such as: the white fish (*Chirostoma humboldtianum*) and several silverside types, e.g. *C. jordani, C. attenuatum* and *C. chapalae*. The population of almost every species within the *Chirostoma* genus is drastically decreased due to overexploitation, pollution, habitat destruction and introduction of foreign species (Rojas, 2013).

The studies conducted on *C. jordani* and *C. humboldtianum* have been focused on the type and selection of food in their wildlife (Navarrete *et al*., 1996; Fernández *et al*., 2008), feed for charal larvae, growth assessment, mortality, survival and jaw development (Navarrete and Contreras, 2011; Sánchez-Merino *et al*., 2006; Figueroa-Lucero *et al*., 2004), helminthic records (Hernández *et al*., 2008), stress control after catching, handling and transportation (Blancas *et al*., 2014), testicular development and maturity (Uria *et al*., 1998), reproductive biology, ontogeny (Ramírez-Sevilla, 2006), structure, oocyte development, ultrastructure, hormone levels (Blancas-Arroyo *et al*., 2008; Cárdenas *et al*., 2008), reproductive aspects (Ibañez *et al*., 2008), intra-

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specific karyotype variation (Urbina-Sánchez *et al*., 2016), among other aspects. However, there is no study describing semen in *C. jordani* and *C. humboldtianum* quality so this information is important to understand the reproductive physiology of males of both species.

Taking this into account, the aim of this study was to assess and to compare *C. jordani* and *C. humboldtianum* semen quality. This information will contribute to obtain parameters intended to optimize their reproduction either in captivity or in the wild.

Materials and Methods

Sampling

C. jordani specimens (n=20) were collected from the Atlangatepec Dam (Tlaxcala, Mexico) with average weight of 5.0 ± 1.2 g and total length of 8.6 ± 0.6 mm whereas *C. humboldtianum* (n=20) were provided by the Aquaculture Experimental Plant of Universidad Autónoma Metropolitana Unidad Iztapalapa, Mexico City with average weight of 14.3 ± 1.1 g and total length of 17.8 ± 1.4 mm.

Water excess was dried in each specimen and semen was obtained by applying slight abdominal pressure in the operculum-caudal sense. The sample was collected by using a 100 µL micropipette and it was placed on microfuge tubes. It was verified that samples did not contained urine, stool, blood or water traces (Bustamante *et al*., 2016a).

Semen evaluation

Semen was collected by using a 100 µL micropipette and volume was expressed in µL. Sperm concentration was expressed as the number of cells per mL and it was assessed from a previously homogenized stock solution consisting of 1:5:1 NaCl, formaldehyde and semen sample. Counting was performed under an Olympus Optical BX41TF® microscope with a Neubauer chamber and by using the Image-Pro 5.1® software (modified from Rodríguez *et al.*, 2016).

Sperm viability was evaluated based on membrane integrity. Briefly, 3 µL of semen were mixed with 1 µL of eosin-nigrosin. Those stained in red or pink were considered dead, whereas those unstained were considered viable (modified from Kuradomi *et al*., 2016).

Motility was assessed by activating 1 µL of semen with 5 µL of water of the environment. Total duration was registered in seconds as observed under an Olympus Optical BX41TF® microscope using a 40X magnification (modified from Rodríguez *et al.*, 2016).

Statistical analysis

Study variables were processed by using descriptive analysis such as: mean, standard deviation (SD), as well as maximum and minimum values. In order to identify significant differences regarding semen quality between both species, a Student's t test was performed with a $P < 0.05$ significance level (Daniel, 2017).

Results

C. jordani semen quality

Mean semen volume was 2.73 ± 1.0 µL for *C. jordani*, displaying maximum and minimum values of 5.29 and 1.23 µL, respectively. Mean sperm concentration was 9.24 ± 4.32 x 10^6 sperms mL^{-1}, whereas maximum and minimum values were 19.50 x 10^6 and 3.75 x 10^6 sperms mL^{-1}, respectively. Viability percentage was 95.10 ± 4.90 % and its maximum and minimum values were 97 and 91 live cells, respectively. Finally, mean motility was 437.85 ± 90.37 s with maximum and minimum values of 524 and 162 s, respectively.

C. humboldtianum semen quality

Mean semen volume was 2.47 ± 1.8 µL for *C. humboldtianum*, displaying maximum and minimum values of 5.61 and 0.06 µL, respectively. Mean sperm concentration was 15.58 ± 13.81 x 10^6 sperms mL^{-1}, whereas maximum and minimum values were 51.00 x 10^6 and 3.75 x 10^6 sperms mL^{-1}, respectively. Viability percentage was 92.10 ± 1.89 %, and its maximum and minimum values were 96 and 93 live cells, respectively. Finally, mean motility was 549.40 ± 31.80 s with maximum and minimum values of 591 and 499 s, respectively.

Semen quality comparison

Semen production and sperm viability of *C. jordani* and *C. humboldtianum* showed no differences ($P > 0.05$). However, differences in concentration and sperm motility were detected between both species ($P < 0.05$) (Fig. 1).

Discussion

Semen quality evaluation is essential for aquaculture and fishing in order to improve the reproductive efficiency of commercially relevant species and also to introduce and or repopulate those species affected by anthropogenic damages. Such quality is correlated with volume, sperm concentration, viability and

morphology, as without enough viable and motile sperms possessing a suitable morphology, oocyte fertilization or embryo production would not occur (Bustamante *et al*., 2016a).

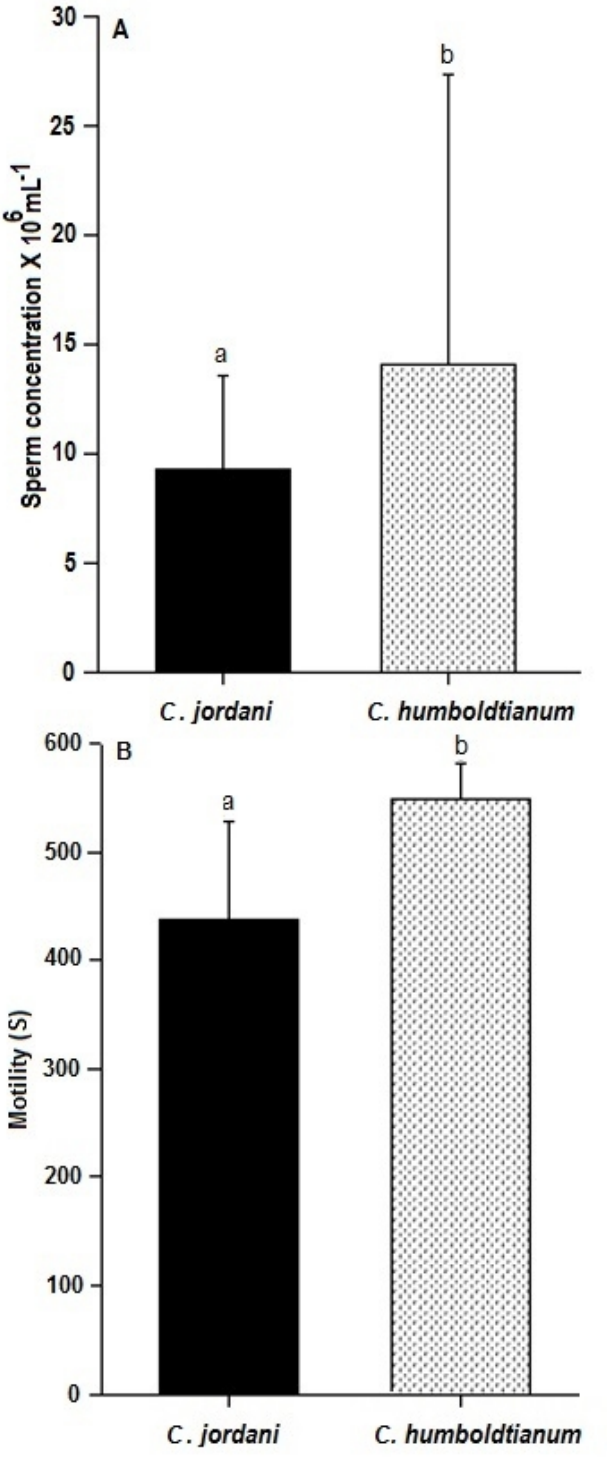

Fig. 1: A) Sperm concentration (mean ± SD); B) Duration of sperm motility (mean ± SD). Upper indexes indicate significant differences (*P* < 0.05) *Student's t test.*

According to the report by Bobe and Labbé (2010) as well as that by Hajirezaee *et al*. (2010), semen quality is defined as the measure of the sperm ability to successfully fertilize the oocyte and any quantifiable physical parameter directly correlated with this ability, which is the main sperm quality marker (Rurangwa *et al*., 2004; Cosson, 2008; Bobe and Labbé, 2010).

Base on the results of the present investigation the volume, sperm concentration, viability and motility; are variables that vary at individual and species level, which coincide with that reported by Piironen (1985) and Bustamante *et al*. (2016b) who point out that the quality of semen varies considerably among individuals of the same species.

Semen quality evaluation is scarce for Atheriniformes in order to perform a comparison. Among these few studies, that published by Blancas-Arroyo *et al*. (2004) reported a maximum and minimum semen volumes of 150 µL and 50 µL, respectively. These are above the values observed in this study for *C. jordani* and *C. humboldtianum* as maximum their respective volumes were 5.29 and 5.61 µL, whereas minimum were de 1.23 and 0.06 µL.

Conversely, Strūssmann *et al*. (1994) reported maximum and minimum volumes of 200 µL and 100 µL, respectively for *Odontesthes bonariensis*. These value is higher when compared to that reported by Miranda *et al*. (2005) for the same species as production was 4.63±1.54 µL. For *C. humboldtianum*, Blancas-Arroyo *et al*. (2004) reported maximum and minimum volumes of 150 µL and 50 µL, whereas in this study maximum volumes were 5.29 and 5.61 µL, whereas minimum were 1.23 and 0.06 µL for *C. jordani* and *C. humboldtianum*, respectively.

Furthermore, when the mean semen production values obtained for *C. jordani* and *C. humboldtianum* in this study (2.73 ± 1.0 and 2.47 ± 1.18 µL, respectively) were compared those for other teleost belonging to different families is lower regarding species as *Oncorhynchus mykiss nelsoni* (355 µL) (Aguilar *et al*., 2014), *Danio rerio* (10.0 ± 0.0 µL) (Jing *et al.,* 2009), *Barbus barbus* (420 µL) (Alavi *et al*., 2008). Conversely, it is higher when compared to the values observed for *Moenkhausia sanctaefilomenae* (2.14 ± 1.55 µL) (Domínguez *et al*., 2015) and *Jenynsia multidentata* (2.0 µL) (Roggio *et al*., 2014).

With regard to sperm concentration, Blancas-Arroyo *et al*. (2004) report 17.760 x 10^6 mL^{-1} sperm in *C. humboldtianum* that surpasses the values of the present investigation for both species. For the same family Mirandas *et al*. (2005) indicate a maximum

concentration of 6.5 x 10^9 mL^{-1} spermatozoa in *O. bonariensis* that exceed that reported.

When comparing the sperm concentration obtained in the present study for *C. jordani* and *C. humboldtianum* 9.24 ± 4.32 x 10^6 and 15.58 ± 13.81 x 10^6 spermatozoa mL^{-1} respectively, with other teleosts is inferior to that obtained in species such as: *C. sanctaefilomenae* 6.8 ± 292,82 x 10^8 (Domínguez *et al*., 2015), *O. mykiss nelsoni* 1.2 x 10^9 (Aguilar *et al*., 2014) and *D. rerio* 7.9 ± 12.4 x 10^7 spermatozoa mL^{-1} (Jing *et al*., 2009) and higher than that of *J. multidentata* 5.5 x 10^6 spermatozoa mL^{-1} (Roggio *et al*., 2014) for both species.

According to Bustamante *et al*. (2016a) and Alavi *et al*. (2008) the variations in volume and sperm concentration between the same or other species could be a function of the reproductive period, size, weight and sexual maturity of the species.

With regard to Rurangwa *et al*. (2004), sperm viability is defined as their ability to be motile and to fertilize an oocyte. However, in this study such variable was measured as membrane integrity based on the absorption of the eosin dye. This method has been used in order to assess the viable percentage of the cells in multiple species such as: *D. rerio* (Gerber *et al*., 2016), *Piaractus mesopotamicys* (Kuradomi *et al*., 2016), *Brycon opalinus* (Viveiros *et al*., 2012) and *Brycon henni* (Tabares *et al*., 2006). The results obtained showed a percentage of viable cells above 80 %, as reported in this study for *C. jordani* and *C. humboldtianum.*

Conversely, the sperm motility value reported in this study was 437.85 ± 90.37 and 549.40 ± 31.80 s for *C. jordani* and *C. humboldtianum*, respectively. This duration value was higher when compared to that obtained by Blancas-Arroyo *et al*. (2004) for *C. humboldtianum* (maximum and minimum mean motility values 86.6 and 36.9 s), to that observed by Alves *et al*. (2016) as they reported a duration of 147.3 ± 10.3 s for *O. bonariensis*. The duration value was also higher regarding that reported for teleost belonging to other families, such as: *Cyprinus carpio* (59.0 ± 7.0 s) (Öğretmen *et al*., 2015), *Oncorhynchus mykiss* (375 s) (Sahin *et al*., 2014), *Pseudoplatystoma metaense* (48.23 ± 4.85s) (Ramírez *et al*., 2011), *Prochilodus lineatus* (88.62 ± 50 s) (Felizardo *et al*., 2010), *Ctenopharyngodon idellus* (36.14 ± 1.15s) (Metwally and Fouad, 2009), whereas it was lower compared to the value obtained for *Pangasianodon hypophthalmus* (632s) (Kuppusamy and Natesan, 2014).

Additionally, the differences regarding motility duration are attributed to the use of an activating solution such as the use of solutions based on salts and sugars, which favors the sperm metabolic activation they must respond to physical-chemical changes of osmotic pressure, ion balance, temperature and pH (Alavi and Cosson 2005; 2006).

However, motility also depends on the amount of mitochondria and the available energy reserves (Browne *et al*., 2015), as they are responsible for the chemical energy derived from ATP hydrolysis and the mechanical energy in order to perform the flagellum movement (Gagnon and de Lamirande, 2006).

According to Blancas-Arroyo *et al*. (2004); Rurangwa *et al*. (2004); Bradshaw and Holpsafel, (2007); Bobe and Labbé, (2010); Hajirezaee *et al*. (2010); Aragón *et al*. (2014) and Bustamante *et al.* (2016b), differences regarding sperm volume, concentration and motility among different organisms or species are attributed to the amount and quality of food, age, geographical conditions, reproductive season, gamete collecting method as well as environmental stimuli such as temperature and photoperiod.

This demonstrates that sperm volume, concentration, viability and motility are parameters defining semen quality and they may be used as markers of their fertilizing ability (Rurangwa *et al*., 2004; Cosson, 2008; Bobe and Labbé, 2010; Hajirezaee *et al*., 2010). Thus, this information contributes to the knowledge of *C. jordani* and *C. humboldtianum* reproductive species. Both of them are Mexican species and this information may be useful for future studies.

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