Genetics of Aquatic Organisms 7(4. Special Issue), GA730
https://doi.org/10.4194/GA730

RESEARCH PAPER

Post-mortem Fertilizing Capacity of Spermatozoid *Chirostoma jordani* (Atheriniformes: Atherinopsidae)

Martha Rodríguez-Gutiérrez[1], Alejandro Ávalos-Rodríguez[2], Javier Lorenzo Olivares-Orozco[1], Jesús Dámaso Bustamante-González[3]

[1]Universidad Autónoma Metropolitana-Xochimilco, Laboratorio de Reproducción, Genética y Sanidad Acuícola. Departamento El Hombre y su Ambiente, Coyoacán, México.
[2]Universidad Autónoma Metropolitana-Xochimilco, Laboratorio de Bioquímica de la Reproducción. Departamento de Producción Agrícola y Animal, Coyoacán, México.
[3]Universidad Autónoma Metropolitana-Iztapalapa, Planta Experimental de Producción Acuícola. Departamento de Hidrobiología, Iztapalapa, México.

How to Cite

Rodríguez-Gutiérrez, M., Ávalos-Rodríguez, A., Olivares-Orozco, J.L., Bustamante-González, J.D. (2023). Post-mortem Fertilizing Capacity of Spermatozoid *Chirostoma jordani* (Atheriniformes: Atherinopsidae). *Genetics of Aquatic Organisms, 7(4. Special Issue), GA730*. https://doi.org/10.4194/GA730

Article History
Received 06 September 2022
Accepted 28 December 2023
First Online 29 December 2023

Corresponding Author
E-mail:
jesusbustamantegonzalez@gmail.com

Keywords
Silverside
Seminal quality
Post-mortem reproduction

Abstract

Post-mortem fertilization is considered a technique in which semen is extracted from a dead male to fertilize the egg of a living female. Therefore, this technique can be an excellent option in the face of the loss of biodiversity of multiple animal species. The objective of this research is to implement post-mortem assisted reproduction in the charal, *Chirostoma jordani*, which is a species of economic, biological and cultural importance in Mexico. Mature males were obtained from commercial capture Atlangatepec Dam, Tlaxcala, Mexico. The mature males were sacrificed and divided into three groups: 1) room temperature 18±1°C, 2) ice 5±1°C and 3) refrigeration 11±1°C. The variables evaluated were: percentage (s), duration of motility (%), and fertilization rate (%) at zero, two, four, six, eight and ten hours in quadruplicate, respectively. The results indicate that sperm viability decreased over time; at room temperature it lasted six hours; in ice and refrigeration 10, denoting significant differences ($P<0.05$). Semen from organisms preserved in ice and refrigeration presented the best fertilization percentages at two and four hours (>90 and ~80%, respectively) ($P<0.05$). For practical purposes, it was demonstrated that semen from post-mortem organisms can be used to develop conservation practices.

Introduction

The *Chirostoma* genus belongs to the Atherinopsidae family, it is endemic to the Trans-Mexican Volcanic Belt, with a discontinuous distribution from the state of Mexico to Nayarit, along the Lerma-Santiago system, where it lives in lake water bodies (Hernández-Rubio et al., 2006; Rodríguez-Gutiérrez et al., 2022), an area that is characterized by a high degree of habitat fragmentation due mainly to urbanization and industrialization.

The result of anthropogenic activity leads to the contamination of water bodies, and the overfishing their populations has severely impacted and decreased some of the species belonging to the genus, for example, *Chirostoma bartoni, C. charari* and *C. riojai* that are cataloged in danger of extinction and *C. labarcae, C. promelas* in the category of threatened species (SEMARNAT-NOM-059, 2019); and *C. jordani* has been cataloged by the International Union for Conservation of Nature in the lower risk category (Rodríguez-Gutiérrez et al., 2022).

Since pre-Hispanic times, *Chirostoma* species have represented the main food and economic resource in various communities in the Valley of Mexico, Michoacán and surrounding areas, which has negatively impacted the size of the populations, which increased due to the introduction of exotic species (Olvera-Blanco et al., 2009; Miller, 2009). With the purpose of sustainable use of the genus, it is proposed to implement post-mortem assisted reproduction, which in teleosts, by presenting external fertilization, facilitates the collection of semen

and oocytes (Dietrich et al., 2005). Post-mortem assisted reproduction is a technique that allows the recovery of genetic material (Dziekońska et al., 2020; Álvarez-Rodríguez et al., 2018; Benítez-González et al., 2018) that can be implemented in multiple species of animals. commercial or biological interest or for the conservation of species.

In addition to the above, the use of animal models is an excellent option to implement protocols related to post-mortem assisted reproduction techniques in commercial species and in species included in some risk category.

To implement post-mortem assisted reproduction, it is important to evaluate the viability and motility sperm of the gametes after the death of the fishes with the possibility of preserving them for a short time (hours) or in the long term through cryopreservation (years) (Dietrich et al., 2005).

Therefore, the aim of this research was to implement post-mortem assisted reproduction in *C. jordani* in Mexico as a model species.

Materials and Methods

Collection of Biological Material

Mature males (fish with semen) were obtained from commercial capture Atlangatepec Dam, Tlaxcala, Mexico, in July 2021, coordinates 9°33'43''N 98°12'03''W, with an average weight of 5.7±0.6 g and total length of 9.2±0.3 mm. The water temperature was 16.8±1°C, pH 8.6±0.2 and dissolved oxygen 6.1±0.6 mg L^{-1}.

Experimental Design

The mature males were sacrificed and divided into three groups: 1) group room temperature 18±1°C (n=30), 2) ice 5±1°C (n=30) and 3) refrigeration 11±1°C (n=30). The variables evaluated were: percentage (s), duration of motility (%), and fertilization rate (%) at zero, two, four, six, eight and ten hours in quadruplicate, respectively.

Semen Collection

The fish were sexed and the males, to extract of semen, was dried the water around the genital pore and with light pressure in the region of the flanks was collected directly with micropipette for avoid contamination of urine or feces (Bustamante et al*.,* 2016).

Estimation of the Percentage and Duration of Sperm Motility

The sperm were activated in a 1:5 ratio (semen: dam water) and were immediately observed under an Olympus Optical BX41TF® microscope at 40X. The percentage of motility was determined considering the initial movement of the sperm expressed as a percentage; and the duration was established as the total time of the flagellar movement (s), each estimation was carried out in triplicate (Bustamante et al., 2016).

Fertilization Rate

Fertilization was carried out in quadruplicate at room temperature (18±1 °C), ice (5±1 °C) and refrigeration (11±1 °C) at zero, two, four, six, eight and ten-hours post-mortem as follows: 1) one microliter of semen obtained at the respective time was extracted and activated with 10 µL of water (temperature 17.6±1 °C, pH 8.3±0.1 and dissolved oxygen 5.8±0.3 mg L^{-1}) as activating solution, 2) the sample semen was poured directly into 60 freshly extracted oocytes. The sperm density was $2x10^4$ sperm per oocyte. The fertilization rate (%) was determined 10 minutes later, with a stereoscopic microscope by the formation of the perivitelline space.

Statistics Analysis

The variables were processed with descriptive analyzes expressed as mean ± standard deviation (SD). To determine if there were differences, the one-way analysis of variance (ANDEVA) was performed; when determining differences, the Tukey test was applied, with a significance level of ($P<0.05$).

Results

Percentage and Duration of Sperm Motility

The percentage of motility in fresh at time zero was one hundred percent and was inversely proportional with respect to time. At 4 hours, significant differences ($P<0.05$) were determined between room temperature *vs* ice and refrigeration, but not between these two latter ($P>0.05$). While at 6 hours in all treatments significant differences were determined ($P<0.05$); and after 8 hours in the room temperature samples, sperm motility was not determined, in ice and refrigeration the percentage of motility was less than 15 percent and not significant differences ($P>0.05$) were determined between 8 and 10 hours (Figure 1).

Sperm motility at time zero had an average duration of 225±37 and was inversely proportional with respect to time. After 2 hours, significant differences were determined between those preserved in refrigeration *vs* room temperature and ice ($P<0.05$); while at 4 hours differences were determined between ice and refrigeration ($P<0.05$); at 6 hours in all treatments, significant differences were determined ($P<0.05$), with motility lasting longer in the ice; at 8 hours in the samples at room temperature, sperm motility was not determined, and in ice and refrigeration the duration was at least 75 seconds, presenting

significant differences (P<0.05) between 8 and 10 hours in both treatments (Figure 2).

Fertilization Rate

The results of the fertilization rate (%) are presented in Figure 3. The semen samples from the specimens kept in ice and refrigeration were those that presented the highest percentages of fertilization. After two hours, in the three groups percentages greater than 90% was obtaining, with significant differences were recorded between room temperature conservation and ice (P<0.05). After four hours, there was a significant decrease in the fertilization rate of those preserved at room temperature, which was maintained until six hours, with significant differences (P<0.05) with respect to those preserved in ice, and refrigeration, after 8 hours, fertilization results were zero in room temperature while in ice it was greater than 25 percent (Figure 3).

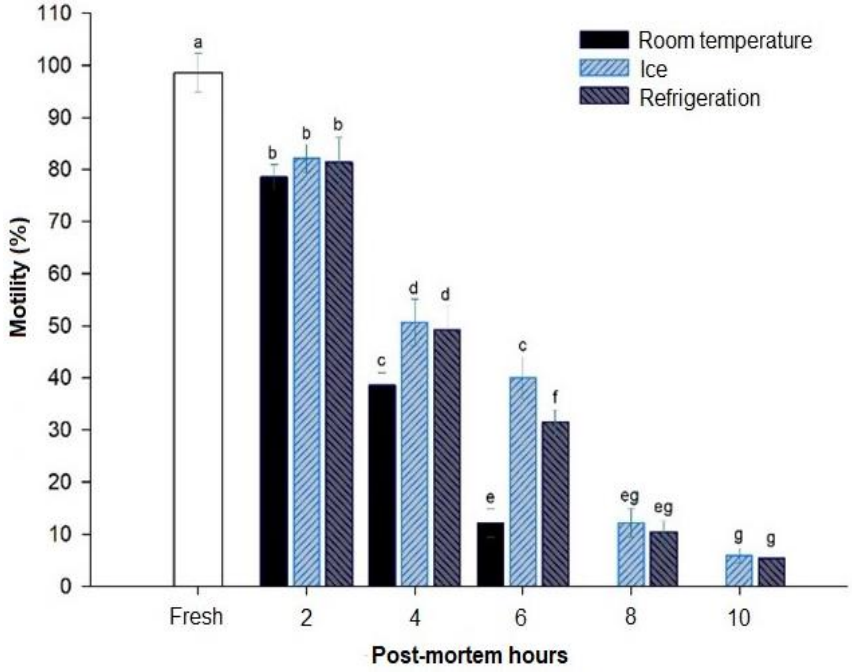

Figure 1. Percentage of sperm motility post-mortem preserved at room temperature (18±1°C), ice (5±1°C) and refrigeration (11±1°C) in *C. jordani*. Bars that do not share the same letter indicate significant differences (P<0.05).

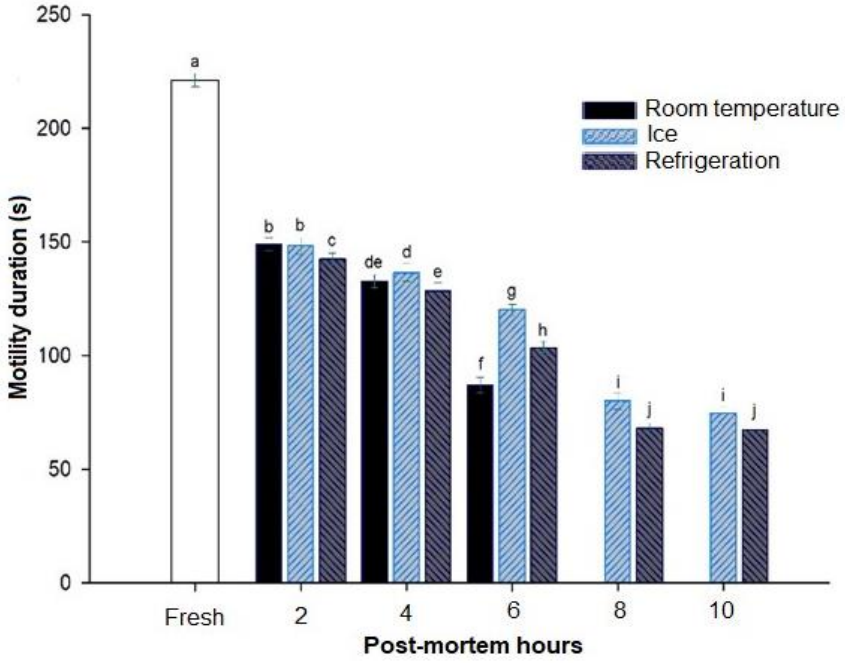

Figure 2. Duration of sperm motility post-mortem preserved at room temperature (18±1°C), ice (5±1°C) and refrigeration (11±1°C) in *C. jordani*. Bars that do not share the same letter denote significant differences (P<0.05).

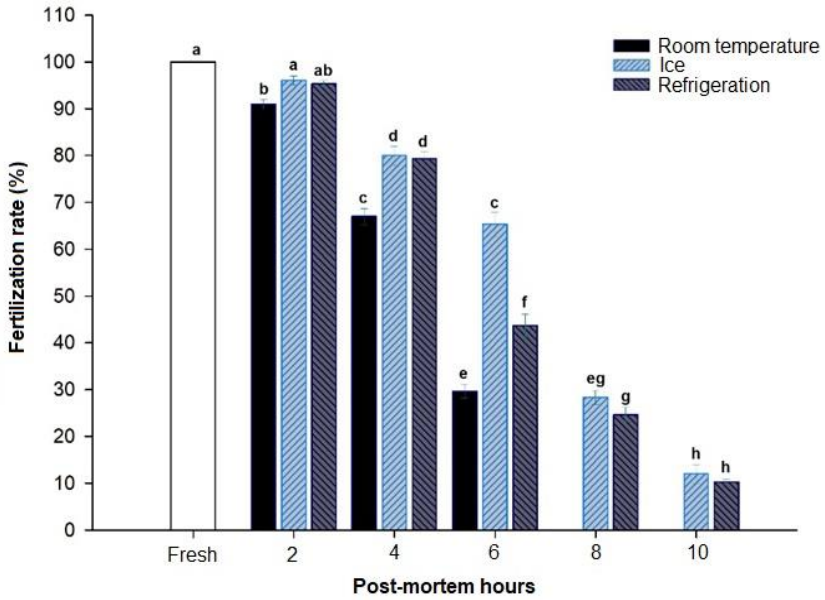

Figure 3. Fertilization rate post-mortem preserved at room temperature (18±1°C), ice (5±1°C) and refrigeration (11±1°C) in *C. jordani*. Bars that do not share the same literal indicate significant differences (P<0.05).

Discussion

In broodstock the quality of semen is determined from the evaluation of the motility and viability of the sperm and is done with the aim of improving the success of controlled fertilization in hatcheries.

In teleost, sperm activate motility upon contact with the external environment, seawater or freshwater, depending on their habitat; the flagella immediately develops waves that propagate at a high frequency of up to 70 beats s^{-1}, which propel them at a speed of 6-10 mm min^{-1}, for short time; the high rate of flagellar energy consumption contributes to the rapid decay of motility and its inability to restore it quickly enough determines that sperm depend on the energy accumulated before activation (Cosson, 2010).

The duration of motility and the percentage of sperm that maintain it play a determining role in the competition for fertilize the egg as demonstrated by the studies carried out by Pizzari and Parker (2009), Gage et al. (2004) in *Salmo salar*, Boschetto et al. (2011) in *P. reticulata* and Bozkurt et al. (2019) in *Salmo trutta macrostigma.*

Short-term sperm conservation studies in fish have focused on semen extraction and refrigeration conservation (Saad et al., 1988; Bozkurt and Secer, 2005); in aerobic conditions to evaluate the effect of oxidative stress on sperm viability (Sadegh-Aramli et al., 2013) and of course its conservation at different temperatures, or in the long term through cryopreservation (Bustamante-González et al., 2021) but unlike mammals (Álvarez-Rodríguez et al., 2018), no studies were found focused on the conservation of semen in post-mortem organisms, which, in the field of aquaculture, is of interest especially in species that, are difficult to handle and/or transport, as is the case of *C. jordani*, which is also of biological importance since, according to the International Union for Conservation of Nature, it is in the Risk category minor.

In male broodstock studies on the short-term conservation of spermatozoa have been carried out in species of commercial interest such as carp, trout, sturgeon; the semen is obtained under aerobic conditions and conserved at low temperatures for short time, or with diluent solutions that keep the sperm immobile, with satisfactory results since it maintains motility with have high percentage of fertilization; or for a long time by cryopreservation (Saad et al., 1988; Tekin et al., 2007; Nynca et al., 2012; Contreras et al., 2019).

According to the results obtained in *C. jordani*, sperm motility averaged 225±37 seconds, a value higher than that most freshwater teleost of commercial interest such as trout, carp, tilapia, which in general only exceed 60 s (Billard, 1986; Cosson, 2010; Bustamante et al., 2016).

Although there is consensus among research that motility is one of the determining qualities in the evaluation of sperm, it is necessary to consider its intensity and duration, since the success of fertilization depends on it (Nynca et al., 2012). Therefore, an additional parameter that will influence the competitiveness of sperm is the percentage that remains mobile in an ejaculate (Snook, 2005).

Regarding the results of the fertilization rate, they are considered adequate, since, in the organisms preserved in refrigeration, the percentage of motility at 6 hours was 40 percent, and the fertilization rate was greater than 45 percent; while those preserved in ice reached 67 percent. As for the organisms kept at room temperature, they barely reached 30 percent, denoting significant differences with respect to other treatments.

The storage of semen for short periods is of interest in farms, where accidents and the death of reproducers occur, when they are manipulated during reproduction or transported to other farms, the present investigation denotes that it is possible to collect their sperm and use it to carry out the fertilization with good results at least for this species.

The results suggest that although cell lysis occurs when the organism dies, each apparatus and organ has a period of differential deterioration and that in sexually mature males, sperm are likely to be preserved for longer, unlike other cells, because during maturity, the production of seminal fluid plays a predominant role in sperm viability determined by osmolarity, which keeps the sperm immobile within the testicle and, once the organism dies, favors conservation for up to 4 hours at temperature environment with a fertilization rate greater than 65 percent, allowing fertilization (Fangzhou et al., 2017).

Conclusion

Reproduction of *C. jordani* is possible from dead mature organisms preserved at room temperature (18±1°C), ice (5±1°C) and refrigeration (11±1°C). For practical purposes, this information will allow fishermen to implement *in situ* post-mortem fertilization for up to 6 hours after fishing, preserving the fish at room temperature.

Ethical Statement

This research was carried out with fish from commercial fishing by native fishermen.

Funding Information

No funding was received for this article.

Author Contribution

MR: Investigation, supervision, manuscript writing. AA: Research, supervision, conceptualization, methodology. JO: Editorial, JB: Investigation, methodology, supervision, analysis.

Conflict of Interest

The authors have no conflict of interest to declare.

Acknowledgements

The authors thank the Atlangatepec aquaculture center, Tlaxcala, Mexico and Universidad Autónoma Metropolitana-Xochimilco for providing the necessary facilities to carry out this work.

References

Álvarez-Rodríguez, M., Álvarez, M., Anel-López, L., Guerra, C., Chamorro, C. A., Anel, L., de Paz, P., & Martínez-Pastor, F. (2018). Effect of length of time post-mortem on quality and freezing capacity of Cantabric chamois (*Rupicapra pirenaica parva*) epididymal spermatozoa. *Animal Reproduction Science*, *198*: 184-192. https://doi.org/10.1016/j.anireprosci.2018.09.018

Benítez-González, E., Chamba-Ochoa, H., Sánchez-Sánchez, E., Luzón-Cevallos, F., & Sánchez-Carrillo, J. (2018). Evaluación comparativa de dos métodos de recuperación espermática de epididímos bovinos post-mortem. *Abanico veterinario*, *8*(1), 59- 74. http://dx.doi.org/10.21929/abavet2018.81

Billard, R. (1986). Spermatogenesis and spermatology of some teleost species. *Reproduction Nutrition Development*. 26(4), 877–920. https://dx.doi.org/10.1051/rnd:19860601

Boschetto, C., Gasparini, C., & Pilastro, A. (2011). Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology, 65*: 813–821. https://doi.org/10.1007/s00265-010-1085-y

Bozkurt, Y., & Seçer, S. (2005). Effect of short-term preservation of mirror carp (*Cyprinus carpio*) semen on motility, fertilization and hatching rates. *The Israeli Journal of Aquaculture-Bamidgeh*, *57*(3), 207-212. https://doi.org/10.46989/001c.20407

Bozkurt, Y., Yavaş, I., Gul, A., Bucak, M. N., Yeni, D., & Avdatek, F. (2019). Effect of extender supplemented with boron on post-thaw motility, viability, DNA damage and fertilization ability of cryopreserved brown trout (*Salmo trutta macrostigma*) spermatozoa. *CryoLetters*, *40*(5), 275-283.

Bustamante-González, J. D., González-Rentería, M., Rodríguez-Gutiérrez, M., Cortés-García, A., & Ávalos-Rodríguez, A. (2016). Methodologies for spermatic evaluation in teleost. *International Journal of Aquatic Science*, *7*(2), 95-106.

Bustamante-González, J.D., Rodríguez-Gutiérrez, M., Cortés-García, A., Arenas-Ríos, E., Figueroa-Lucero, G., & Ávalos-Rodríguez, A. (2021). Cryopreservation of *Chirostoma jordani* sperm, fish model for conservation of the genus in Mexico. *Journal of Applied Ichthyology*, *37*, 278-284. https://doi.org/10.1111/jai.14151

Contreras, P., Dumorné, K., Ulloa-Rodríguez, P., Merino, O., Figueroa, E., Farias, J. G., Valdebenito, I., & Risopatrón, J. (2019). Effect of short-term storage on sperm function in fish semen: a review. *Reviews in Aquaculture*, *12*, 1373-1389. https://doi.org/10.1111/raq.12387

Cosson, J. (2010). Frenetic activation of fish spermatozoa flagella entails short-term motility, portending their precocious decadence. *Journal of Fish Biology*, *76*(1), 240–279. https://doi.org/10.1111/j.1095-8649.2009.02504.x

Dietrich, G. J., Kowalski, R., Wojtczak, M., Dobosz, S., Goryczko, K., & Ciereszko, A. (2005). Motility parameters of rainbow trout (*Oncorhynchus mykiss*) spermatozoa in relation to sequential collection of milt, time of post-mortem storage and anesthesia. *Fish Physiology Biochemistry, 31*, 1-9. https://doi.org/10.1007/s10695-005-3527-4

Dziekońska, A., Niedźwiecka, E., Ewa, N. M., Koziorowska-Gilun, M., & Kordan W. (2020). Viability longevity and

quality of epididymal sperm stored in the liquid state of European red deer (*Cervus elaphus elaphus*). *Animal Reproduction Science*, *213*, 1-10. https://doi.org/10.1016/j.anireprosci.2019.106269

Fangzhou, H., Kang, X., Yunfan, Z., Chang, W., Shi, W., Jun, X., Min, W., Rurong, Z., Kaikun, L., Min, T., Wei, D., & Shaojun, L. (2017). Different expression patterns of sperm motility-related genes in testis of diploid and tetraploid cyprinid fish. *Biology of Reproduction*, *96*(4), 907–920. https://doi.org/10.1093/biolre/iox010

Gage, M. J. G., Macfarlane, C. P., Yeates, S., Ward, R. G., Searle, J. B., & Parker, G. A. (2004). Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. *Current Biology*, *14*(1), 44–47. https://doi.org/10.1016/j.cub.2003.12.028

Hernández-Rubio, M., Figueroa-Lucero, G., Barriga-Sosa, I. D. L. A., Arredondo-Figueroa, J. L., & Castro-Barrera, T. (2006). Early development of the shortfin silverside *Chirostoma humboldtianum* (Valenciennes, 1835) (Atheriniformes: Atherinopsidae). *Aquaculture*, *261*(4), 1440–1446. https://doi.org/10.1016/j.aquaculture.2006.08.048

Miller, R. R. (2009). *Peces Dulceacuícolas de México*. CONABIO, Sociedad Ictiológica Mexicana, Colegio de la Frontera Sur y Colegio de Peces del Desierto, México-Estados Unidos. Ciudad de México, México. 559 p.

Nynca, J., Dietrich, G. J., Dobosz, S., Kuzminski, H., & Ciereszko, A. (2012). Sperm motility rate al pH 6.5 as a useful parameter for the evaluation of rainbow trout sperm quality and usefulness for short time storage. *Journal of Applied Ichthyology*. *28*, 930-933. https://doi.org/10.1111/jai.12053

Olvera-Blanco, M. Y., Gómez-Márquez, J. L., Peña-Mendoza, B., Gaspar-Dillanes, M. T., & Pérez. C. (2009). Reproductive biology of *Menidia jordani* (Atheriniformes: Atherinopsidae) in Xochimilco Lake, México. *Ciencia Pesquera*, (2), 65-75.

Pizzari, T., Parker, G. (2008). Sperm competition and sperm phenotype pp 207- 245 In: *Sperm Biology an Evolutionary Perspective* (Pitnick, S.S., Hosken, D.J. & Birkhead, T.R. eds.) Academic Press, 674p.

Rodríguez-Gutiérrez, M., Pineda-Escobar, M. E., & Mata-Sotres, J. A. (2022). Cambios en la captura pesquera en la presa Atlangatepec, Tlaxcala, México. *Ciencia Pesquera*, *29*(1-2), 11- 18.

Saad, A., Billard, R., Theron, M. C., & Hollebecq, M. G. (1988). Short -term preservation of carp (*Cyprins carpio*) semen. *Aquaculture*, *71*(1-2), 133-150. https://doi.org/10.1016/0044-8486(88)90280-3

SEMARNAT (Secretaría del Medio Ambiente y Recursos Naturales). (2019). Norma Oficial Mexicana NOM-059-SEMARNAT-2019, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario Oficial De La Federación. 14 de noviembre de 2019, México. https://www.dof.gob.mx/nota_detalle.php?codigo=5578808&fecha=14/11/2019

Snook, R. R. (2005). Sperm in competition: not playing by the numbers. *Trends in Ecology and Evolution*, *20*(1), 46–53. https://doi.org/10.1016/j.tree.2004.10.011

Tekin, N., Seçer, S., Akcay, E., Bozkurt, Y., & Kayam, S. (2007). Effects of glycerol additions on post-thaw fertility of frozen rainbow trout sperm, with an emphasis on interaction between extender and cryoprotectant. *Journal of Applied Ichthyology*, *23*(1), 60-63. https://doi.org/10.1111/j.1439-0426.2006.00792.x