Neotropical Ichthyology

Original article

https://doi.org/10.1590/1982-0224-2023-0109

Relationship between seminal plasma composition and sperm quality parameters of the catfish *Pseudoplatystoma reticulatum*

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Sperm quality is a fundamental parameter for the effective reproduction of fish in captivity and the development of reproductive techniques, such as semen cryopreservation. This study aimed to determine the composition of the seminal plasma of *Pseudoplatystoma reticulatum* and analyze the relationships between plasma components and sperm characteristics. Nine males were induced to spermiation with carp pituitary extract in the reproductive period of the species (November and December/2019). Semen characteristics were evaluated: subjective sperm motility, motility, duration, released sperm volume, sperm concentration, pH, osmolality, and seminal plasma composition, including levels of calcium, chloride, sodium, magnesium, potassium, glucose, fructosamine, triglycerides, and total protein. To determine the relationship between seminal plasma components and sperm motility parameters, a principal component analysis (PCA) was performed. The seminal plasma of *P. reticulatum* is composed mainly of the Na^+ ion and organic components such as protein and glucose. Through PCA, it was observed that sperm motility had a strong positive correlation with motility time, sperm concentration, and total protein and a negative correlation with osmolality and fructosamine.

Keywords: Ionic composition, Fish reproduction, PCA, Siluriformes, Sperm characteristics.

Submitted October 16, 2023
Accepted June 3, 2024
by Bernardo Baldisserotto
Epub July 22, 2024

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Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 22, no. 2, Maringá 2024

A qualidade espermática é parâmetro fundamental para a reprodução eficaz de peixes em cativeiro e para o desenvolvimento de técnicas reprodutivas, como a criopreservação de sêmen. Este estudo teve como objetivo determinar a composição do plasma seminal de *Pseudoplatystoma reticulatum* e analisar as relações entre os componentes do plasma seminal e as características espermáticas. Nove machos foram induzidos à espermiação com extrato de hipófise de carpa no período reprodutivo da espécie (novembro e dezembro/2019). As características do sêmen foram avaliadas: motilidade espermática subjetiva, motilidade, duração, volume espermático liberado, concentração espermática, pH, osmolalidade e composição do plasma seminal, incluindo níveis de cálcio, cloreto, sódio, magnésio, potássio, glicose, frutosamina, triglicerídeos e proteína total. Para determinar a relação entre os componentes do plasma seminal e os parâmetros de motilidade espermática, foi realizada uma análise de componentes principais (PCA). O plasma seminal de *P. reticulatum* é composto principalmente pelo íon Na^+ e componentes orgânicos como proteínas e glicose. Através da PCA, observou-se que a motilidade espermática apresentou forte correlação positiva com tempo de motilidade, concentração espermática e proteína total e correlação negativa com osmolalidade e frutosamina.

Palavras-chave: Composição iônica, Característica espermática, PCA, Reprodução de peixes, Siluriformes.

INTRODUCTION

Brazil is the country with the greatest powers in the world for the development of fish farming due to its climate and diversity of species. The growth in recent years in the aquaculture sector has contributed to the decrease in fish catches in the natural environment (FAO, 2020). With the increase in the production of fish in captivity, it becomes necessary to develop effective protocols that optimize artificial reproduction. In this sense, it is fundamental to obtain biological and technical knowledge that allows the evaluation of the reproductive parameters of the fish, allowing the development of biotechnology that will make it possible to optimize the reproduction in captivity of these species, such as protocols for manipulation and storage of gametes.

The use of high-quality gametes from both males and females is an essential prerequisite to achieving high fertilization success and hatching, both for aquaculture and scientific purposes (Bozkurt *et al*., 2008; Yoshida, Asturiano, 2020). In the evaluation of fish semen quality, one of the most important criteria used in the literature is sperm motility, generally expressed as the percentage and duration of sperm motility after activation. (Lahnsteiner *et al*., 1996; Gallego, Asturiano, 2018).

Seminal plasma has a unique species-specific composition, containing substances that support sperm cells (Ciereszko *et al*., 2011). In addition, it plays an important physiological role in sperm maturation, having a biochemical composition that supports and protects the viability, motility, and fertilizing capacity of sperm, creating an ideal environment for their storage (Ciereszko, 2008; Bozkurt *et al*., 2011).

In fish, unlike other vertebrates, seminal plasma is composed mainly of mineral compounds (Na^+, K^+, Mg^{2+} and Ca^+), being characterized by low concentrations of proteins, as well as other organic substances, such as hormones and pheromones, cholesterol, glycerol, vitamins, free amino acids, sugars, citric acid and lipids (Linhart *et al*., 1991; Ciereszko *et al*., 2000; Cosson, 2004). The determination of seminal plasma composition has a great influence on the biological quality of the semen. It can help to understand the design requirements to prepare the appropriate artificial seminal plasma solutions (Ciereszko, 2008).

Knowledge of the relationships between seminal characteristics and the chemical composition of sperm is a prerequisite for successfully assessing the reproductive capacity of different fish species (Ciereszko *et al*., 2011; Hussain *et al*., 2018). Also, these would provide knowledge for the preparation of artificial plasma solutions, which can be used for the dilution of semen for short-term storage or cryopreservation (Billard, Cosson, 1992).

In the last years, the interest of researchers in studies on seminal plasma in fish has been growing, especially in publications that somehow cover correlations between seminal plasma components and sperm motility. However, descriptions of plasma components and possible relationships between sperm motility parameters in species of neotropical fishes need further investigation. And, referring to fish species belonging to the Siluriformes order, some works related to this theme can be highlighted in species such as *Clarias gariepinus* (Burchell, 1822) (Steyn, van Vuren, 1986), *Clarias macrocephalus* Günther, 1864 (Tan-Fermin *et al*., 1999) and *Rhamdia quelen* (Quoy & Gaimard, 1824) (Borges *et al*., 2005), however, they are still very few.

The catfish species *Pseudoplatystoma reticulatum* Eigenmann & Eigenmann, 1889, popularly known as "Surubim cachara" (Silva *et al*., 2015) is among the species of Brazilian ichthyofauna that stand out with potential for fish farming. In the literature, no previous published works are describing the composition of seminal plasma and its relationship with sperm physical parameters. As it is a species that has high commercial value as it has characteristics that meet the fish consumer market (Kubitza, 1998). Its natural populations can be affected over time as a result of indiscriminate capture, as well as the destruction of their natural habitats.

Thus, it is essential to have in-depth knowledge of the seminal characteristics of the species, thus generating basic knowledge for future cryopreservation studies that could help to maintain the genetic viability of their populations. Given the above, the present study aimed to characterize the composition of seminal plasma and determine the relationships between its components and the characteristics of the semen of *P. reticulatum* in captivity.

MATERIAL AND METHODS

Fish handling. Nine adult males of *P. reticulatum* (Fig. 1.) from the breeding stock of the company Piraí Fish-farming, located in Terenos, Mato Grosso do Sul, Brazil (20°25'05"S 55°17'11"W) were used for the development of the present work. The experiments were carried out between the late spring and early summer (November and December) of 2019, corresponding to the peak of the species' reproductive season.

No vouchers of the target species were deposited in a scientific collection, since they were all adults belonging to the breeding stock, and there were no juveniles at the station that could be sent as vouchers. At the end of this experiment, the specimens were released into their natural environment, the Miranda River, in the upper Paraguai River basin.

Sperm collection and analysis of seminal characteristics. After capturing the specimens of *P. reticulatum* in the excavated ponds, they were placed in a concrete tank for hormonal induction and semen collection. Adult breeders have an average body mass of 2.5 kg and were identified with microchips. The animals were hormonally induced by applying a single dose of carp pituitary extract at 3 mg/kg of fish. The semen was collected after 232 hours/degree (t = 8 h; T = 29 °C) by gentle abdominal pressing and stored in graduated sterilized falcon tubes and kept in a styrofoam box at approximately 4 degrees until analysis. The semen obtained from each specimen, color, and seminal volume (Sem. Vol.) was determined, considering the maximum volume obtained via abdominal massage until the beginning of the presence of blood, avoiding contamination with urine, feces, and blood. Subjective sperm motility (Mot) was determined under light microscopy based on the scale proposed by Fribourgh (1966). Sperm was activated with 0.45% NaCl in a proportion of 1:10 (semen: activator). Motility duration (Mot.tm) was measured from sperm activation to observation of only 10% of motile sperm. The pH of fresh semen was measured using a pHmeter (Checker®). For osmolality (Osm), the semen was centrifuged at 3000 rpm for 15 min, the supernatant was removed and analyzed in a cryo-osmometer (OSMOMAT® model 030, Berlin, Germany). To determine the sperm concentration (Conc), the semen was diluted in formalin-saline solution at a proportion of 1:1000 (semen: solution), and the count was performed in a Neubauer hemocytometer chamber, the results were obtained according (Kavamoto *et al*., 1985).

FIGURE 1 | Adult male specimen of *Pseudoplatystoma reticulatum*, belonging to the Piraí fish farming, Terenos, Mato Grosso do Sul, Brazil.

Analysis of seminal plasma components. To obtain seminal plasma, the semen was centrifuged at 3000 g for 15 min. After centrifugation, the supernatant was transferred to sterile polyethylene tubes, properly identified, and stored in an ultra-freezer at -80 °C until biochemical analysis. Aliquots were thawed at room temperature (~25 °C) for biochemical measurements of seminal plasma. The concentrations of calcium (Ca^{+}), chlorides (Cl^{-}), sodium (Na^{+}), magnesium (Mg^{2+}), potassium (K^{+}), glucose (Glic), fructosamine (Frut), triglycerides (Trig), and total proteins (Tot. pro). The tests were performed using commercial kits (LABTEST® Diagnostica S.A.), following the instructions for each analysis (Leite *et al.*, 2018). All analyzes were determined using a SpectraMax Plus 384 spectrophotometer.

Statistical analysis. Data on sperm characteristics and respective components of seminal plasma were presented through a descriptive analysis expressed as mean ± SEM (Standard Error of the Mean). To determine the relationships between seminal plasma components and sperm characteristics of the species, a Principal Component Analysis (PCA) was performed to reduce the redundancy of the observed variables and identify patterns in the dataset. First, the data were standardized, allowing variables measured on different measurement units to be compared. The first two eigenvectors with the highest percentage of accumulated variance were considered to construct the PCA graph. For this analysis, six copies of the total analyzed were used. All analyzes were performed using the R Software, "FactoMineR" (R Development Core Team, 2020).

RESULTS

Sperm characteristics of *Pseudoplatystoma reticulatum*. Spermatological parameters of the sperm of *P. reticulatum* were found rather variable and they are shown in Tab. 1. The parameters of osmolality, motility, and motility time were where the greatest variability was found, with pH and concentration being the least variable.

TABLE 1 | Sperm characteristics of *Pseudoplatystoma reticulatum* (n = 9). Minimum, maximum, and mean values. SEM = Standard error of the mean.

Variable	Minimum	Maximum	Mean	SEM
Seminal volume (mL)	2.90	12.00	7.80	1.04
Sperm motility (%)	70.00	95.00	86.25	3.09
Time motility (s)	75.00	112.00	89.25	3.92
Concentration (spz.10^{9}/mL)	12.70	18.10	15.71	0.72
pH	7.33	8.00	7.75	0.08
Osmolality (mOsm/kg)	173.66	276.00	211.62	11.01

Seminal plasma composition of *Pseudoplatystoma reticulatum*. The results of the analysis of the seminal plasma are shown in Tab. 2. The seminal plasma of *P. reticulatum* is mainly composed of the Na^+ ion, followed by the Cl^-, Ca^+ and K^+ ions, while the Mg^{2+} ion showed the lowest values. Within the organic components, total protein and glucose stood out; on the other hand, fructosamine and triglycerides had low values.

Correlation between seminal plasma components and seminal characteristics of *Pseudoplatystoma reticulatum*. In the principal component analysis (PCA) to determine the possible relationships between the seminal plasma components and the sperm characteristics of this species, the first two components were chosen: the first component (PC1) explained 47.1% of the data variance and the second component (PC2) explained 21.42%, totaling 68.52% of the total variation of the data (Fig. 2).

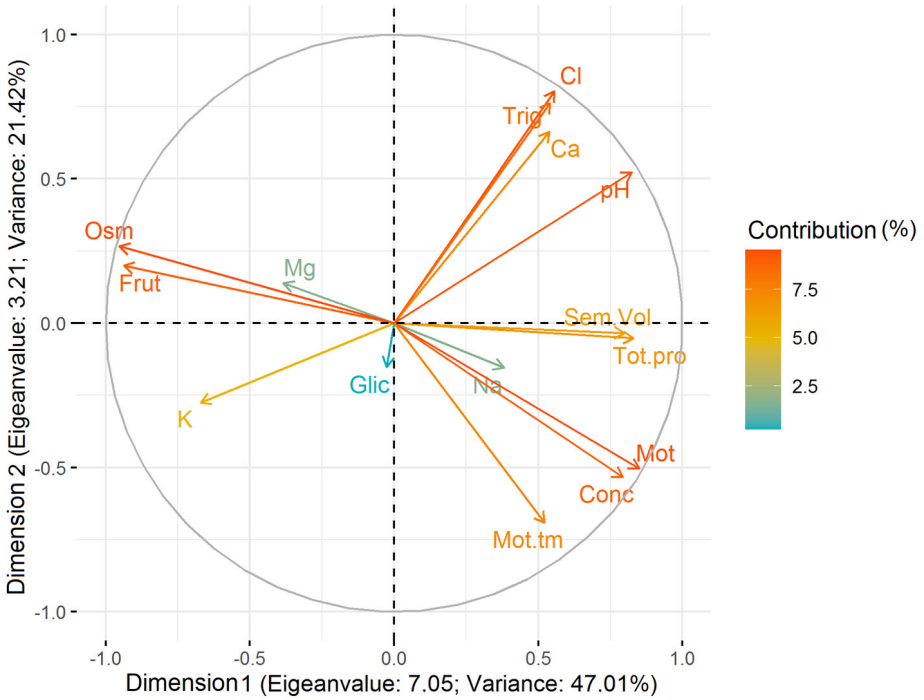

FIGURE 2 | Representation of the first two axes obtained by PCA analyzing the relationships between the components of the seminal plasma and the sperm characteristics of *Pseudoplatystoma reticulatum* (n = 6). Motilidade total (Mot), Tempo de motilidade (Mot. tm), Concentração (Conc) Calcium (Ca^+), Chlorides (Cl^-), Sodium (Na^+), Magnesium (Mg^{2+}), Potassium (K^+), Glucose (Glic), Fructosamine (Frut), Triglycerides (Trig) and Total proteins (Tot.pro).

TABLE 2 | Seminal plasma components of *Pseudoplatystoma reticulatum* (n = 6). Minimum, maximum, and mean values. Standard error of the mean (SEM), Calcium (Ca^{+}), Chlorides (Cl^{-}), Sodium (Na^{+}), Magnesium (Mg^{2+}), Potassium (K^{+}), Glucose (Glic), Fructosamine (Frut), Triglycerides (Trig), and Total proteins (Tot. Pro).

Variable	Min	Max	Mean	SEM
Ca^{+} (mg/dL)	7.94	26.42	12.67	2.15
Cl^{-} (mM/L)	2.83	4.95	4.24	0.25
Na^{+} (mM/L)	108.78	140.97	124.87	3.81
Mg^{2+} (mEq/L)	0.36	2.14	1.15	0.24
K^{+} (mM/L)	0.01	6.09	3.15	0.75
Glic (mg/dL)	124.11	365.24	205.67	39.63
Frut (µmol/L)	23.93	131.63	64.75	15.67
Trig (mg/dL)	1.86	11.60	4.97	1.24
Tot. Pro (g/dL)	0.00	0.85	0.39	0.12

The first two axes are obtained through the PCA and represented in Fig. 2, which can be observed in the colors from light blue to orange that indicate the percentage of contribution in the explained variance, with light blue for the lowest value and orange for the highest percentage. The graph shows a cluster between variables such as Mot, Mot.tm, and Conc, indicating a strong positive correlation between them and Sem.vol and Tot.pro. On the other hand, Frut and Osm had a high positive correlation, but a negative relationship with Mot, Mot.tm, Conc, Tot.pt, and pH. The Cl^{-}, Ca^{+}, Trig, Sem. Vol, and Tot.pro variables had a strong positive correlation with pH, while this variable had a strong negative correlation with K^{+}, which in turn had a positive correlation with Osm and Frut. The variables Glic, Na^{+} and Mg^{2+} contributed little to the variance of the analyzed data, but the Na^{+} and Mg^{2+} had a strong negative correlation between them.

DISCUSSION

Seminal plasma is an important component of fish semen, playing a vital role in sperm metabolism, function, survival, and motility (Mojica-Rodríguez, Cruz-Casallas, 2005; Navarro *et al.*, 2019). Numerous components of seminal plasma are directly linked to these functions. This study to determine the main plasmatic components of *P. reticulatum* semen and its relationship with parameters of seminal quality is unprecedented and will serve as a basis for studies of the reproductive aspects of other fish species, in addition to providing important information for the development of extenders, extender immobilizers, and cryoprotective solutions.

The data obtained in the evaluation of the characteristics of the physical parameters of the species were variable among the individuals. Variations in seminal characteristics are related to physiological variations of the specimens, linked to their genetics and how they react to the "environmental conditions" to which they are subjected. Semen quality, particularly in aquaculture species, depends on several external factors, such as feeding regime, feed quality, temperature, and male reproductive season (Bozkurt *et al.*, 2008).

The seminal plasma in fish, in contrast to that of other vertebrates, is characterized by low concentrations of proteins and other organic substances, containing mainly mineral compounds such as Na^+, K^+, Ca^+, and Mg^{2+} (Ciereszko, 2008). In the present study, the seminal plasma was composed of Na^+ ions like that reported for *Clarias macrocephalus* (Tan-Fermin *et al*., 1999).

Usually in seminal plasma sodium concentrations are 10 times higher than potassium concentrations (Tan-Fermin *et al*., 1999). In membrane permeability processes, ions play a fundamental role, according to the variation of their concentrations in the extra- and intracellular medium, some theses involucred inhibitory effects on the initiation of sperm motility. The increase in Na^+ may be related to the high secretory activity in the sperm ducts (Lahnsteiner *et al*., 1993), for *P. reticulatum* it seems to be one of the fundamental ions in the maintenance of seminal plasma function. For other species of the order Siluriformes, similar concentrations to those obtained in this work were observed, *i.e*., *Clarias gariepinus* 125.25±9.80 (Steyn, van Vuren, 1986); *Clarias macrocephalus* Günther, 1864 164.4±0.6 (Tan-Fermin *et al*., 1999), *Rhamdia quelen* 153.7±2.4 (Borges *et al*., 2005), but the deference may be due to differences in feeding conditions, age, environ-mental factors within the same species due to their reproductive processes. The Na^+ ion can act directly on the osmotic balance, motility, morphology, and metabolism of sperm (Rodríguez *et al*., 2013).

Potassium (K^+) presented low concentration values in the present study, compared to other freshwater species, such as *Hypophthalmichthys molitrix* (Valenciennes, 1844), *Clarias macrocephalus* (Tan-Fermin *et al*., 1999; Rahman *et al*., 2011). It has been reported that low concentrations of K^+ in seminal plasma may be associated with a high percentage of motility and better seminal quality in salmonids (Billard, Cosson, 1992). It has also been documented that in several species of the order Siluriformes, K^+ levels are generally low, but this variability is species-specific and not always low rates of this ion are related to low sperm quality since depending on the concentrations of the other ions which can also influence the motility mechanisms (Dziewulska, 2020).

Ca^+ and Mg^{2+} ions contribute significantly to the composition of fish sperm seminal plasma. These cations are effective as antagonists of the inhibitory effect of K^+ on the motility of the Na^+ ion (Billard, Cosson, 1992). In this study, the values of Ca^+ and Mg^{2+} were similar to those compared with other Siluriformes species (Tan-Fermin *et al*., 1999; Borges *et al*., 2005).

The concentration of glucose in the seminal plasma has a primordial function for the protection of spermatozoa, providing high energy during the process of spermiogenesis, as well as in sperm motility. Different sperm glucose concentrations may indicate differences in sperm metabolic energy from numerous fish species (Hussain *et al*., 2018). In the present study, glucose showed a high concentration in seminal plasma, which coincides with what was detected for other fish species (Lahnsteiner *et al*., 1995), which may suggest that *P. reticulatum* spermatozoa also have a need for higher amounts of energy for its functions, compared to a species of the same genus, *Pseudoplatystoma metaense* Buitrago-Suárez & Burr, 2007, for which (Ramírez-Merlano *et al*., 2011). In turn, the presence in seminal plasma of glucose and high levels of fructosamine, which is described as a glycated protein that is not commonly investigated in seminal plasma of animals, may indicate the inefficient use of glucose by the organism (Armbruster, 1987). In humans, high fructosamine levels may indicate infertility (Tomaszewski *et al*., 1992).

However, it is also known that fructosamine is consumed by superoxide dismutase (SOD), indirectly used to determine the activity of this antioxidant enzyme in the seminal plasma of dogs (Lopes *et al*., 2011). Considering this information, together with the data obtained from the PCA analysis in this study, we can consider that fructosamine has a negative correlation with the parameters of sperm motility.

The PCA is an interesting tool, as it is a type of exploratory analysis that can help in understanding the results obtainedresenting positive correlations between motility mainly with the concentration and motility time corresponding to that reported for freshwater species such as *Rhamdia quelen*, *Salmo trutta* Linnaeus, 1758, *Barbus grypus* (Heckel, 1843) (Borges *et al*., 2005; Bozkurt *et al*., 2011; Güllü *et al*., 2015).

However, in the particular case of motility and concentration, negative correlations have already been reported for species such as *Prochilodus lineatus* (Valenciennes, 1837) (Viveiros *et al*., 2019), *Oncorynchus mykiss* (Walbaum, 1792) (Ciereszko, Dabrowski, 1994), *Cyprinus carpio* Linnaeus, 1758 (Bozkurt *et al*., 2009). It can be considered that a higher sperm concentration is not always related to a higher motility and fertilization rate (Williot *et al*., 2000). It is understood that sperm concentration is species-specific and may be related to gonadal development and maturation, which in turn will depend on changes in temperature, nutritional quality, enzymatic activity, and age, which varies between individuals (Piros *et al*., 2002; Shaliutina-Kolešová *et al*., 2020).

Total proteins also play an important role in energy allocation in sperm, which may be the cause of a positive relationship with motility in the present study. Lahnsteiner *et al*. (2004) report that proteins in seminal plasma prolong sperm viability in *O. mykiss.* Lipids in seminal plasma are also used by spermatozoa as energy reserves while they remain in the spermatic ducts, and the synthesis of triglycerides may be a response to distinct physiological changes during artificial sperm storage (Lahnsteiner *et al*., 1993).

In the present study, triglycerides did not directly link with motility but showed a positive correlation with pH, Cl^-, and Ca^+. Specific physiological relationships between these variables should be studied in more detail in the future, however, Lahnsteiner *et al*. (1998) reported that low triglyceride concentrations may indicate inadequate energy sources, which may reduce motility rates and fertilizing capacity in *O. mykiss*. Cl^- and Ca^+ ions also showed no apparent relationship with motility in the present study.

The pH was not correlated with motility in this study. However, other authors have reported the effects of pH on sperm motility in several fish species, suggesting its importance in seminal plasma characteristics and membrane potential as well as motility (Billard, Cosson, 1988; Gallego *et al*., 2014; Baradaran *et al*., 2019; Pérez *et al*., 2020; Silva Pinheiro *et al*., 2020). According to previous studies, during the passage of sperm from the testis to the spermatic duct, an increase in external pH may be responsible for the acquisition of motility in some salmonid fish (Morisawa, Morisawa, 1986, 1988) and, therefore, the pH of the seminal fluid can also affect the final maturation of sperm (Lahnsteiner *et al*., 1998).

Several authors have reported the importance of the Na^+ ion in the beginning of sperm motility. For this reason, it is widespread to observe activating solutions that contain Na^+, *i.e*., for *Esox lucius* Linnaeus, 1758 (Hadi-Alavi *et al*., 2009) *Prochilodus lineatus* (Viveiros *et al*., 2009), *Brycon insignis* (Orfão *et al*., 2011), *P. lineatus* and *Brycon orbignyanus* (Valenciennes, 1850) (Viveiros *et al*., 2019). In the present study, sodium contributed little to the variance of the data, but it also had a trend in the graph very close to motility, so it could be positively related to this variable.

Knowledge of the physical and chemical constituents of sperm and seminal plasma is a prerequisite for success in assessing the reproductive capacity of different fish species. It is important to emphasize that the composition of seminal plasma can vary not only according to the characteristics of each species but also due to other external factors such as age, reproductive period, nutrition, and many others.

The seminal plasma of *P. reticulatum* has the predominant ions and sugars in its composition, Na^+ and glucose, respectively, in addition to proteins. When applying PCA to the data, we observe positive relationships between motility and the parameters of motility time, sperm concentration, total protein, and negative relationships with osmolality and fructosamine. These data can serve as a basis for creating diluting or cryoprotectant solutions in the sperm cryopreservation protocols of the species.

ACKNOWLEDGMENTS

We would like to thank Piraí Piscicultura for providing the experimental fish. Special thanks to Dr. Juan F. Asturiano, Professor at the Polytechnic University of Valencia, for the final review of this work. This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROEX N°88887.302629/2018–00), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq N°200452/2022–3), the Brazilian fostering agencies Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP N°2020/15020–0).

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AUTHORS' CONTRIBUTION

Malbelys Padilla Sanchez: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing-original draft, Writing-review and editing.

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ETHICAL STATEMENT

All procedures used with the animals for the development of this experiment follow the standards approved by the Ethics Committee for the Use of Animals (CEUA-FEIS/UNESP 04/2021).

COMPETING INTERESTS

The author declares no competing interests.

HOW TO CITE THIS ARTICLE

- **Sanchez MP, Borges LP, Lobato SIR, Carneiro-Leite L, Kasai RYD, Ribeiro CS, Verissimo-Silveira R, Ninhaus-Silveira A.** Relationship between seminal plasma composition and sperm quality parameters of the catfish *Pseudoplatystoma reticulatum*. Neotrop Ichthyol. 2024; 22(2):e230109. https://doi.org/10.1590/1982-0224-2023-0109

Neotropical Ichthyology

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