



Reproductive aspects of the Amazon giant paiche (*Arapaima gigas*): a review

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Abstract

Paiche (*Arapaima gigas*), is a colossal freshwater fish native to the Amazon basin. Its geographic distribution spans various regions, including Brazil, Peru, Colombia, and Guyana, making it a significant component of the aquatic ecosystems in this area. Beyond its ecological role, the paiche holds substantial importance as a valuable fish resource for local communities, providing sustenance and economic opportunities. This review provides a comprehensive analysis of the reproductive aspects of the paiche, based on information published from January 2000 to January 2022. It encompasses a wide range of reproductive characteristics, including sexual differentiation, age at first maturity, and identification techniques. Additionally, it offers an evaluation of various mating behaviors, highlighting their respective advantages and disadvantages. The review also explores genetic and behavioral traits observed in both wild and captive specimens, offering valuable insights for the effective management of breeding programs.

Keywords: Fish reproduction, Sex determination, Amazon, Aquaculture

Introduction

Arapaima gigas (Schinz, 1822) is an iconic fish in the Amazon region (Fig. 1), capable of growing up to 2.5 m in length and weighing up to 180 kg (Chu-Koo et al., 2009). It is one of the most economically significant species in the Amazonian fauna, known as “paiche” in Peru and “pirarucu” in Brazil. Despite having been exploited for decades, studies on its biology and behavior are still scarce (Castello et al., 2015). Its importance in the Amazonian fishery industry is well recognized, but over-



Fig. 1. *Arapaima gigas* specimen.

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fishing has led to a decline in the population, especially among large individuals (Reis et al., 2003). Although it is not classified as an endangered species, it was included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES II) in 1975, and in 2004, it was added to the Brazilian list of fish and aquatic invertebrates threatened by overexploitation (Farias et al., 2019). To address this, measures such as a prolonged ban from October 1 to February 28 of the following year have been established in Peru (García Vasquez & Montreuil Frías, 2003). The species cannot be commercialized without a certificate of origin, which confirms that the fingerlings come from aquaculture. Thus, captive reproduction has become an essential requirement for its cultivation and commercialization (Alcántara et al., 2002). However, an established procedure for the reproduction of the species is still lacking, as the results reported in various studies are variable and dependent on seasonal conditions (Burgos-Morán et al., 2019). Therefore, this review aims to present the main findings related to the reproduction of *A. gigas*.

Reproductive Aspects

The process of gonadal differentiation in *A. gigas* provides insight into its functional biology and ecology. This process occurs prior to testicular differentiation, during which epithelial cells undergo changes in the region of the future lamellae, while germ cells form nests of oogonia due to their concentration in this area. After spatial arrangement, the oogonia become oocytes and initiate the meiotic prophase. Testicular differentiation occurs much later, with germ cells initially dispersed throughout the gonad, and spermatogonial cysts restricted to the central zone of the testicles. Since meiosis in *A. gigas* only begins in individuals over 1 meter long, it is likely that the testes only undergo allometric growth during youth (Godinho et al., 2005). It has been reported that the growth and development of oocytes may differ between individuals from different places, possibly due to environmental or genetic differences or temperature (da Costa Amaral et al., 2020).

The gonadal morphology and reproductive features in *A. gigas* females show the absence of the ventral portion of the ovarian capsule, the absence of an oviduct, and a funnel-shaped coelomic cavity, indicating that oocytes are released into this cavity before being released into the environment (Helfman et al., 2009). Male individuals have a single functional testicle measuring 1 to 1.5 cm in diameter, which is connected to the genital

papilla via a sperm duct. Sexual maturity in females is typically reached at 5 years of age, with a size of 1.7 meters and a weight of 45 kilograms (Lüling, 1964). In Peru, sexual maturity has been described in individuals of 1.85 meters (Guerra, 1980). In a study conducted in the Tocantins river basin, a female length of 1.45–1.54 meters and a male length of 1.15–1.24 meters were recorded during the gonadal maturity stage (Godinho et al., 2005).

Until the year 2022, there are no scientific publications pertaining to the histological description of paiche testicles. There is only a description available regarding gonadal differentiation in males (da Costa Amaral et al., 2020). This underscores the lack of detailed investigations into the specific histological structure of paiche testicles up to the mentioned date.

Regarding sexual maturation, Godinho et al. (2005) first proposed a five-stage system, which was later expanded to a six-stage maturation scale for *A. gigas* females, four of which were already well documented. In contrast, macroscopic observations of different stages of development have led to the description of three stages of sexual maturation in males (Lopes, 2005). However, further histological studies are necessary due to the subjective variations in the macroscopic observations described (Lopes & Queiroz, 2009). To determine female maturation, 11-ketotestosterone (11KT) levels can be measured, since high levels are present near the time of spawning and could be related to sexual behavior. 17α -hydroxyprogesterone, which seems to play an important role as a pheromone in the final stages of the reproductive cycle, has also been reported to be present in the cephalic fluid (Amaral et al., 2019).

In terms of external characteristics, males have an orange spot located in the lower region of the head, indicating high concentrations of testosterone in their hormonal profile. Additionally, it has been reported that females between the ages of 3 and 4 years have greater physiological preparation for reproduction, as indicated by primarily 17 beta-estradiol hormone concentrations, unlike females over 4 years of age (Monteiro et al., 2010). As the population increases, the time to maturity can decrease from 4–5 years to approximately 3 years (Arantes et al., 2010).

In 2013, the effects of gonadotropin-releasing hormone (GnRHa) on *A. gigas* males and females were studied, revealing an increase in testosterone and 11-ketotestosterone during sexual maturity (Faria et al., 2013). However, despite hormone induction, a lack of spontaneous spawning was observed, potentially due to inadequate stimulation of breeding behavior,

such as courtship swimming, nest building, and parental care. This suggests that other GnRHa variants may be involved in modulating reproductive behavior (Okubo & Nagahama, 2008).

Techniques for Sexual Identification

Early sexual identification is critical for larval production in aquaculture and the effective management of broodstock feeding (Saavedra Rojas et al., 2005). In species without clear sexual dimorphism, such as *A. gigas*, dissection for observation of gonadal morphology and confirmation by histology is the most accurate method for sex identification. However, for specimens intended for aquaculture, minimally invasive methods have been developed (Ramírez-Arrarte et al., 2014).

Molecular biology and genetic techniques are widely used for sex identification (Acosta Villota, 2018). Marques et al.'s (2006) study was the first in this field, but it was found that the species lacks a cytologically distinguishable sex chromosome, and both sexes have 56 chromosomes, rendering sexual identification impossible using this method.

Sexual identification based on female-specific plasma vitellogenin is achieved using an enzyme immunoassay (EIA) that can detect very low concentrations of vitellogenin. Since vitellogenin is a specific molecule of females, sexual identification is possible in fish from three to five years of age, corresponding to the initiation of sexual maturation (Dugué et al., 2008).

The use of vitellogenin for sexual identification of *A. gigas* was demonstrated by Chu-Koo et al. (2009), who achieved sexual identification in 100% of the fish analyzed using vitellogenin levels in 6-year-old *A. gigas* adults. Another technique used for sexual identification in immature individuals is the determination of plasma levels of 17 beta-estradiol and 11-ketotestosterone, achieving 95% efficacy in sex identification. Moreover, the data obtained allowed the identification of distinct color patterns around the reproductive period of adult male and female fish (6 years old).

Lopes & Queiroz (2009) evaluated the ability of a group of fishermen from the Mamirauá Reserve, Brazil to differentiate sex by the color pattern. However, of the 109 individuals collected, only 64 were correctly sexually identified by the fishermen, representing an efficacy of 58.71%. The differentiation of males can be achieved by the identification of an orange spot located in the lower region of the head related to the high concentration of testosterone in the hormone profile of these specimens (Monteiro et al., 2010). In a study by Lima et al. (2020), an

efficacy of around 80% was obtained with the observation of patterns. However, they reported that factors such as territorial behavior, water transparency, and seasonality can affect the efficiency of this determination, and thus, the use of other methods such as endoscopy is recommended.

The study by Carreiro et al. (2011) demonstrated that the use of coloration patterns was ineffective for the determination of sex. These authors proposed laparoscopy as a highly reliable method with which they were able to determine sex in 3-year-old animals with 100% efficacy. They also reported that the use of gaseous chloroform was adequate as anesthesia to perform a correct procedure in *A. gigas*. The images obtained allowed differentiating males with a long, opaque, smooth, and milky testicle found on the left side of the body, while females present a fusiform ovary and oocytes easily observable due to their semitransparent green color.

In contrast to the 100% efficacy achieved by Carreiro et al. (2011) with the laparoscopic method, the plasma vitellogenin method used by Chu-Koo et al. (2009) obtained an efficacy of 95% for sex determination. However, with the latter method, 100% efficacy was obtained in immature and adult individuals, and 95% efficacy was obtained, with the 17 beta-estradiol/11-ketotestosterone ratio playing a role in the lower value achieved. Likewise, in a subsequent study, endoscopy and cannulation were used considering the labor intensiveness and invasiveness of the laparoscopic method (Torati et al., 2019).

In another study using endoscopy to determine the sex of 19-month-old *A. gigas* juveniles, females were identified by the presentation of pale-colored, leaf-shaped ovaries, after approximately 2 minutes of observation. In relation to males, in some cases, the gonopore could not be located, and sex determination had to be indirect (Torati et al., 2019).

Apart from these methods for the determination of sex, Du et al. (2019) report that biopsies or observation through gonoducts are difficult and laborious to perform, and genetic markers would better facilitate identification. Furthermore, according to Núñez et al. (2011), only the left lobe of the gonad is functional, and the right gonad is atrophied in both males and females.

In order to identify a simple and inexpensive method to determine sexually immature specimens of *A. gigas*, Almeida et al. (2013) analyzed male and female DNA pools with 56 randomly amplified polymorphic DNA primers, generating 2,609 fragments with 1,341 segregating polymorphic markers with a spacing of 714 kb, which corresponds to less than 0.1% of the genome. In this study, it was not possible to identify evident

genomic differences between males and females, suggesting that the species had undergone a recent evolutionary loss of the SDL-carrying gene, as seen in two rodents of the genus *Ellibus*, which had presented the loss of the SRY gene and the Y chromosome itself over time (Marshall Graves & Shetty, 2001).

When performing a transcriptome analysis based on pyrosequencing, 105 genes were found in the liver and 204 in the skin with differentiated profiles, in which 95 were expressed in females and 214 in males. Gene expression in the liver was found to be similar in the two sexes. One of the genes differentially expressed in the skin of males and females was protein 4, which is associated with the acrosome membrane of spermatozoa. In this study, the gene responsible for vitellogenin was present in both the skin of males and females. However, this protein was absent in the liver, which could be due to the fact that they were juvenile individuals and, therefore, this type of analysis should not be recommended for sex determination (Watanabe et al., 2018).

One year after the previously mentioned study, the complete genome of the species was published, describing 254 female- and 281 male-specific scaffolds, which represented 0.12% (Du et al., 2019), being a value greater than the 0.01% obtained by Almeida et al. (2013). Likewise, Vialle et al. (2018) suggested that the *amdhd1* and *cd48* genes were “potential male-specific genes”. Among the proposals related to marker for sex identification in *A. gigas*, Cavalcante et al. (2020) evaluated methods based on sequences of *k* consecutive nucleotides (*k*-mer), demonstrating that this interesting and low-complexity sexual identification technique searches for repeat regions on represented or under-represented nucleotides in one of the two sexes (Ezaz & Deakin, 2014). The first method for determining genetic markers that met the necessary accuracy and was minimally invasive was that of Adolphi et al. (2021), who performed a transcriptome analysis and protein structure predictions, which confirmed that the *id2bbY* gene is the main gene candidate for sex determination, since it is expressed in gonads of juvenile individuals. The method used was minimally invasive and only required the extraction of a small sample of the fin. Regarding adult specimens, Santos et al. (2021) described different genes responsible for biological processes linked and not linked to sex. In addition, marked dimorphism was demonstrated in the gene expression patterns in brain and gonadal tissue, which could serve for future research focused on the gonadal development processes of this species.

In order to identify a simple and cost-effective method for

determining sexually immature individuals of *A. gigas*, Almeida et al. (2013) analyzed male and female DNA pools using 56 randomly amplified polymorphic DNA primers. They generated 2,609 fragments with 1,341 segregating polymorphic markers with a spacing of 714 kb, which represents less than 0.1% of the genome. However, this study was not able to identify clear genomic differences between males and females, suggesting that the species had experienced a recent evolutionary loss of the SDL-carrying gene, similar to what was seen in two rodents of the *Ellibus* genus. These rodents experienced the loss of the SRY gene and the Y chromosome itself over time (Marshall Graves & Shetty, 2001).

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One year after the previously mentioned study, the complete genome of the species was published. It described 254 female-specific and 281 male-specific scaffolds, which represented 0.12% of the genome (Du et al., 2019), a higher value than the 0.01% obtained by Almeida et al. (2013). Vialle et al. (2018) proposed that the *amdhd1* and *cd48* genes were “potential male-specific genes”. In addition, Cavalcante et al. (2020) evaluated methods based on sequences of consecutive nucleotides (*k*-mers), demonstrating that this technique could search for repeat regions on represented or under-represented nucleotides in one of the two sexes, making it a simple and low-complexity method for sexual identification (Ezaz & Deakin, 2014).

The first minimally invasive and accurate method for determining genetic markers was presented by Adolphi et al. (2021). They performed a transcriptome analysis and protein structure predictions, which confirmed that the *id2bbY* gene is the main candidate gene for sex determination since it is expressed in the gonads of juvenile individuals. This method required only a small sample of the fin. Santos et al. (2021) described different genes responsible for biological processes linked and not linked to sex in adult specimens. They also demonstrated marked di-

morphism in the gene expression patterns in brain and gonadal tissue, which could be useful for future research focused on gonadal development processes of this species.

Furthermore, López-Landavery et al. (2022) implemented an innovative approach that harnessed the recently available genome sequence of paiche, employing a state-of-the-art next generation sequencing (NGS) method known as genome differences by unmapped reads. This strategy facilitated the identification of a male-specific region (MSR_3728), found exclusively in males, laying the groundwork for the subsequent development of a duplex qPCR method. The groundbreaking nature of this qPCR method, validated across gonad, fin, and mucus samples, became evident as it provided a swift and accurate means of sex determination at any developmental stage. Notably, both primer sets, MSR_107 and MSR_129, demonstrated an exceptional 100% accuracy in detecting males. López-Landavery et al. (2022) duplex qPCR method, incorporating a reference gene, not only exhibited effectiveness in gonads and fins but also demonstrated utility in non-invasive sampling through gill mucus. The outcomes of this study introduce a novel pipeline for identifying DNA sex markers, culminating in a rapid, non-invasive, and cost-effective solution for sexing *A. gigas*.

Breeding Stock Management

The study of *A. gigas* ethology has become a priority in recent years due to the limited knowledge available on its behavior in both wild and captivity, which affects its reproduction (Arantes et al., 2010). Despite evidence of monogamous behavior (Goossens et al., 1998), it has been found that in artificial or semi-artificial environments, these animals seek to increase their chances of preserving offspring by fertilizing more than one nest. However, they only care for one of these nests as a couple (Farias et al., 2015). Having unrelated fry, which will share a nest with another male's offspring, reduces the chances of all hatchlings being preyed upon (Wisenden, 1999). This indicates a kind of alloparental care in which other pairs spawn and fertilize the nest of a caretaker male. This information could lead to the development of new reproduction techniques, reduce the costs of artificial reproduction, and better contribute to conservation and intensive cultivation (Farias et al., 2015).

The literature describes *A. gigas* as an annual spawner which performs its main spawning during the rainy season in Peru (Guerra, 1980) and Brazil (Imbiriba et al., 1991). In addition, the maximum survival of fry has been observed at

the beginning of these rainy seasons (October to December), decreases with the reduction in rainfall. Likewise, feeding is considered an important factor in the number of fry present in breeding areas (Núñez et al., 2011). When feeding is inefficient, fewer spawnings have been observed, being a key factor in the breeding season (Saavedra Rojas et al., 2005). On the other hand, there does not seem to be a positive correlation between the presence or absence of floating vegetation in *A. gigas* ponds and reproductive success (Núñez et al., 2011). However, Gurdak et al. (2019) found that 90% of the nests within the study area were under woody vegetation and reported that these plant species should be considered for the conservation and management of the species.

The number of spawnings and survival between farms is highly variable. These differences could be explained with the hypothesis that fry feeding depends mainly on the productivity of the ponds themselves, since in nature they feed on zooplankton, small gastropods and insect larvae (Oliveira et al., 2005). For this reason, it is believed that the population dynamics of plankton or other food sources could represent a variable for the survival of the larvae, and limnological studies are needed to evaluate this idea (Núñez et al., 2011). Besides the diet provided by the ecosystem, the nutrition supplied through the cephalic fluid of the parents of *A. gigas* is crucial for the life of the fry, and is, therefore, a key factor in reproduction that has begun to be studied. In the work of Chong et al. (2006) proteome and peptidome techniques were applied to characterize the composition of the cephalic fluid of parental and non-parental fish. Peptides have been found in the inner ear, eyes, and central nervous system. In addition, a total of 422 proteins and 28 secreted extracellular proteins have been identified, as well as 2 hormones (prolactin and stanniocalcin) and 12 proteins associated with immunological processes (serotransferrin, α -1-antitrypsin homologue, apolipoprotein A-I, and others).

The protein concentration levels found were relatively low compared to *Symphysodon equifasciata* whose mucus is a source of protein for the fry, which could indicate that the cephalic fluid of *A. gigas* plays a secondary role as a source of protein for its offspring (Torati et al., 2017). However, Du et al. (2019) described genetic expression in the cephalic secretory organ, linked to fry nutrition and pheromone-based signaling that attracts females, reaffirming the idea that cephalic fluid is involved in the reproductive process of the species. Regarding the distribution and movements of the species, it presents low reproduction rates, nesting sites, sedentary lifestyle and parental

care; that is, the species has a type K strategy (Braga & Rebêlo, 2017). The characteristics of the ecosystem allow the generation of strategies in captivity. In breeding ponds, the sex ratio has been found to affect reproductive behavior. When analyzing three models: 1 male: 1 female; 2 males: 1 female and 1 male: 2 females, the best results were achieved with the model resembling monogamous reproduction; that is, 1 male for 1 female. Another factor to take into account in the reproductive behavior is the size of the ponds. Reduced spaces are not suitable for polygamy, and thus, when working in captivity conditions it is recommended to maintain protocols that include one pair per pond at the time of spawning (Lima, 2018). Using radio telemetry, movement patterns in wild *A. gigas* have been identified, finding that they usually place their nest near the coast (Imbiriba et al., 1991). Likewise, the distribution in the pond was linked to the position of the nest and that the presence of the male near the nest was greater than that of the female, thereby demonstrating a caretaker behavior. This telemetry technique allows studying *A. gigas* in its spatio-temporal occupation in the body of water and male-female interactions before, during and after laying (Núñez-Rodríguez et al., 2018). The formation of nests of 50 cm in diameter, the lack of interest in food and courtship fights have been described as part of the reproductive behavior of *A. gigas* (Imbiriba et al., 1991). This species performs small migrations to areas at the mouth of whitewater rivers to spawn, since in their initial stages the larvae carry out gas exchange through the gills and therefore need dissolved oxygen in the environment (Imbiriba et al., 1991). What is known to date is that in the natural state *A. gigas* does not perform migration for reproduction but does carry out courtship behaviors before spawning that can last several days, consisting of the swimming in pairs, circling, chasing each other, building the nest and guarding the nest after spawning. This entire process is influenced by hormones that are correlated with specific events, such as reproduction in this case (Amaral et al., 2019).

A. gigas has been observed to have a high degree of fidelity to certain lakes, with a significant proportion of individuals returning annually to the same protected lake. This behavior is beneficial for the justification of protection efforts made by surrounding communities (Campos-Silva et al., 2019). However, this does not align with the genetic mixture that occurs in the Amazon due to migration (Cynthia Watson et al., 2016). In the case of *A. gigas*, genetic mixture does not occur randomly and it has been hypothesized that these fish seek lakes with high anthropogenic noise as a signal to determine the level of protec-

tion of the lake. This behavior is called the “fear landscape”, and it has been important in understanding predator-prey dynamics (Gallagher et al., 2017). This could explain the large population growth observed in protected lakes year after year, as it is unlikely that this has occurred solely through internal recruitment and reproduction. For example, a 31-fold increase in population size was reported in the protected lakes of the Juruá river study area in just one year (Araripe et al., 2013).

The historical connectivity levels and genetic variability of *A. gigas* have been studied by examining the variation of 11 microsatellite DNA markers in individuals from 22 locations in Brazil, Colombia, and Peru. Despite being sedentary, small migration events between locations occur through the network of lakes and flooded channels that provide abundant food for the fry (Castello, 2007). However, the degree of genetic divergence indicates that most of these localities have demographically independent subpopulations (Farias et al., 2019). The Araguaia river shows little genetic diversity of *A. gigas*, consistent with the results of another study carried out in four localities of the same river and Tocantins (Vitorino et al., 2017). The results suggest the presence of genetic bottlenecks that have been enhanced by demographic decrease and low numbers of reproducers, making both captive and wild populations unable to effectively respond to possible selective pressures caused by environmental or anthropogenic changes (Fazzi-Gomes et al., 2021).

Final Remarks

The text discusses various studies conducted on the reproductive biology of *A. gigas*. These studies include morphological and maturity scale descriptions of testicles and ovaries, sexual dimorphism, and sex determination methods. The *id2bby* gene identification is considered the most accurate method for sex determination in immature individuals. Other findings include the importance of natural feeding and cephalic fluid in the fry stage, the sexual ratio of 1:1 for pair formation, and the significance of courtship for effective spawning. The text also highlights the reduced genetic variability of the species, necessitating further studies on spawning induction. Information on wild and captive specimens is important for establishing effective reproductive management strategies.

Competing interests

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Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals.

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