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Efficiency of color pattern as a method for sex identification in *Arapaima gigas* (Schinz, 1822)

ADRIANA FERREIRA LIMA, ROSIANA RODRIGUES ALVES & LUCAS SIMON TORATI
Embrapa Pesca e Aquicultura, Avenida NS 10, cruzamento com a LO 18, Loteamento Água Fria, Palmas, Tocantins, Brasil, CEP: 77.008-900.

*Corresponding author: adriana.lima@embrapa.br

Abstract. This study evaluated the utility of color pattern as a tool for sex identification in broodstocks of *Arapaima gigas*. To do so, 110 individuals from three study sites had their sex determined with vitellogenin (VTG). Then, presence of red pigmentation was systematically evaluated scoring from the head (0) counting scales from the base of pectoral fin towards fish tail (1, 2, 3,…). Sex and study site were the variables that had significant effects on the zero-inflated negative binomial model (p<0.01) to assess color pattern. Discriminant function analysis (DFA) yielded 82 % of correct classification for females and 76 % for males. With such method, both sexes could be identified with similar hit rates. Differences were seen in sexual color pattern comparing the different study sites. Therefore, application of the method should be restricted to site-specific broodstocks rather than be a general rule for all populations. In conclusion, sex identification in Arapaima broodstocks through color pattern may be considered a cheap alternative for broodstock management but should be used in combination with other available methods to yield optimal rates for sex identification (i.e. endoscopy, cannulation and/or vitellogenin).

Key words: Broodstock management, fish pigmentation, sex identification, sexual dimorphism.

Resumo: Padrão de Coloração como um Método para Identificação Sexual em Arapaima gigas (Schinz, 1822). Este estudo avaliou a eficiência do padrão de coloração como uma ferramenta para identificação sexual em reprodutores de *Arapaima gigas*. Um total de 110 indivíduos de três locais de estudo tiveram o sexo determinado por meio da análise de vitelogenina (VTG). Adicionalmente, o padrão de coloração vermelha foi sistematicamente avaliado a partir da identificação da posição da primeira escama apresentando essa pigmentação, iniciando a contagem a partir da cabeça (0) e seguindo a linha de escamas na base da nadadeira peitoral até a nadadeira caudal (1, 2, 3 ...). No modelo binomial negativo inflado de zero, as variáveis sexo e local de estudo foram significativas (p<0.01) na avaliação do padrão de coloração dos peixes. A análise discriminante (DFA) classificou corretamente 82% das fêmeas e 76% dos machos, sem diferença significativa entre a taxa de acerto para machos e fêmeas. Diferenças foram observadas no padrão de coloração dos peixes nos diferentes locais de estudo. Por isso, esse método não deve ser aplicado como uma regra para todos os peixes de diferentes populações, mas sim para animais submetidos às mesmas condições ambientais. Por fim, a identificação do sexo de reprodutores de Arapaima através da avaliação do padrão de coloração pode ser considerado um método alternativo de baixo custo, mas deve ser utilizado em combinação com outros métodos disponíveis, de forma a aumentar a sua eficiência na identificação do sexo (p. ex. endoscopia, vitelogenina).

Palavras-chave: manejo de reprodutores, pigmentação em peixes, identificação sexual, dimorfismo sexual.

Introduction

Color pattern in teleosts is influenced by several factors including nutritional status (Kalinowski et al. 2005), environmental conditions (Maan et al. 2010), mating season (Kodric-Brown 1998), and social interactions (Korzan et al. 2008). In aquaculture, such morphological variability is often used to identify sex and even sexual maturity allowing broodstock management. In teleosts, sexual dimorphisms can remain constant throughout lifetime (*i.e.* wrasses, blennies and parrot fishes) or be seasonal or ephemeral demarking the breeding season as in *Culea inconstams* (Kodric-Brown 1998, Erisman and Allen 2005, Price et al. 2008). In an evolutionary perspective, sexual dimorphisms displayed in color patterns can influence mating success, thus conferring adaptive advantages in natural and/or sexual selection processes (Maan and Sefc 2013), even though displaying intense colors is energetically costly during the mating season when gamete production is intense and fish are more prone to predation and diseases (Price et al. 2008).

Arapaima gigas (Schinz, 1822) is an Amazon osteoglossid with enormous potential for aquaculture, though lack of basic information on its reproductive biology is hindering control of captive reproduction. Therefore, the commercial production of *A. gigas* is limited and recent research effort has been done to allow sex identification in the species, yet it remains an issue for research and production (Chu-Koo et al. 2009, Núñez et al. 2011, Almeida et al. 2013, Torati et al. 2016). In farms and study sites over the Amazon, there is a lack of reliable and cheap tools for its sex identification. Sexual dimorphism on the color pattern has been suggested to *A. gigas* and this is currently an empirical method used for sex identification when pairing fish to stimulate captive reproduction (Chu-Koo et al. 2009, Núñez et al. 2011). It is believed that color intensity changes in *A. gigas* throughout the year, though red spot pigmentation is apparently permanent throughout adulthood (Monteiro et al. 2010). However, thus far no systematic study evaluated color pattern in different stocks either to further understand this important sexual dimorphism or its potential application for sex determination in *A. gigas*. Capitalizing on the recent development of a vitellogenin (VTG) enzyme immune assay for sex identification in *A. gigas* (Dugue et al. 2008, Chu-Koo et al. 2009), this study aimed to systematically investigate color pattern in different captive populations of *Arapaima* to estimate the extent to which color can be applied to discriminate sex in the species.

Material and Methods

Study specimens: A total of 110 broodstock fish were analyzed in three study sites in Brazil. In site 1 (Amajari - Roraima, Brazil, 3°17'17,49''N, 61°26'31,15''W), 39 individuals known to be over 4-5 years of age and measuring (mean ± s.d.) 149 ± 15 cm in Total Length (TL) were examined on May 2014. In site 1, broodstock fish were reared in earthen ponds (600 m^2) and fed with foraging fish and commercial ration for carnivorous fish species (40% crude protein). In site 2 (Pentecoste – Ceará, Brazil, 3°46'10''S, 39°16'38''W), 47 individuals known to be over 6-7 years of age and measuring 171 ± 22 cm in TL were analyzed on January 2014. In site 2, broodstock were reared in earthen ponds (330 m^2) with foraging fish and a typical ball made to feed *Arapaima* broodstocks composed by commercial feed (38% crude protein, Aquamix, Brazil) and grounded tilapia *Oreochromis niloticus*. In site 3 (Taipas – Tocantins, Brazil, 12°09'38''S, 46°51'26''W), 24 pirarucu known to be over 7-8 years of age, measuring 172 ± 10 cm in TL were analyzed in June 2016. Broodstock in site 3 were reared in 300 m^2 earthen ponds and fed with foraging fish and a commercial feed (40% crude protein, Socil, Brazil).

Fish were individually removed from earth ponds using a dragging net, and kept on a wet mattress following handling procedures and welfare recommendations described in Lima et al. (2015). Each fish was measured to the nearest 0.1 cm, weighted to the nearest 0.1 kg, and a photograph taken from the left lateral region for color pattern evaluation as in Chu-Koo et al. (2009). Sex was confirmed through VTG analysis using an enzyme immune assay kit (Acobiom, Montpellier - France) developed specifically for *Arapaima gigas* according to Dugue et al. (2008) and following kit protocol. To assess color pattern, the distribution of the red pigmentation was observed and coded according to its position starting from the head (0) following scale line demarked by the pectoral fin (1, 2, 3 and so on) towards fish tail (Fig. 1). Therefore, for each fish a number was attributed representing how close to the head the red pigmentation was. This study complied with the Brazilian guidelines for the care and use of animals for scientific and educational purposes – DBCA – Concea (CEUA CNPASA protocol n° 05/2015).

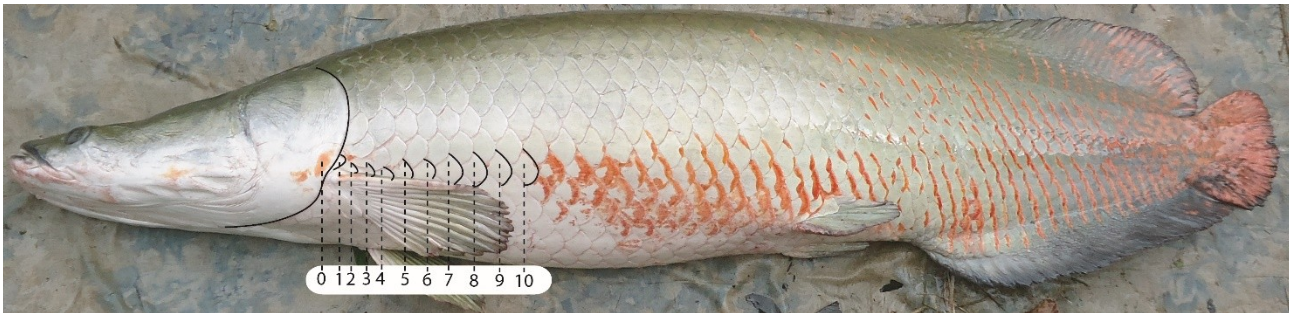

Figure 1. Direction and position in which scales were counted (arrow) in pirarucu broodstock, *Arapaima gigas* (Schinz, 1822).

Statistical analysis: Sexual dimorphisms on color patterns were compared in broodstocks from different sites. Firstly, the correlation analysis was performed for weight (r=0.20; P=0.08) and TL (r=0.24; P=0.01) *versus* color pattern. Following, weight, TL, sex and study site on color pattern were assessed through a zero-inflated negative binomial (ZINB) regression (package: pscl). As the best fit data was a model with sex and study site on color pattern, a discriminant function analysis (DFA) was used to classify fish sex using leave-one-out cross-validation (package: MASS) considering study sites and color pattern. Finally, a binomial test was used to assess the rates provided by the discriminant analysis. All analyzes were performed in R software (R-Core-Team 2016) with the level of significance set in p<0.05.

Results

Sex and study site were the variables that had significant effects on the zero-inflated negative binomial model (p<0.01) to assess color pattern. The discriminant analysis correctly classified 79% of the total fish sample (p<0.01; 72% of males, 82% of females), and the red pigmentation of the fifth scale was the cut-off separating males (0-5) and females (6 onwards) (Fig. 2). The hit rates were similar in the three farms analyzed for both sexes (p>0.05).

Considering the study sites (different populations) affected the lateral red pigmentation of broodstocks, a discriminant function analysis was performed for each study site. It was observed that in the study site 1 the color pattern evaluation correctly classified only 69% of individuals (p<0.05; 59% of males, 77% of females). In the study site 2, the highest classification rates (83%) were observed, (p<0.01; 81% of males, 85% of females) while in the study site 3, 79% of the analyzed fish were correctly classified (p<0.01; 75% of males, 83% of females). The different sites had different cut-offs for sex discrimination, being the red pigmentation on scales at position one, seven and four the cut-off values for sites 1, 2 and 3, respectively (Fig. 2).

Discussion

Previous studies have suggested a sexual dimorphism on the color pattern of adult *Arapaima gigas* (Chu-Koo et al. 2009, Núñez et al. 2011), although no systematic study have evaluated this on a wide range of broodstocks or natural populations. Thus, this study investigated color pattern in different *Arapaima* captive populations to estimate the extent to which color pattern can be used to discriminate sex. We found that color pattern can correctly classify 79% of fish, suggesting that it can be considered a cheap alternative for broodstock management especially if used with other tools such as vitellogenin assays, endoscopy or cannulation (Torati et al., 2019). Find out sexual dimorphism in color pattern such as the observed in this study for *Arapaima gigas* has been reported for other fish species, such as the *Fundulus heteroclitus* (Newman 1907), *Poecilia reticulata*, *Phalloceros caudimaculatus* (Endler 1984), *Sparisoma viride* (Cardwell and Liley 1991), and this particularity can be considered widespread among several teleost families. In comparison to other populations of *Arapaima*, the color pattern observed in study site 2 was similar to the description for pirarucu from Solimões River (Queiroz 2000), whereas the patterns observed in study sites 1 and 3 were similar to those described previously in Chu-Koo et al. (2009) and Monteiro et al. (2010) though all were based on a limited number of analyzed fish. In captive populations of pirarucu, we detected that color patterns vary between sites and may not always represent the natural populations which originated these captive populations, but also different environmental conditions and feeding regimes, factors difficult to unravel in comparative analyzes such as the current study.

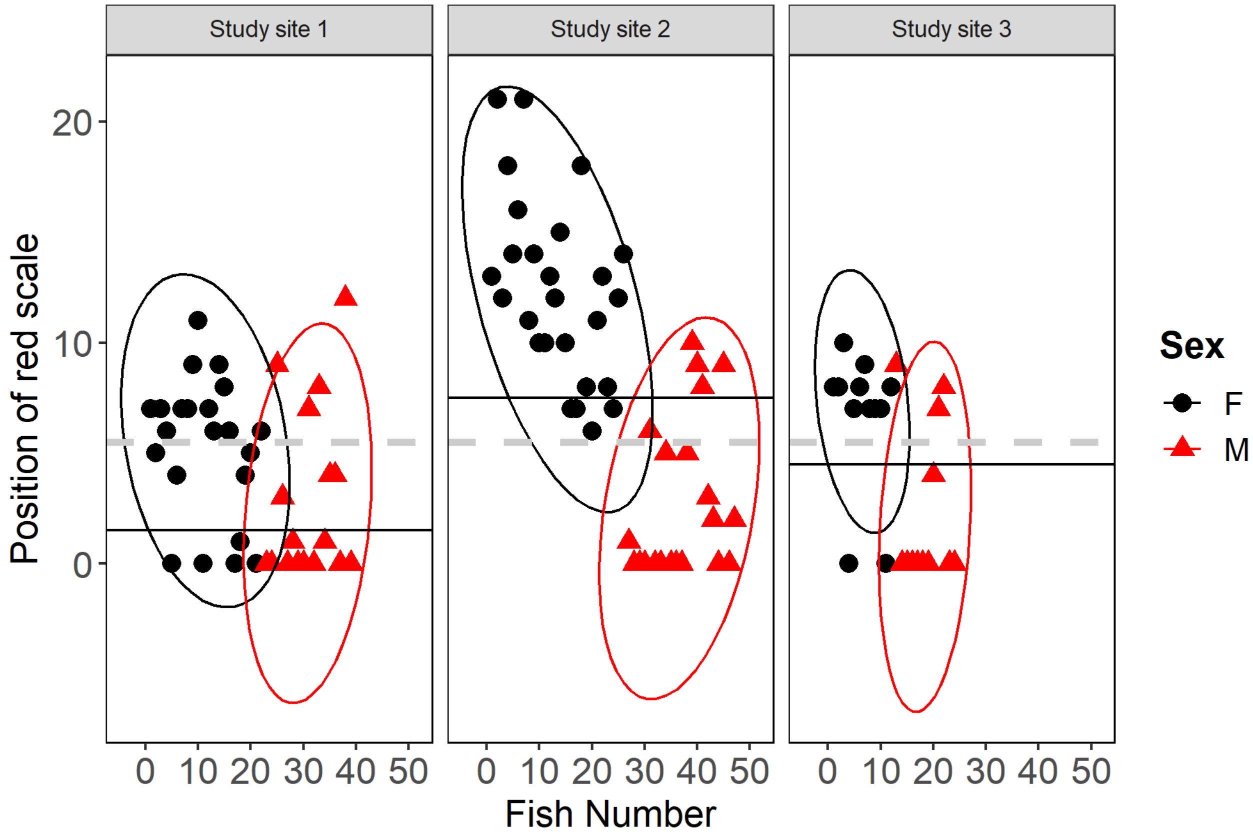

Figure 2. Biplot of the Linear Analyses Discriminant of the position of color pigmentation in scales in broodstock pirarucu, *Arapaima gigas* (Schinz, 1822). Data are shown for each study site (farms). F designates females and M, males. The dashed lines indicate the discriminant position for all fish sampled, while the solid lines indicate the discriminant position for each study site.

Color patterns of a species may vary between individuals of different populations or even within the same population, depending upon age, social status, environmental conditions, populational isolation, reproductive season and feeding (Newman 1907, Endler 1984, Cardwell & Liley 1991, Queiroz 2000, Maan & Sefc 2013), thus it may not always reflect exactly the sex of the individual. This may explain why color pattern did not reflect with total efficiency the sex of all the individuals tested in the present study and the differences observed in fish from different study sites. Considering that, the differences in color pattern observed in the present study may be related to different feeding regimes and environmental responses of the individuals. It is well-known that carotenoids are responsible for the red coloration in fish and that they have to be provided in the diet (Chatzifotis et al. 2005, Price et al. 2008). Since feeding of farmed *Arapaima* is different from the wild, and coloration may be influenced by the diet, farmed individuals may lose the natural coloration patterns (Chatzifotis et al. 2005, Kalinowski et al. 2005), as possibly occurred in sites 1 and 3 if compared to site 2 which is similar to those reported for natural populations. Thus, different feeding regimes could be contributing either to fade or to stress a coloration pattern in the red pigmentation of pirarucu. Additionally, other factors such as water transparency and seasonality can influence coloration (Seehausen et al. 1999, Maan et al. 2010). These are factors that stress the importance of comparing individuals under similar environmental conditions.

Color pattern as a tool for sexing individuals presented similar hit rates in the three analyzed farms, although the position of the first red scale differed between farms. This may demonstrate that even under different environmental conditions and feeding regimes, pirarucu broodstock is still able to maintain a coloration pattern that allows identification as males or females within the same stock. However, some few males and females presented coloration pattern of the opposite sex, which affected negatively the hit rate of the test. This may suggest that there are others factors directly influencing individual coloration. Considering that specimens at the farms were the (1) same age, (2) subjected to similar environmental conditions and feeding regime, and that (3) they were analyzed at the same time, possible social interactions may have influenced coloration pattern in pirarucu, as likewise observed in the cichlid *Astatotilapia burtoni* (Korzan et al. 2008). Furthermore, it has been observed that in a population of *Cyprinodon pecosensis* only a small number of males change coloration prior to reproduction and this was related to territory defense (Kodric-Brown 1998). For *Arapaima gigas*, very little information is available on color patterns and social behavior although it has been described that males of *Arapaima gigas* can fight for females and for territory defense during the breeding season (Farias et al. 2015, Lima 2018), which could be related to the observed color intensification in males either to attract females or to deviate rival males (Kodric-Brown 1998). Nevertheless, this does not seem to occur uniformly for all males kept in the same environment.

In conclusion, sex identification in Arapaima broodstocks through color pattern can be considered a cheap alternative for broodstock management, especially if used in combination with other available methods for sex identification to improve its accuracy.

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