Gender determination in *Arapaima gigas* (Pirarucu) using a vitellogenin detection kit

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Abstract

Arapaima gigas (Pirarucu or Paiche) is an emblematic species of the Amazonian basin. From the beginning of the 18th century, this species has been intensively targeted by fishing and is presently depleted over almost all its distribution area. This situation led it to be inscribed in the red list of the threatened species (CITES II), and its fishing and international trade are now strictly regulated.

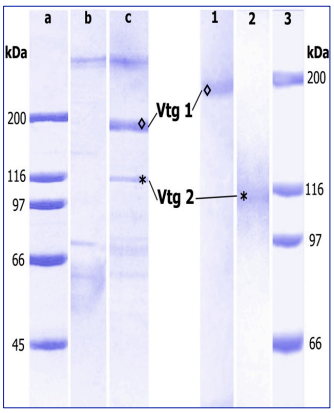
In order to satisfy a commercial demand constantly increasing, its aquaculture began to develop principally in the Peruvian, Colombian and Brazilian Amazonia. Owing to its particular reproductive biology, it remains difficult to produce significant amounts of fingerlings for on-growing of this species and fry production is the principal bottleneck for aquaculture development of Pirarucu in South America.

The knowledge of its behavior and specifically the optimum conditions for the formation of mating pairs is a prerequisite for efficient breeding management in captivity. Nevertheless it is practically impossible to distinguish males and females out of the period of reproduction.

The investigations carried out the last few years allowed us to determine the sex of the Pirarucu by means of a simple blood sample using a sexing kit, based on plasma vitellogenin detection, which can be implemented in the field by the farmers themselves in less than 3 hours.

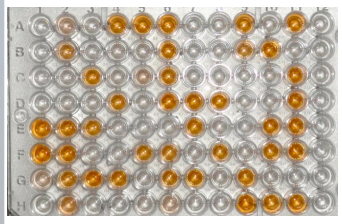
Results

Pirarucu Vitellogenin Purification



Arapaima gigas vitellogenin has been purified by electrophoresis of Vtg enriched plasma (17ß-estradiol treated fish) on polyacrilamide gels (lane c) and the Vtg bands were cut-off and electro-eluted immediately. Two vitellogenin bands were isolated (Vtg 1 and Vtg 2) and specific antibodies were raised in rabbits using the two electro-eluted Vitellogenins (lanes 1 & 2). As Vtg 1 was the most abundant Vtg in sexually mature female plasma, we used the Vtg1 antibody to develop the enzyme immunoassay and the membrane sexing kit. Lane b corresponds to a control of Vtg-free plasma from immature fish. Lanes a and 3 correspond to high molecular mass markers, values correspond to kilo Daltons (kDa).

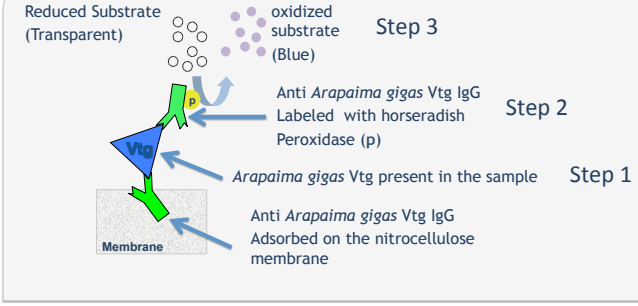
Specific Pirarucu Vitellogenin Immunoassay

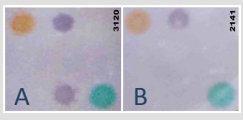


Vtg Antibodies have been tested using conventional Enzyme immuno Assay (EIA) techniques in 96 well plastic plates. This allowed us to set up a specific EIA for *Arapaima gigas* Vitellogenin and as Vtg is a female specific plasma protein (in normal conditions) the optic density of each well reflects directly the Vtg concentration in the plasma sample.

This first sexing method for Pirarucu developed with this methodology had the inconvenient to require expensive laboratory equipment, trained personal and a minimum of 24 hours delay to get the results.

Principle of the sexing Kit





Typical staining of the nitrocellulose membranes after incubation with a Female blood sample (A) and (B) with a Male blood sample. Orange and green spots are pre-stained allowing the correct orientation of the membrane. All the procedure lasts 2:30 hrs. This kit has been successfully tested in field conditions in the Peruvian amazon and allowed gender determination in three-year old and above breeders.

Conclusions

The development of an immune assay to determine plasma vitellogenin allowed the sexing of sexually mature *Arapaima gigas*. The setup of a field sexing kit should allow large scale gender determination by the fish farmers themselves and should contribute to enhance the reproductive success and substantially increase fry production. This methodology will allow the formation of more Pirarucu mating pairs and finally significantly improve the management of available breeders. As the environmental and social factors leading to the constitution of stable mating pairs is not known, additional work should be undertaken to optimize broodstock management.

Acknowledgements

This work was carried out as part of the scientific collaborations between the IIAP and the Institut de Recherche pour le Développement (IRD), both part of the research network known as RIIA (Red de Investigación sobre la Ictiofauna Amazónica, http:// www.riiaamazonia.org) and funded by IRD, IIAP and INCAGRO.