

Purification and assay of *Arapaima gigas* vitellogenin: potential use for sex determination

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Figure 1. A : Young *Arapaima gigas* (6 kg, 1 year old) at the Quistococha IAP's Research facilities, Iquitos, Perú. B: Adult *Arapaima gigas*. (approx. 180 kg and 2.5 m).

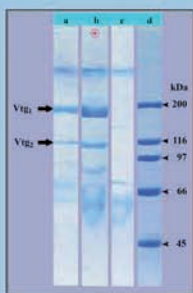


Figure 2. SDS-PAGE of various *A. gigas* plasma samples. a: plasma of an 17β-Estradiol treated fish; b: same as a except that sample was prepared with β-mercapto-ethanol (reducing conditions). c: Vtg-free plasma from a control juvenile d: Molecular Mass Markers, Molecular Mass is expressed in kiloDaltons (kDa).

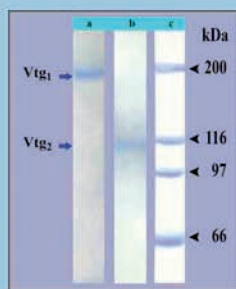


Figure 3. SDS-PAGE of electro-eluted *A. gigas* Vitellogenins. a: Vtg₁, b : Vtg₂; c: Molecular Mass Markers (kDa).

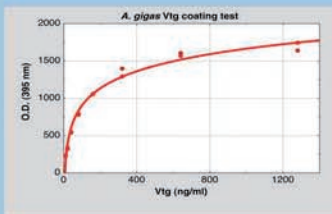


Figure 4. Vtg₁ coating test of *A. gigas* Vitellogenin using anti Vtg₁ antibody diluted 1:40,000.

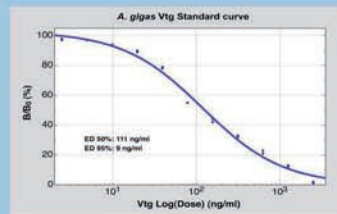


Figure 5. Standard curve of *A. gigas* Vtg₁. Coating, 400 ng/ml; antibody dilution, 1:40,000; incubation time, 2 hours at 37 °C.

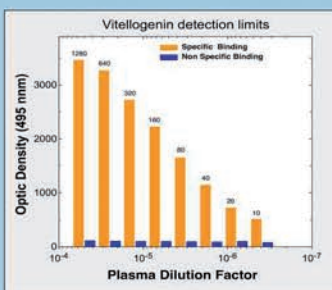


Figure 6. Vitellogenin detection by direct coating of serial dilutions of male and Female plasma (from $1 \cdot 10^{-4}$ to $2.5 \cdot 10^{-9}$). Male and female plasma gave very different signals for all the dilutions tested with anti Vtg₁ antibody diluted 1:40,000. Superscript values indicate the corresponding Vtg concentrations of female plasma dilutions in ng/ml.

Introduction: The “Paiche” (Perú) or “Pirarucu” (Brasil) is an ancient, air-breathing, giant fish of Amazonian rivers and one of the largest freshwater fishes in the world, *A. gigas* attaining nearly 3 meters and more than of 200 kg. Its a valuable species for local communities and for professional fishermen. Due to its economic importance the fishing pressure has already dramatically decreased *Arapaima* populations. The development of the aquaculture of this species is actually a good alternative to face the decline of wild populations.

This species reproduces probably more than once a year but there are only very few incomplete studies in the wild. From observations in captivity, natural reproduction occurs only once a year and the fecundity is extremely low, only a few thousands of fingerlings per female for each spawning event. Generally a male and a female occupy an exclusive territory where a nest is built, and where the fry is guarded approximately for one month.

Objectives: Improving reproduction performance in captivity implies the sex determination of the breeders and the constitution of mating couples in a controlled pond environment. As no external distinctive sex characters are observed in this species, we planed two different approaches, one based on sex steroids for juvenile fish and a other based on plasma Vitellogenin detection in maturing females. We present here this second approach.

Material and Methods: Vitellogenin purification was performed by electro-elution after polyacrilamide gel electrophoresis (PAGE) from plasma of 17β-Estradiol treated *A. gigas* juveniles. The Vtg preparations obtained were used as antigen to obtain specific polyclonal antisera from rabbits. Antibody specificity and affinity was tested by Enzyme Immuno-Assay methodology (EIA).

Results: Using SDS-PAGE in non-reducing conditions, the Estradiol treatment induced two major bands (Vtg₁ and Vtg₂) with 184 and 112 kDa apparent molecular masses respectively which were absent from control plasma. These bands correspond to 2 different vitellogenin molecules since they also migrate as two separate bands under SDS-PAGE in reducing conditions (with β-mercapto-ethanol). The electro-eluted Vtg preparations were used as coating and standard antigen to set up a specific EIA for each vitellogenin. Antibody-Antigen working concentrations, have been determined by cross dilution tests and they are similar for both vitellogenins, (400 ng.ml⁻¹ for coating and 1:40,000 for antibody dilution). This assay allowed us to quantify plasma Vtg in different plasma samples with a sensitivity around 10 ng.ml⁻¹.

Conclusions: We have set up a Vtg EIA for *A. gigas*, which can detect very low Vtg concentrations in the plasma. As Vtg is a female specific molecule in normal rearing conditions, we might be able to sex the 3 to 5 year-old fish since this age corresponds to the reported age of first maturation in captivity. At this time Vtg levels in females will be higher than the assay sensitivity (10 ng/ml). To confirm this first results, blood sampling of pre-adult *A. gigas* specimens has been undertaken. The feasibility of early sexing (when fish are still immature) with sexual steroids like 11-Keto Testosterone, 17β-Estradiol and Testosterone is being evaluated. But, as killing fish from breeding stock was not possible, we will confirm sex determination by Vtg measurement using the EIA methodology during the first reproduction period and thus validate the hormonal method.