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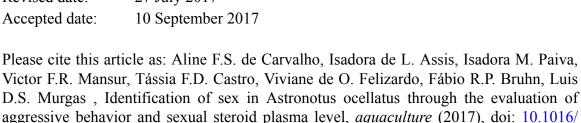
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## **ACCEPTED MANUSCRIPT**

Identification of sex in *Astronotus ocellatus* through the evaluation of aggressive behavior and sexual steroid plasma level

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#### **Abstract**

Identification of sex in fish is essential for captive breeding particularly in species that do not exhibit sexual dimorphism and heteromorphic sex chromosomes, including Astronotus ocellatus. This study aimed at identifying sex in adult A. ocellatus by evaluating their aggressive behavior (frequency of total attacks) and sexual steroid dosing (T/E<sub>2</sub> ratio). Mirror test was used to assess such aggressive behavior and quantify latency, specific attacks and total attacks. Testosterone (T) and 17β-estradiol (E<sub>2</sub>) levels, in turn, were determined by ELISA test. We used the gold standard sexing to analyze the gonads histologically, validate the techniques proposed, and determine the stages of sex maturation. Choen's Kappa was used to evaluate the concordance between such sexing methods. Student"sor Mann-Whitney"s t tests were applied to assess likely differences in behavior and hormone profile. Identifying sex through aggressive behavior was not possible (p=0.613 and concordancerate of 0.069). On the other hand, determining a T/E<sub>2</sub> ratio made it posible to sex identify with an 80.56% accuracy (p=0.001 and concordancerate of 0.571). We also identified significant differences (p<0.05) in T and  $E_2$  levels between male and female individuals. In conclusion, the method of dosing of the sex steroids was validated for the identification of sex in A. ocellatus. This method can be used to predict in advance the sex in A. ocellatus, through the  $T/E_2$  ration.

**Key-words:** Aggressiveness. Androgens. Estrogens. Sexing. Oscar.

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