HETEROGENEOUS DISTRIBUTION OF OOCYTES IN THE OVARIES OF *PROCHILODUS LINEATUS*

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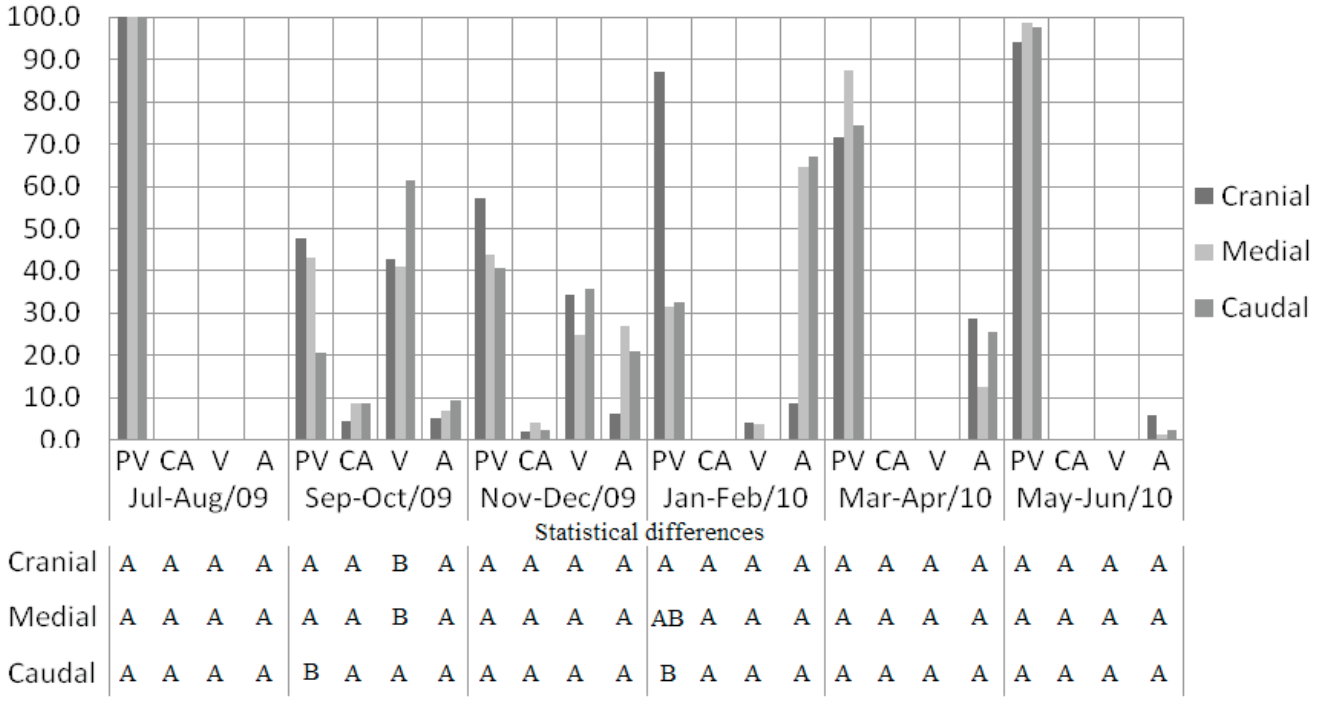
Introduction:

Brazil, despite being the bearer of most species of freshwater fish in the world, due economic, cultural and production knowledge has a production constituted mainly by introduced species, among which are trouts, tilapia and carp fish, while native species are produced on a smaller scale [3]. *Prochilodus lineatus* is of great scientific importance, very popular in fish farms in Brazil, and is a rheophilic species, which reproduce in captivity by induction with hormone [9], which often gives adverse results obtained in spawning induction in rheophilic fish [1][2][4-9]. This study was carried out in order to determine the best way to get a more representative sample from a fish ovary, by microscopic analysis of different portions of ovaries of females throughout gonadal development along the year, thereby establishing a standard method to avoid misleading results.

Methods:

In March 2009, 150 P. lineatus specimens (12 months of age, average length 31.48 + 1.03 cm, and average weight 394.85 + 29.85g) were housed in Aquaculture Center (CAUNESP), São Paulo State University, Jaboticabal, São Paulo, Brazil. The animals were distributed in three earthen ponds (EP) of 50m^2, of 1.50m average depth, and 18 L/min flow. Initial stocking densities of 1 fish.m-2 (0.27 kg.m-3). Water conditions were monitored during experimental period: pH (7.7±0.45), dissolved oxygen (3.77±1.6mg.L-1), conductivity (69.34±15.94µS.cm-1), transparency (68.3cm), ammonia (129.8±80.8µ.L-1) and temperature (23.22± 3.1ºC). Fish were kept in these conditions for a period of 440 days; 360 days of experimental period after 80 days of acclimation. During the experimental period samples were taken every 60 days. Only one EP unit was used in each sampling to avoid excessive management over the specimens, so that breeders were manipulated only every six months. 5 females from EP were randomly selected, sacrificed by a lethal dose of benzocaine(0.15g.L-1) for collection of gonads. All procedures used followed approved guidelines for the ethical treatment of animals and national laws. Experimental protocols were submitted to, and approved

Fig 1. Frequency of the different oocyte types. PV: Pre-vitellogenic; CA: Cortical Alveoli; V: Vitelogenic; A: Atretic; Capital letters show statistical differences between regions.



by, the Animal Ethics and Welfare Committee CEBEA, (Comite de Ética e Bem-Estar Animal) of The Faculdade de Ciências Agrárias e Vetrinárias (FCAV), Unesp, Jaboticabal, SP, Brazil. Ovarian samples (cranial, medial and caudal regions) were collected and fixed in a solution of 2.5% glutaraldehyde for 24 hours. The material was embedded in paraplast, cut into 3-5 μm thick sections, submitted to hematoxylin and eosin staining. Histological sections were utilized to determine the frequency of different oocyte types of each region. All oocytes present in thirty fields (ten fields per region/ 5x magnification) were considered. Previously, the maximum diameter of vitelogenic oocyte was established to determine the section thickness that avoids counting the same oocyte twice. Data were subjected to the test of homogeneity and normality. Statistical differences between values obtained were detected by ANOVA followed by Tukey's test, using the computer program SAS 9.1.

Results:

Analysis of frequency of the different oocyte types (Fig. 1) showed that at the first sampling (Jul-Aug/09), during winter all the regions had the same oocyte types (pre-vitellogenic stage). Two months later (Sep-Oct/09), at the beginning of vitelogenic phase, data show that caudal region is in a earlier process of maturation compared with the other two regions and therefore, pre-vitellogenic oocyte of cranial region progressively increased up to the end of breeding season. On the third sampling during summer, medial and caudal region begins to show some atretic oocytes, at Jan-Feb/10 medial and caudal regions shows advanced stage of atresia. At the last two samplings data show that all three regions began to have similar aspects.

Conclusion:

The present results showed that during the beginning of vitelogenic phase to the reproductive cycle end, spring and summer, each region has its own profile. Thus, the best way to obtain a sample that truly reflects the state of the ovary is to collect at least three samples (cranial, middle, caudal) and calculates the average.

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