NOTE

Reproductive aspects of the flag cichlid *Mesonauta insignis* in várzea lakes of the Central Brazilian Amazon

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ABSTRACT: We describe the most important reproductive aspects of the flag cichlid *Mesonauta insignis* in the floodplain (várzea) lakes of the Central Brazilian Amazon. Between September 2005 and August 2006, fish were collected monthly from mattresses of aquatic macrophytes. Biometric measurements as well as macroscopic and microscopic analyses of the gonads were performed. Standard body lengths ranged from 2.4 to 9.3 cm, and weight–length relationships were indicative of isometric growth patterns. Reproduction events were detected in all months, but the gonadosomatic index indicated a reproductive season from October to February (during low and rising water periods), reaching a reproductive peak in February. Average fecundity was 1184 ± 40.82 (mean ± SD) mature oocytes. *M. insignis* exhibits an asynchronous oocytal development, characteristic of multiple spawnings. Morphological and histological analyses indicated 5 gonadal maturation stages for females. Average standard length at first sexual maturation was 5.18 cm for females and 5.5 cm for males. *M. insignis* shows late sexual maturation. Taken together, these aspects suggest that a life history strategy of 'equilibrium' predominates in the species; consequently, *M. insignis* can be considered vulnerable to high extraction rates, and can only be exploited sustainably if adequate precautionary management procedures are adopted. Some management measures are suggested for the exploitation of this species in the Central Amazon.

KEY WORDS: Fecundity · Gonad histology · Management · Life history strategies · Sexual maturation · Spawning

INTRODUCTION

The flag cichlid *Mesonauta insignis* is widespread in the Amazon River and the upper Negro River basins in Brazil, and in the Orinoco River basin in Colombia and Venezuela (Kullander 2003). This species is benthopelagic, omnivorous, lays its eggs on submerged leaves near the surface and shows long and intense bi-parental care (Keith et al. 2000), a widespread behavior among members of this family (Keenleyside 1991, Turner et al. 2001).

M. insignis is one of the most dominant species in the water bodies of the middle Solimões River floodplain (i.e. várzea lakes) (Hercos et al. 2013), and is also important in the aquarist industry. Although it is not listed among those fishes frequently exported (Prang 2007, Anjos et al. 2009), it is very similar in appearance to *Mesonauta festivus*, and can be easily misidentified by those involved in the exportation of ornamental fish. *M. insignis* was recently included in the official list of Brazilian species that are permitted to be exploited for ornamental purposes

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(MPA-MMA 2012). In spite of its ecological and economic importance, the biology of this cichlid remains largely unknown, which makes it difficult to sustainably exploit its natural population. Studies on the reproductive biology of Amazonian ornamental fish species in their natural environments are relatively rare (Tlusty 2002).

There have been no published studies on the reproduction of *M. insignis* inhabiting the várzea environment. The results of such an investigation could aid in the sustainable use of the species and contribute to its management in this region. Therefore, this work was aimed at characterizing the main reproductive aspects of *M. insignis* in the várzea environment of the Central Amazon, and to provide some of the biological bases for its sustainable use.

MATERIALS AND METHODS

This study was conducted in 5 lakes in the floodplains of the Central Brazilian Amazon, an extension of lowlands annually flooded by white waters (sediment, nutritient-rich waters originating from the foothills of the Andes) (Neill et al. 2006). These floodplain lakes, Tracajá, Juruá Grande, Juruazinho, Araçazinho and Pagão (Fig. 1), belong to Mamirauá Sustainable Development Reserve (MSDR), a protected area limited by the Japurá and Solimões Rivers and the Auati-Paraná channel in Amazonas State, Brazil. MSDR encompasses 1 124 000 hectares (Queiroz & Peralta 2006), and is strongly influenced by the flood

pulse (Junk et al. 1989), a periodic oscillation of the water level that defines hydrological periods and contributes to all major phenological processes in this ecosystem. These hydrological periods or phases are hereafter referred to as 'low water', 'rising water', 'high water' and 'dropping water' (Ramalho et al. 2009).

Fish were collected monthly during a complete hydrological cycle from September 2005 to August 2006. Every month, fish were sampled at 5 randomly chosen points of aquatic vegetation in each lake. Sampling occurred in 4 × 4 m blocks of aquatic macrophytes encircled by multifilament seine nets (0.3 cm mesh size, 6 m wide, 30 m long). All specimens of *Mesonauta insignis* present in these blocks were removed from the seine nets and transported to a field laboratory. Fish were fixed in 10 % formalin solution for 24 h, then transported to the laboratory in the nearby town, washed with running water and stored in 70 % alcohol solution.

All fish were measured for total and standard length (SL; mm) and weighed (g). Sex and gonadal maturation stages were visually determined and classified as 'immature', 'maturing', 'mature', 'spawned' (for females) and 'resting', based on an adapted system of maturation stages (Núñez & Duponchelle 2009). After the macroscopic analysis, samples from 150 ovaries were removed and submitted to routine histological processing for embedding in paraffin (Prophet et al. 1995) as follows: dehydrated with increasing solutions of ethyl alcohol (70 to 100 %), diafanized in xylol and placed in paraffin to allow cuts of 5 µm thick slices. Standard hematoxylin and eosin (H&E) staining was performed, and a total of 100 slides were prepared, analyzed and photographed under a microscope (Axiostar Plus 1169-151, Carl Zeiss).

The reproductive period was determined using the gonadosomatic index (GSI). GSI is a quantitative indication of the reproductive period. The variation in GSI for females (Vazzoler 1996) was defined as:

$$GSI = (W_o / W_t) \times 100 \qquad (1)$$

where W_o and W_t are the weight of the ovary and total body weight, respectively.

ANOVA and post-hoc Tukey's tests were performed to detect differences in ΔGSI among months of the hydrological cycle. Fecundity was determined by the gravimetric method (Vazzoler 1996).
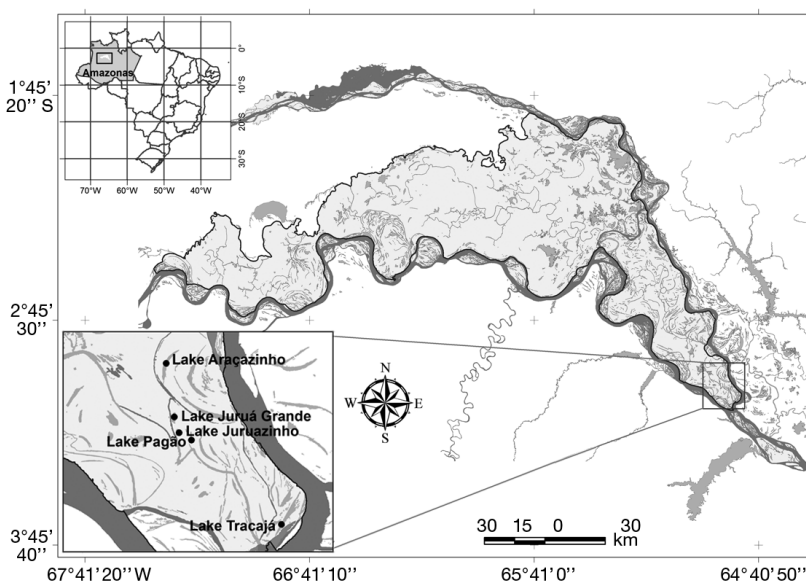

Fig. 1. Mamirauá Reserve and the lakes where *Mesonauta insignis* were collected

Oocytal development was determined based on the frequency distribution of oocyte diameters, and the number of classes found (diameters were measured using digital calipers and a stereomicroscope) in 5 mature ovaries. Modal groups were interpreted in diagrams (Vazzoler 1996). Spawning strategy was determined by analysis of oocyte development pattern and by histology (Núñez & Duponchelle 2009).

The average size at first sexual maturation was determined from the frequency of mature fish in specific length classes. These were depicted in a graph and adjusted to a logistic equation:

$$F = \frac{1}{1+\exp[-R(\text{SLC}-L_{50})]} \qquad (2)$$

where F is the ratio of mature fish in each length class; R is the rate of change from the non-reproductive to reproductive state; SLC is the mid-value of the length class and L_{50} is the size at first maturation when 50% of the adult fish are sexually mature (King 1995).

The precocity in reproductive development was calculated as the relationship between L_{50} and asymptotic length (L_{∞}), obtained from the maximum length in the sample (Pauly 1984). Therefore, reproductive precocity of *M. insignis* was calculated by the formula:

$$D = \frac{L_{50}}{L_{\infty}} \qquad (3)$$

All statistical tests were performed with BioEstat 5.0 (Ayres et al. 2007).

RESULTS AND DISCUSSION

In total, 380 individual *Mesonauta insignis* were collected and used in the analysis of reproductive biology. Microscopic analysis of the ovaries confirmed 5 developmental stages (immature, maturing, mature, spawned and resting) (Fig. 2), corroborating the stages identified macroscopically. Immature ovaries exhibited oogonias and numerous oocytes in stages I (previtellogenic) and II (early vitellogenesis). The maturing ovaries contained large amounts of oocytes in stages I, II and III (vitellogenic); mature ovaries had a small amount of oocytes in stages I, II, III and many oocytes in stage IV (vitellogenic). These cells presented acidophilic cytoplasm and full yolk granules. In the spawned ovaries, the parenchyma was characterized by the presence of postovulatory follicles and atresic oocytes, and by a small amount of oocytes in stages I and II. In the resting ovaries, numerous stage I and II oocytes were found, sur-

rounded by a fibrillar network. This indicates an asynchronous pattern of gonadal development, characteristic of multiple spawners (as evidenced by the presence of oocytes in stages I and II and postovulatory follicles, indicating the removal of mature oocytes). The stages of gonadal development of *M. insignis* exhibited similar characteristics to those observed in other cichlids, such as *Cichla pleiozona*, and other Amazonian species, such as *Plagioscion squamosissimus* (Núñez & Duponchelle 2009).

The frequency distribution of oocyte diameters was also indicative of multiple spawnings. The graphs indicate a distribution in 2 modes, and a synchronic development of oocytes in 2 groups (Fig. 3), confirming the histological analyses. Microscopic analysis revealed that these 2 modes occur in one reproductive season. This result is similar to that obtained by Favero et al. (2010a) for the same species living in black waters (nutrient-poor waters originating in terrains of the Guianean or Central Brazil shields) at the igarapés of Amanã, which was also determined to be a multiple spawner.

A seasonal trend was observed in *M. insignis* reproduction, suggesting a period of high reproductive activity, or a reproductive season, starting during the low water phase (October) and lasting until the first part of the rising water phase (March), reaching a peak in the early months of the rising water phase (GSI = 4.7) in February. Variation in GSI during this period was significantly higher than during the non-reproductive season ($F = 4.382$; $p = 0.002$).

Reproductive activity of most Amazonian species intensifies as environmental conditions favor their recruitment (Lowe-McConnell 1999, Barletta et al. 2010). This starts when food abundance is higher, and autochthonous prey are concentrated in water bodies during the low water phase. In the present study, at the end of the low water phase, *M. insignis* showed intense feeding and ripe gonads. Rising water provides the ideal nutritional conditions for most Amazonian fish species, and thus favorable conditions for the conclusion of sexual maturation, gametogenesis and spawning (Vazzoler 1996), perhaps explaining the close relationship between water levels and GSIs reported for a large number of Amazonian fish species (Barthem & Fabré 2003)

Average fecundity recorded for *M. insignis* was 1184 ± 40.82 (mean ± SD) vitellogenic oocytes (>0.6 mm diameter), ranging from 840 to 1840 oocytes female^{-1}. Favero et al. (2010a), investigating this species at a different site, reported lower fecundity: an average of 609 ± 178.3 oocytes female^{-1}, ranging from 386 to 920 mature oocytes female^{-1}. Species that ex-
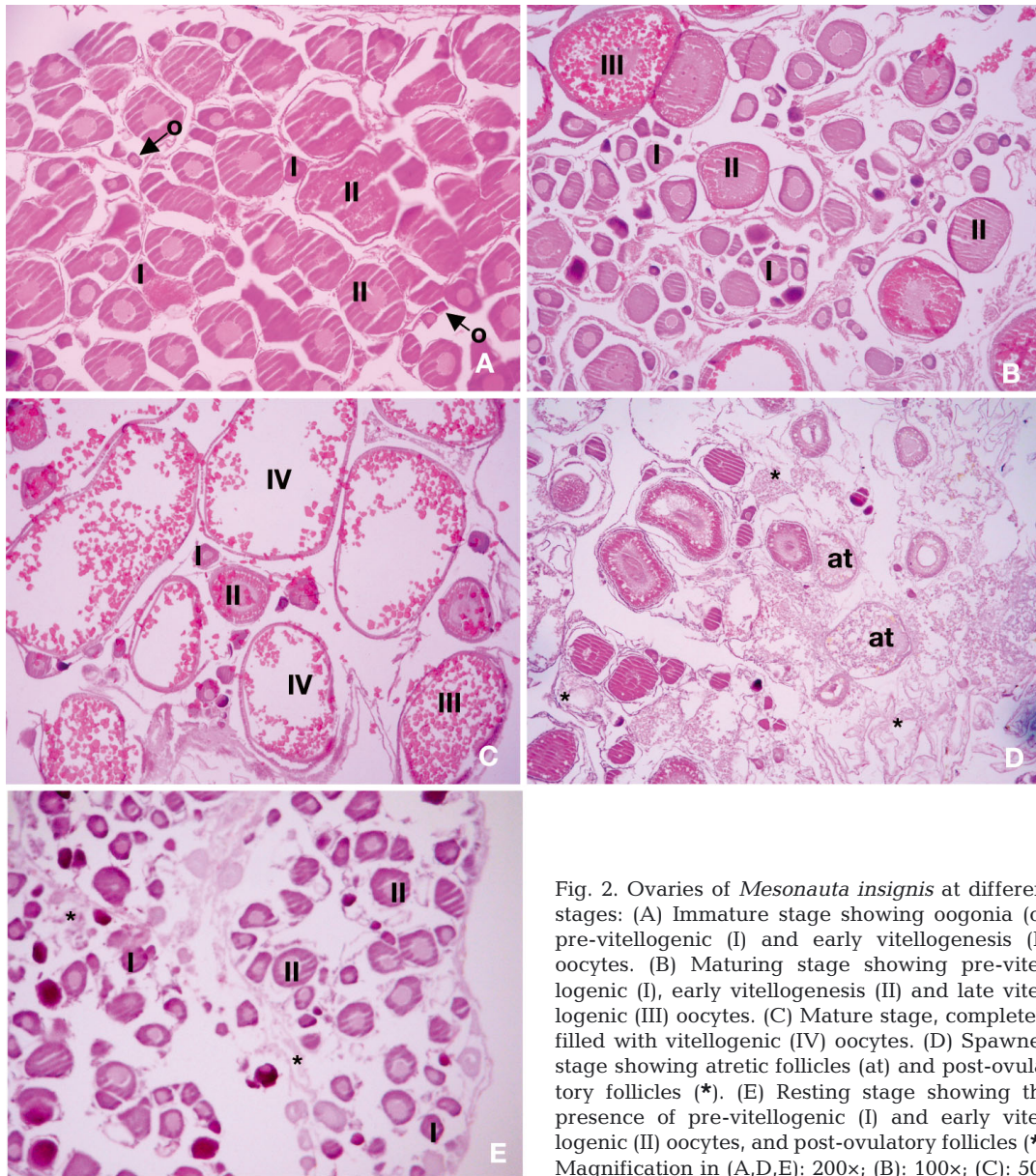
Fig. 2. Ovaries of *Mesonauta insignis* at different stages: (A) Immature stage showing oogonia (o), pre-vitellogenic (I) and early vitellogenesis (II) oocytes. (B) Maturing stage showing pre-vitellogenic (I), early vitellogenesis (II) and late vitellogenic (III) oocytes. (C) Mature stage, completely filled with vitellogenic (IV) oocytes. (D) Spawned stage showing atretic follicles (at) and post-ovulatory follicles (*****). (E) Resting stage showing the presence of pre-vitellogenic (I) and early vitellogenic (II) oocytes, and post-ovulatory follicles (*****). Magnification in (A,D,E): 200×; (B): 100×; (C): 50×

hibit parental care usually show lower fecundity compared to species that do not (Sato et al. 2003). Some neotropical members of the Cichlidae family that are similar in size to *Mesonauta* show similar or higher fecundity, such as *Heros efasciatus*, with 2502 oocytes female^{-1} (Favero et al. 2010b), or *Acarichthys heckelli,* with 1341 oocytes female^{-1} (Favero et al. 2010a).

In our study site, *M. insignis* females reached sexual maturation at 5.18 cm SL and males at 5.5 cm SL (Fig. 4). The average length at first sexual maturation is related to the growth pattern of each species, and inter-specific variations in space and time can have an important influence on the population (Lowe-McConnell 1999). The same species, living in black water environments, averaged 6.41cm SL at first reproduction (Favero et al. 2010a), which is significantly higher than in MSDR. This suggests that when living in richer environments, *M. insignis* grows faster and individuals mature earlier. Most fish species exhibit a decrease in size at first sexual maturation (along with a reduction in other growth patterns) as a result of changes in population densities. White-water bodies, such as várzea lakes, harbor many species of aquatic predators that might add new sources of mortality (Henderson & Crampton 1997). The increase in mortality rates may cause a reduction in size at first sexual maturation as a compensatory response (Rose et al. 2001).
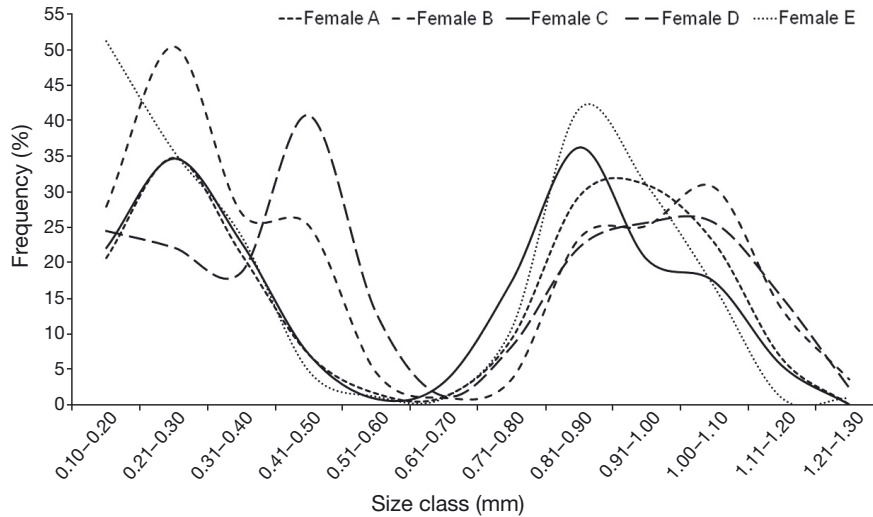
Fig. 3. Frequency distribution of oocyte diameters (mm) for 5 mature *Mesonauta insignis* females from the study site

The relationship between L_{50} and L_∞ (representing the precocity of sexual development) differed between males (0.54) and females (0.67); however, these rates suggest late sexual maturation for *M. insignis* (Vazzoler 1996). Late maturation might improve overall reproductive success, with higher fry survival resulting from additional parental investment, such as production of larger oocytes, longer autotrophic periods, and more efficient parental care (Zeug & Winemiller 2007). During field work, we observed pairs of *M. insignis* in the wild performing bi-parental care, actively guarding their offspring.

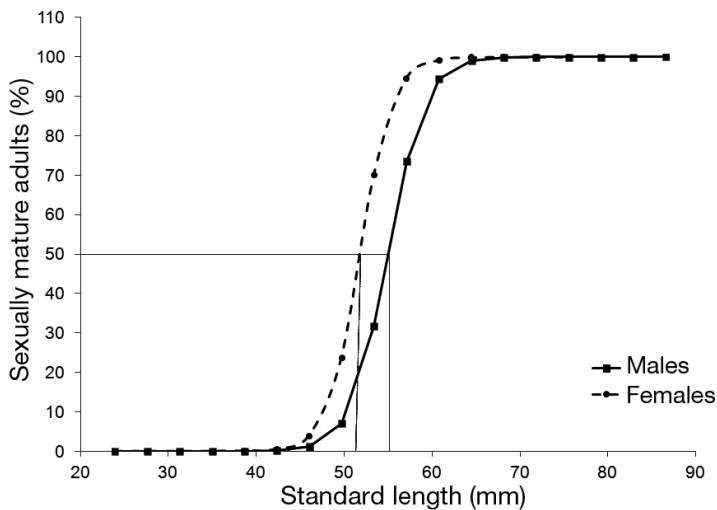Cichlid species are usually considered equilibrium strategists (Winemiller 1989). However, at our study site, while some reproductive aspects of *M. insignis* appeared to confirm this life history strategy (such as late sexual maturation, low fecundity, high survival rates and multiple spawning), others more closely resembled a seasonal or opportunistic strategy, such as small size and a seasonal peak of reproductive activity. Thus, some Amazonian cichlid species, such as *M. insignis*, probably show a more intermediate life history strategy (i.e. an equilibrium–opportunistic strategy; Winemiller & Rose 1992).

Increased knowledge of the reproductive biology of this species is fundamental for the establishment of management criteria to regulate its exploitation. Minimal size at capture, regulation of the maximum amount of specimens caught, and closed seasons are all potential measures to ensure sustainable exploitation (Hilborn & Walters 1992). Based on the results presented here, we suggest that 5.3 cm SL be adopted as the minimal size for capture in this area, and to avoid captures during the reproductive period (January to March). Given the predominance of an equilibrium life strategy, it is clear that *M insignis* can only be sustainably exploited if adequate precautionary management measures are taken.

Fig. 4. Average size at first sexual maturation (L_{50}) for *Mesonauta insignis* females and males

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