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Influence of cage farming on feeding and reproductive aspects of *Pimelodus maculatus* Lacépède, 1803 (Siluriformes: Pimelodidae) in the Chavantes reservoir, Brazil

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ABSTRACT. This study evaluated the diet and reproductive aspects of the population of *Pimelodus maculatus* around net cage fish farming in order to assess the possible impacts of this activity. Monthly collections were performed from March 2008 to February 2009 on two populations: one close to the net cages (NC) and one from an area not influenced by these cages denominated the "reference site" (RS). Results of the Alimentary Index (AI), Gonadosomatic Index (GSI), reproductive potential and histological analysis were obtained for both NC and RS populations. The population from NC used leftover food (ration) that escapes from net cages as the main food item (99.3%). For the RS population, the detritus item was the more important food source (51.7%). The Detrended Correspondence Analysis (DCA) showed that the use of food resources was different between the two sites. The reproductive period of the species (indicated by the GSI) revealed that the population of the NC showed an extended reproductive period compared to RS. The histology of the ovaries indicated that the specimens in the NC were spawning capable. This study indicates that fish farming activities influence the species *P. maculatus* in the Chavantes reservoir by adding a new resource to the food web.

Keywords: fish, biology, neotropical reservoir, Paranapanema river.

Influência de uma piscicultura em tanques-rede sobre a alimentação e aspectos reprodutivos de *Pimelodus maculatus* Lacépède, 1803 (Siluriformes: Pimelodidae) no reservatório Chavantes, Brasil

RESUMO. O objetivo deste estudo foi avaliar a dieta e os aspectos reprodutivos da população de *Pimelodus maculatus* residentes ao redor de tanques-rede. Os peixes foram coletados mensalmente, de março/2008 a fevereiro/2009, a partir de duas populações: uma próxima ao ambiente de tanques-rede (NC) e a outra em uma área não influenciada pelos tanques-rede, denominada área de referência (RS). As análises do Índice Alimentar (AI), Índice Gonadossomático (IG), Potencial Reprodutivo e Histologia das gônadas foram realizadas para as populações das duas áreas amostradas. As populações de NC utilizaram-se de restos de ração que escapam dos tanques-rede, como principal fonte alimentar (99,3%). Para a população do RS, os detritos foram a fonte mais importante (51,7%). A análise de Correspondência Destendenciada (DCA) mostrou diferenças na utilização dos recursos alimentares entre as duas populações. O período reprodutivo das espécies (indicado pelo IG) mostrou que a população do NC apresentou maior período reprodutivo em relação às populações do RS. A histologia dos ovários indicou que os espécimes de NC estavam aptos à desova. Este estudo adverte que as atividades de piscicultura influenciam a referida espécie na represa de Chavantes pela adição de um novo recurso para a cadeia alimentar.

Palavras-chave: peixes, biologia, represa neotropical, rio Paranapanema.

Introduction

Globally, as a food-producing sector, aquaculture has surpassed both capture fisheries and terrestrially farmed meat production in terms of average annual growth. However, a number of biosecurity concerns arise due to the risks posed to sustainable aquaculture development. As aquaculture is very diverse in terms of species, nvironments, systems and practices, the range of hazards is broad and the perceived risks are complex (ARTHUR; BONDAD-REANTASO, 2012). Nevertheless, the possibility to increase aquaculture production in the future seems to be very high (GJEDREM, 2012).

Net cage fish farming is a fast-growing activity, but has a potential impact on the biota of both freshwater and marine environments (DIAS et al., 2011; MIRTO et al., 2012; MORATA et al., 2013). As examples, we mention the possible deterioration of water, caused by external inputs to the system, and introduction of non-native species, caused by escapes from net cages, which, in the medium to long term, may cause impacts in local biota. In Brazil, since the 1990´s, a commercial expansion of aquaculture has occurred mainly in the southeast, where the most commonly farmed fish species is *Oreochromis niloticus* (ONO, 1998) and is the dominant model of fish farming in cages.

The problem is that this activity produces a considerable amount of unused food (rations), and other products (BEVERIDGE, 2004; PILLAY, 2004) which are often lost to the aquatic environment. Furthermore, the food ingested by cage fish farming that is eliminated in the form of excreta (feces and metabolites), are also utilized by resident fish fauna occupying areas close to fish farming cages (HÅKANSON, 2005). Thus, part of this food is not fully exploited by fish in the farming cage and is, therefore, lost to the aquatic environment and can be used by the local biota (BEVERIDGE et al., 1991). The availability of food may cause changes in the potential of aquatic organisms through trophic interactions (FRAGOSO JR. et al., 2009). Additionally, the input of fish food into the ecosystem may confer greater advantage in the diet of opportunistic native fish species in relation to non-opportunistic species (STRICTAR-PEREIRA et al., 2010; CARVALHO et al., 2012). Thus, knowledge of fish movement around farms and feeding behavior studies using stomach contents are key factors to achieve a global idea of their role in the dynamics of fish farms (BOYRA et al., 2004).

The increasing inclusion of net cages in littoral areas of large rivers can lead to modification of these important spaces that serve as protection, feeding and breeding areas for many species of fish and other organisms, and, consequently, change the dynamics of the natural environment. For this reason, the technological development and negative potential impacts caused by fish farming have been assessed in various regions of the world across marine and freshwater environments (BRIGOLIN et al., 2009; DEMPSTER et al., 2002; GIANNOULAKI et al., 2005; MACHIAS et al., 2005; MENEZES; BEYRUTH, 2003; NICKELL et al., 2003; TUYA et al., 2006; ROMANA-EGUIA et al., 2010; WETENGERE, 2011). This demonstrates the importance of these studies.

The choice of *Pimelodus maculatus*, commonly known as “mandi amarelo”, to perform this study was based on the following characteristics: (i) the species is widely distributed in South America (Brazil, Guyana, Venezuela, Peru, Bolivia, Argentina, and the Amazon Plate and Uruguay River basin (BRITSKI et al., 1988; REIS et al., 2003), and is one of the most abundant species of the Paraná River basin and an important constituent of the fish fauna of rivers and streams (LIMA-JUNIOR; GOITEIN, 2006; LOLIS; ANDRIAN, 1996), lentic waters and also large reservoirs (AGOSTINHO et al., 1997a and b; ALVES et al., 1998; SUZUKI et al., 2005) and (ii) has continuously increased in the surrounding area of the fish farming since its construction (personal observation).This study aimed to evaluate the influence of fish farming in Chavantes reservoir on the diet and reproductive aspects of the population of *P. maculatus* captured around a cage fish farming system, compared to a reference site. For this, we aimed to respond the following questions: (1) Does the population of this species around the net cage fish farming have a different diet and feeding activity compared to the reference site? (2) Do changes occur in the reproductive aspect of the species due to the fish farming activities?

Material and methods

The Chavantes reservoir (23º 22´ S; 49º 36´ W) is located in the middle stretch of the Paranapanema River, on the border of São Paulo and Paraná States. The reservoir is at 480 m above sea level, with a maximum depth about 70 to 90 m, a total volume of 9410 x 106 m^3 and an area of 400 km^2 (DUKE ENERGY, 2002).

This study was conducted in a private company for breeding tilapia, *Oreochromis niloticus*, in a lentic area of the reservoir between the municipalities of Ipaussu and Chavantes, São Paulo State. The fish farming studied is operating since the beginning of 2008. It is classified as medium-sized and has about 200 net cages, each with a volume ranging from 6 to 18 m^3. Two sites were selected for the study - one close to the fish farming, designated the net cage site (NC) and the other, located in a stretch below the NC, designated the reference site (RS) (Figure 1). The two study sites were bordered by rocks, fragments of mesophytic forests and areas of aquatic macrophytes.

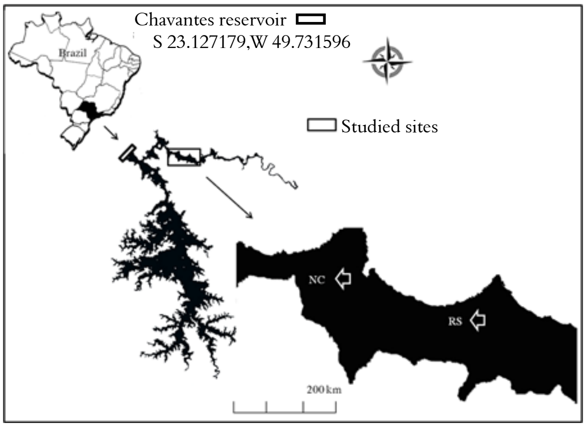

Figure 1. Location of the Chavantes reservoir in the middle stretch of the Paranapanema river SP/PR. White arrows indicate study sites: Net Cage fish farming (NC), and Reference Site (RS). (Source: Satellite images from GoogleEarth - DigitalGlobe).

Fish were collected monthly (Ibama License number: 2629349) at the two sites, from March 2008 to February 2009, using gill nets, grouped into three sets with five nets each (mesh sizes = 3 to 14 cm, between opposite knots and height from 1.44 to 2.20 m). Nets were set at 5:00 pm and removed at 6:00 am (exposure time of 13 hours). Right after the capture all individuals were measured and weighed.

Hydrological and environmental data of this reservoir, such as rainy and dry season (cumulative monthly rainfall), were obtained from the Department of Hydrobiology of Duke Energy - Generation Paranapanema.

In the field, the gonads were removed and weighed on an analytical balance (accurate to 0.0001 g), and the stomachs were removed and transferred to labeled bottles containing 10% formalin for transport to the laboratory.

Stomach contents of all individuals containing some type of food were analyzed under a stereomicroscope, identified to the lowest possible taxonomic level and weighed (wet weight) on an analytical balance (accurate to 0.0001 g).

The results were expressed by the frequency of occurrence and gravimetric methods, generating an index that shows the main food items (KAWAKAMI; VAZZOLER, 1980), adapted by Hahn et al. (1998): $AI = Fi \times Wi \times 100 / \sum Fi \times Wi$, when: AI = alimentary index; i = 1, 2 ... n, food items, Fi = frequency of occurrence of item i (%); Wi = wet weight of item i (%). The identification of food items was performed based on identification keys (COSTA et al., 2006; LEHMKUHL, 1979; MERRITT; CUMMINS, 1996; STRIXINO; STRIXINO, 1982).

The degree of stomach repletion was rated visually on a scale from 0 to 4, according to Walsh and Rankine apud Marçal-Shimabuku and Peret (2002), in which: 0 = empty stomach, 1 = less than 25%; 2 = between 25 and 50%, 3 = between 50 and 75% and 4 = more than 75%. To detect possible spatial patterns between the NC and RS areas in the distribution of food resources consumed, a Detrended Correspondence Analysis (DCA) was employed based on the weight data of the food items and tested by the Multi Response Permutation Procedure (MRPP) using the PCORD4 program (McCUNE; GRACE, 2002).

To evaluate the occurrence of reproductive stages, gonads were examined using the Gonadosomatic Index (GSI), where $GSI = Wg / Wt * 100$ where: Wg = weight of gonads and Wt = total weight (VAZZOLER, 1996).

All female individuals with mature gonads had their ovaries selected for the reproductive potential analysis. Then, oocyte counts were performed in 437 previously fixed ovary samples from the NC and 219 from the RS using a stereomicroscope. The samples were weighed (sub-sample) and oocytes were dissociated using forceps and stilettos after fixing in Gilson solution. Thus, with the information of the sample weight (w), the number of oocytes in each sample (n) and the weight of the ovaries (W_g), it was possible to estimate the total number of oocytes in the ovaries (N) using a rule of three, as follows: $N = n * W_g / w$. For the morphometry of the oocytes, 10 units of oocytes from five specimens from each month were randomly selected to determine the average area, and horizontal and vertical diameter, using the programs QWin Lite 3.1 and LAZ V3 (Leica Application Suite).

The histological analysis was performed using samples of 50 ovaries of *P. maculatus* (42 from the NC and eight from the RS). To confirm the data from the Gonadosomatic Index (GSI), the gonads that showed a higher peak for the GSI were selected and fixed in 4% formaldehyde solution for at least 24 hours. Later were dehydrated in increasing ethanol solutions (70-95%) and embedded in Historesin (Leica) in an incubator (60º) for 24 hours. Afterwards, the material was sectioned with 3-5 μm of thickness on a rotating microtome equipped with a glass blade. The sections were stained with haematoxylin and eosin. The oocyte classification and ovarian development followed Brown-Peterson et al. (2011).

For all statistical analyses in this study, differences between variables were considered significant when $p < 0.05$, with significance level of 5%. Voucher specimens were deposited at the Laboratório de Biologia e Genética de Peixes (LBP), Instituto de Biociências, UNESP, Botucatu, São Paulo State, Brazil.

Results

In total, 888 individuals of *P. maculatus* were captured in the NC (biomass of 142.4 kg) and 112 individuals were captured in the RS (biomass of 17.8 kg). For the NC, 644 specimens had their stomach contents analyzed, with ration being the most consumed item (AI = 99.3%), followed by detritus (AI = 0.3%) (Figure 2). For the RS, 75 specimens had their stomach contents analyzed, with detritus (AI = 51.7%) being the main item, followed by gastropods (AI = 24%) (Figure 3) The other individuals in both study areas had empty stomachs. The Repletion Degree (GRm) was higher in the NC site (GRm = 1.5) than in the RS (GRm = 1.2).

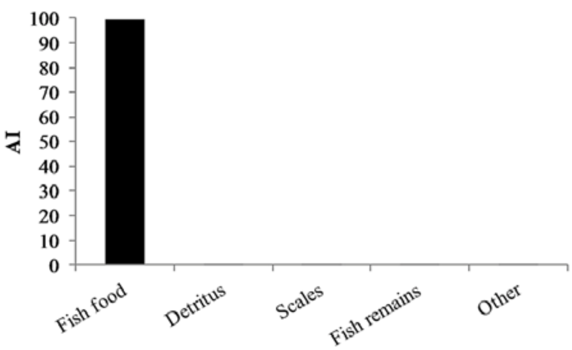

Figure 2. Alimentary Index (AI) of *Pimelodus maculatus* collected in the NC site in Chavantes reservoir.

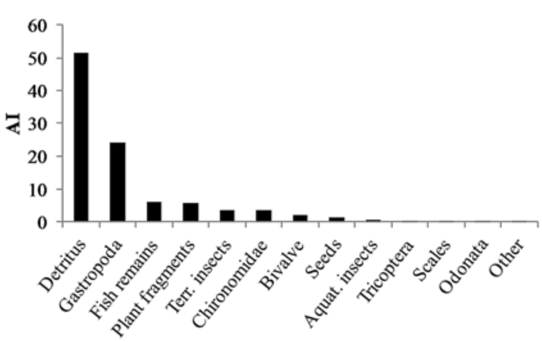

Figure 3. Alimentary Index (AI) of *Pimelodus maculatus* collected in the RS site in Chavantes reservoir.

The ordination of food resources used by the two populations, calculated by a Detrended Correspondence Analysis (DCA), showed that the axis 1 (eigenvalue = 0.71) separated the item ration for the NC population. The axis 2 (eigenvalue = 0.48) grouped other food items in the RS site, showing that the distribution and use of food resources were different between the two populations studied (Figure 4). The diet similarity was verified by a Multiresponse Permutation Procedure (A = 0.022; $p < 0.05$).

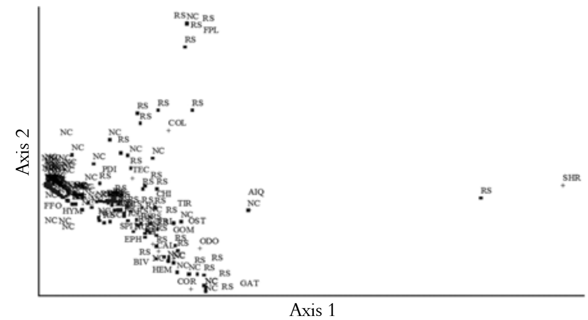

Figure 4. Detrended Correspondence Analysis (DCA) of food resources consumed by *Pimelodus maculatus* in the NC and RS sites in Chavantes reservoir. Legend: Aquatic insect = AIQ, Bivalve = BIV, Ceratopogonidae = CER, Chaoboridae = CAL, Chironomidae = CHI, Corixidae = COR, Coleoptera = COL, Detritus = DET, Ephemeroptera = EPH, Fish = FIS, Fishfood = FFO, Plant Fragment = FPL, Gastropoda = GAT, Gomphid = GOM, Hemiptera = HEM, Hymenoptera = HYM, Odonata = ODO, Ostracod = OST, Pupa Diptera = PDI, Shrimp = SHR, Spider = SPI, Tecameba = TEC, Terrestrial insect = TIR, Trichoptera = TRI.

Female specimens from the NC showed the highest peaks of Gonadosomatic Index (GSI) in December, January and February (0.09, 0.06 and 0.87% respectively). In the RS, only females were recorded, with the highest values of GSI in December, January and February (0.41, 0.31 and 1.01%, respectively) (Figure 5).

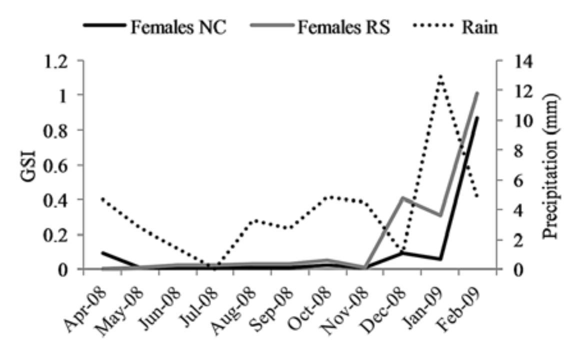

Figure 5. Relationship between monthly rainfall (mm) and variation in the Gonadosomatic Index (GSI) of females of *Pimelodus maculatus* in the NC and RS sites in Chavantes reservoir. (Source: Duke Energy – Paranapanema Generation).

Smaller oocytes were recorded in the NC, with monthly mean values ranging from 0.54 to 0.78 mm in horizontal diameter, and 0.50 to 0.81 mm in vertical diameter. The area ranged from 0.20 to 0.46 mm^2. In the RS, oocytes ranged from 0.67 to 0.79 mm in horizontal diameter, and 0.69 to 0.77 mm in vertical diameter. The area ranged from 0.34 to 0.43 mm^2. The maximum reproductive potential in the NC population ranged from 115,853 to 432,820 oocytes, and the fertility in the RS ranged from 23,215 to 81,361 oocytes (Table 1).

In the histological analysis, the ovaries from the NC and RS showed oocytes at different stages of development (Figure 6A and B): oocytes in the primary growth or pre-vitellogenic stage and oocytes in secondary growth also referred to as vitellogenic oocytes. Secondary growth is marked by the beginning of yolk deposition. The oocyte gradually increases its size in this stage. The secondary growth oocytes were divided into early vitellogenic oocytes, mid-vitellogenic oocytes and late vitellogenic oocytes. Only ovaries in two distinct reproductive phases were found in both NC and RS areas. They were classified according to Brown-Peterson et al. (2011) into:

- *Developing* (Figure 6A): the ovaries showed primary growth or pre-vitellogenic oocytes with basophilic cytoplasm, which indicates RNA deposition; early vitellogenic oocytes, mid-vitellogenic oocytes and a smaller amount of late vitellogenic oocytes were also observed.

- *Spawning Capable* (Figure 6B): the ovaries showed a predominance of oocytes in advanced yolk deposition (late vitellogenic oocytes) with cortical alveoli located in the oocyte periphery; primary growth oocytes were also present, although in a smaller amount.

The ovaries analyzed for both the NC (Figure 7A, B and C) and RS (Figure 7D, E and F) individuals presented a decreased amount of primary growth oocytes in December 2008, January 2009 and February 2009. On the other hand, the number of late vitellogenic oocytes increased in the same period. Thus, most of the ovaries analyzed in these three months were classified as spawning capable.

Discussion

The population of *Pimelodus maculatus* analyzed showed differences in diet and reproductive aspects between the reference site (RS) and the net cage fish farming (NC) area. During the study, a larger number of individual and higher biomass was observed in the net cage site than in the RS, which might be related to the availability of food resources at the NC.

Table 1. Reproductive potential of *Pimelodus maculatus* in the net cage area (NC) and in the reference site (RS) in Chavantes reservoir. Where: (N) = number of individuals; (HD) = horizontal diameter of oocytes, (VD) = vertical diameter of oocytes, and (Area) = area of oocytes. Biometry performed by the programs QWin Lite 3.1 e LAZ V3 (Leica Application Suite). All measurements are in mm.

Month	N	Minimum number of oocytes	Maximum number of oocytes	Mean number of oocytes	Standard Deviation	HD	VD	Area
				Net Cage				
Apr/08	4	11,070	250,842	107,193	114.990	0.54	0.50	0.20
Nov/08	9	11,214	432,820	109,228	138.129	0.54	0.54	0.21
Dec/08	7	15,394	78,272	34,441	21.601	0.68	0.71	0.37
Jan/09	15	7,649	63,765	30,947	20.039	0.67	0.68	0.32
Feb/09	24	4,292	115,853	26,299	22.415	0.78	0.81	0.46
				Reference Site				
Mar/08	1	-	33,957	-	-	-	-	-
Dec/08	3	44,951	81,361	53,136	37.964	0.79	0.77	0.43
Jan/09	2	26,291	79,980	17,075	7.100	0.73	0.72	0.37
Feb/09	3	9,300	23,215	16,250	9.840	0.67	0.69	0.35

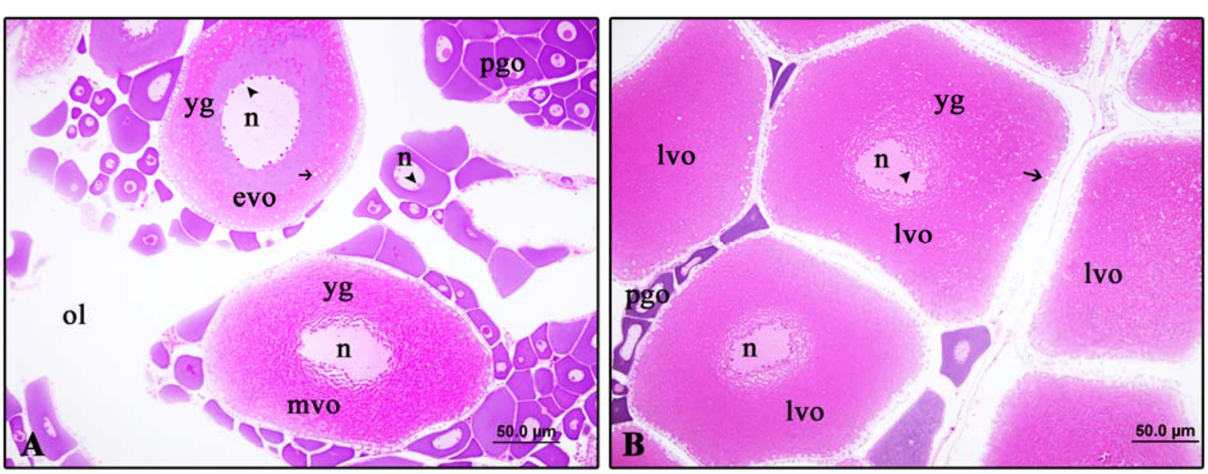

Figure 6. Histological analysis of ovaries and oocytes of *Pimelodus maculatus*: A) developing and B) spawning capable. Note the presence of oocytes in primary growth or pre-vitellogenic (pgo) in greater number in a developing ovary (a) than in a spawning capable ovary; (b) and oocytes in secondary growth in early vitellogenesis (evo), mid-vitellogenesis (mvo) and late vitellogenesis (lvo). Note the increase of late vitellogenic oocytes in the spawning capable ovary. Legend: arrow: cortical alveoli, arrowhead: nucleoli, n: nuclei, ol: ovarian lumen, yg: yolk globule.

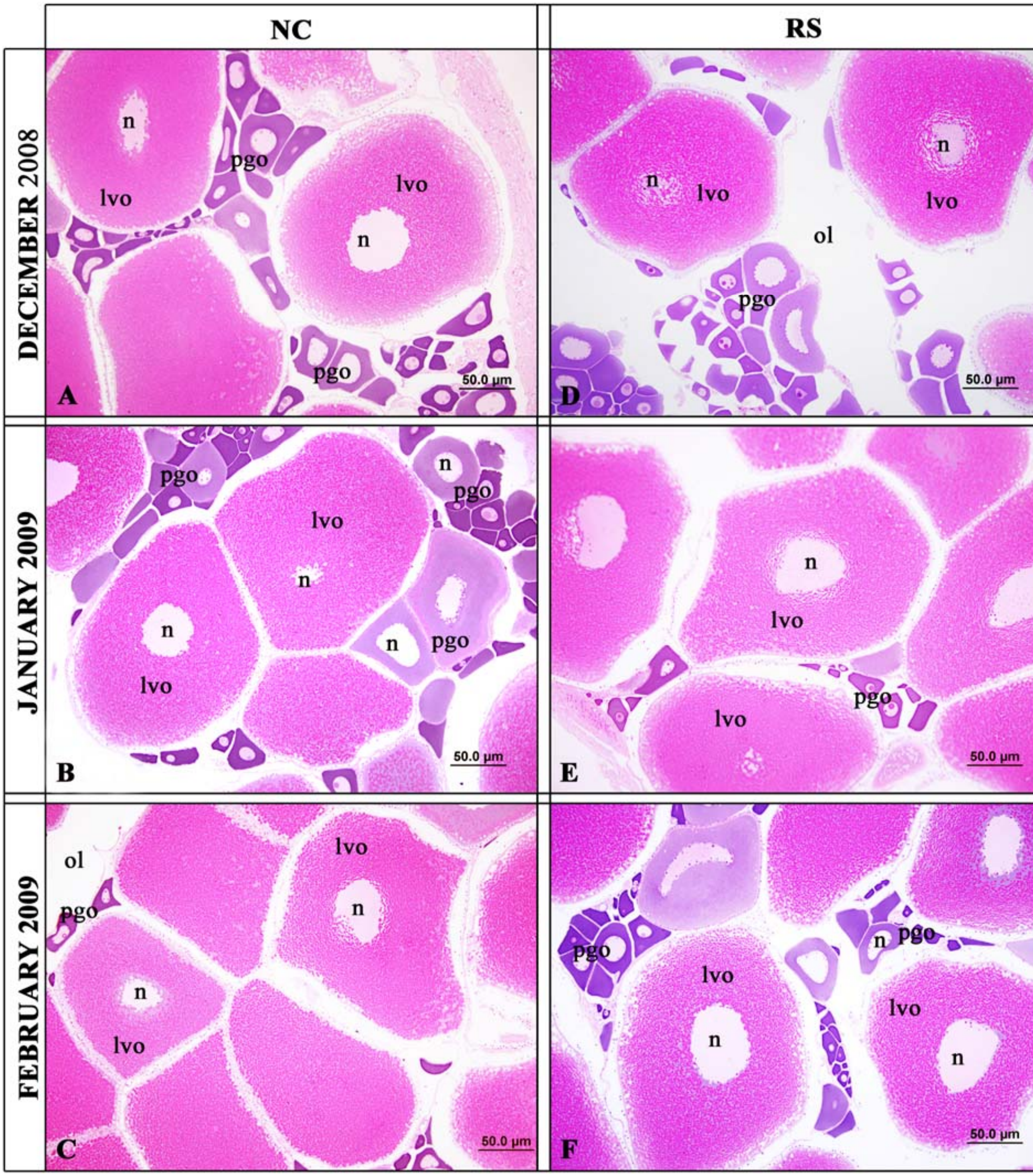

Figure 7. Histological analysis of ovaries of *Pimelodus maculatus* for the NC (A, B, C) and RS (D, E, F) individuals in December 2008, January 2009 and February 2009. Note the decrease in the amount of oocytes in primary growth or pre-vitellogenic (pgo) from December 2008 to February 2009 in both the NC and RS, and unlike the predominance of oocytes in secondary growth into late vitellogenesis (lvo) in the same period. Legend: n: nuclei, ol: ovarian lumen.

Feeding activity was more intense in the net cage site, which is directly related to fish farming activities due to the high amount of fish food that escapes to the surrounding aquatic ecosystem. This can be explained by the predominant use of fish ration by this species, demonstrating trophic opportunism. The opportunistic behavior may contribute to the success of the species in adjusting to the new artificial environment provided by the facilities and zootechnical management. Additionally, we found that *P. maculatus* may exploit almost every trophic level of the aquatic ecosystem, showing a wide flexibility in ingesting practically all organisms available, as described by other authors (LOBÓN-CERVIÁ; BENNEMANN, 2000; SILVA et al., 2007).

During the study period it was clear that *P. maculatus* was able to supply energetic requirements using fish food from the area around the net cages. Conversely, at the reference site, the individuals needed to search for diverse food sources, shaping the diet according to the trophic category, and according to what is abundant in the environment during a certain period of time. In addition, this fact is evident when observing the lower number of food items in the NC *versus* a higher number in RS.

Our calculations showed that the population of the NC presented a longer reproductive period compared to RS population (indicated by the GSI). This also suggests that the fish farming population might have some reproductive advantages. The histological analysis of the ovaries confirmed an increase in oocyte volume during secondary growth, due to advanced yolk deposition, which reflects an increase in the GSI from December 2008 to February 2009. Moreover, histologically, a large number of gonads showed oocytes that had completed the vitellogenesis phase. These oocytes are also called late vitellogenic or “full-grown” (*sensu* GRIER et al., 2009). Thus, according to the classification of Brown-Peterson et al. (2011), these individuals can be spawning capable. One hypothesis is that these populations could be utilizing the NC only to feed and later migrate, searching for closer tributaries to spawn (DEITOS et al., 2002). Moreover, Braga (2001) observed only feeding and growth activity for this species at the Volta Grande reservoir (Minas Gerais State), but an absence of breeding activity. This reinforces our hypothesis that this species uses the fish farming area for feeding, but still needs to search for tributaries to spawn.

The assessment of the reproductive potential indicated that the number of the oocytes was greater in NC population. However, the diameter of the oocytes was smaller in NC than in RS female population. Perhaps these differences in oocyte diameter may be related to the reproductive strategy of the species, by producing small oocytes that can be fast released and in great amounts. Agostinho et al. (2007) argue that small eggs and rapid development are components of the reproductive strategy of fishes in artificial reservoirs. This strategy seems to occur with this species, indicating a high degree of adaptability in ecosystems strongly influenced by human activities. The nature and intensity of the impact of food escape from aquaculture to the aquatic environment and their incorporation into the food chain still require more detailed investigation, at the individual level (physiology) as well as higher levels of organization (communities) (DEMÉTRIO et al., 2012). At this point, this study contributes to the knowledge of the possible impacts of fish farming in net cages installed in continental waters, and demonstrates the possibility of the presence of cages in the aquatic ecosystem influencing the diet and reproductive aspects of *maculatus*.

The impact of adding artificial nutrients into trophic webs is not well understood. Therefore, studies about the diet and reproductive aspect of fish, which are adjusting to an ecological niche around the cages, may contribute to an understanding of species dynamics and the functioning of the trophic webs of these complex ecosystems. According to Vita et al. (2004), the trophic role of wild fish should be considered when evaluated the environmental impact of fish farming. The consumption of aquaculture wastes by wild fish populations may be important in nutrient removal or re-distribution in certain marine environments (FELSING et al., 2005), and also in freshwater ecosystems. The ability of *maculatus* to feed on ration around the net cages makes it an important agent in the cycling of matter and energy, absorbing the excessive food that escapes from the cages and can reach the sediment. However, this is not a solution to the problem. The conservation and sustainable use of continental waters for fish farming in cages requires knowledge and understanding of the aquatic ecosystem in which they have been installed.

Necessarily, for achieving long-term aquaculture potential to increase global fish supplies and to provide food for the world's growing population will require a shared vision between the public and private sectors. Governments can support research and development of environmentally benign systems, eliminating implicit subsidies for ecologically unsound fish production, and establishing regulatory measures to protect coastal ecosystems (NAYLOR et al., 2000).

Conclusion

This study demonstrates that the presence of net cages in an aquatic ecosystem influences the diet and reproductive aspects of *Pimelodus maculatus*, indicated by quantitative differences in abundance and biomass, diet, period and higher reproductive potential in the fish sampled in the net cage farming area (NC) relative to a reference site (RS), that is not influenced by fish farm.

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