INFLUENCE OF CAGE FISH FARMING ON THE DIET AND BIOLOGICAL ATTRIBUTES OF *Galeocharax knerii* IN THE CHAVANTES RESERVOIR, BRAZIL*

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ABSTRACT

The aim of the present study was to evaluate the diet and biological attributes of the population of *Galeocharax knerii* residing near net cage fish farming activities in the Chavantes reservoir (Paranapanema River, Brazil) to check their possible impacts. Samples were collected from two populations: one near the net cages (NC) and one from an area not influenced by these cages denominated the "reference site" (RS). Monthly sampling was carried out from March 2008 to February 2009. Fish were caught with a standardized effort using gill nets deployed for 14 hours. The alimentary index (AI) and degree of repletion (RD) were calculated to determine diet composition. Analyses of the sex ratio and the gonadosomatic index (GSI) were also performed. The calculations of AI revealed that fish wastes constituted the most frequent food item in the diet in both study areas (NC = 70.43; RS = 87.55), followed by the consumption of *Apareiodon affinis* (AI = 29.56), which was abundant near the NC, and prawn at the reference site (AI = 12.28). The sex ratio differed from 1:1 and mature individuals were only found in the population near the NC. The findings demonstrate that *G. knerii* indirectly benefits from the input of organic matter, using small fish as its main food resource. We conclude that the activities of fish farming influence diet and biological attributes of the species *G. knerii*, evidenced mainly by higher feeding activity, numerical abundance and biomass in the area of the cages.

Keywords: Fish; Characidae; impact of fish farming; teleost

INFLUÊNCIA DE UMA PISCICULTURA EM TANQUES-REDE NA DIETA E ATRIBUTOS BIOLÓGICOS DE *Galeocharax knerii* NA REPRESA DE CHAVANTES, BRASIL

RESUMO

O objetivo deste estudo foi avaliar a dieta e atributos biológicos das populações de *Galeocharax knerii* residentes ao redor de uma piscicultura em tanques-rede no reservatório de Chavantes, para verificar seus possíveis impactos. Para isto, foram coletadas amostras da população em torno dos tanques-rede (TR) e, em uma área sem influência da piscicultura, denominado "controle" (CT). As amostragens ocorreram mensalmente, de março 2008 a fevereiro de 2009. Os peixes foram capturados com rede de espera com esforço padronizado, expostas por 14 horas. Para determinar a composição da dieta, foi calculado o índice alimentar (IAi) e o grau de repleção (GR). Foi analisada a razão sexual e índice gonodossomático (IG). O cálculo do índice alimentar (IAi) revelou que restos de peixes constituiu o item mais frequente na dieta em ambas as áreas de estudo (TR = 70,43; CT = 87,55) seguido pelo consumo da espécie de peixe *Apareiodon affinis* (IA = 29,56), abundante em torno dos tanques, e camarão, na área controle (IAi = 12,28). A razão sexual diferiu de 1:1 e indivíduos maduros na população foram encontrados somente em torno dos tanques-rede. Os resultados mostram a habilidade da espécie em se beneficiar da entrada de matéria orgânica de forma indireta, utilizando como fonte de alimento principal, peixes de pequeno porte. Conclui-se que as atividades da piscicultura influenciam a dieta e os atributos biológicos da espécie *G. knerii*, evidenciado, principalmente, pela maior atividade alimentar, abundância numérica e biomassa na área dos tanques-rede.

Palavras chaves: Peixes; Characidae; impacto dos tanques-rede; teleósteo

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INTRODUTION

As a food-producing sector, aquaculture has surpassed both traditional catch fisheries and farmed meat production in terms of mean annual growth rate. However, a number of biosafety concerns arise with regard to aquaculture development due to the diversity in species, environments, type of systems and practices employed (ARTHUR and BONDAD-REANTASO, 2012). Despite the broad range of hazards and the complexity of perceived risks, the potential for the expansion of aquaculture production 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; DEMÉTRIO *et al.*, 2012; MIRTO *et al*., 2012; MORATA *et al*., 2013), for example, the possible deterioration of water, caused by external inputs to the system, introduction of non-native species, caused by escapes from net cage and alterations in local biota may cause impacts in the medium to long term. This activity produces a considerable amount of unused food (rations), feces and other products, such as medicines and pesticides (KUBITZA and ONO, 2003; BEVERIDGE, 2004; PILLAY, 2004).

The input of rations into an ecosystem may lead to changes in the diet of native fish, which could favor opportunistic species (STRICTAR-PEREIRA *et al*., 2010; CARVALHO *et al*., 2012). Thus, knowledge on fish movements around farms and the study of feeding behavior through the analysis of stomach contents are key factors to achieving an overview of the role native species play in the dynamics of fish farms (BOYRA *et al*., 2004). According to VITA *et al*. (2004), the trophic role of wild fishes should be considered when evaluating the environmental impact of fish farms. Ecological studies offer important information that can used in the establishment of effective management measures designed to mitigate the negative effects of human production systems and ensure the sustainable development of aquaculture projects.

The impact of adding artificial nutrients into trophic webs is not yet well understood. Therefore, studies on the diet and biological attributes of fish species that reside in or are adjusting to the ecological niches around net cage activities may contribute to our understanding of the dynamics of species and the functioning of trophic webs in these complex ecosystems. The increase in net cage activities on the banks of large rivers can lead to the modification of important spaces that serve as shelter, feeding and breeding grounds for a large number of species of fish and other organisms, with a consequent impact on the dynamics of the natural environment.

The choice of the species *Galeocharax knerii* Steindachner, 1879 (Actinopterygii), commonly known as the "peixe cadela", to perform this study was based on the following characteristics: (i) the species in Brazil, is widely distributed in rivers and lakes in the Neotropical region (REIS *et al.*, 2003); (ii) The population of *G. knerii* has increased in the area surrounding the fish farm since its construction and has grown continuously in the area near the net cages (personal observation).

The aim of the present study was to evaluate the influence of fish farming in the Chavantes reservoir (Paranapanema River, Brazil) on the diet and biological attributes (abundance, biomass, size class and reproductive period) of the population of *G. knerii* caught near a net cage fish farming system and compared to a reference site (not influenced by a cage farm system) to test the following hypothesis: (1) Does the population of *G. knerii* around net cage fish farming activities have a different diet than that of the population at the reference site? (2) Do changes occur in the biological attributes of this species due to fish farm activities?

MATERIALS AND METHODS

Study Area

The Chavantes reservoir (23°22'S 49°36'W) is located in the medium stretch of the Paranapanema River on the border of the states of São Paulo (SP) and Paraná (PR) (southeastern and southern Brazil, respectively). The reservoir is 480 m above sea level, with a maximum depth of 70 to 90 m, a total volume of 9,410 x 10^6 m^3 and an area of 400 km^2 (DUKE ENERGY, 2002).

This study was conducted at a private enterprise for the breeding of tilapia, *Oreochromis niloticus,* in a net cage system located in a lentic segment of the reservoir between the municipalities of Ipaussu and Chavantes (SP). Two sites were selected for the study - one close to the fish farm, designated the net cage site (NC), and one located in a stretch above the NC, designated the reference site (RS). The RS was located at geographic coordinates 23º7'56.89"S and 49º36'13.24"W, approximately three km from the NC (Figure 1). The two study sites were bordered by rocks, fragments of mesophytic forests and areas of aquatic macrophytes.

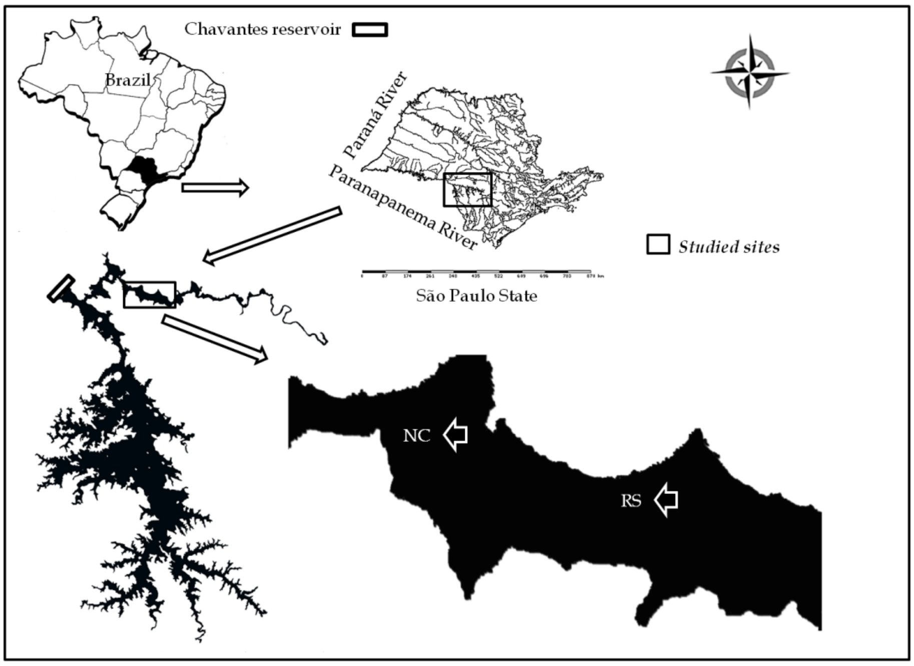

Figure 1. Location of Chavantes reservoir on the Paranapanema River (SP/PR); White arrows indicate study sites – net cage fish farming site (NC) and reference site (RS). (Source: Satellite images from Google Earth - Digital Globe)

The fish farm studied has been operating since 2008 and is classified as a medium-size farm, with about 200 net cages, each ranging in volume from 6 to 18 m^3. Fish were sampled monthly (License nº Ibama Register: 2629349) from March 2008 to February 2009 at the two sites. Fish were caught with gill nets grouped in three sets of five nets each (mesh sizes = 3 to 14 cm between opposing knots and height from 1.44 to 2.20 m). The nets were deployed at 5:00 PM and removed at 7:00 AM (exposure time: 14 hours). In field, all individuals were measured and weighed immediately after capture. The stomachs of the specimens were separated by a section of the intestine immediately anterior to the pyloric caeca, weighed and transferred to labeled vials containing 10% formalin for transport to the laboratory.

Hydrological and environmental data on the reservoir for the rainy and dry seasons were obtained from the Department of Hydrobiology of Duke Energy, Paranapanema – Generation. Regarding the biometric data of the species, standard length in centimeters (L_s) and total weight in grams (W_t) were determined using an ichthyometer and precision scale, respectively. Stomach samples were separated by sectioning the intestine immediately prior to the pyloric caeca; weighed on a centigram scale, transferred to duly labeled bottles containing 10% formalin and transported to the laboratory.

Differences in mean standard length for the population of *G. knerii* in both study areas were evaluated with the non-parametric test of Mann-Whitney, after checking assumptions of normality

(Lilliefors test), with the aid of the BioEstat 5.0 (AYRES *et al.*, 2007).

Stomach contents were analyzed under a stereomicroscope, identified to the most detailed taxonomic level possible and weighed (wet weight) on a scale with accuracy of 0.0001 g. The identification of food items was performed using identification keys (LEHMKUHL, 1979; STRIXINO and STRIXINO, 1982; MERRITT and CUMMINS, 1996; COSTA *et al.*, 2006).

The results were determined based on frequency of occurrence (FO) and gravimetry weight (%), and then applying the alimentary index (AI) to demonstrate the main food items of the diet in each sampling site (NC and RS) (KAWAKAMI and VAZZOLER, 1980), as adapted by HAHN *et al.* (1998):

$$AI = Fi \times Wi \times 100 / \sum Fi \times Wi,$$

in which AI = alimentary index; i = 1.2 ... n, food items, Fi = frequency of occurrence of item i (%); Wi = wet weight of item i (%).

The degree of repletion of the stomachs was rated visually on a scale of 0 to 4, following the method proposed by Walsh and Rankine *apud* MARÇAL-SHIMABUKU and PERET (2002), for which 0 = empty stomach, 1 = less than 25% full; 2 = between 25 and 50% full; 3 = between 50 and 75% full; and 4 = greater than 75% full. To detect possible spatial patterns between the NC and RS areas regarding the distribution of food resources used by the species, Detrended Correspondence Analysis (DCA) was employed based on the weight data of the food resources and tested by the Multi Response Permutation Procedure (MRPP) using the PCORD4 program (McCUNE and GRACE, 2002).

The distribution of the specimens according to size classes was performed considering the population of each site separately, based on STURGES (1926). The relative frequency of each size class considered grouped sexes (females and males). Differences were tested using the Kolmogorov-Smirnov test ($p<0.05$) with the aid of the BioEstat 5.0 program (AYRES *et al.*, 2007).

Sex was determined by macroscopic visualization of the gonads and related traits, such as color, transparency and vascularization, according to VAZZOLER (1996) and VEREGUE and ORSI (2003). For such, the abdominal cavity was exposed through ventral sectioning and the gonads were removed for analysis.

For the analysis of reproductive stages, seasonal variations in the gonads were determined using the gonadosomatic index (GSI):

$$GSI = Wg/Wt * 100,$$

in which Wg = weight of gonads and Wt = total weight (VAZZOLER, 1996).

Voucher specimens were deposited at the Laboratório de Biologia e Genética de Peixes (LBP), Instituto de Biociências, UNESP Botucatu, Brazil (LBP 9180).

RESULTS

A total of 451 specimens of *G. knerii* were caught. The comparison between the NC and RS regarding the number of individuals caught and biomass revealed that the population around the fish farming system was far more numerous than that of the reference site (Figure 2).

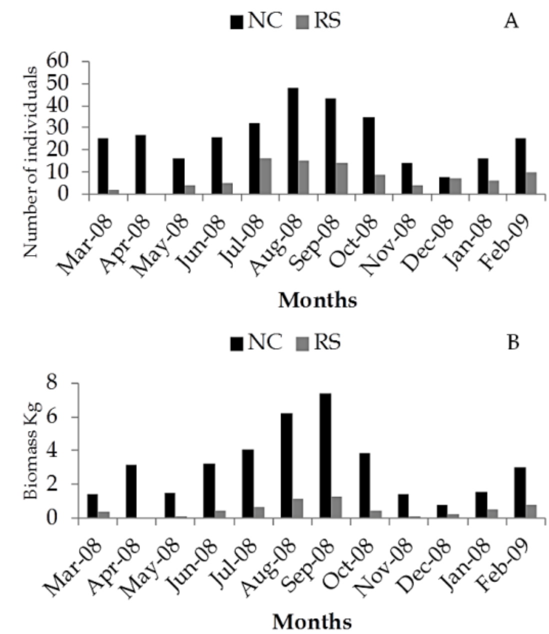

Figure 2. Number of individuals (A) and biomass (B) of *Galeocharax knerii* caught monthly at NC and RS in the Chavantes reservoir, Paranapanema River, SP/PR, Brazil.

A total of 346 individuals were caught at the NC (biomass: 49.5 kg) and the stomach contents of 101 individuals were analyzed. A total of 105 individuals were caught at the RS (biomass: 10.6 kg) and the stomach contents of 16 specimens were analyzed. The other individuals in both study areas had empty stomachs. Food items were grouped into four categories: The first category consists only of the species *A. affinis*; second: Fish wastes, consisting of remains of muscle, bones, and scales; the third: Crustaceans, with the identification of the genus *Macrobrachium* sp., and the fourth category: Insects, composed by Hymenoptera larvae, non-identified remains; Diptera larvae and pupae.

Fish wastes constituted the most consumed item at both the NC and RS (AI = 70.4% and 87.5%, respectively), followed by *Apareiodon affinis* (commonly known as "canivete" in Brazil) at the NC (AI = 29.6%) and prawn at the RS (AI = 12.3%) (Figures 3 and 4). The analysis of the degree of repletion (DR) revealed higher values at the NC (DR = 1.1) in comparison to the RS (DR = 0.4).

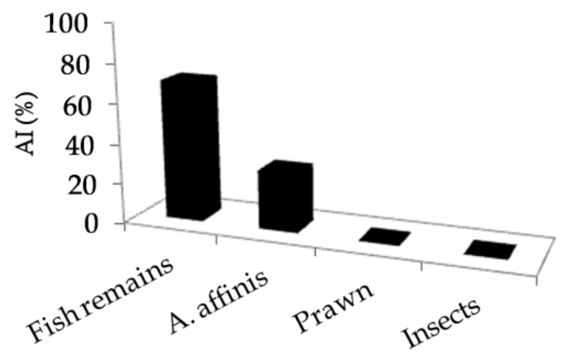

Figure 3. Alimentary index (AI) of *Galeocharax knerii* caught at NC in Chavantes reservoir, Paranapanema River SP/PR, Brazil.

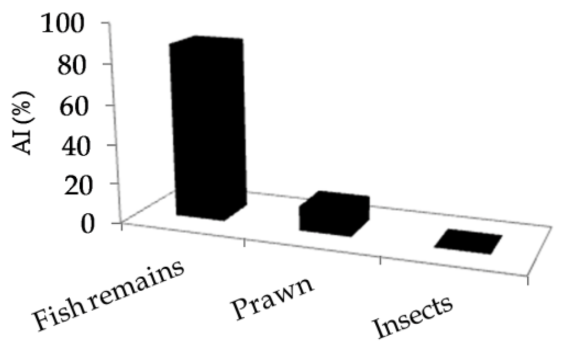

Figure 4. Alimentary index (AI) of *Galeocharax knerii* caught at RS in Chavantes reservoir, Paranapanema River SP/PR, Brazil.

In the detrended correspondence analysis for the ordination of food resources, Axis 1 (eigenvalue = 1.00) separated the item *A. affinis* for the NC population and Axis 2 (eigenvalue = 1.00) grouped other food items in the RS site, demonstrating the ability of *G. knerii* to consume *A. affinis*, which was abundant around the cages (Figure 5).

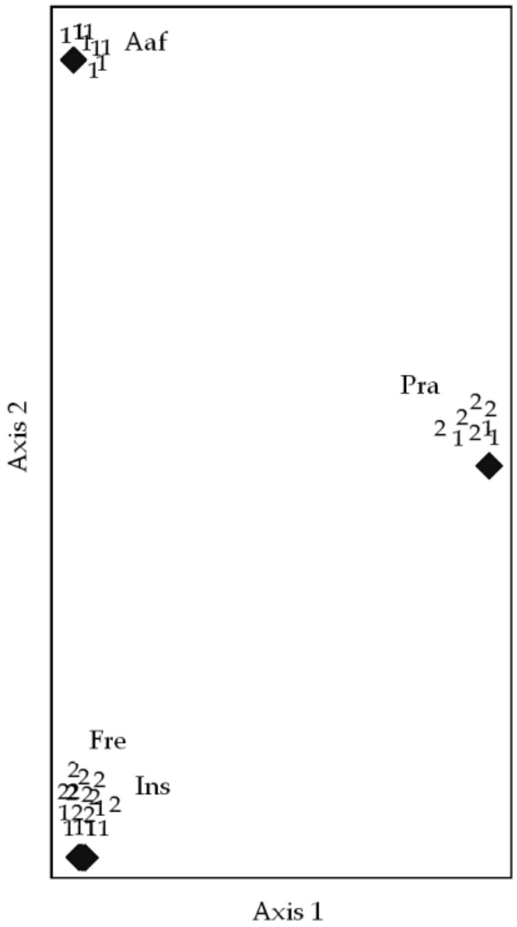

Figure 5. Detrended Correspondence Analysis of food items in the diet of of *Galeocharax knerii* at NC and RS in Chavantes reservoir, Paranapanema River SP/PR, Brazil; in which: 1 = NC, 2 = RS, Fre = Fish wastes, Aaf = *Apareiodon affinis*, Pra = prawn and Ins = Insects. Diet similarity was verified using the Multi Response Permutation Procedure (A = 0.022; $p<0.05$).

Was observed through of analysis of Mann-Whitney ($p<0.0001$) statistically significant differences in standard length between the *G. knerii* populations in the two study areas (Table 1).

Table 1. Standard length (cm) of populations of *Galeocharax knerii* at NC and RS in Chavantes, Paranapanema River SP/PR, Brazil; (n) = number of individuals; (**X**) = mean; (SD) = standard deviation.

Site	n	X	SD	Mann-Whitney
NC-total	346	18.2	4.8	$p < 0.0001$
RS-total	105	14.6	4.8	
NC-female	221	20.5	3.8	$p < 0.0001$
NC-male	55	15.7	2.2	
RS-female	49.0	17.6	5.1	$p = 0.0012$
RS-male	21	13.7	2.7	
NC-female	228	20.5	3.8	$p < 0.0001$
RS-female	49	17.6	5.1	
NC-male	54	15.7	2.2	$p = 0.0011$
RS-male	21	13.7	2.7	

The Kolmogorov-Smirnov test revealed significant differences ($p<0.05$) in the distribution of length classes in the two sites with the sexes grouped and when comparing females and males separately. In the NC the 22 to 24.1 cm class was the most frequent and RS 8.8 to 10.9 and 11 to 13.1 cm were the most frequent (Figure 6-A). In the analysis of females and males separately at NC, 21 to 23.3 cm class was the most frequent in the females and the 16.2 to 18.5 cm class was the most frequent in the males (Figure 6-B). In the RS the most frequent class was 21.5 to 23.1 cm for females and 11.9 to 14.2 cm for males (Figure 6-C).

The analysis of the reproductive period of *G. knerii* was performed only at the NC, as no mature animals were caught at the RS. GSI peaks at the NC were recorded in November and February, following periods of greater precipitation (Figure 7 A and B).

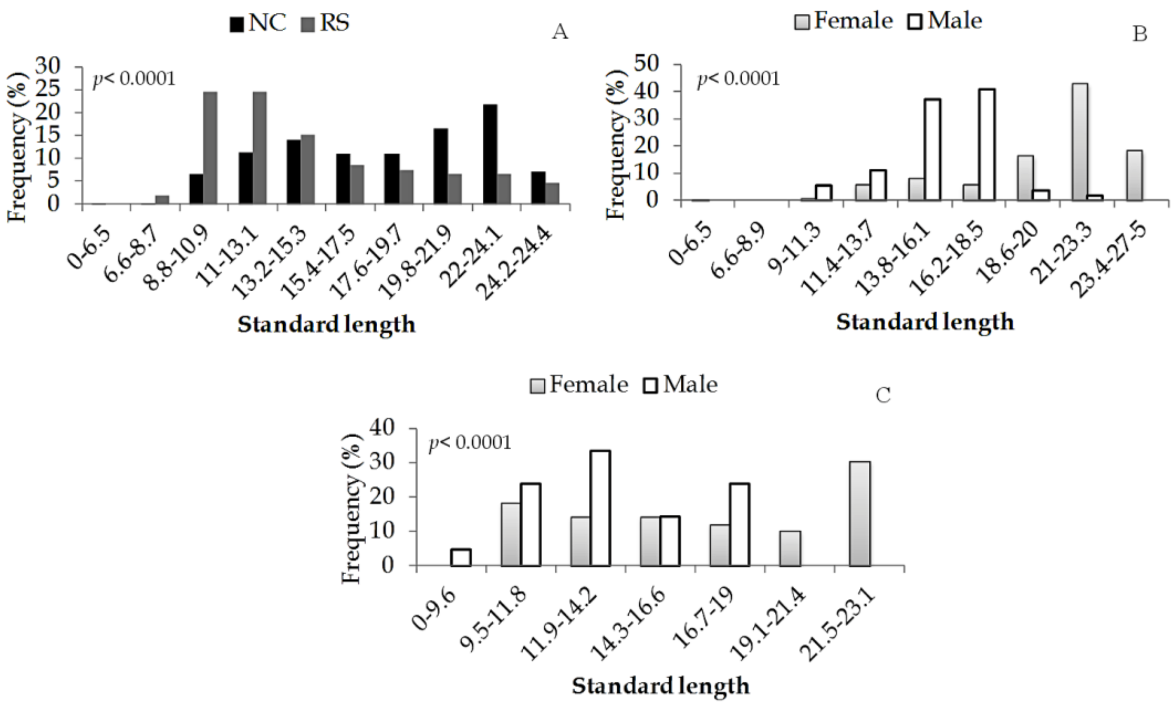

Figure 6. Frequency (%) of standard length classes of *Galeocharax knerii* collected from Chavantes Reservoir. Paranapanema River SP/PR, Brazil; A) both sexes at NC and RS; B) females and males at NC; and C) females and males at RS. Values significantly different to $p<0.0001$.

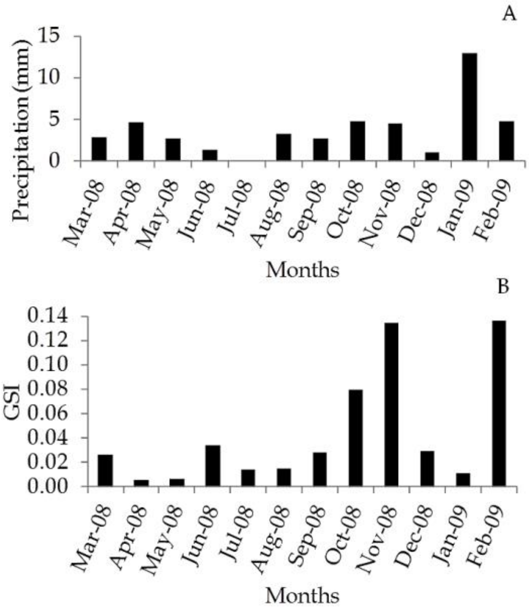

Figure 7. A) Monthly rainfall (mm) (Source: Duke Energy – Paranapanema Generation); B) Monthly variation in gonadosomatic index of *Galeocharax knerii* females at NC in Chavantes reservoir. Paranapanema River SP/PR. Brazil.

DISCUSSION

Differences in abundance and biomass were found between the population of *G. knerii* sampled around the net cages and that sampled from the reference site. The diet at both sites was based on fish, with greater feeding activity around the net cages. Net cage fish farming leads to the input of matter and energy into aquatic food webs, attracting a large number of organisms (BOYRA *et al*., 2004; HÅKANSON, 2005; RAMOS *et al*., 2008) due to release of leftover ration and indirectly contributing to the growth of algae (MANNINO and SARA, 2008). These effluents have different degrees of impact on the aquatic ecosystem, depending on the amount released, dilution, time of release and dispersal capacity in the water column (CARROLL *et al*., 2003; YOKOYAMA, 2003).The input of leftover ration and feces from fish farming activities becomes the primary source of nutrients in the surrounding aquatic ecosystems (KUBITZA and ONO, 2003) and the increased availability of food resources can cause changes in the potential productivity of aquatic organisms through trophic interactions.

The analysis of stomach contents suggests that *G. knerii* indirectly uses the remains of ration from the fish farming activity by consuming the species *A. affinis* using it as their main food source. This is confirmed by high abundance of *A. affinis* feeding on leftover the ration around the net cages (BRANDÃO, 2010; CARVALHO *et al*., 2012), which explains the greater feeding activity of *G. knerii* in this area. Moreover, the detrended correspondence analysis allowed identifying *A. affinis* as main food resource of the diet at the NC, confirming the piscivorous feeding habits of *G. knerii*. Fish farming has the potential to attract wildlife (DEMPSTER *et al*., 2004) and as small fishes are the most abundant in the majority of aquatic environments, these organisms become an available food resource for piscivorous fishes (HAHN and FUGI, 2008).

Opportunistic consumption is considered an important tactic for successful colonization in habitats affected by anthropogenic activities allowing species to maximize energy input due to the supply and quality of available food (RAMOS *et al*., 2008). The evidence suggests that this tactic is used by the species studied (*G. knerii*) around the cages. Moreover, greater abundance and biomass of *G. knerii* were recorded at the NC in comparison to the RS.

The analysis of standard length of specimens *G. Knerii* revealed that the residents around fish farming exhibit larger number of individuals in largest size classes within in the population. Analysis of the length structure of a population provides qualitative information regarding its development, as growth indicates favorable environmental conditions (BENEDITO-CECILIO and AGOSTINHO, 1997). Thus, the results on the distribution of length classes in the populations of *G. knerii* suggest that this species was successful the first year of activity of fish farming, regarding the exploitation of food resources around the net cages.

In addition, mature individuals of *G. knerii* were only recorded around the cages, with the reproductive period occurring in November and February, based on the highest GSI peaks. Regardless of the strategy used, the reproduction of Neotropical fish species is highly seasonal (AGOSTINHO *et al*., 2007), as observed in the present study, in which the presence of mature females was synchronized with the greatest amount of rainfall. However, few mature individuals were found and further studies are needed to confirm the reproductive period of this species. Moreover, the fact that mature specimens were only recorded at the NC suggests that fish farming activities exert an influence on reproductive aspects of this species through the maturation of gonads, possibly favored by the large supply of food surrounding area.

The potential negative impacts caused by fish farming have been assessed in different marine and freshwater environments throughout the world (BEVERIDGE, 1996; DEMPSTER *et al.*, 2002; MENEZES and BEYRUTH, 2003; NICKELL *et al.*, 2003; GIANNOULAKI *et al.*, 2005; MACHIAS *et al.*, 2005; TUYA *et al.*, 2006; KUTTI, 2008; BRIGOLIN *et al.*, 2009; ROMANA-EGUIA *et al.*, 2010; DIAS *et al.*, 2011, WETENGERE, 2011). For the effective development of aquaculture in both ecosystems (marine and freshwater) experts agree that the sustainable management requires the involvement and cooperation of the government, academia, private sector, investors, communities and society (DEVOE and HODGES. 2002; AGOSTINHO *et al*., 2007).

This study showed that fish farming system activities located in reservoir Chavantes causes changes in natural conditions of wild fish, evidenced in this study across species *G. knerii*. The distribution of food items between cage farms and reference site demonstrated that the items waste of fish and *A. affinis* were more associated with cage farms areas. It may be interpreted that there was an increased abundance of these items, allowing a greater exploration by *G. knerii* in these areas.

Moreover, the ability of *G. knerii* to feed on small fish residing around net cages constitutes an important agent in the cycling of matter and energy controlling the population explosion of species of small fish possibly attracted by fish farming activities in this Neotropical reservoir. So, the trophic role of wild fishes should be considered when evaluating the environmental impact of fish farms, as also suggested by VITA *et al*. (2004), in marine ecosystems. However, further studies are required to determine the extent of this effect in medium and long term and the consequences which may occur in all biota.

CONCLUSION

The cage fish farm system causes changes in the diet and biological attributes of *G. knerii*, due to high feeding activity, quantitative differences in diet, greater standard length, and the presence of mature females only in the net cage are compared to the reference area. The conservation and sustainable use of continental waters for fish farming in cages require knowledge and understanding of aquatic ecosystem in which they have been installed. We suggest that population closely associated to net cage should be permanently monitored as part of a continuing environmental assessment.

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