UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

BACHARELADO EM CIÊNCIAS BIOLÓGICAS

Trabalho de Conclusão de Curso

HISTORY OF LIFE OF A NEW SPECIES OF THE GENUS *Gymnotus* (GYMNOTIFORMES: GYMNOTIDAE) AN ENDANGERED SPECIES IN THE SOUTHERN BRAZIL

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Porto Alegre, Novembro de 2015

History of life of a new species of the genus *Gymnotus* (Gymnotiformes; Gymnotidae): an endangered species in the Southern Brazil

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Porto Alegre, Novembro de 2015

Agradecimentos

Com o término de mais esse ciclo só posso agradecer a tudo e a todos que me acompanharam nessa jornada, sem os quais a conquista de mais essa etapa não seria possível.

Agradeço à minha família de sangue e coração por todo suporte e incentivo dado em todas as fases da minha vida. Agradeço por todo amor e confiança, pela certeza de tê-los como meu porto seguro, como exemplo de persistência e dedicação. Obrigada também pela paciência e compreensão nos momentos difíceis e pelos risos compartilhados em todas as ocasiões. Essa conquista é nossa! Amo vocês!!

Agradeço também à família pela qual eu fui escolhida, aos amigos/irmãos que me acolheram e estiveram do meu lado nesses anos de faculdade. Obrigada, pelas noites/dias/anos de estudo, de festas, de risos, 'de mimimis', de companheirismo, de apoio e acima de tudo de muito amor. Gustavo, Nicolle, Pedro, Carol, Thais, Michele, Tashe, Gabriel, Fábio, Lagoa e Irina meus irmãos, amigos e colegas de curso e profissão, vocês são com certeza uma das melhores consequências dessa jornada, vocês são pra sempre! A todos os colegas de curso agradeço pelo companheirismo e lealdade, pela ajuda dada em todos os momentos. Cada um de vocês foi especial do seu próprio jeito, cada um com algo a acrescentar e a compartilhar. Agradeço por todos esses ganhos, vou carrega-los comigo pra sempre! Agradeço à vida por ter me presenteado com tantos anjos no meu caminho, por todas as pessoas e vivências maravilhosas que hoje fazem parte de mim e me tornam o que sou.

Agradeço a toda equipe do departamento de Ictiologia, por serem muito mais que colegas, pela boa vontade em ajudar, ensinar, por estarem sempre presentes e por me acolherem tão bem ao longo desses anos de Ictio. Às minhas orientadoras, Clarice e Júlia, só tenho a dizer que não poderia pedir por algo melhor, sou muito grata por todo apoio dado a cada escolha que fiz, por estarem sempre presentes em cada fase desses anos de aprendizado no lab. E a ti Laísa, muito obrigada pela especial ajuda na elaboração desse trabalho.

Agradeço à ‘mãe UFRGS’ pelo acolhimento e por todas as oportunidades de crescimento profissional e principalmente pessoal que obtive. Agradeço aos mestres que cruzaram meu caminho deixando tanto conhecimento, deixando tanto de si para a minha formação como pessoa e futura bióloga.

Manuscrito formatado conforme

as normas editoriais da revista

PLOS ONE.

As tabelas e figuras necessárias para a compreensão do trabalho foram inseridas no próprio texto para melhor visualização.

History of life of a new species of the genus *Gymnotus* (Gymnotiformes; Gymnotidae): an endangered species in the Southern Brazil

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Resumo

O presente estudo descreve a história de vida de *Gymnotus* sp. n., uma espécie classificada como Em Perigo na Lista das Espécies Ameaçadas de Extinção do RS, enfocando a biologia alimentar e reprodutiva da espécie. As coletas ocorreram mensalmente entre Março/2011 e Fevereiro/2012 no Refúgio da Vidal Silvestre Banhado dos Pachecos (RVSBP), Rio Grande do Sul, Brasil. *Gymnotus* sp. n. apresenta período reprodutivo ocorrendo de novembro até fevereiro com picos mais acentuados do índice gonadossomático (IGS) no mês de outubro para machos e fêmeas. Fêmeas histologicamente classificadas como aptos à desova foram registradas entre agosto e fevereiro, enquanto machos nesta mesma fase foram registrados em agosto e de novembro a fevereiro. A espécie possui desova do tipo parcelada e a menor fecundidade relativa registrada entre as espécies de Gymnotiformes até então estudadas. O IGS de machos e fêmeas é positivamente relacionado com o índice de repleção estomacal (IR). Os baixos valores de fecundidade e a queda nos valores de IR de machos após o ápice reprodutivo sugerem a existência de cuidado parental para a espécie. Analises indicam um padrão sazonal na dieta, associando a predação de insetos autóctones ao inverno, insetos alóctones ao outono e matéria orgânica digerida à primavera. De acordo com a análise dos itens alimentares e do quociente intestinal estimado, *Gymnotus* sp. n. pode ser classificado como invertívoro se alimentando principalmente de insetos aquáticos, característica também encontrada para a maioria dos Gymnotiformes já estudados.

Palavras-chave: Peixe-elétrico, espécie ameaçada, reprodução, dieta.

Abstract

The present study describes the life history of *Gymnotus* sp. n., a species classified as Endangered in the last published list of threatened species of fauna of Rio Grande do Sul state, regarding the species reproductive biology and feeding habits. The sampling occurred once a month from March/2011 to February/2012 in the Refúgio da Vida Silvestre Banhado dos Pachecos (RVSBP), Rio Grande do Sul, Brazil. The reproductive period of *Gymnotus* sp. n. was estimated from November to February with peaks of gonadossomatic index (GSI) occurring in October for both males and females. Females were histologically classified as spawning capable from November to February; whereas males in the same phase of gonadal maturation were observed in August and from November to February. The species presented fractional spawning with the lowest average relative fecundity among the Gymnotifomes species studied at the present. The GSI of both males and females was significantly related to the stomachal replation index (RI). The low values of fecundity and the decrease in males RI values after the reproductive period point to the existence of a parental care, as already observed for other *Gymnotus* species. The analyses showed a seasonal pattern on the species diet, associating the predation on autochthonous insects to winter, allochthones to autumn and digested organic material (DOM) to spring. According to food items analysis and estimated intestinal quotient, *Gymnotus.* sp. n. was classified as invertivorous, feeding mainly on autochthonous insects, matching previous studies regarding other species of Gymnotiformes.

Key words: Electric-fish, Endangered, reproduction, feeding.

Introduction

The order Gymnotiformes is composed by freshwater weakly electric fishes which are able to produce and detect electric fields with the function of localization and intra and interspecific communication [1]. They occur from southern Mexico to Argentina, and in the Caribbean island of Trinidad [2]. The genus *Gymnotus* is the representative with the larger distribution among the order, occurring along its entire distribution area [3]. Environmental conditions (i.e. water flow, temperature, dissolved oxygen, conductivity and vegetation) are important factors influencing the distribution of electric fishes [4]. These fishes also have several strategies allowing adaptations to environments with a large range of abiotic and biotic factors [4-9], including air breathing adaptations to cope with prolonged periods of hypoxia or anoxia [4-10].

The new species of the genus *Gymnotus* (Giora & Malabarba, in prep.), belongs to the species group *G*. *pantherinus* due to a set of characters of coloration and body proportions, and was formerly misidentified as the species *G*. *pantherinus*. *Gymnotus* sp. n. is found inhabiting the interior of wetlands and edges of swamp forests compound by dense and floating vegetation [11], and is distributed from coastal rivers of southern Rio Grande do Sul state to southern Santa Catarina state (Giora & Malabarba, in prep.). According to the last published list of threatened species of fauna of Rio Grande do Sul state, *Gymnotus* sp. n. (quoted as *Gymnotus pantherinus*) is labeled as EN (Endangered) according to the UCN criteria [12].This classification is based on the species distribution in the state, which is estimated in only 24km² and restricted to streams and fragmented areas subjected to anthropic actions, and on its high habitat specificity. Up until now, *Gymnotus* sp. n. is found occurring more abundantly only in two conservation areas of the Rio Grande do Sul: “Refúgio da Vida Silvestre Banhado dos Pachecos” and “Parque Estadual de Itapeva”, being rare and scarce in all the other places where it has been registered (Giora & Malabarba, in prep.).

A disturbed ecosystem can affect directly the dynamic, seasonality and behaviour of species. In order to develop conservation efforts toward threaten species it is essential to understand the biology and the ecological interaction between these organisms and their habitat. Therefore, the present study aims to describe the reproductive biology, gonadal maturation, and feeding habits of a population of the endangered species *Gymnotus* sp. n.

Material and Methods

Study area

The sampling area is located in the "Refugio da Vida Silvestre Banhado dos Pachecos" (30º05’S; 50º50’W) (Fig. 1) in Viamão municipality, state of Rio Grande do Sul, Brazil. The RVSBP is a conservation unit created in 2002, covering 2.560ha inserted in the Gravataí river basin. The area also guards the headwater of Gravataí river which medium and low segments are extremely impacted by human activities [13]. According to Oliveira et al. [14], the fragments of swamp forest surrounding the Gravataí river are the last remaining of semideciduous forest permanently undergoing on the river basin. The stream where *Gymnotus* sp. n. was sampled is characterized by murky water, abundant floating vegetation and muddy substrate.

Sampling

Field work and sampling were executed according to the Authorization for Scientific Activities (#25542-1) concede by the Sistema de Autorização e Informação em Biodiversidade - SISBIO of the Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio. The ICMBio is the Brazilian Government Department responsible for analysing research projects that involve field work, capture and sampling of specimens of native fauna and flora, conceding authorizations which regulate the area and period of sampling, as well as the species and number of specimens which can be capture and/or sacrificed during a study. ICMBio Authorization for Scientific Activities is obligatory for any research project that includes field work in the Brazilian territory. Ethical approval was obtained through the Comissão de Ética no Uso de Animais - CEUA of the Universidade Federal do Rio Grande do Sul - UFRGS, where the study was conducted. The CEUA is the University division responsible for the analysis and approval of all research projects conducted at UFRGS that involve the use of animals (Chordata: Vertebrata). Any research project concerning animals can be conducted at UFRGS without CEUA approval.

The specimens of *Gymnotus* sp. n. were monthly sampled from March/2011 to February/2012 between 9am and 5pm. Fishes were collected using a dip net under floating vegetation and an electric fish finder [15]. In the field, the specimens sampled were euthanized by immersion in 10% eugenol solution and then fixed in 10% formalin solution. Water and air temperature, water pH, dissolved oxygen and conductivity were recorded at the time and place of collection. The monthly amounts of rainfall (in millimeters) and time of sunshine (in hours) was obtained at the 8th District of Meteorology of Porto Alegre (available at Meteorological databank for Education and Research of the National Institute of Meteorology, Brazil, station 83967) [16].

Data analysis

In the laboratory, fishes were transferred to 70% ethanol solution and, afterward, the total length (Lt) in millimeter and total weight (Wt) in grams were measured. Individuals were dissected to register liver (Wl) and stomach (Ws) weight, and intestine length (Li). Stomach repletion index (RI) and gonadosomatic index (GSI) were estimated following the formula adapted from Santos [17]. These indexes represent the percentage organ weight related to fish total weight: RI = Ws x100/Wt and GSI = Wg x100/Wt. Ws corresponds to stomach weight, Wg to gonad weight, and Wt to total weight. The intestinal quotient (IQ) represents the ratio of the intestine length related to the fish total length: IQ = Li/Lt. Li corresponds to the intestine length and Lt to the total length.

The reproductive period for males and females was established through the analyses of monthly variation of the mean GSI values. The multiple regression with analysis of variance (ANOVA) with Tuckey's post-test was applied to verify possible differences between the monthly values of both GSI and RI of males and females separately. A simple linear regression was applied to verify possible correlation between the RI and GSI of males and females. A multiple linear regression was applied to verify possible correlation between the GSI and RI of females and males with the abiotic factors (photoperiod, rainfall, water and air temperature, water conductivity, pH, and dissolved O2).

To corroborate the macroscopic characterization and define the gonadal maturation phases, male and female gonads were selected for histological analysis being dehydrated in ethanol series, and infiltrated and embedded in glycolmethacrylate resin. Sections of 3μm were performed on a Leica RM2245 microtome with glass knifes, and were stained with Toluidine blue. The slides were photographed under a Nikon AZ 100mm microscope. The phases of gonadal maturation were classified according to Brown-Peterson et al. [18]. Absolute fecundity was estimated by counting all vitellogenic oocytes present in the ovaries of seven females with the highest GSI values recorded. The relative fecundity was determined by the number of vitellogenic oocytes counted per female milligram of total weight [19]. The same gonads selected for fecundity analysis were used for the determination of the spawning type. A sub-sample of 150 oocytes was randomly removed from each selected gonad and the largest possible oocyte diameter was obtained by examination under a stereomicroscope equipped with a millimetred ocular [20].

The sex ratio was determined by the distribution of male and female frequency during the sample period. The χ^2 test (α <0.05) was applied to determine the existence of significant differences between the proportion of males and females in the population. The χ^2 test (α <0.05) was also applied to the distribution of relative frequencies of males and females in different total length classes in order to test sexual dimorphism related to total length.

Stomach content analysis was performed with the help of a stereomicroscope and the items in the stomach contents were identified to the lowest possible taxonomic level. The alimentary items were analyzed by the frequency of occurrence method [21], including all food items, and by percentage composition method [22] where the number of times that each item has occurred was treated as the percent of total occurrence number of all items. For the percent composition method and statistical analyzes, the food items were grouped in eight broad categories according to their ecological characteristics and origin: allochthonous insects (Al), autochthones insects (Au), digested organic material (DOM), fish (Fis), crustacea (Crus), plant material (PM), sediment (Sed), and others (Other).

The factors sex, seasonality and length classes were tested on the diet composition of the species through the Permutational Multivariate Analysis of Variance (PERMANOVA) ($p \leq 0.05$) with the Bray-Curtis dissimilarity matrix. In order to test the influence of seasonality in the diet of *Gymnotus* sp. n., the months of the year were separated in different seasons established as: autumn the period from March to May, winter from June to August, spring from September to November and summer from December to January. The length classes were determined according to the Sturges rule [23]. The Principal Coordinate's Analysis (PCoA) was applied to visualize possible variations in the categories consumed with the factors considered. The Indicator Value Index (IndVal) was applied to visualise which alimentary categories might be related to sex, seasonality, and length class. The IndVal results were also expressed in terms of specificity (A) and fidelity (B), both ranging [0-1] and representing respectively the abundance of a category over all groups, and the presence or absence of a category within the site group. All analyses were performed through the software R Project for Statistical Computing 3.0.1.

Results

Reproduction

A total of 123 specimens of *Gymnotus* sp. n., 62 females (55.8mm – 200.8mm) and 61 males (63mm – 240.7mm), were sampled. The estimated reproductive period lasted from August to March, with GSI peak occurring in October for females and August and October for males (Fig. 1). According to the analysis of variance (ANOVA) with Tuckey's post-test, male and female mean GSI values differ significantly between the months of sampling ($F= 5.34$, $p< 0.05$ for males; $F= 3.75$, $p< 0.05$ for females). For females, mean GSI value obtained in October differed from April to July and from January. For males, mean GSI value obtained in October differed significantly from May to July and December differed from August, October and November. The mean GSI values of *Gymnotus* sp. n. females ($F= 1.3$; $t= 13.74$; $p< 0.05$) and males ($F=11.1$; $t=6.16$; $p< 0.05$) were significantly related to the mean repletion index (RI) according to the test of simple linear regression. Although the monthly data for dissolved oxygen, pH and water temperature were not completely recorded for each month (Table 1), the multiple linear regression indicated no correlation between the abiotic factors and the GSI of both males and females ($F= 0.62$; $p= 0.72$).

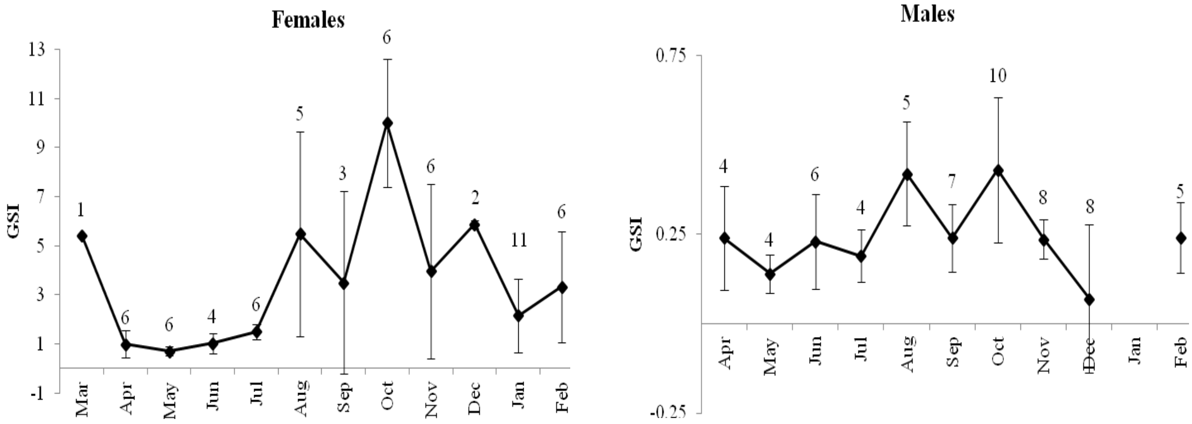

Figure 1: Gonadosomatic index.

Monthly variation of mean gonadosomatic index (GSI) for *Gymnotus* sp. n. from March/2011 to February/2012 (females) and April/2011 to February/2012 (males).Vertical bars represent the standard deviation. Numbers above the bars correspond to the numbers of specimens included in the analysis.

Table 1. Abiotic factors at sampling site.

Monthly variation of the water conductivity (µS/cm), dissolved oxygen (mg/l), pH, water and air temperature (°C), photoperiod (hours) and rainfall (mm) at the sampling site in Refúgio da Vida Silvestre Banhado dos Pachecos, Rio Grande do Sul state, from March/2011 to February/2012.

	Conductivity	Diss. O^2	pH	Temp. (H_20)	Temp. (air)	Photoperiod	Rainfall
Mar	43.5				23	215	83.1
Apr	36.4		4.63	23.7	25.5	162.7	172.7
May	19.7	4.1	4.96	22.6	24	122.4	50.1
Jun	18.7	5.6	4.9	14.7	17.5	107.5	109.6
Jul	26.5	3.5	4.8	18.6	24	101.3	225.7
Aug	18.5	3.5	5.4	19.3	29	118.6	182.3
Sep	41.1	3.4	5.4	19.2	16.7	180.3	53
Oct	43.5	2.8	5.25	25.5	27	200	123.7
Nov	22.1			23.5	34	262	13.7
Dec	48.6	3.6		31.3	22.8	233.2	52.1
Jan	47.2	2.3	4.68	23	26	298.4	166
Feb	28	5.7	4.63		27.4	226.5	139.5

For the monthly variation of the gonadal maturation phases of females and males (Fig. 2), the developing subphases were grouped and classified as ‘developing’, whereas the histological results permitted a more detailed classification, including the subphases early developing, mid developing, and late developing. The histological analysis of female gonads demonstrated all gonadal maturation phases and subphases as proposed by Brown-Peterson et al. [18]. Immature individuals occurred only in October, ovaries showing predominance of oogonia and primary growth oocytes (Fig.3-A). Females in the developing phase were captured during the whole sampling period except by March, being more predominant from May to September. During this period, the female gonads presented primary growth oocytes, more abundant in the early developing phases (Fig.3-B), primary and secondary vitellogenic oocytes, increasing in oocytes size and amount of yolk in the cytoplasm as the gonad maturation reached the mid (Fig.3-C) and late developing phase (Fig. 3-D).

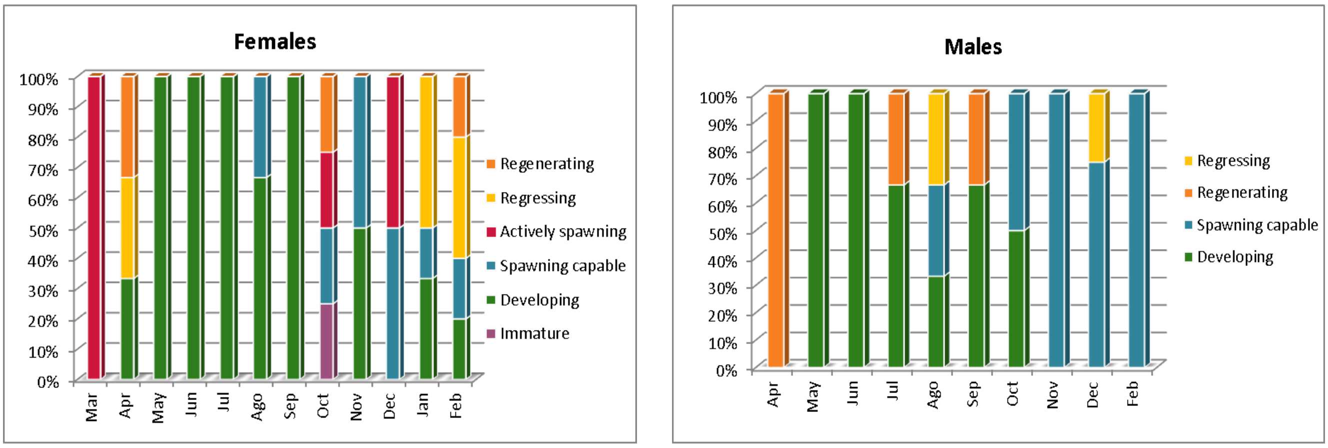

Figure 2: Gonadal maturation phases.

Monthly variation of the gonadal maturation phases of males and females of *Gymnotus* sp. n. histologically analyzed.

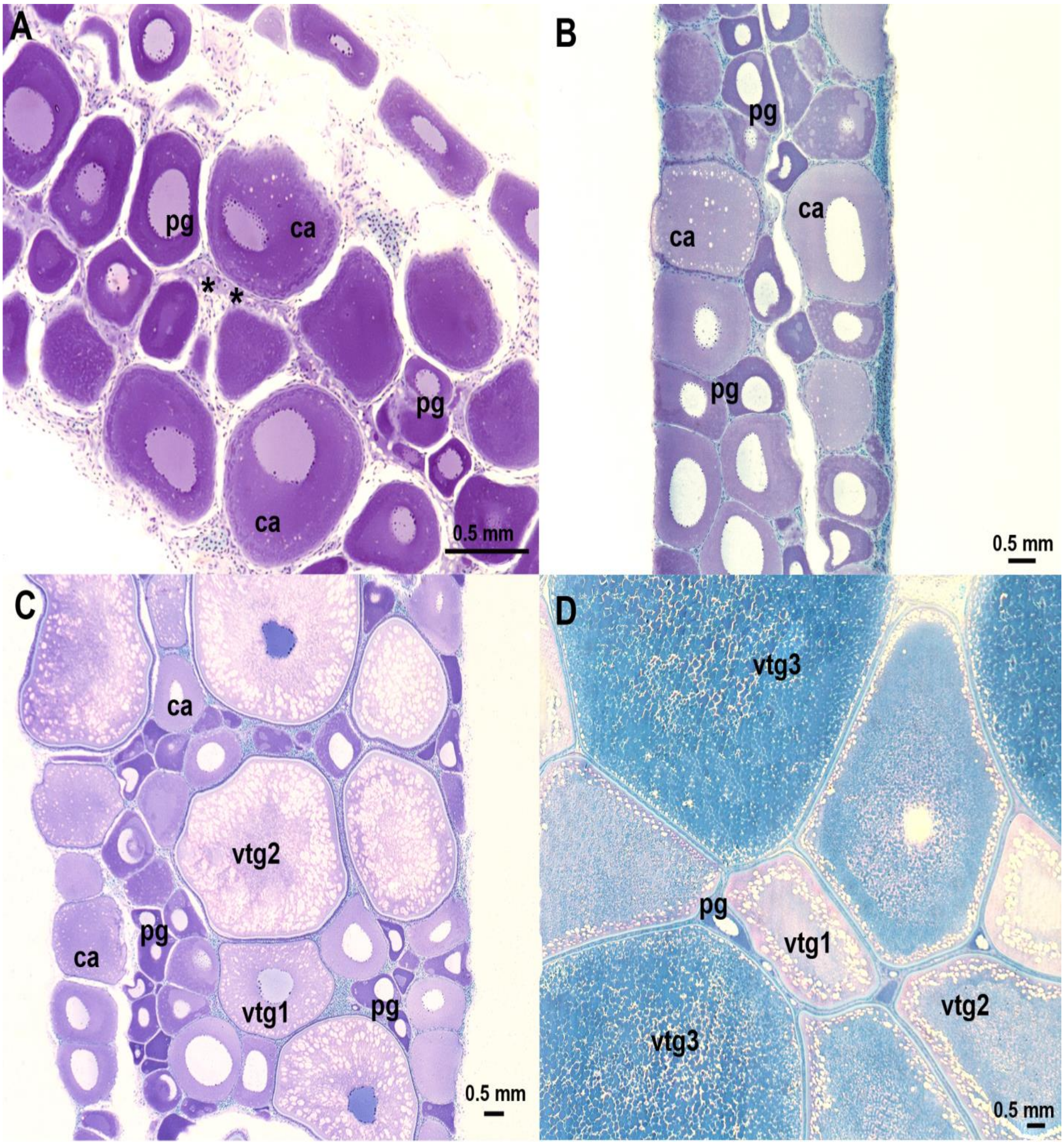

Figure3: Histology of ovaries.

Histological sections of *Gymnotus* sp. n. ovaries in different gonadal maturation phases. A: Immature; B: Early developing; C: Mid developing; D: Late developing. *: oogonias; pg: primary growth oocytes; ca: cortical alveolar oocytes; vtg1: primary vitellogenic oocytes; vtg2: secundary vitellogenic oocytes; vtg3: tertiray vitellogenic oocytes.

The spawning capable phase was observed from August to February (Fig4-A); whereas actively spawning females were collected in October, November and March (Fig.4-B). These two phases differed from each other due the presence of postovulatory follicle complex in the actively spawning phase indicating oocytes released. Regressing individuals were sampled in April, January and February, when the histological analysis demonstrated unorganized gonads with abundant blood vessels, postovulatory follicle complexes and enlarged ovarian lamelae (Fig.4-C). The regenerating phase observed in April, October and February was characterized by the presence of blood vessels, oogonias and primary growth oocytes (Fig.4-D), indicating a reorganization in the structure for a new beginning of the gonad maturation process.

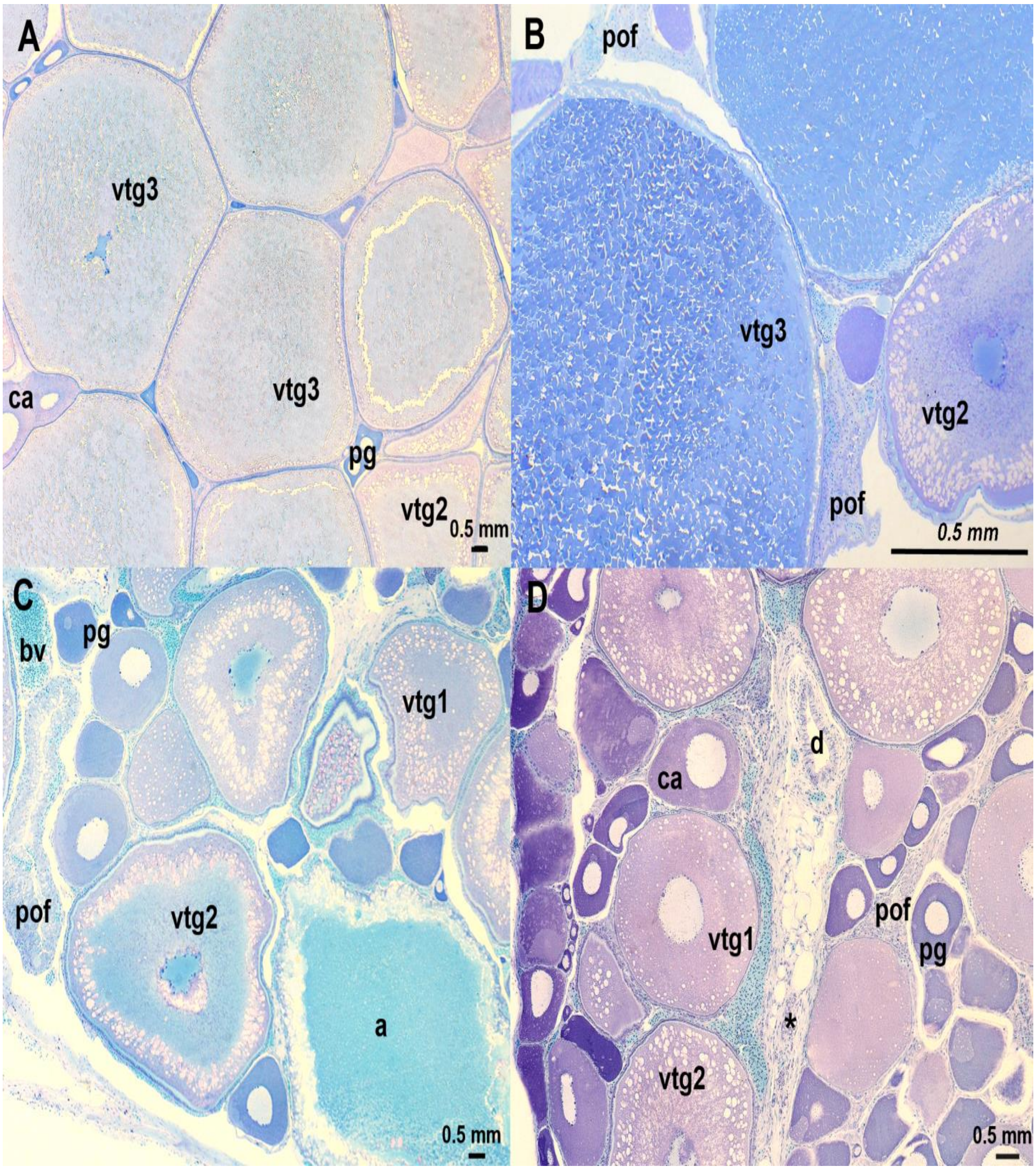

Figure 4. Histology of ovaries.

Histological sections of *Gymnotus* sp. n. ovaries in different gonadal maturation phases. A: Spawning capable; B: Actively spawning; C: Regressing; D: Regenerating. *: oogonias; pg: primary growth oocytes; ca: cortical alveolar oocytes; vtg1: primary vitellogenic oocytes; vtg2: secundary vitellogenic oocytes; vtg3: tertiray vitellogenic oocytes; d: ovarian duct; bv: blood vessels; a: atresia; pof: postovulatory follicle complexes.

The histological analysis of male gonads showed the following phases of gonadal maturation: developing, spawning capable, regressing and regenerating (Fig.3). Males in the developing phase were captured throughout most of the sampling period, except by November and February. Histologically, these gonads showed active spermatogenesis and presence of great amount of primary and secondary spermatocytes, crescent number of spermatids and spermatozoa and scarse sprematogonia (Fig.6-A,B). Spawning capable individuals were sampled in August and from November to February showing high amount of spermatozoa in lumen of sperm ducts, the other spermatogenic cells being poorly scatter in de gonade (Fig.6-C). Regressing individuals occurred in August and December, showing depleted stores of spermatozoa in sperm ducts and the lumen of the lobules, cessation of spermatogenesis, and a increasing number of spermatogonias. (Fig.6-D). Fishes remain in the regressing phase for a relatively short time, moving to the regenerating phase which was observed in April, July and September for *Gymnotus* sp. n. This phase is characterized by the massive presence of spermatogonia, and residual spermatozoa in sperm ducts (Fig.6-E). Fish in this phase are sexually mature but reproductively inactive. There are no results for males in March/2011 and January/2012, since only females were sampled in these months.

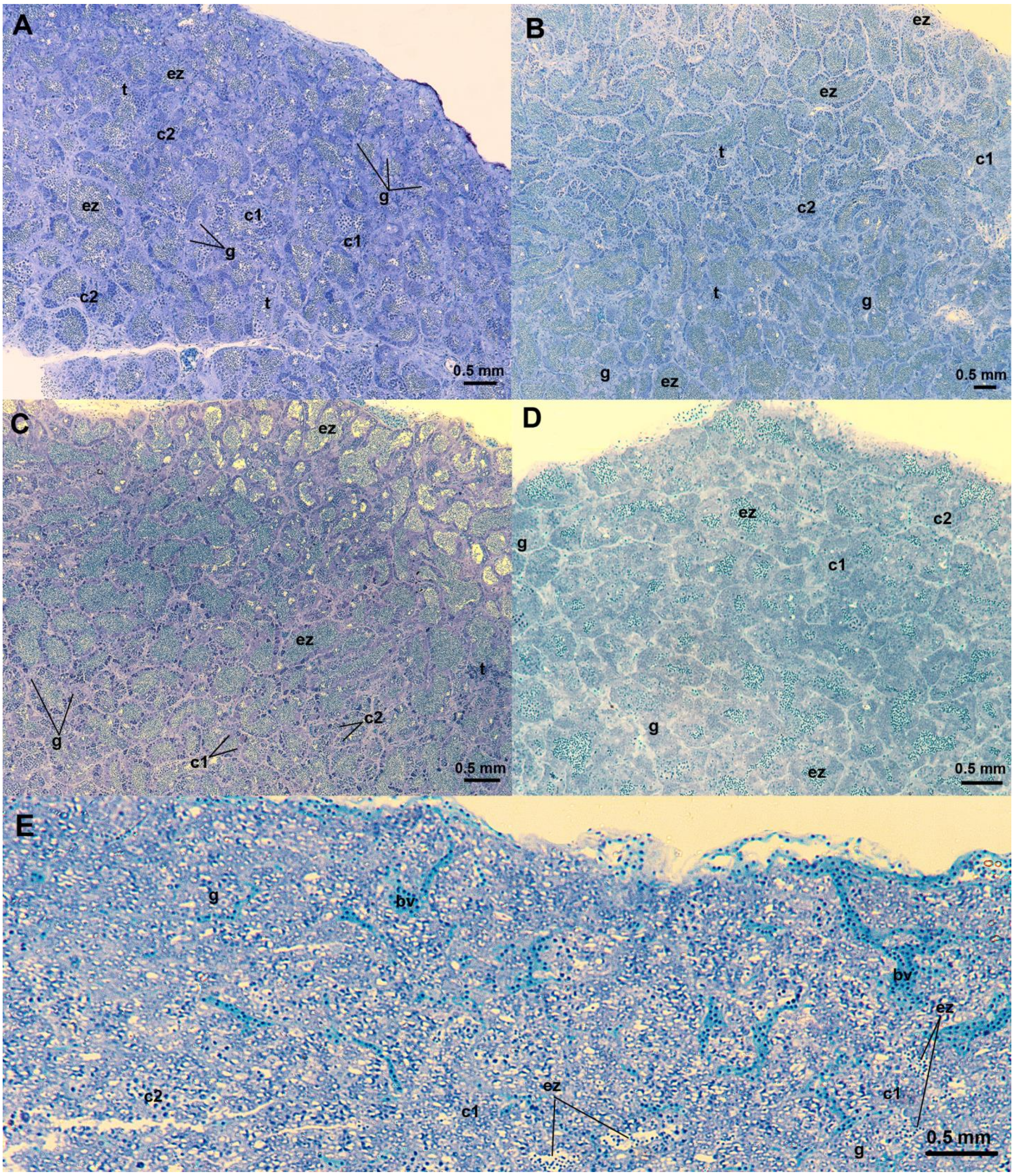

Figure. 5. Histology of testes.

Histological sections of *Gymnotus* sp. n. testes in different gonadal maturation phases. A: Mid developing; B: Late developing; C: Sapawning Capable; D Regressing; E: Regenerating. g: spermatogonia; c1: primary spermatocytes; c2: secondary spermatocytes; t: spermatids; ez: spermatozoas. bv: blood vessels.

The absolute fecundity of *Gymnotus* sp. n. had an average value of 419 oocytes ranging from 0.15mm - 2.85mm of diameter (Table 2) for females with total length varying from 121.3mm to 196.8mm, while the average relative fecundity was estimated as 0.05 oocytes per mg total weight. Analyzes of absolute frequency distribution of oocytes diameters show that the specie has oocyte development synchronic in more than two groups, iteroparity, and fractional spawning (Fig. 6).

Table 2. Fecundity data.

Total length (Lt), total weight (Wt), gonadosomatic index (GSI), absolute fecundity (Fa), and relative fecundity (Fr) of 7 females of *Gymnotus* sp. n.

	Lt (mm)	Wt (g)	IGS	Fa	Fr
	121.32	4.64	11.1	286	0.06
	135.33	5.56	12.4	330	0.05
	148.91	8.06	11.4	409	0.05
	164.84	9.87	8.2	452	0.04
	165.74	8.16	9	238	0.02
	170.36	11.06	7.7	518	0.04
	196.81	9.13	10.9	698	0.07
Mean	157.62	8.07	10.1	419	0.05

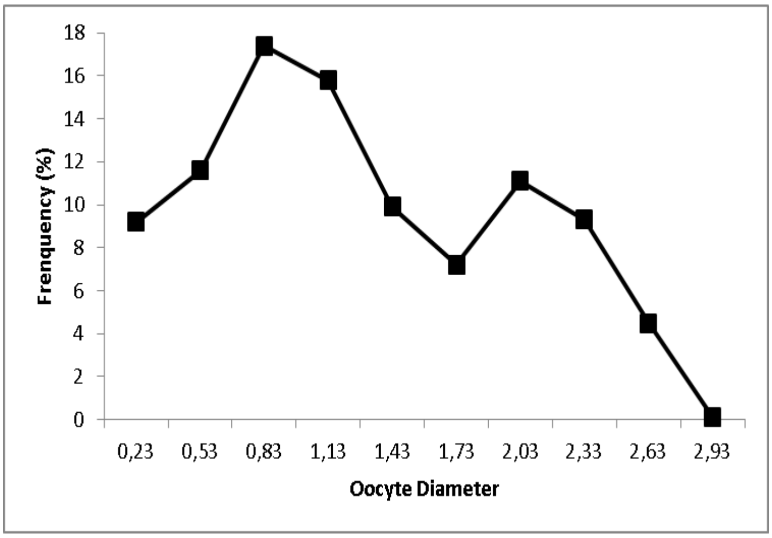

Figure 6. Spawning type.

Distribution of relative frequency of 150 oocyte diameters of the seven females of *Gymnotus* sp. n. with the highest registered GSI values.

The chi-square test ($p<0.05$) did not show significant differences in total length related to sexual dimorphism for *Gymnotus* sp. n.; however, males reached the largest length class established (Fig. 7). According to the same test ($p<0.05$), the sex ratio established for *Gymnotus* sp. n. was 1:1 in each sampled month, and also in the total sample.

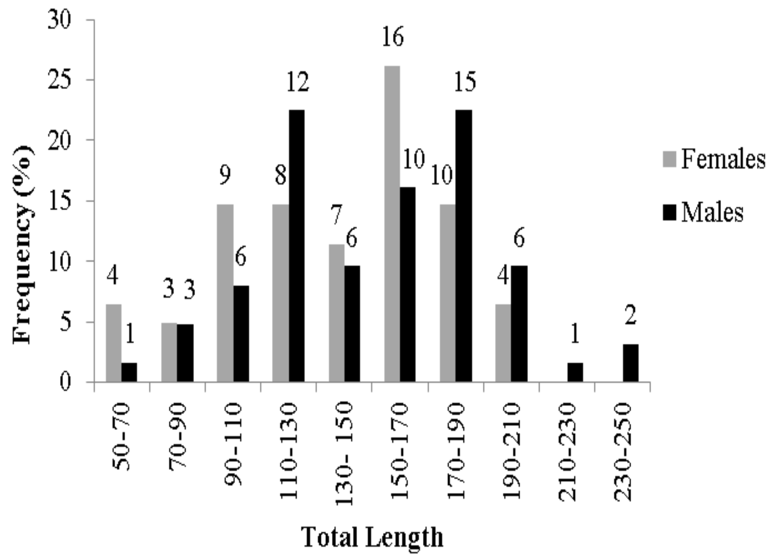

Figure 7. Total length classes.

Distribution of relative frequency (%) of *Gymnotus* sp. n. males (n= 61) and females (n= 62) for total length classes (mm). Numbers above the column represent the number of specimens in each length class.

Feeding

A total of 22 food items were identified in the 120 stomach analysed. Three empty stomachs were registered, one stomach in April and two in September these being excluded from the analysis. The most frequent items observed were larvae of diptera, odonata and trichoptera, and digested organic material (Table 3).

Table 3. Frequency of occurrence.

Frequency of occurrence of alimentary items for *Gymnotus* sp. n. (*= allochthonous origin).

Alimentary Items	Mar	Apr	Mai	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Crustaceae	0	0	10	11	20	10	22.5	18.7	14.3	0	0	18.2
Amphipoda	0	0	0	11	20	10	11	18.7	0	0	0	18.2
Cladocera	0	0	0	0	0	0	0	0	14.3	0	0	0
Copepoda	0	0	10	0	0	0	0	0	0	0	0	0
Digested organic material	100	33.3	30	11	0	90	75	75	100	60	20	91
Fish	0	0	0	0	0	0	0	0	0	10	0	0
Insecta	100	100	100	100	100	100	100	93.7	100	100	100	81.8
Acari	0	0	0	0	0	0	12.5	0	14.3	10	0	27.3
Coleopetra (adult)*	0	0	20	0	0	0	0	0	0	10	0	0
Coleoptera (larvae)	0	0	10	0	0	0	37.5	0	0	0	70	0
Diptera (larvae)	100	88.8	100	100	100	100	87.5	93.7	100	100	100	63.6
Diptera (nymph)	0	0	20	11	20	50	0	12.5	7	0	20	0
Ephemeroptera	0	44.5	10	0	0	0	25	6.25	35.7	20	0	27.3
Hemiptera	0	0	0	0	0	0	12.5	0	0	0	10	0
Hymenoptera*	100	0	0	0	0	0	0	0	7	0	0	9
Odonata	100	66.6	70	88.8	60	70	37.5	50	57	30	10	18.2
Plecoptera	0	11	0	11	0	0	0	0	0	0	0	0
Trichoptera	100	33.3	80	66.6	60	40	25	12.5	7	30	70	45.5
Non-identified-allochthonous insect*	0	0	0	0	0	0	25	0	0	0	20	0
Non-identified-autochthonous insect	0	0	0	0	0	0	0	0	0	10	57	18.2
Other	0	0	10	20	0	0	12.5	6.25	0	0	10	0
Fish egg	0	0	0	0	0	0	12.5	6.25	0	0	10	0
Insect egg	0	0	10	20	0	0	0	0	0	0	0	0
Plant material	0	55.5	20	33.3	30	60	62.5	56.2	43	70	80	9
Sediment	0	11	0	0	0	0	0	0	0	20	0	0

In terms of percent composition, the category autochthonous insects reached the highest values (100%), followed by digested organic material and plant material (91.6% each), allochthonous insects, crustacea and the others category (66.6% each), whereas the categories sediment and fish reached the lowest values (16.6% and 8.3% respectively) (Fig.8)

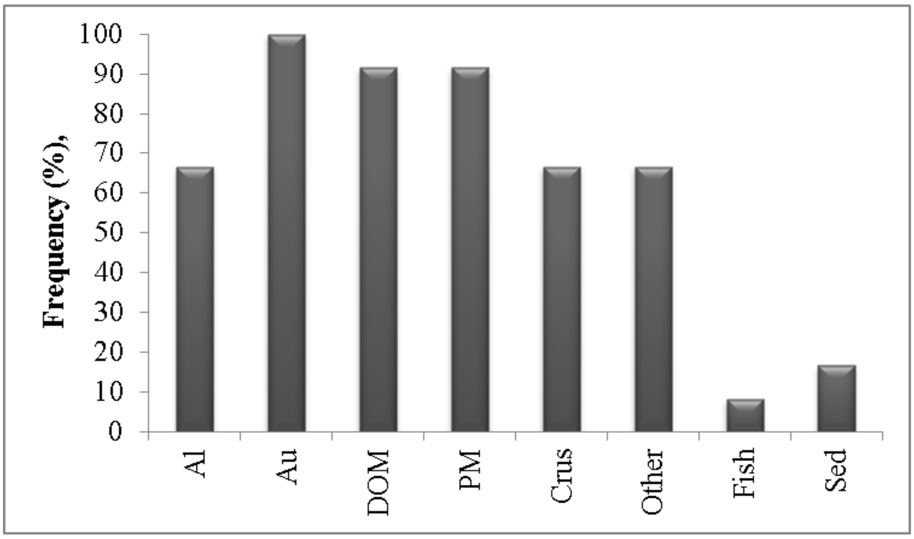

Figure 8. Percent composition.

Percent composition of the alimentary categories established for *Gymnotus* sp. n. Al: allochthonous insects; Au: autochthonous insects; DOM: digested organic material; PM: plant material; Crus: crustacea; Other: other items; Fish: fish consumed; Sed: sediment.

The distribution of mean values of RI (Fig.9) show significant differences throughout the sampling period only for males ($F=10.35$; $p <0.05$), December differing of all the other months except of May, and February differing of August and September. There was no significant difference between the mean RI values of males and females and the abiotic factors analysed.

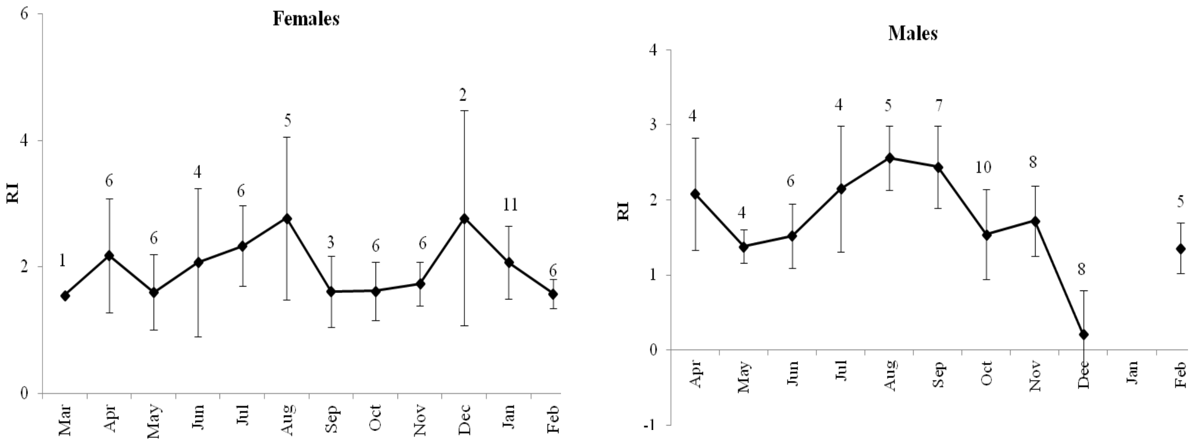

Figure 9. Repletion index.

Monthly variation of mean repletion index (RI) for *Gymnotus* sp. n. males and females from March/2011 to February/2012 (females), and April/2011 to February/2012 (males).Vertical bars represent the standard deviation. Numbers above the bars correspond to the numbers of specimens included in the analysis.

The PERMANOVA results indicate a seasonal pattern in the species diet (F=16.5 p= 0.001), as demonstrates through the PCoA (Fig. 10). The food categories were not associated with either ontogeny (F= 0.72 p = 0.49) and sex (F= 2.05 p= 0.12) The IndVal values show feeding specificity, associating predation on autochthonous insects during winter (Stat: 0.55 p= 0.038), allochthonous insects during autumn (Stat: 0.39 p= 0.025) and ingestion of digested organic material during spring (Stat: 0.57 p= 0.001). Summer was not associated with any alimentary category in particular. In addition, the terms A and B (IndVal) indicate the occurrence of autochthonous insects in the stomachs of all specimens sampled on winter, although the category is not exclusive of the season (A: 0.3; B: 1). The same is shown for digested organic material found in almost all stomachs in the spring (A:0.36; B: 0.9). Allochthonous insects were consumed mainly in autumn, not occurring in all stomachs analysed in the season (A:0.76 B:0.2). The intestinal quotient for *Gymnotus* sp. n. was estimated as 0.29, with a standard deviation of 0.05.

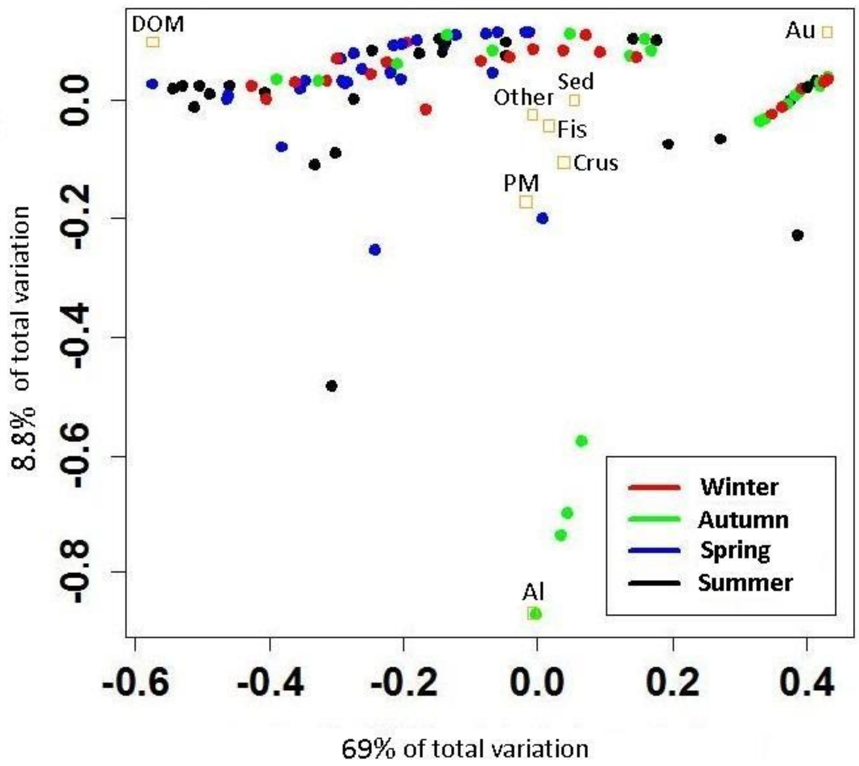

Figure 10. **Seasonal distribution of food categories.**

Seasonal distribution of the alimentary categories identified for *Gymnotus* sp. n. according to the principal coordinates analysis (PCoA). Al: allochthones insects; Au: autochthones insects; Crus: crustacea; DOM: digested organic material; Fis: fish; Other: other items; PM: plant material; Sed: sediment.

Discussion

Understanding the patterns that shape the life history of a species is important not only for the comprehension of its behaviour and ecosystem interactions but also for the conservation of these organisms and their habitats as a whole. Similarly to other species of gymnotiforms inhabiting the southern South America [24-27], *Gymnotus* sp. n. presented a long reproductive period lasting from August to March. Furthermore, histological analysis shown females in the actively spawning and spawning capable phases of the gonadal maturation cycle from October to March, while males had spawning capable gonads from August to February, matching the GSI peaks observed in October for females and August and October for males. In addition, the higher means of males GSI in August suggests that males were ready for reproduction before the females. Although no correlation was observed between the species GSI and the abiotic factors, the reproductive period matched the months with the highest photoperiod and water temperature registered [16]. Similar results were described for other gymnotiforms inhabiting Southern Brazil and Uruguay, which shown reproduction positively associated with both temperature and photoperiod [27-31]; therefore, *Gymnotus* sp. n. fits the pattern proposed for all gymnotiforms from high latitudes [27].

Microscopical analysis demonstrated a fast process of oocyte development in females, in which the store oocytes (primary growth oocytes) and vitellogenic oocytes in its many developing stages were continuously observed through the maturation phases. Even though the reproductive period was well defined for males, the histology of the testes demonstrated a fast and continuous spermatogenesis process throughout the sampling period, impeding the differentiation of the actively spawning subphase integrating the spawning capable phase [19]. This fast and continuous spermatogenesis can be proven by the presence of spermatozoa in almost all phases of the maturation cycle. The smallest male (63mm) and female (59mm) collected were both in different phases of gonadal development, being the female immature and the male in the mid-developing phase. This observation indicate that the process of gonadal maturation for *Gymnotus* sp. n starts when individuals reach lengths shorter than 60mm, pointing to a fast development of the juveniles. Although the samples were performed with specific equipment for the capture of electric fishes, larvae agglomeration and juveniles were not either observed or sampled in this study. Therefore, it is possible to presume that the species uses the flooded interior of the riparian forest, inaccessible for samples, as a nursery for breeding and early development of the juveniles.

According to the obtained data, *Gymnotus* sp. n. present very low relative fecundity estimated as 0.05 oocytes/mg of body weight. Available information concerning relative fecundity of other gymnotiform genus points out the species low fecundity value, *Brachyhypopomus gauderio* reaching 0.2 [27], *Brachyhypopomus draco* 0.17 [26], *Brachyhypopomus bombilla* 0.21 [31], and *Eigenmannia virescens* 0.27 [7], as well as other *Gymnotus* species such as *Gymnotus* aff. c*arapo* reaching 0.20 oocytes/mg body weight [24]. Conforming to Vazzoler [20], fecundity depends on the availability of female body cavity and on the size of oocytes produced. In line with it, the maximum diameter of a vitellogenic oocyte of *Gymnotus* sp. n. was estimated as 2.85mm, while Cognato & Fialho [24] estimated for *Gymnotus* aff. c*arapo* a maximum diameter of vitellogenic oocytes of 1.7mm. Parental care is a behaviour that improves the offspring fitness, and is often related to low fecundity values. This behaviour is largely described among Gymnotiformes, for which larvae guarding and nest building by males were already described for different species of the order [1,20,24,25, 32-34]. Although evidence of parental care (i.e detection of larval agglomeration protected by an adult, and/or nests) was not record in field for *Gymnotus* sp. n., the species low fecundity registered is consonant with this hypothesis. Moreover, males demonstrated significant variation in their monthly RI values, where the lowest mean value of December indicate a decrease in the feeding activity after the peaks of reproductive period, reinforcing the hypothesis that *Gymnotus* sp. n. might also present parental care, in which males would reduce their feeding activity in order to invest in offspring protection.

The species spawning was defined according to the distribution of oocytes diameter and corroborated by histological analysis. As a result, the analysis demonstrated the existence of an actively spawning subphase in females which are spawning capable, classifying the species as fractional spawner. Moreover, the distribution of vitellogenic oocytes with different diameters (i.e. maturation classes) indicates that oocytes are released in different moments until the end of reproductive period. The same spawning strategy is described for all gymnotiformes studied at the present time [1, 24 - 27, 31, 33- 35]. Although fractional spawning can require a higher reproductive effort than a single spawning [36], species with this strategy tend to be better adapted to unfavourable environmental conditions and may solve competition issues for spawning sites between the females of the same population [37]. The sex ratio 1:1 defined for *Gymnotus* sp. n. is also commonly observed in natural population of fishes and may diverge when one of the sexes has particular advantage [38]. According to Vazzoler [20], since females have higher growth taxa than males, females tend to be more predominant in a population when analyses are made by total length classes. On the other hand, when comparing the body length of *Gymnotus* sp. n. males and females, the highest length classes were reached by males. Similarly, species of different Gymnotiformes genus, such as *Egeinmannia* [7, 25] and *Brachyhypopomus*, [27, 31] had the same pattern of sex ratio and total length distribution described for both males and females.

The feeding ecology of a species is directly linked to its population dynamics as well as to a set of factors such as foraging behaviour, competition, and habitat preference that provide valuable information for conservation of species and ecosystems [39]. Even though the direct influence of abiotic factors on the species diet was not significant, the seasonal variations observed on the diet of *Gymnotus* sp. n. might be a result of environmental changes defining each season. The high incidence of autochthones insects consumed during winter follows the increasing RI of males and females from May to August, period in which the rainfall is more intense in the state [40] increasing the availability of resources and facilitating their catch by fishes as the water level rises. In addition, the predominance of aquatic insects, mainly Diptera larvae, on the species diet is probably related to the great abundance of these organisms in streams [41].

The continuous distribution of riparian forests surrounding the streams contributes indirectly with the allochthonous items in fishes diet, serving as shelter as well as food resource for the invertebrates preyed [42]. The floating vegetation in the sampling site not only shelters the fishes inhabiting the stream, but also provides a variety of allochthonous insects associated with that. Thus it is possible to suppose that the plant material consumed is not an essential component of the species diet, but an abundant item associated with the capture of insects.

Gymnotiform fishes are characterized by a crepuscular behaviour, hunting actively at night [3]. Once all sampling occurred at the daylight, part of the items was already digested at the moment the specimens were fixed, compromising the identification of food items and explaining the high frequency of digested organic material observed. Even though previous studies [2] claim that species of the genus *Gymnotus* can prey on small fishes, there are no direct evidence of this behaviour in studies regarding the feeding biology of these species [43– 47]. For *Gymnotus* sp.

n., the only sign of piscivory observed is related to a single specimen among all the individuals sampled, suggesting an opportunistic predation on this alimentary item.

According to the results obtained concerning the diet composition of *Gymnotus* sp. n., the species can be classified as invertivorous with tendency to insectivory. Moreover, the low intestinal quotient estimated for *Gymnotus* sp. n. corresponds to the values of intestine length estimated for species consuming animal origin alimentary resources as the scale proposed by Fryer & Iles [48] to compare trophic category and intestine length. In comparison to other studies regarding the feeding habits of gymnotiform species [27, 31, 43, 45 – 47] it is possible to conclude that *Gymnotus* sp. n. conform the order pattern of diet as it was proposed by Giora et al. [27].

Anthropic activities, such as pollution, habitat degradation and the expansion of urban areas have contributed to the depletion of fish populations worldwide [49-52], increasing the need to discover our biodiversity in order to preserve it [53]. Therefore, the results obtained herein suggest that the *Gymnotus* sp. n is a territorial species, with very low fecundity, and presenting habitat specificity, which possibly explain its status of an Endangered [EN] species in Rio Grande do Sul state. Although gymnotiform species present diverse characteristics to cope with environmental conditions, it is possible to infer that *Gymnotus* sp. n. is extremely dependent on the condition of its habitat. The riparian forest surrounding the species habitat influence directly on the species ecology, contributing with the food items consumed and may serve, in its flooded interior, as a nursery and shelter for the species during dry periods, explaining the absence juveniles sampled and larvae agglomeration in field.

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