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Reproductive aspects of the one-sided livebearer *Jenynsia multidentata* (Jenyns, 1842) (Cyprinodontiformes) in the Patos Lagoon estuary, Brazil

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Abstract. *Jenynsia multidentata* (Jenyns, 1842) is a viviparous fish found year-round in the estuary zone of Patos Lagoon and represents one of the dominant species in its shallow areas. Although biological and ecological aspects of the J. multidentata's population occurring in Patos Lagoon estuary have been previously investigated, there is no published information regarding its reproductive biology in this estuarine area. The reproductive cycle of this species in the estuarine zone of Patos Lagoon occurred from September to May, showing a positive correlation with water temperature (r = 0.76). The sexual ratio in the estuary was 1.98/1 (female/male) and female size and fecundity had a positive correlation. It was observed that *J. multidentata* presented a second pregnancy during January and February months, which has not been described in previous studies.

Key words: Anablepidae, reproduction, fish, viviparous, estuaries.

Resumo. Aspectos reprodutivos do barrigudinho *Jenynsia multidentata* (Jenyns, 1842) (Cyprinodontiformes) no estuário da Laguna dos Patos, Rio Grande do Sul, Brasil. *Jenynsia multidentata* (Jenyns, 1842) é um peixe vivíparo lecitrotófico residente do estuário da Lagoa dos Patos e dominante nas zonas rasas. Estudos sobre a reprodução desta espécie já foram realizados em ambientes límnicos, e nesse estudo se pretendeu caracterizar sua reprodução em águas mixohalinas. Sua proporção sexual no estuário foi 1,98: 1 (fêmea/macho) e constatou-se que quanto maior o comprimento da fêmea, maior será sua fecundidade. Neste ambiente o ciclo reprodutivo da espécie estende-se de setembro a maio, havendo correlação positiva com a temperatura da água (r = 0,76). Observou-se que *J. multidentata* apresenta segunda gestação nos meses de janeiro e fevereiro, fato este ainda não descrito para a espécie.

Palavras-chave: Anablepidae, reprodução, peixe, vivíparo, estuários.

Introduction

Due their reproductive aspects and characteristic mating, small viviparous fishes from Cyprinodontiformes Order, specially from Poecilidae family, have greatly contributed for the understanding of many biological and ecological aspects of ichthyology in general, particularly related with the evolution of viviparity, internal fertilization, growth, maturation, reproductive behavior (court) and many other aspects related to populational dynamics of fishes (Farr 1977, Reznick 1983, Constantz 1984, Meyer & Lydeard 1993, Endler 1995).

Despite of that, there is very few published information related to Anablepidae family (Ghedotti 1998), which is closely related to poecilids (Nelson 1984). This family is comprised of only 13 species, being the one-sided livebearer *Jenynsia multidentata* the most widespread within the group; occurring from Argentina (Rio Negro) up to Brazil (Rio de Janeiro) (Ghedotti & Weitzman 1996, Ghedotti 1998).

In the shallow estuarine zones of Patos Lagoon, this small fish (< 9 cm of total length) represents an important component of the fish assemblage (Chao *et al.* 1985). Several features such as its high abundance and occurrence year-round in the estuary, its great resistance to salinity and temperature variations, and the fact that is easy to catch and to maintain in aquarium, make this species a suitable model for laboratorial studies (Mai *et al.* 2005).

Although studies about reproduction and life history strategies of *J. multidentata* in the Patos Lagoon estuary have been already carried out (Betito 1984), there is little published articles concerning its biology in this system (Garcia *et al*. 2004, Mai *et al.* 2005). For instance, Garcia *et al*. (2004), based exclusively on recruitment patterns (abundance per size class plots), suggested that *J. multidentata* has an annual reproductive cycle in Patos Lagoon estuary that is composed by two cohorts: one comprised of individuals born from December to March that started reproducing during late winter and spring, and a second cohort comprised of individuals born from September to November that started reproducing during late summer and fall. However, there is no current published information regarding the reproductive biology of *J. multidentata* in this estuary that has been based on direct evidences obtained by dissection and analysis of the individuals. Moreover, there is still certain debate about which factors drive the markedly sexual dimorphism in size (females being larger than males), the larger number of females in relation to the males and the amplitude of reproductive season for this species (Bisazza *et al.* 2000, Garcia *et al.* 2004).

Aiming to contribute to the current knowledge about the biology of *J. multidentata* in Patos Lagoon estuary, the present work examine some reproductive aspects of this species, such as the reproductive season, period of gonad maturation and sexual ratio and their correlations with abiotic factors (salinity, temperature e water transparency).

Materials and methods

Sampling was conducted in the estuarine zone of Patos Lagoon, Rio Grande, RS, Brazil. The specimens analyzed where caught in three location within the shallow bays (< 2m) of the estuary: Saco da Mangueira (32°05'S; 52°07'W), Marambaia (31°59'S; 52°05'W) and Prainha (32º 09'S; 52º 06'W) (Fig. 1). The sampling was part of a larger long-term ecological project called 'Pesquisas Ecológicas de Longa Duração (PELD)', which have been carrying out monthly fish sampling in several locations within the estuary since 1999.

From March 2000 to February 2001, the specimens were collected each month using a beach seine net (locally known as 'picaré') with the following dimensions: 9 × 1.5m net with 13mm bar mesh in the wings and 5mm in the center 3-m section. The samples were preserved in formalin 10% after caught and then were, kept in alcohol 70%, then stored at the FURG Fish Collection (http://www.ictio.furg.br/).

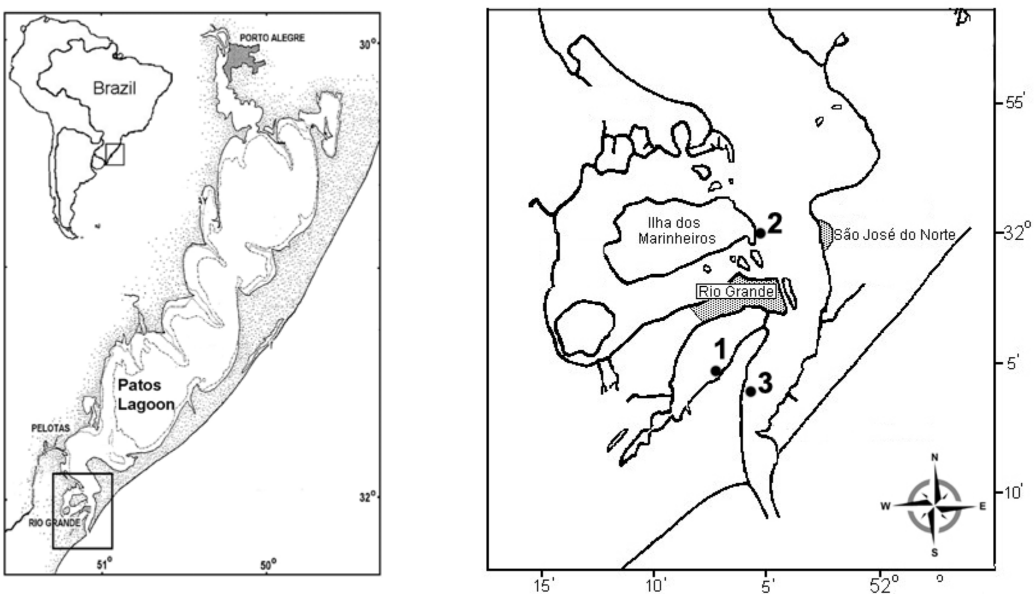

Figure 1. Map of Patos Lagoon estuary and Rio Grande City (32°S). Black dots denotes the sampling locations: (1) Saco da Mangueira; (2) Marambaia; and (3) Prainha.

A total of 247 females were dissected in laboratory, and for each individual we recorded: total length (TL, mm), total weight (WT, g), weight of the full and empty gonad (g) and reproductive stage (rest, with eggs or embryos), number and weight (g) of eggs and embryos. These measurements were obtained using a microscopy stereoscopic, a caliper rule (precision on decimals of millimeters) and a precision balance Kern 410 (precision of 10^{-3} grams).

The maturation stages of gonads were distinguished as: rest, with eggs and with embryos. The reproductive season was estimated based on analyses of monthly frequencies of gonadal maturation stages (Santos 1978), and also by variation in the average values of the gonadossomatic relation (GSR) (Vazzoler 1996). The GSR was obtained for each individual using the total gonad weight/WT*100 and for each month we calculated the value's average and its respective deviation. This relation was used to balance the subjectivity of data over the stages of maturity (Vazzoler 1996). The *Spearman* test (Hamilton *et al.* 1977) was performed to verify possible correlations between GSR and abiotic factors (salinity, temperature and water transparence) collected simultaneously with fishes sampling.

The monthly variation in abundance and sexual ratio (number of females/males) was calculated based on the total of number specimens collected in the sampling locations (n= 1330). The Chi-Square test (χ^2) was performed in order to verify the significant differences in the sex ratio. At last, we analyzed the distribution of female with embryos, broke down by length classes of 5mm. The fecundity was calculated based on distribution of average number of eggs and embryos per month and also by the number of embryos per female divided in 5 mm size classes (SC).

Results

The periods of highest abundance of *J. multidentata* in the Patos Lagoon estuary occurred between March-April and November-December, when, in most cases, females predominate in relation to males. The sex ratio showed monthly variations with peaks in January (♀:♂: 13.5:1, n= 86) and June (♀:♂: 11:1, n= 12) and had an average of 2.02:1 during the sampling period (Tab. I). The Chi-Square test showed a significant difference in this ratio (♀:♂: χ^2 = 1171.42; d.f. = 48; p < 0.01).

The average number of eggs and embryos changed along the year. September and January had the largest number of eggs per female (Fig. 2). In winter (June, July and August) all captured specimens were in sexual rest stage, but in September 42.9% of specimens presented eggs, and in the following month, 63.5% of females were found with embryos. The females with embryos were dominant along spring and summer (October until March), showing a decline in fall (April and May) (Fig. 3a). A temporal variation of gonadossomatic relation (GSR) showed an increase tendency starting in September and, although variable, it seemed to extent until February and March (Fig. 3b). The species reproductive cycle presented a larger correlation with water temperature (r = 0.76) than to water transparency (r= 0.52) and salinity (r= 0.04) (Fig. 3c).

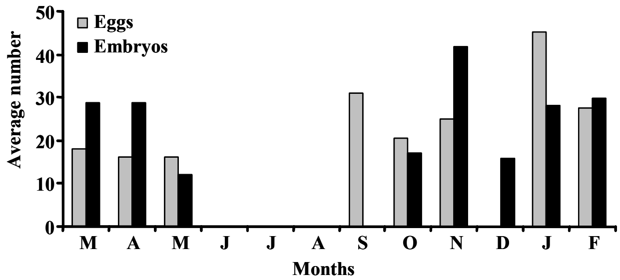

Figure 2. Average number of eggs and embryos per month for females of *Jenynsia multidentata* colleted in the estuary of Patos Lagoon, between March 2000 and February 2001.

Based on the monthly average distribution of number of eggs and embryos, frequencies (in percentage) of gonad maturation stages and average values of the gonadossomatic index, we may ascertain that this species has a reproductive season between September and May, which is coincident with local higher water temperatures (> 15°C) (Fig. 2 e 3).

The fecundity, estimated from the number of embryos, was directly proportional to the length of females (Fig. 4). The fecundity average was estimated in 30 embryos per female, and the female that presented the lowest number of embryos (n=9), measured 51 mm in Total Length (TL). The most fecund female (74 eggs, TL = 72 mm) was collected in January 2001 at Saco da Mangueira, and the smallest mature female observed measured 43 mm TL.

Pregnant females were recorded in January and February, with growing gonads and the abdominal region extended, presenting an empty space, which characterize a second pregnancy in the reproductive period.

The largest and smallest reproductive females (SC = 72.5 e 77.5 mm e SC = 42.5 mm, respectively) were observed in reproduction in January (Fig. 5). This suggests that females born in

October-November achieved maturation fast, probably due the abundant food in spring, and they reproduce altogether with their mothers (in 2nd pregnancy at this moment) in January-February. The females born in January-February have taken nine months to achieve the maturity and they have shown the first pregnancy in October-November, only in the next reproductive period and, consequently, their 2nd pregnancy will occur just in the following year.

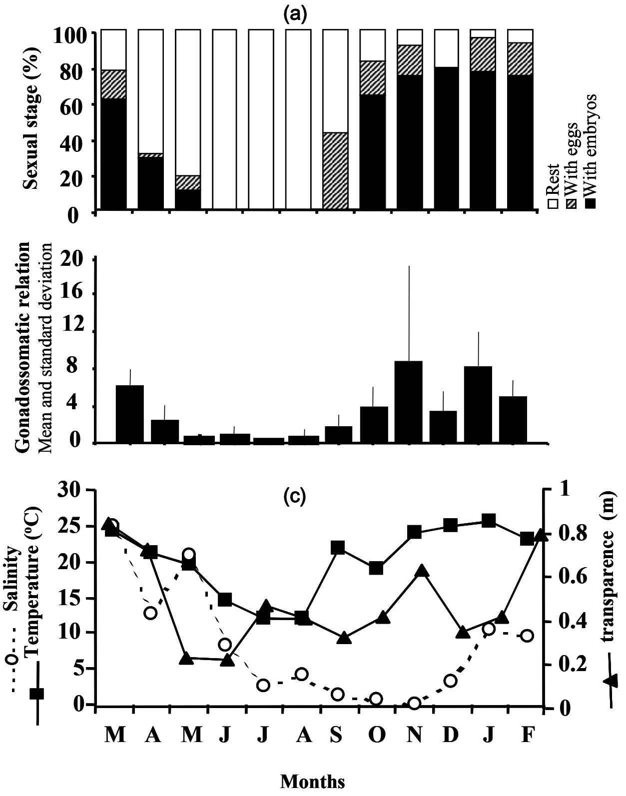

Figure 3. (a) Monthly variation of frequency of occurrence (in percentage) in each stage of gonad maturation of *Jenynsia multidentata* colleted in the estuary of Patos Lagoon, between March 2000 and February 2001; (b) Monthly variation of average values of gonadossomatic index for females of *J. multidentata* in the estuary of Patos Lagoon; (c) Variation of abiotic variables in the sampled period.

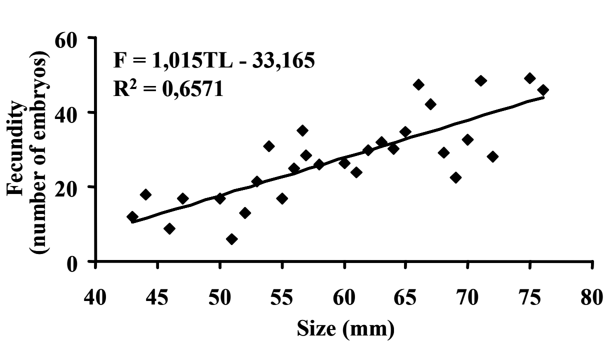

Figure 4. Relationship between the number of embryos and the total length (TL) *Jenynsia multidentata*'s females colleted in the estuary of Patos Lagoon, between March 2000 and February 2001.

Table I. Number of females' specimens, males and sexual ratio of *Jenynsia multidentata* collected at Patos Lagoon estuary between March 2000 and February 2001.

Period	**♀ (n)**	**♂ (n)**	**♀:♂**
January	81	6	13.5
February	30	10	3
March	205	82	2.5
April	235	156	1.51
May	47	45	1.04
June	11	1	11
July	---	---	---
August	2	---	---
September	28	38	0.74
October	4	1	4
November	105	76	1.38
December	141	26	5.42
Total	**889**	**441**	**2.02**

Discussion

Reproductive season and relationship with abiotic factors

Fontoura *et al.* (1994) and Garcia *et al.* (2004), for a freshwater lake and for the Patos Lagoon estuary, respectively, suggested this species has two reproductive peaks, in which females born in December to March comprises the first cohort, reproducing at the end of winter and beginning of spring (September-November). The second cohort (September-November) will reproduce for the first time in summer and fall (December-May). Our results indicate a reproductive period extending from September to May, which encompass both reproductive peaks reported by the above mentioned studies. The low GSR value we observed in December did not fit this hypothesis but it could be explained by the absence of females with eggs and to the low fecundity (average of 16 embryos, n= 5) showed in this month.

Temperature seemed to be the factor that better explained the GSR variations along the year, which have been previously noted by Garcia *et al.* (2004). In contrast, there were no significant correlations among water transparency and salinity with reproductive aspects. The fact that temperature was the only abiotic factor measured showing significant correlation with GSR is not surprising, considering that temperature variation is much more predictable in this region when compared with water transparency and salinity. These latter factors have a more stochastic pattern along the year due to their relationships with wind patterns (Costa *et al.* 1988).

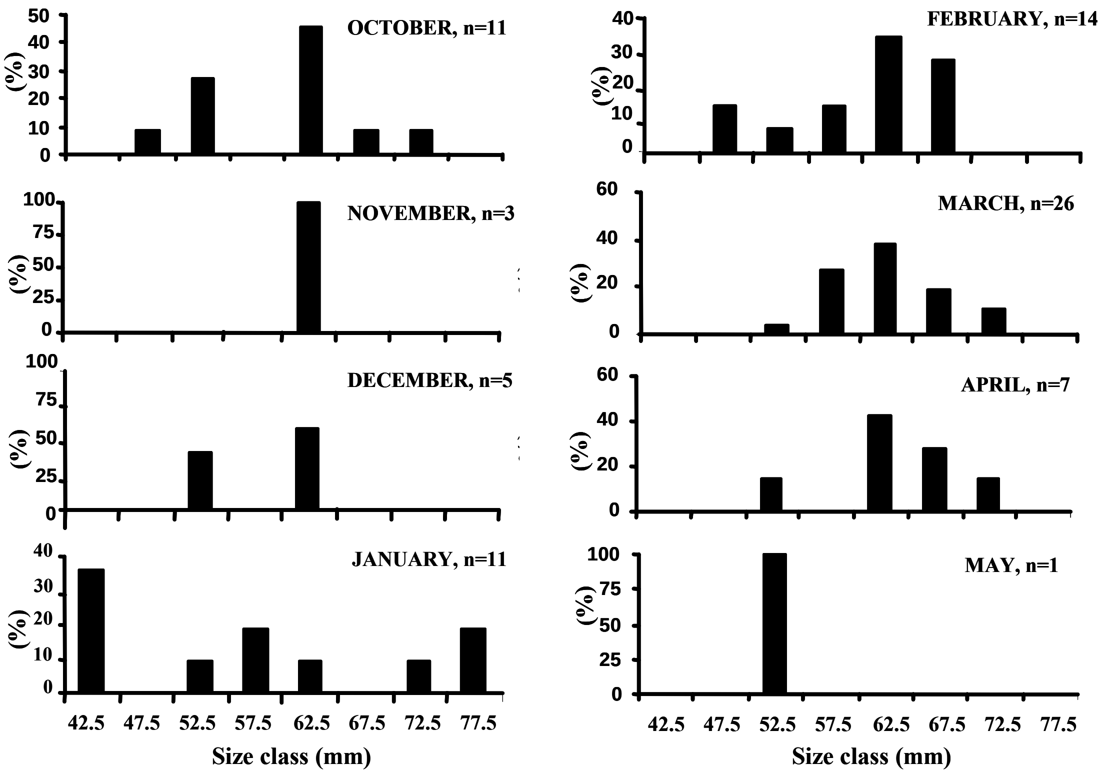

Figure 5. Monthly distribution (in percentage) *Jenynsia multidentata*'s females with embryos per 5 TL mm size classes, colleted in the estuary of Patos Lagoon between March 2000 and February 2001.

The second pregnancy

We observed a second pregnancy for some females along the reproductive period. In January, we verified the occurrence of the smallest reproductive females, probably born in October-November. Also in this month, we observed the occurrence of large reproductive females and they have showed a large empty space inside the abdominal region, which suggests that these females would be in their second pregnancy in the reproductive period. Or rather, females that have given birth in October-November would be in their second reproductive cycle in January-February.

This second pregnancy has not been reported in the previous reproductive study of this species in the same estuary by Garcia *et al.* (2004). Probably, this is because their studied was based on indirect reproductive evidences (abundance per size class plots) that did not involved dissection and examination of reproductive status of each specimen. Another confounding factor in this previous study was the higher variance in their dataset, since the analyzed individuals came from a larger number of sampling stations, encompassing not only the estuary but also freshwater sites in the northern reaches of the lagoon.

Fontoura *et al.* (1994) have observed that *J. multidentata* reaches smaller sizes in fresh water environments when compared with brackish ones. Experimental studies tested this hypothesis, confirming that individuals reared in brackish water presented higher growth and less mortality than those reared in freshwater (Mai *et al.* 2005). It seems plausible to expect, therefore, that higher growth and survival rates in brackish water would allow a second pregnancy for those females inhabiting an estuarine zone, as we have reported in this study.

Fecundity and sex ratio

The positive relation between the number of embryos and the total length of females corroborates the previous analysis of Betito (1984), which suggest that females limit the space of ovaries and, consequently, their fecundity. The minimum female size (43 mm) observed in this work was similar to the values described by Betito (1984) and Fontoura *et al.* (1994) of 42 e 45 mm, respectively.

Previous studies using this species have shown a higher number of females in relation to the males (Fontoura *et al.* 1994, Betito 1984, Garcia *et al.* 2004). Many hypotheses have been suggested to explain this pattern. For example, some authors have hypothesized the existence of an asymmetric predation between genders to due the peculiar reproductive behavior of this species. The copulation in the one-sided livebearer happens without the consent of female. The males take position along the female posterior side and try to introduce their gonopod into the female urogenital opening as fast and furtively as they can (Bisazza *et al*. 2000). The males expend a great part of their daily time in this activity. This behavior probably make them less careful (compared with females) regarding avoiding predator's attacks by piscivorous fishes and birds. This differential behavior between genders could result in a higher male predation rate, resulting in larger numbers of females in the population (Magurran & Nowak 1991, Magurran & Seghers 1991). An alternative hypothesis could be associated with differences in capture of males and females by the fish gear (beach seine hauls) employed during our sampling. In this case, the males could be simply under captured due their smaller size, resulting in a higher ratio of females in the samples. More studies should be conducted in controlled conditions at the laboratory and in natural environment in order to test these hypotheses.

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