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Research Article

Establishing evidence of a non-native species *Pachyurus bonariensis* Steindachner, 1879 (Perciformes, Sciaenidae) in Mirim Lagoon, Rio Grande do Sul (Brazil)

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Abstract

The La Plata croaker (*Pachyurus bonariensis*) family Sciaenidae, is a small (< 23 cm) benthopelagic fish that originated in the Paraná-Paraguay-Uruguay river systems in South America. Its first record in the Patos Lagoon drainage area, Rio Grande do Sul (Brazil) occurred in 2001. This research reports for the first time, the occurrence of both juvenile and adult specimens of *P. bonariensis* in the São Gonçalo Channel (a 75 km long waterway between Patos and Mirim lagoon) and in the Mirim Lagoon, in 2005 and 2007 respectively. A total of 434 gonads were analyzed histologically from specimens captured by artisanal fishermen at Capilha, Mirim Lagoon between 2008 and 2010. Gonadal development was observed and described. *Pachyurus bonariensis* probably dispersed from northern Patos Lagoon in 2001 to the São Gonçalo Channel by 2005. The occurrence of adults in limnic waters in the São Gonçalo Channel and further south in the Mirim Lagoon in 2007, confirm that *P. bonariensis* is now an established species in Mirim Lagoon and could become a commercially important species to the artisanal fishermen of the area. Likewise at the limnetic northern parts of Patos Lagoon, *P. bonariensis* is now abundant at Mirin Lagoon and the ecological impacts of this range extension must be investigated.

Key words: invasive species, Mirim Lagoon basin, reproduction, ovarian development

Introduction

The introduction of alien aquatic organisms has intensified with the increase of global trade and human mobility in recent centuries (Gozlan 2008; Gozlan et al. 2010). Species introductions from one watershed to another, within the same continent are capable of threatening local biodiversity (Vitule 2009). Ricciardi and Cohen (2007) suggest that invaders that colonize a large area and spread rapidly are not, on average, also those that cause large impacts on native species populations and therefore the term "invasive' should not be used to connote negative environmental impacts. To evaluate the impact invasive species can have on native biota, it is necessary to understand all aspects of their biology, ecology and population dynamics (Souza et al. 2009).

The ability of Sciaenidae species to adapt to different temperatures enables their wider distribution in both tropical and temperate waters (Flores and Hirt 2002). In South America, sciaenids occur in marine, brackish, and freshwater habitats. True freshwater sciaenids encompass the genera *Pachyurus*, *Pachypops*, *Plagioscion* and *Petilipinnis* that inhabited continental waters and eventually estuarine habitats (Casatti 2003; Lima and Behr 2010; Dufech and Fialho 2007). With the exception of *Petilipinnis*, all of these genera can be found in Brazil (Flores and Hirt 2002). The genus *Pachyurus* has the greatest number of species and the broadest geographic distribution, including the river systems of Orinoco, Amazonas, Paraná-Paraguay-Uruguay and São Francisco, east coastal Brazilian rivers, Guyana rivers and also French Guiana rivers (Dufech and Fialho 2007). The La Plata croaker (*Pachyurus bonariensis* Steindachner, 1879), popularly known in Brazil as "corvina-de-rio" or "Maria-luiza" (Figure 1), is a species associated with lentic ecosystems (Flores and Hirt 2002). It is most prolific in tropical water, especially those subject to human impacts (Agostinho et al. 1993, Flores and Hirt 2002). It is a small (<23 cm) bentho-pelagic fish, originating from Paraná-Paraguay-Uruguay rivers systems and down-stream of the Itaipu reservoir (Flores and Hirt 2002; Dufech and Fialho 2007; Fugi et al. 2007; Santin et al. 2009).

Pachyurus bonariensis invasion into the Patos Lagoon drainage area, Rio Grande do Sul (Brazil), was initially recorded by Pinto et al. in 2000. The mechanism of its introduction is uncertain; the prevailing hypothesis is the relative proximity of the Ibicuí (Uruguay River basin) river to an area of intense rice farming, where water and effluents are pumped and discharged to the Patos Lagoon basin. Its presence could also be as a consequence of its introduction by fishermen and/or fish farmers (Dufech and Fialho 2007; Barletta et al. 2010).

The occurrence of reproducing mature females of *P. bonariensis* in the limnetic northern part of Patos Lagoon basin and its high relative abundance (Dufech and Fialho 2007) suggests the establishment of this allochthonous species in the system. Indeed, Milani and Fontoura (2007) stated that according to artisanal fishermen in Casamento Lagoon (northeast section of the Patos Lagoon) (Figure 2) *P. bonariensis* is already considered a commercially important species for the area.

The occurrence of both juvenile and adults in the lower reaches of the Patos Lagoon system, the São Gonçalo Channel and in the Mirim Lagoon are recorded for the first time, and this study intended to register the establishment of *P. bonariensis* through monitoring the ovarian development of specimens collected in Mirim Lagoon.

Material and methods

Study area

The Mirim Lagoon basin is located between 31° and 34° S and 52° and 54° W, in the eastern part of the South American central plains (Figure 2). The basin covers an area of 54,000 km^2, with 24,000 km^2 in southern Brazil and the remaining 30,000 km^2 in Uruguay (Fia et al. 2009; Gouvêa et al. 2010). The main geographical feature of this basin is the Mirim lagoon itself, with an approx. area of 6,000 km^2 (Bracco et al. 2005).

During periods of high waters and intense rainfall, Mirim Lagoon and the adjacent wetland system drains through the natural channel of São Gonçalo (75-km long; 200 to 500-m wide; 6-m deep) into Patos Lagoon, which is connected to the Atlantic Ocean (Figure 2). Water generally flows northwards from Mirim to the Patos Lagoon (70% of the time), but episodes of reverse flow can occur during drought periods and altered wind activity (usually between November to May). In 1977, a dam (Watergate) was built in the São Gonçalo Channel to prevent the entrance of saline waters from Patos Lagoon estuary into Mirim Lagoon (Burns et al. 2006). This allowed an important constant supply of uncontaminated freshwater for rice irrigation, which is cultivated in large amounts along the lagoon's margins. Mirim lagoon also represents a valuable fishery resource (Steinke and Saito 2008).

Sampling

Since 2005, the Laboratory of Ichthyology of FURG take monthly samples from above and below the São Gonçalo dam, using both bottom and beach seine trawling (30 m length; 1.5 m height; 12 mm mesh manufactured with monofilament nylon wire) methods. From September 2008 to May 2010 (with the exception of the closed fishery season: November - January IBAMA 2008), specimens were purchased monthly, from gillnet fishermen from the Capilha region (Figure 2). Additionally, a few individuals were caught by beach seine and bottom trawling in the São Gonçalo Channel and Mirim Lagoon. Exemplary testimonies have been preserved for the Ichthyology Laboratory of FURG (FURG 2644, 2646, 2647, 2648, 2649).

Biometrics and dissection

Fish were either immediately fixed in buffered formalin or frozen and kept in a freezer until dissection. Total body length (TL) was measured in mm. Gonads were extracted and fixed in buffered formalin for 72 h. After fixation, gonads were kept in 70% alcohol for posterior histological processing.

Morphometry and gonadal classification

Gonad fragments were taken for histological analysis. The gonads were transversely sliced in 7 µm, stained with Hematoxylin-Eosin (HE), analyzed and images taken using an Olympus BX-51 microscope, mounted with an Olympus DP-72 camera. Morphometric analyses were made by measuring the greatest diameter of twenty oocytes for each ovary. Measurements were made using software image analysis ImageJ version 1.43.

Figure 1. *Pachyurus bonariensis* (Steindachner, 1879). Photograph by Cyntia A. Y. Harayashiki.

Figure 2. Study area: Mirim Lagoon. (*) Capilha region; (●) Casamento Lagoon.

Results

Six individuals were caught using bottom trawling in the estuarine area downstream of the São Gonçalo dam between December 2005 (n = 1) and January 2006 (n = 5). Total lengths ranged from 34 to 227 mm. In 2007, forty-one individuals were captured in the limnetic area upstream of the São Gonçalo dam, and only one specimen downstream (Table 1). The first occurrence of *P. bonariensis* in the Mirim Lagoon was reported in 2007, when several individuals were captured by artisanal fishers at Capão Alto beach, near the Taim Ecological Reserve. A total of 434 specimens of *P. bonariensis* were captured between 2008 and 2010 by artisanal fishers in the Capilha region; TL ranged between 50 and 255 mm. These specimens were used for histological processing,

Table 1. Number of *Pachyurus bonariensis* captured by bottom trawling and beach seine trawling between 2005 and 2007 in estuarine and limnic areas of São Gonçalo Channel.

		Estuarine Area		Limnic Area	
		Bottom Trawl	Beach Seine Trawl	Bottom Trawl	Beach Seine Trawl
2005	Summer	0	0	0	0
	Fall	0	0	0	0
	Winter	0	0	0	0
	Spring	1	0	0	0
2006	Summer	5	0	0	0
	Fall	0	0	0	0
	Winter				
	Spring	0	0	0	0
2007	Summer	0	0	33	1
	Fall	0	1	0	7
	Winter	0	0	0	0
	Spring	0	0	0	0

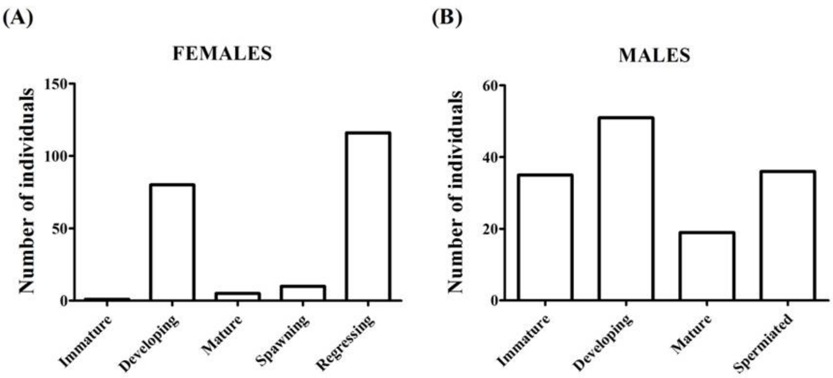

Figure 3. Proportion of gonadal development stages observed in (A) females and (B) males of *Pachyurus bonariensis* collected in Mirim Lagoon between September 2008 and May 2010.

namely 252 females, 148 males and 34 of non-identified gender. Histological analysis showed the presence of both females and males from all developmental stages (Figure 3).

The histological analysis from ovaries allowed the identification of four phases of oogenesis.

Phase I – CHROMATIN-NUCLEOLUSOO CYTES: The first phase of follicle development (Figure 4A) was characterized by small cells organized in nests, with large nuclei compared to cytoplasm volume, and having only one nucleolus that was highly basophilic, and usually located in the central region of the nucleus.

Phase II – PERINUCLEOLAR OOCYTES: In this phase (Figure 4A and 4B) the number of nucleoli increased and they migrated to the nuclear periphery.

Phase III – INITIAL FORMATION OF YOLK SAC: This phase (Figure 4B) was characterized by the accumulation of reserve substances (lipid) and accelerated increments of cytoplasmic volume and cytoplasmatic vacuolization; for *P. bonariensis* vacuolization occured in centripetal vitellogenesis.

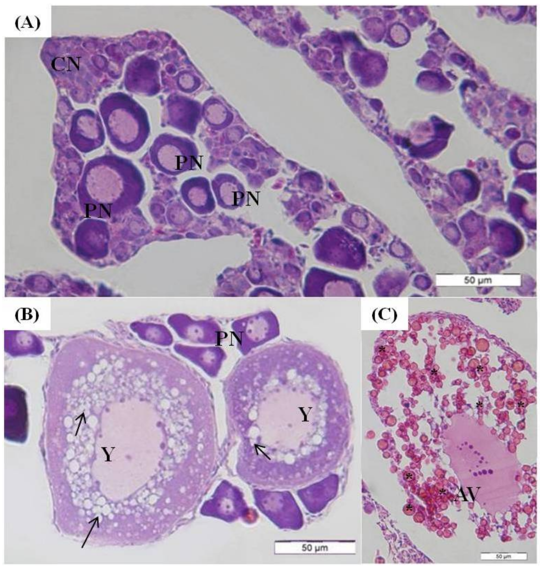

Figure 4. Phases of development of *Pachyurus bonariensis* oocytes. (A) chromatin-nucleolus oocytes (CN); perinucleolar oocytes (PN); (B) perinucleolar oocytes (PN) and perinucleolar oocytes with initial formation of yolk sac (Y); (C) advanced vitellogenesis (AV). (Arrow) vacuoles characteristic of lipid deposition; (*) protein depositions. Scale bar 50 µm. HE, obj. 40x

Phase IV – ADVANCEDVITELLOGENESIS: The last phase (Figure 4C) began with the deposition of proteins in the form of acidophilic granules in the cytoplasm. During this process, the granules advanced centripetally, pushing the vacuoles to the center of the cell.

Histological analysis noted several phases of germ cell development; ovaries were classified into separate developmental stages: 1. "immature" - nuclear chromatin (Phase I) was the only phase found; 2. "developing" oocytes had initial yolk sac formation; 3. "mature" oocytes had advanced vitellogenesis (phase IV); 4. "spawning" ovaries had hemorrhagic regions, atretic follicles and oocytes with initial yolk sac formation; 5. "regressing" several hemorrhagic regions and an elevated number of atretic follicles.

Testicles were examined and classified as follows: 1. "immature" where there was an absence of spermatozoa but others germ cells present (i.e. spermatogonia, spermatocytes and spermatids); 2. "developing" where all phases of germ cell development existed; 3. "mature" where testis showed only spermatozoa; 4. "spermiated" where germ cells were practically absent or may have had some remaining spermatozoa or there were early developing cells with spermatogonia.

Besides these four phases, histological analyses also showed hemorrhagic regions and atretic follicles. Hemorrhagic regions were characterized by a light homogeneous basophilic coloration between the follicles. The atretic follicles were oocytes undergoing a periodic degenerative process, being disintegrated and reabsorbed; they could be distinguished by an amorphous, more acidophilic mass.

The frequency of oocyte diameters in different stages of gonadal development (Figure 5) showed the presence of small oocytes (1–100 µm) in all stages of development. Oocyte size range in the mature stage was broad, 15 to 420 µm, with an average diameter of 165.95 µm. Post maturity, oocyte size tended to reduce, probably due to spawning. The stages "developing" and "regressing" had the same pattern, but the average oocyte diameter for "regressing" was bigger than "developing" (developing – 57.18 ± 0.76 µm; regressing – 63.58 ± 1.2 µm) oocytes.

March was the only month where immature females were present (Figure 6A) while males began their gonadal maturation in February (Figure 6B). Other differences found were in relation to the commencement of spawning, with males able to spawn in August, while females began a month later (Figure 6).

Discussion

Although sampling size was significant (n = 434), several months presented sampling problems when data was separated on a monthly basis. Sampling began with the early invasion of *P. bonariensis* in Mirim Lagoon, yet populations were not sufficient for intensive capture by local fishermen. By 2010 however, there was a significant increase in the number of captured fishes. This consistently harvested fishery and the presence of juveniles (Ceni and Vieira 2013), mature adults and spawners in Mirim Lagoon are evidence that this species is now established in the region.

Considering reproduction as a process whereby species perpetuate, the ability to reproduce in diverse environments is one of the most important factors that allow the success of a species (Vazzoler 1996). The study of gametogenesis in female teleost is important in order to clearly understand reproductive activity and accurately determine the breeding season (Agostinho et al. 1982; Spadella et al. 2005). According to Vazzoler (1996), fish spawning is determined by the interaction between the dynamics of oocytes development, the frequency of spawning during the reproduction period and the number of these periods during a life cycle. This knowledge can be used as a support tool in monitoring the settling of non-native species. Flores and Hirt (2002) classified the ovarian development of *P. bonariensis* in just 3 stages, namely pre-vitellogenesis, vitellogenesis and post-spawning; average maximum diameter of oocytes (354 µm) occurred during the vitellogenesis stage and minimum diameter of 17.62 µm was noted in the pre-vitellogenesis stage. In the present study, the gonadal development of females were separated into five stages (immature, developing, mature, spawning and regressing), the average minimum diameter measured was 21.2 µm in the immature stage and the average maximum diameter measured was 165.95 µm in mature ovaries, being the maximum individual diameter measured (420 µm) fairly close to values measured by Flores and Hirt (2002).

Intensive ongoing measurements of oocyte diameter showed the gradual growth of oocytes from gonadal development right through to the mature stage, where varied oocytes sizes were present. Even at the spawning stage oocytes of different sizes existed. According to the characteristics observed in specimens from Mirim Lagoon, the same sequence of oocyte development is also found in the Yacyretá dam (Argentina), which suggest that spawning was

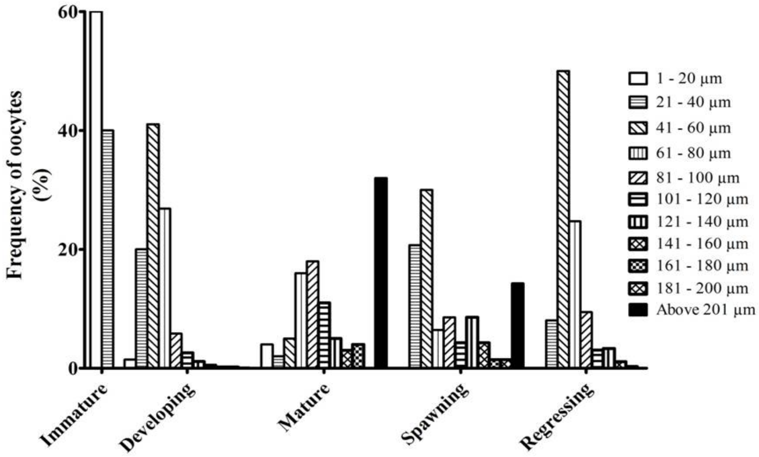

Figure 5. Frequency distribution of the diameter of oocytes of female *Pachyurus bonariensis* sampled in Mirim Lagoon at different developmental stages.

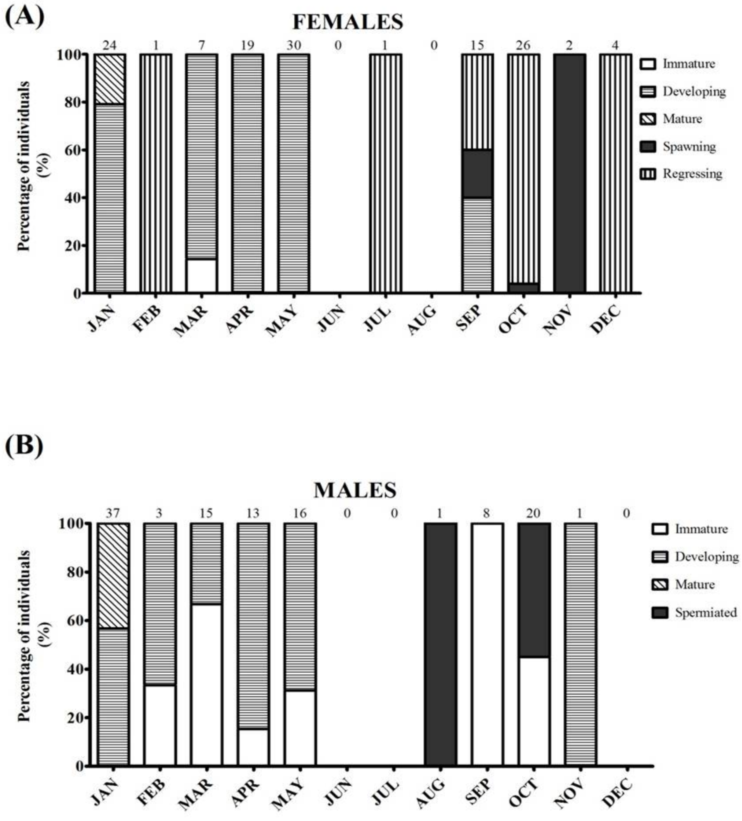

Figure 6. Monthly percentage contribution of *Pachyurus bonariensis* gonadal development stages (A) Females; (B) Males. Numbers above bars indicate the number of fish captured in each month.

synchronous in more than two groups (Flores and Hirt 2002).

The occurrence of spermatogenesis did not show a well-defined pattern, with immature and developing testes being present during different seasons of the year. Reproductive males matured before females. Males had a longer period of intense reproductive activity than females. This is a common phenomenon with many other fish species, probably because of the exaggerated differences in the process of gamete production between males and females.

Data analysis showed that reproduction can occur during spring (September–November) and summer (December–February). Since the only month with immature females was March, it is most likely that ovarian development begins around this time and consequently that September heralds the beginning of the spawning period. A similar pattern was observed by Flores and Hirt (2002) in Yacyretá Dam, where maximum reproductive activity occurred during the spring. Studies made by Santin et al. (2009), based on larval development of this species in Sinhá Mariana Bay (Brazil) suggest the same reproductive period. These authors however did not discard the possibility of a continuous reproductive cycle, where larvae occur only when water temperatures are greater than 21ºC. In Mirim Lagoon, average water temperatures have a broad seasonal fluctuation with minimum of 10–12ºC during the winter, and maximum of 24–31ºC during the summer months (Lopes and Vieira 2012; Moura et al. 2012). This observation suggests that reproduction at Patos Lagoon is probably restricted to spring and summer.

There is a predominance of the "developing" and "regressing" phases in females captured and of "developing" and "spermiated" phases in males examined. However, all stages of gonadal development in both sexes are present. The complete life cycle and the high number of fish captured in 2010 strongly indicate the settlement of the species in the region.

The negative effects of introduced species are widely recognized and are having major impacts on biodiversity in aquatic ecosystems (Agostinho et al. 2005; Vitule et al. 2006), but most of their specific ecological and evolutionary consequences remain unexplored (Olden et al. 2004). Fish introductions of both alien and native species are common in Brazil, and result from irresponsibility on the part of people involved in recreational fisheries, fish stocking, and aquaculture (Agostinho et al. 2005). The mechanism of Uruguay basin species invasion in the Patos-Mirim drainage is unknown, but prevalent hypothesis falls over the relative proximity of the upper Vacacaí (Jacuí basin) and Ibicuí (Uruguay basin) rivers in an area of intense rice farming and probable water pumping from both drainages (Barletta et al. 2010).

Is *P. bonariensis* really an introduced species? The answer of this question is that *P. bonariensis* is definitely a non-native, once this species has been recognized as introduced from the Uruguay basin by other authors (Pinto et al. 2000; Dufech and Fialho 2007; Milani and Fontoura 2007) and was not reported in Malabarba's (1989) review of fish species related to Patos-Mirim lagoon basin and Jacuí River, in which 106 freshwater fish species was reported, but not *P. bonariensis*. *Pachyurus bonariensis* is a freshwater fish that had never previously been reported in the well studied Patos Lagoon estuary (Chao et al. 1982; Vieira et al. 2010). This rationale discards the possibility that this species was rare or unknown in the Patos-Mirim system, proving that it is a non-native species.

According to some authors, the majority of freshwater fish introductions are not identified as having an ecological impact, but instead give great societal benefits and facilitation of native species by non-indigenous species through direct (habitat modification, trophic subsidy) and indirect (competitive release and predatory release) interactions, which is frequently overlooked (Rodriguez 2006; Gozlan 2008). Dufech and Fialho (2007) noted that *P. bonariensis* is not an aggressive invader species, but monitoring of this population into Mirim Lagoon is nevertheless necessary, not just because of its unknown ecological impact on the environment, but also because this species can become a fishery resource for local artisanal fisherman, as occurred in Casamento Lagoon, in the northeast section of the Patos Lagoon (Milani and Fontoura 2007).

Considering the short period in which it took *P. bonariensis* to move from the northern Patos Lagoon in 2000 (Pinto et al. 2000; Dufech and Fialho 2007; Milani and Fontoura 2007) to the São Gonçalo Channel (spring and summer of 2005 and 2006) and the occurrence of adults in the limnic area of São Gonçalo Channel and Mirim Lagoon in 2007, it is plausible to state that *P. bonariensis* is now a settled inhabitant of Mirim Lagoon. It may become a commercially important species to the artisanal fishermen of the local area and the ecological impacts of this range extension must be investigated.

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