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Original article

Defining the reproductive period of freshwater fish species using the Gonadosomatic Index: a proposed protocol applied to ten species of the Patos Lagoon basin

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This contribution records the reproductive periods of ten dominant freshwater fish species from the Patos Lagoon and Guaíba Lake (*Astyanax fasciatus*, *Cyphocharax voga*, *Hoplias malabaricus*, *Oligosarcus jenynsii*, *Oligosarcus robustus*, *Hoplosternum littorale*, *Loricariichthys anus*, *Parapimelodus nigribarbis*, *Trachelyopterus lucenai*, *Pachyurus bonariensis*). Data were derived from monthly samples in Casamento Lake (northern Patos Lagoon; Nov. 2002 to Apr. 2004) and Guaíba Lake (Jun. 2005 to May 2006). The reproductive period was determined according to the monthly variation of the gonadosomatic index (GSI). Fish reproduction was identified during all months of the year. *Oligosarcus jenynsii* started reproduction in winter, but extended spawning to spring (early warming-water reproduction). Three species also presented reproduction during warming water months, but beginning in spring and finishing in summer (late warm-water reproduction): *P. nigribarbis*, *T. lucenai* and *P. bonariensis*. Three species presented relatively short reproduction periods on summer (spotted warm-water reproduction): *H. malabaricus*, *H. littorale* and *L. anus,* and only one species reproduces almost continuously during warmer waters (long-season warm-water reproduction): *A. fasciatus*. Finally, two other species presented a very distinct reproductive pattern, starting reproduction on late summer but increasing GSI values along autumn and winter (long-season cooling-water reproducers): *C. voga* and *O. robustus*.

Keywords: GSI, Maturation, Maturity Criteria, Reproductive Cycle.

Esta pesquisa registra o período reprodutivo de dez espécies de peixes dulcícolas dominantes na região límnica da Laguna dos Patos e Lago Guaíba (*Astyanax fasciatus*, *Cyphocharax voga*, *Hoplias malabaricus*, *Oligosarcus jenynsii*, *Oligosarcus robustus*, *Hoplosternum littorale*, *Loricariichthys anus*, *Parapimelodus nigribarbis*, *Trachelyopterus lucenai*, *Pachyurus bonariensis*). Os dados derivam de amostras mensais realizadas na Lagoa do Casamento (Nordeste da Laguna dos Patos; Nov. 2002 a Abr. 2004) e no Lago Guaíba (Jun. 2005 a Maio 2006). A reprodução de peixes foi identificada durante todos os meses do ano. *Oligosarcus jenynsii* iniciou a reprodução no inverno, mas prolongou a desova até a primavera (reprodução no início do ciclo de aquecimento da água). Três espécies também apresentaram reprodução durante os meses de aquecimento da água, mas começando na primavera e terminando no verão (reprodução tardia do ciclo de aquecimento da água): *P. nigribarbis*, *T. lucenai* e *P. bonariensis*. Três espécies apresentaram períodos de reprodução relativamente curtos no verão (reprodução concentrada em período de água quente): *H. malabaricus*, *H. littorale* e *L. anus*, e apenas uma espécie se reproduz quase continuamente durante águas mais quentes (reprodução de longa duração em período de água quente): *A. fasciatus*. Finalmente, duas outras espécies apresentaram um padrão reprodutivo muito distinto, iniciando a reprodução no final do verão, mas aumentando os valores do IGS ao longo do outono e inverno (reprodutores de longo período com águas em resfriamento): *C. voga* e *O. robustus*.

Palavras-chave: Ciclo Reprodutivo, Critério de Maturidade, IGS, Maturação.

Introduction

Fish reproductive modes are extremely diverse, which includes variations concerning partner choice, spawning grounds and periods, and distinct schemes for parental care (Wootton, Smith, 2015). To understand those reproductive strategies in relation to the size at maturity, reproductive period and spawning grounds is essential to management of both fisheries and aquatic ecosystems.

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Although the freshwater fish species of the Patos Lagoon basin are relatively well known taxonomically, with more than 200 recorded limnetic species (Malabarba, 1989; Reis *et al.*, 2003; Bertaco *et al.*, 2016; Fontoura *et al.*, 2016), information concerning reproductive aspects of these species is still needed. Although recent reviews have treated aspects of the conservation (Fontoura *et al.*, 2016) and fishery (Ceni *et al.*, 2016) of the Patos basin, the only general overview of the reproductive biology of fish species was by Marques *et al.* (2007), who estimated the size at first maturity for some dominant species.

Usually, defining the reproductive period of a fish species depends on some degree of morphological interpretation, including choosing between maturity scales of ovaries (macroscopically) or oocytes (microscopically). Although microscopic analysis provides a better understanding of the full reproductive cycle, it is clearly time-consuming and difficult to apply to entire, highly diverse fish communities. Even so, in a recent review, Schemmel *et al.* (2016) compared the evaluation of seasonal spawning peaks of 57 fish species across the Hawaiian Islands by using both community-collected GSI data and scientifically (histologically) assessed information. Both approaches gave similar results, suggesting that the Gonadosomatic Index (GSI) approach could be applied in data-poor fisheries for which morphological analysis is not possible due to logistic constraints.

The problem with using GSI information is that setting a cut-off value to identify an individual as involved or not in reproductive activities is necessarily subjective. Fontoura *et al.* (2009) evaluated the use of GSI values to estimate the size at first maturity (L_{50}) for four fish species of the Patos basin, testing different GSI cut-off values to set an individual as involved or not in reproduction. In the present study, we will focus on the identification of fish reproductive periods, testing clear operational criteria based on female GSI values to delimit the fish reproductive periods. The proposal was evaluated using data for ten dominant fish species inhabiting the limnetic area of the lower Patos basin.

Material and Methods

Study area. The drainage basin of the Patos Lagoon covers 30% of Rio Grande do Sul State (approximately 88,000 km^2) (Fig. 1). This catchment is responsible for the formation of a massive body of water, the Patos Lagoon, the world's largest choked lagoon, with a surface area of approximately 10,000 km² (Kjerve, 1986). In this system, the largest freshwater input comes from Guaíba Lake, with an area of approximately 468 km^2 (50 km long, 1 to 19 km wide), mean depth of 2 m and maximum depth of over 30 m. The main tributaries of Guaíba Lake are the Jacuí, Caí, Sinos and Gravataí rivers. Casamento Lake, in the northeast region of the Patos system, is an important area for artisanal fishery (Milani, Fontoura, 2007), with a surface area of approximately 272 km^2 and 4.1 m depth (Villwock, 1978); the Capivari and Palmares rivers are the main tributaries. These two sites, Guaíba Lake and Casamento Lake, were sampled in the present study.

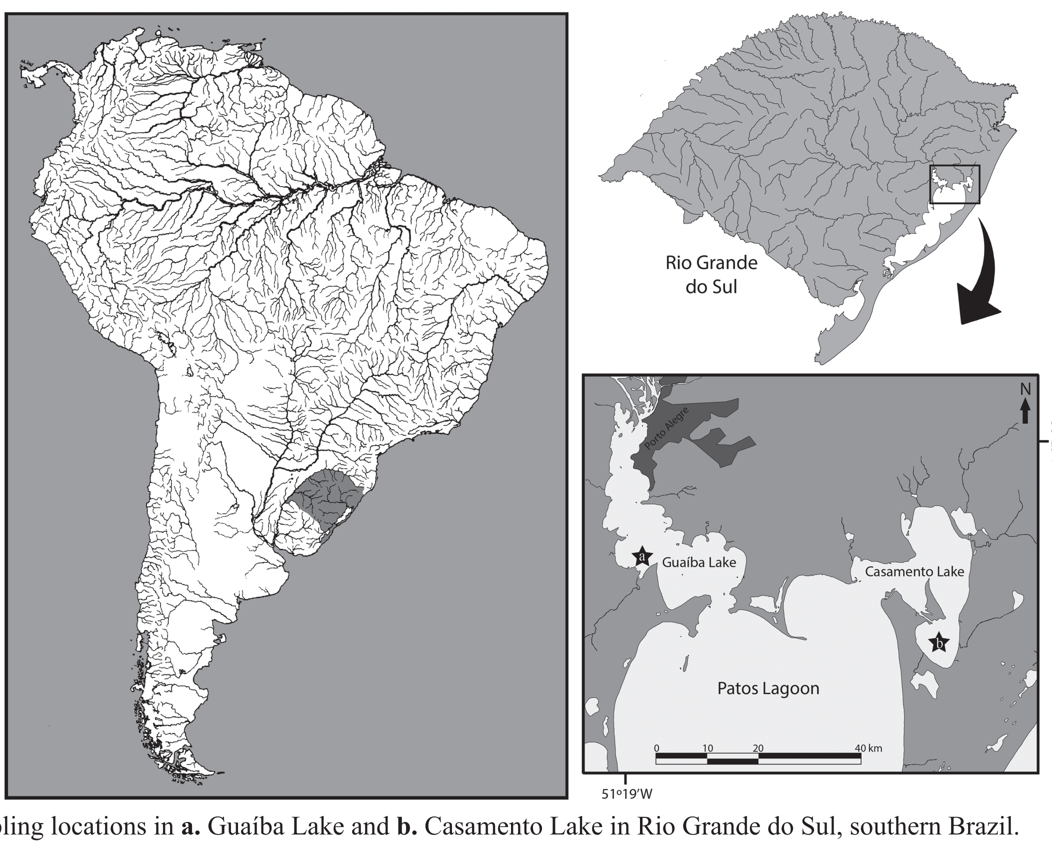

Fig. 1. Sampling locations in **a.** Guaíba Lake and **b.** Casamento Lake in Rio Grande do Sul, southern Brazil.

The region has a humid climate: rainfall varies between 1,200 and 1,500 mm per year, most intense in late winter and early spring. During the 2002 sampling program, rainfall increased during a moderate *El Niño* phenomenon, with an Oceanic *El Niño* Index above 1.0 (CPC, 2017). Summer has a mean temperature of 25°C in January; in winter the mean temperature is 14°C in July (see Fontoura *et al.*, 2016, for a general characterization of the area).

Sampling. Samples were taken monthly at both sampling sites. In Casamento Lake the sampling period extended from November 2002 to April 2004 (with no sample in October 2003), while in Guaíba Lake sampling continued from June 2005 to May 2006. Fish were caught with a set of gillnets (mesh sizes 15, 20, 25, 30, 35, 40, 50, 60 and 70 mm, square measure), each 30 m long. The gillnets were set at 4 p.m. and removed at 10 a.m. the next day (18 h effort). All fish were fixed in 4% formalin, and the larger individuals (>25 cm TL) also received an injection of 5 ml of 40% formalin (commercial solution) in the visceral cavity. Voucher specimens, deposited in the Museu de Ciências e Tecnologia da PUCRS (MCP), are as follows: *Astyanax fasciatus* (MCP 25845), *Cyphocharax voga* (MCP 25837), *Hoplias malabaricus* (MCP 25851), *Oligosarcus jenynsii* (MCP 25838), *Oligosarcus robustus* (MCP 25835), *Hoplosternum littorale* (MCP 10541), *Loricariichthys anus* (MCP 10539), *Parapimelodus nigribarbis* (MCP 16359), *Trachelyopterus lucenai* (MCP 17174), *Pachyurus bonariensis* (MCP 48997).

Data analysis. Species caught in the largest numbers by the gillnets were selected for the present study. Females were measured (total length, 1 mm precision) and weighed (0.01 g precision). Ovaries were weighed (0.0001 g precision) and the Gonadosomatic Index (GSI) was calculated as follows:

$$GSI = \frac{Wg}{Wt} \, . \, 100$$

where Wg is the gonad weight (g) and Wt is the total weight (g) of the individual.

Only adult females, according to the size at first maturity as estimated by Marques *et al.* (2007), were included to identify the reproductive period. For each species, the annual reproductive cycle was described based on monthly female GSI distributions. To identify the cycles, three GSI cut-off values were proposed and tested in relation to the maximum GSI recorded for each species: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}). For example, if the maximum-recorded GSI value for a species is 10%, G_{20}, G_{30} and G_{40} will be 2%, 3% and 4% respectively.

Aiming to define the reproductive cycle, three distinct periods were proposed: (1) months with recorded reproduction, when at least one female reached a GSI value equal to or greater than the G_{20}, G_{30} or G_{40} cut-off value; (2) months with core reproduction, when at least one sample site (Guaíba or Casamento) showed a median GSI value equal to or greater than the G_{20}, G_{30} or G_{40} cut-off value; and (3) non-reproductive months, when no female showed a GSI value equal to or greater than these cut-off values. Year seasons are considered as follow: summer (December, January and February), autumn (March, April, May), winter (June, July, August), and spring (September, October, November).

Results

Months with recorded reproduction, when at least one individual showed a GSI value equal to or greater than 20% (G_{20}), 30% (G_{30}) or 40% (G_{40}) of the maximum recorded GSI for each species, are presented in Tab. 1. The core reproductive months, when at least one sample (Guaíba or Casamento) showed a female median GSI equal to or greater than 20% (G_{20}), 30% (G_{30}) or 40% (G_{40}) of the maximum recorded GSI for each species are presented in Tab. 2. By inspecting both tables, we can divide the species into two large groups. The first group includes species that stop reproducing in some cooler (winter) months. This group was termed "warming-water reproduction" because inspection of the monthly GSI values (Figs. 2, 4, 7-11) revealed a pattern of increasing GSI medians from spring to summer. The second group, termed "cooling-water reproduction", includes species that stop reproducing in some warmer (summer) months, showing median GSI values that increase from summer/autumn to winter. Among the warming-water reproducing species, the length of the reproductive period could be relatively short (focal), restricted to 3-4 months, to very long (almost year-round). All species with cooling-water reproduction have very long reproductive periods, exceeding seven months according to all criteria. Detailed information for each species is presented as follows (grouped by order).

Characiformes. *Astyanax fasciatus* (Cuvier, 1819). A total of 577 females were caught, with total lengths ranging from 5.4 to 16.4 cm. The highest recorded GSI value was 19.4%. The recorded reproduction (Tab. 1; G_{20}, G_{30}, G_{40}) extended to all months of the year, except June (and November with no catch) and July according to the G_{40} criterion. This almost continuous reproduction seems to show two reproductive cycles with increasing GSI medians (Fig. 2), the first from February to May, and the second from September to December. Core months of reproduction (Tab. 2) are the same as the recorded reproduction for the G_{20} criterion (no core reproduction in June), but shows the same two reproductive cycles (February to May and September to December) by the G_{30} criterion. Following the G_{40} criterion, core months of reproduction are restricted to December, February and March.

Cyphocharax voga (Hensel, 1870). A total of 596 females were caught, with lengths ranging from 7.5 to 25.9 cm. The highest GSI value was 31.5%. The recorded reproduction (Tab. 1; G_{20}, G_{30}, G_{40}) extended to all months of the year, except November, and October according to the G_{30} and G_{40} criteria respectively. The median GSI values (Fig. 3) increased from January to August, with an off-trend high median in December. Core months of reproduction (Tab. 2) are the same for the G_{20} and G_{30} criteria, and reveal a long core season, from February to September, and an off-trend core month in December. By the G_{40} criterion, the core months of reproduction are restricted to April-August, still keeping December as an off-trend core month.

Hoplias malabaricus (Bloch, 1794). A total of 297 females were caught, with lengths ranging from 13.7 to 38.4 cm. The highest GSI value was 11.9%. Recorded reproduction (Tab. 1) ranged from November to February for all cut-off criteria. Core reproductive months are restricted to December and February for the G_{30} and G_{40} criteria, and begin earlier, in November, by the G_{20} cut-off value. Inspecting the seasonal variation of GSI values (Fig. 4) shows that the two different core periods (December and February) occurred at different sites and in different sampling years, indicating not a two-step reproductive cycle, but rather reproductive plasticity in relation to time and/or site.

Oligosarcus jenynsii (Günther, 1864). A total of 845 females were caught, with lengths ranging from 8.2 to 26.5 cm. The highest GSI value was 27.3%. The recorded reproduction according to G_{20} criteria (Tab. 1) extended to most months of the year, except January, October and December, the last two with no catch. By the G_{30} and G_{40} cut-off values, there was also no reproduction in February (G_{30} and G_{40}) and in November (G_{40}). This long reproductive period shows a cycle of increasing GSI values from February to August (Fig. 5), indicating that the species is a typical cooling-water reproducer. The core months of reproduction (Tab. 2) extend from March to September using the G_{20} or G_{30} criteria, but end one month earlier, in August, using the G_{40} cut-off value.

Oligosarcus robustus Menezes, 1969. A total of 225 females were caught, with total lengths ranging from 7.5 to 33.6 cm. The highest GSI value was 24.2%. The recorded reproduction according to G_{20} criteria (Tab. 1) comprised almost all months of the year except November, for all cut-off criteria; October-November using G_{30}; or October-December using the G_{40} cut-off value. As for *O. jenynsii*, a long reproductive period was observed (cooling-water reproduction), with increasing median GSI values from January to August (Fig. 6). Core months of reproduction (Tab. 2) extend from January to September, using the G_{20}; and from February to September, using the G_{30} or G_{40} cut-off values (except March for G_{40}).

Tab. 1. Months with recorded fish reproduction based on GSI values, when at least one individual showed a GSI value equal to or greater than 20% (G_{20}), 30% (G_{30}) or 40% (G_{40}) of the maximum recorded GSI value recorded for each species. Data for dominant fish species in the Patos Lagoon basin (Guaíba Lake and Casamento Lake, Rio Grande do Sul, Brazil).

Strategy		Species	Cut-off	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Warming-Water Reproduction	Very Long ↕ Focal	*Astyanax fasciatus*	G_{40}	■	■	■	■	■			■	■	■	-	■
			G_{30}	■	■	■	■	■		■	■	■	■	-	■
			G_{20}	■	■	■	■	■		■	■	■	■	-	■
		Parapimelodus nigribarbis	G_{40}		■							■	■	■	■
			G_{30}	■	■							■	■	■	■
			G_{20}	■	■						■	■	■	■	■
		Pachyurus bonariensis	G_{40}	■	■							■		■	■
			G_{30}	■	■	■						■		■	■
			G_{20}	■	■	■	■					■	■	■	■
		Trachelyopterus lucenai	G_{40}	■	■									■	■
			G_{30}	■	■									■	■
			G_{20}	■	■	■								■	■
		Hoplias malabaricus	G_{40}	■	■									■	■
			G_{30}	■	■									■	■
			G_{20}	■	■									■	■
		Hoplosternum littorale	G_{40}	■									■	■	■
			G_{30}	■									■	■	■
			G_{20}	■									■	■	■
		Loricariichthys anus	G_{40}	■										■	■
			G_{30}	■										■	■
			G_{20}	■	■									■	■
Cooling-water Reproduction	Very long	*Cyphocharax voga*	G_{40}	■	■	■	■	■	■	■	■	■			■
			G_{30}	■	■	■	■	■	■	■	■	■			■
			G_{20}	■	■	■	■	■	■	■	■	■	■		■
		Oligosarcus jenynsii	G_{40}			■	■	■	■	■	■	■	-		-
			G_{30}			■	■	■	■	■	■	■	-	■	-
			G_{20}		■	■	■	■	■	■	■	■	-	■	-
		Oligosarcus robustus	G_{40}	■	■	■	■	■	■	■	■	■			
			G_{30}	■	■	■	■	■	■	■	■	■			■
			G_{20}	■	■	■	■	■	■	■	■	■	■		■

Tab. 2. Months with fish core reproduction based on GSI values, when at least one sample showed a median GSI equal or greater than 20% (G_{20}), 30% (G_{30}) or 40% (G_{40}) of the maximum GSI value recorded for each species. Data for dominant fish species in the Patos Lagoon basin (Guaíba Lake and Casamento Lake, Rio Grande do Sul, Brazil).

Strategy		Species	Cut-off	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Warming-Water Reproduction	Very Long ↔ Focal	*Astyanax fasciatus*	G_{40}		■	■								-	■
			G_{30}		■	■	■	■				■	■	-	■
			G_{20}	■	■	■	■	■		■	■	■	■	-	■
		Pachyurus bonariensis	G_{40}											■	■
			G_{30}	■	■									■	■
			G_{20}	■	■							■	■	■	■
		Parapimelodus nigribarbis	G_{40}												
			G_{30}									■			
			G_{20}									■	■	■	■
		Trachelyopterus lucenai	G_{40}	■											■
			G_{30}	■	■									■	■
			G_{20}	■	■	■								■	■
		Hoplias malabaricus	G_{40}		■										■
			G_{30}		■										■
			G_{20}		■									■	■
		Hoplosternum littorale	G_{40}											■	■
			G_{30}	■										■	■
			G_{20}	■										■	■
		Loricariichthys anus	G_{40}	■											
			G_{30}	■											■
			G_{20}	■										■	■
Cooling-water Reproduction	Very long	*Cyphocharax voga*	G_{40}				■	■	■	■	■				■
			G_{30}		■	■	■	■	■	■	■	■			■
			G_{20}		■	■	■	■	■	■	■	■			■
		Oligosarcus jenynsii	G_{40}			■	■	■	■	■	■		-		-
			G_{30}			■	■	■	■	■	■	■	-		-
			G_{20}			■	■	■	■	■	■	■	-		-
		Oligosarcus robustus	G_{40}		■		■	■	■	■	■	■			
			G_{30}		■	■	■	■	■	■	■	■			
			G_{20}	■	■	■	■	■	■	■	■	■			

Siluriformes. *Hoplosternum littorale* (Hancock, 1828). A total of 321 females were caught, with total lengths from 11.6 to 22 cm. The highest GSI value was 17.1%. The recorded reproduction according to all criteria (Tab. 1) showed the same length, from October to January. This well-defined spring-summer reproduction starts with a rapid GSI increase from September to November, although with only one reproducing female caught in October (Fig. 7). Core months of reproduction (Tab. 2) extend from November to January, using the G_{20} or G_{30} criteria; but end earlier, in December, using the G_{40} cut-off value.

Loricariichthys anus (Valenciennes, 1835). A total of 504 females were caught, with lengths ranging from 11.7 to 41 cm. The highest GSI value was 12%. The species is a focal reproducer, with recorded reproduction from November to January, according to the G_{30} or G_{40} criteria, and extending to February if using the G_{20} criterion. As for *H. littorale*, reproduction starts with a rapid mean GSI increase from September to November, although with only one individual caught in October (Fig. 8). Core months of reproduction (Tab. 2) extend from November to January using the G_{20} criterion, but are restricted to December-January using the G_{30} cut-off value, or only January by the G_{40} criterion.

Parapimelodus nigribarbis (Boulenger, 1889). A total of 480 females were caught, with lengths ranging from 7.8 to 22.5 cm. The highest GSI value was 11%. The species shows a long warming-water reproductive cycle, with recorded reproduction from August to February according to the G_{20} criterion; and starting one month later, in September, using the G_{30} or G_{40} criteria (with no reproduction recorded in January for G_{40}). As for other warming-water reproducers, a rapid GSI increase was observed, with an almost explosive rise from August to September (Fig. 9). Core months of reproduction (Tab. 2) extend from September to December using the G_{20} criterion, are restricted to September, under the G_{30} cut-off; and with no core reproductive month under the G_{40} cut-off value. These low median GSIs during the reproductive period suggest that the species shows reproductive migration, with mature individuals moving outside the sampled areas to spawn.

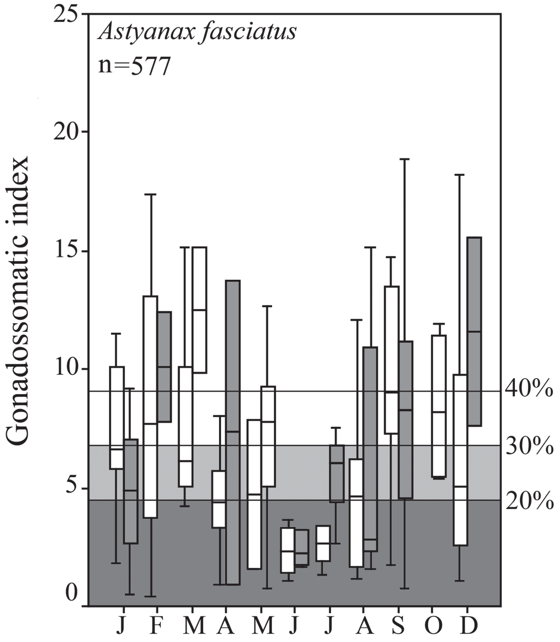

Fig. 2. Monthly variation of *Astyanax fasciatus* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

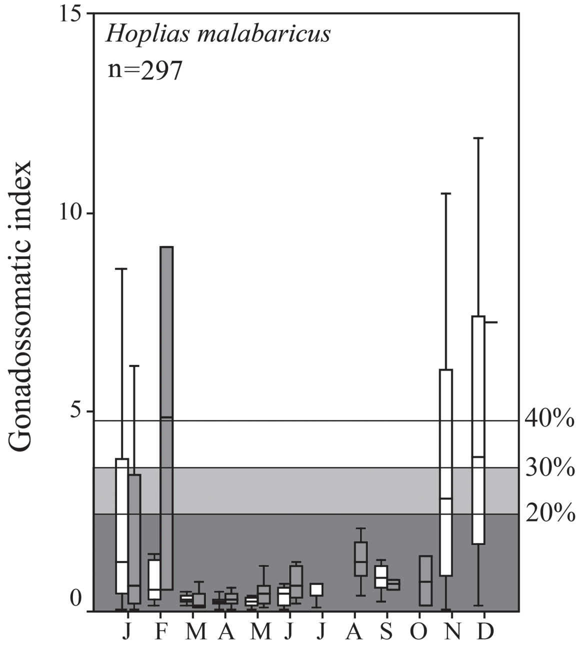

Fig. 4. Monthly variation of *Hoplias malabaricus* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

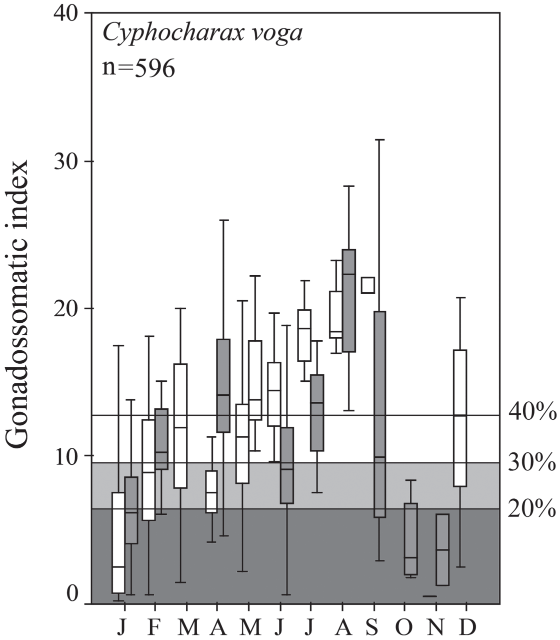

Fig. 3. Monthly variation of *Cyphocharax voga* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

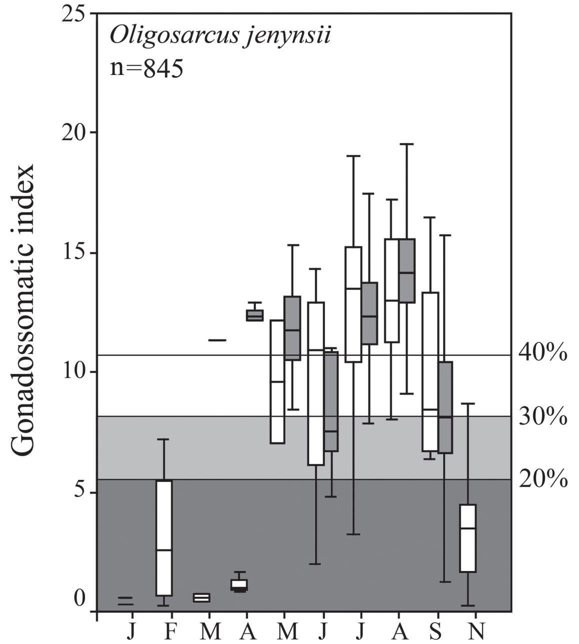

Fig. 5. Monthly variation of *Oligosarcus jenynsii* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

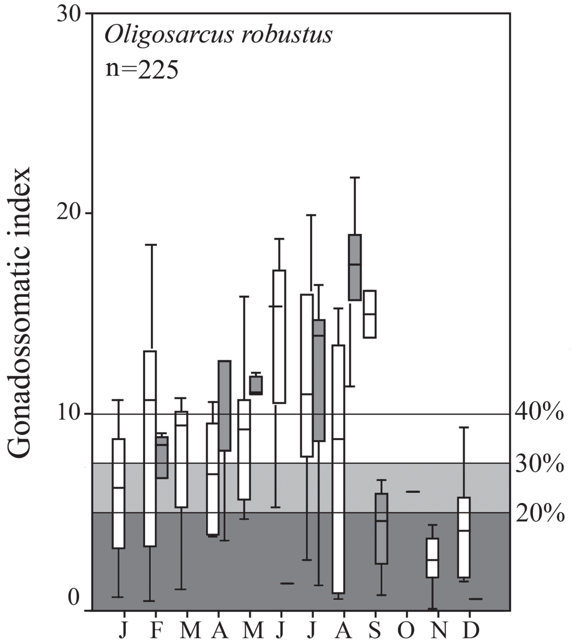

Fig. 6. Monthly variation of *Oligosarcus robustus* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

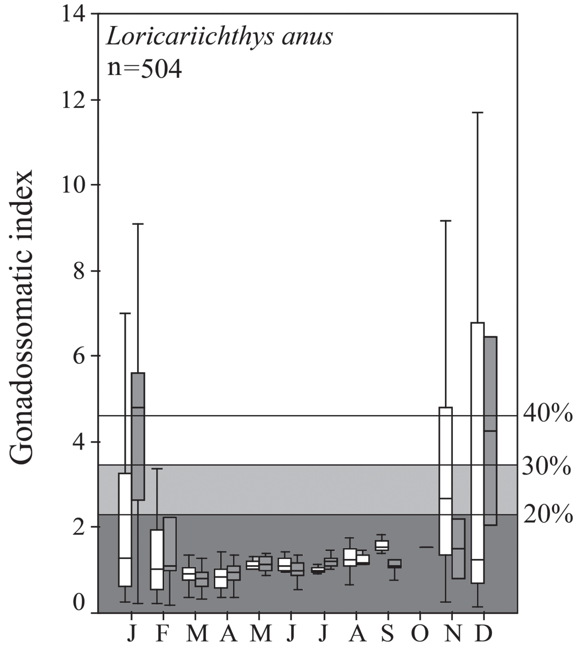

Fig. 8. Monthly variation of *Loricariichthys anus* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

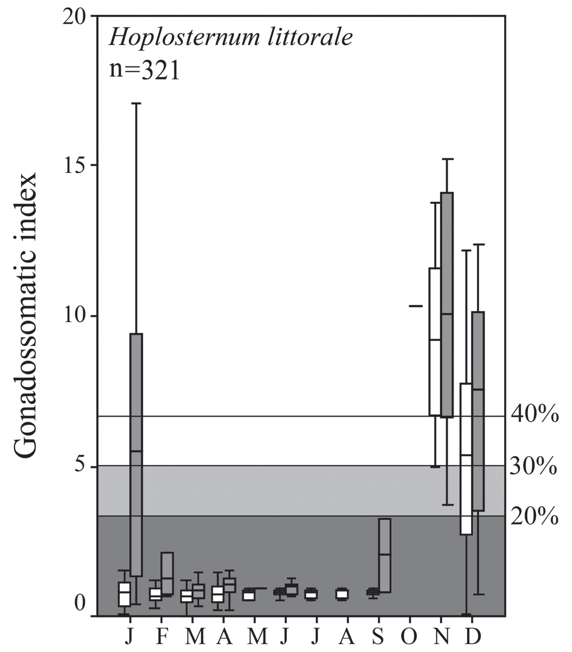

Fig. 7. Monthly variation of *Hoplosternum littorale* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

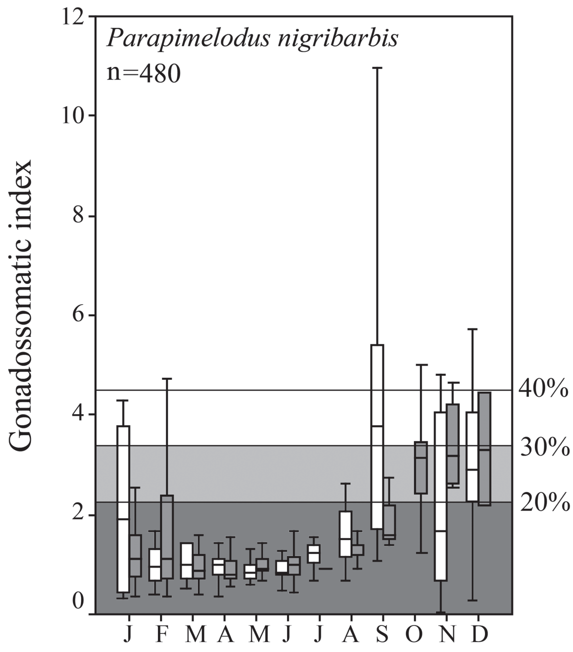

Fig. 9. Monthly variation of *Parapimelodus nigribarbis* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with rspect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

Trachelyopterus lucenai Bertoletti, da Silva & Pereira, 1995. A total of 232 females were caught, with lengths ranging from 10.2 to 22.2 cm. The highest GSI value was 26.2%. The observed reproduction (Tab. 1) is very similar to *H. malabaricus*, ranging from November to February for all cut-off criteria, and extending to March if using the G_{20} cut-off. Core reproductive months extend from November to March, November to February, or December to January for G_{20}, G_{30} or G_{40} respectively. The seasonal variation of GSI values (Fig. 10) showed an almost perfect sinusoidal variation.

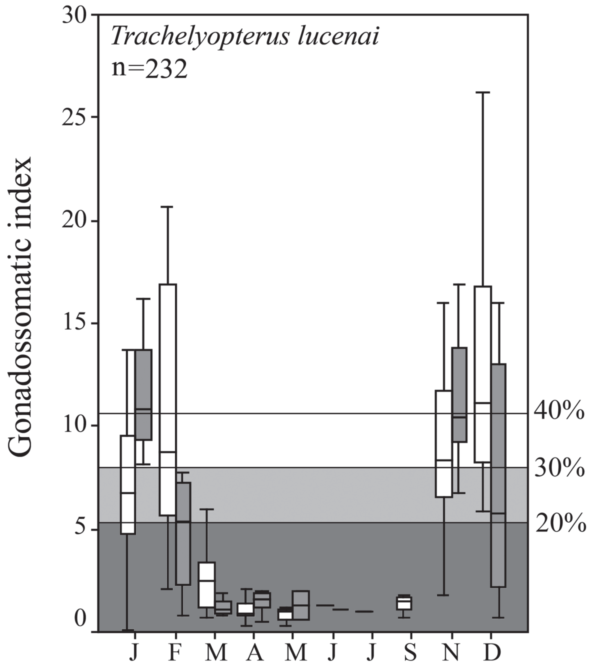

Fig. 10. Monthly variation of *Trachelyopterus lucenai* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

Perciformes. *Pachyurus bonariensis* Steindachner, 1879. A total of 318 females were caught, with lengths ranging from 7.6 to 26.4 cm. The highest GSI value was 6.6%. As for *T. lucenai,* the species showed a smooth cycle of annual variation for GSI values, following a sinusoidal pattern (Fig. 11). The species showed a long warming-water reproductive cycle, with reproduction from September to April according to the G_{20} criterion, and stopping in March and February for the G_{30} and G_{40} criteria respectively. Core months of reproduction (Tab.) extend from September to February, using the G_{20} criterion; from November to February applying the G_{30} cut-off value; and are restricted to November-December, using G_{40}.

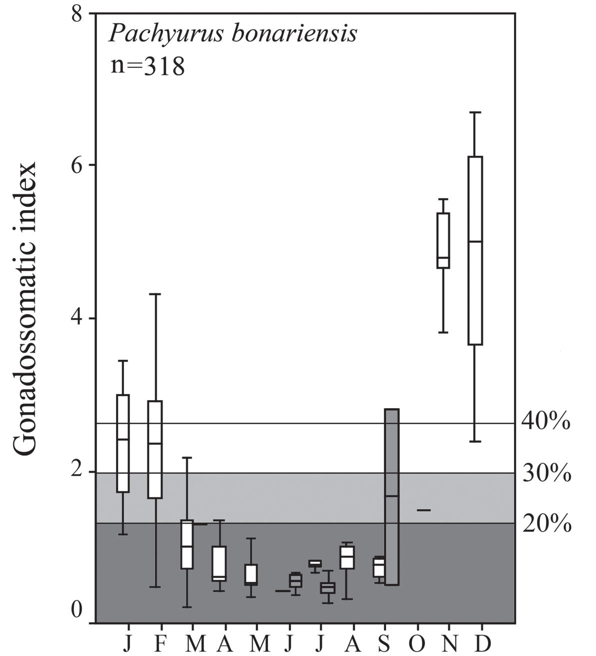

Fig. 11. Monthly variation of *Pachyurus bonariensis* GSI values (adult females only) in Guaíba Lake (white) and Casamento Lake (dark gray), Rio Grande do Sul, Brazil (median, 25-75% quartiles and lower-upper GSI limits by month and site). Three GSI cut-off values were tested with respect to the maximum GSI recorded for each species, for delimitation of reproductive months: 20% (G_{20}), 30% (G_{30}) and 40% (G_{40}).

Discussion

Concerning the proposed protocol to identify reproductive cycles, the use of GSI and numerical threshold values presents some practical advantages for a large dataset in comparison to morphological analysis, as it is less time-consuming than a histological analysis (Schemmel *et al.*, 2016). However, the use of a standard protocol has some weaknesses. The first aspect concerns the use of an arbitrary GSI value as a cut-off point to discriminate animals as involved or not in reproductive activities. Although it is an objective and easily assessed criterion, the selection of a cut-off point can be a methodological trap. Three questions arise: (1) Which cut-off point for the maximum GSI (G_{20}, G_{30}, G_{40}) is the most appropriate? (2) Should we attempt to determine the reproductive period based on only one cut-off value, or should we use more than one? (3) How representative is the maximum recorded GSI value, as it depends largely on the sample size (n)?

Fontoura *et al.* (2009) used GSI values to estimate size at first maturity for four fish species in the Patos Lagoon basin. As a first attempt, these authors tested values ranging from 5% to 30% of the maximum recorded GSI as cut-off marks to set any individual as reproductive or non-reproductive (see Fontoura *et al.*, 2009, Fig. 4). The surprising result was that it makes no difference: estimates of size at first maturity were essentially the same for all cut-off values.

Nevertheless, to identify the reproductive period at population scale is more challenging. A female with a GSI value of 5% of the maximum will show almost no change in length until the ovary is fully developed. Consequently, this cut-off value is good enough to identify a female that is beginning the maturation cycle, and the estimation of size at first maturity is not affected by the selected value of GSI as identified by Fontoura *et al.* (2009).

On the other hand, the time lag for a GSI to increase from just 5% of the maximum GSI to a fully developed ovary is unknown for most species, although expected to be a few weeks to a few months. In this regard, any value chosen is compromising. If we set a low cut-off value, the estimated reproductive period will be extended. If we set it too high, we will limit the reproductive period to only one, two, or even no months. Hence, the proposed 20-40% cut-off range is merely a practical proposal, to be revised with larger data sets.

Nevertheless, observing the temporal progression of GSI values (Figs. 2-11) makes it clear that the GSI variation (as a proxy for gonadal maturation) could be an almost explosive process, from almost nothing in a month to fully developed ovaries one or two months later (Figs. 4, 7-11). On the other hand, maturation could be a gradual process, taking several months for the median GSI to attain maximum values (Figs. 3, 6). Depending on the maturation strategy, the results from different GSI cut-off values could vary widely.

Although any binary classification based on cut-off values will always be biased at some point, some general patterns could be identified from our data set. Concerning the recorded period of reproduction, when at least one individual reached a GSI value equal to or greater than the G_{20}, G_{30} or G_{40} cut-off values, the results were the same for all cut-off thresholds for *H. malabaricus* and *H. littorale.* In relation to the G_{30} criteria, the recorded reproduction was one month longer if the G_{20} cut-off value was used for *P. nigribarbis, P. bonariensis, L. anus, C. voga, O. jenynsii* and *O. robustus.* By comparing the G_{30} and G_{40} criteria, the periods of recorded reproduction were the same for *T. lucenai, L. anus* and *C. voga,* and shorter by one month for *A. fasciatus, P. bonariensis, O. jenynsii* and *O. robustus.*

Examining species with more explosive maturation such as *H. malabaricus* (Fig. 4), *H. littorale* (Fig. 7) or *L. anus* (Fig. 8) shows clearly that the G_{20} cut-off value is well above any threshold level, separating females that are not reproducing at all from those that are already involved in reproduction. Also, inspecting Tab. 2 reveals that only the G_{20} criterion showed no interruption in the middle of the reproductive cycle (as for *P. bonariensis*), suggesting that this level is more stable for small samples. On the other hand, as a species' reproductive period could change from site to site or year to year, as did that of *H. malabaricus* in the present study (Fig. 4), it is better to be conservative when setting the overall reproductive period of a species.

On the other hand, if the objective is to set a core reproductive period, as to establish a closed fishing season, we should also look for stable results, with no break due to small sample sizes (such as the G_{40} criterion for *O. robustus*), but able to assign the months when a significant proportion of the females have well-developed ovaries. The G_{30} criterion appears, then, as a well-balanced proposal, neither excessively extending nor restricting the proposed core reproductive period of a species.

Following these considerations, we pass to the third point. How representative is the maximum GSI value recorded for a species? Of course, the value obtained depends strongly on the sample size. Larger samples increase the probability of capturing a female just before egg laying, increasing the reference point for cut-off values. Therefore, 20% or 30% of some value depends on the sample size and is clearly a moving target. How can we trust it?

As could be observed by the different cut-off values, the results are not much different and sometimes are even equal if any cut-off criterion is used, showing possible fluctuations at the edges, especially for small samples. Especially, species with short reproductive periods and explosive maturation will provide more stable results, less sensitive to the maximum GSI reference or cut-off criterion. On the other hand, species with long reproductive periods and slower ovary development will be more sensitive to both the maximum reference GSI and the selected cut-off value. For these species, GSI estimates of the reproductive period should be considered merely a first approximation until morphological analyses can be completed.

Of course, blind tests would be useful to validate the GSI method and especially the cut-off thresholds, based on the same large sample and defining the reproductive period with both GSI and morphological methods. Although such reviews are not available, we can compare our estimates with the available literature (Tab. 3), excluding estimates from distant hydrographic basins (*e.g.*, Nomura, 1975; Barbieri, 1989; Araújo-Lima, Bittencourt, 2001; Mesones *et al.*, 1998; Sá-Oliveira, Chellappa, 2002; Flores, Hirt, 2002; Lagemann, Fialho, 2014). Although these authors all worked with the same nominal species as in the present study, the geographical distances imply not only very different environmental pressures on each species, but also the possibility of different evolutionary units within the same taxon designation.

Available data for the Patos/Guaíba system or the nearby coastal lagoons of Rio Grande do Sul provide very similar or very divergent information (Tab. 3; Hartz, Barbieri, 1994; Fialho *et al.*, 1998; Schifino *et al.*, 1998; Becker, 2001; Nunes *et al.*, 2004; Maia *et al.*, 2013). One example is *C. voga.* For this species, published information on the reproductive season varies from a long warm season, excluding winter (September to April; Hartz, Barbieri, 1994) to a winter-spring estimate (Schifino *et al.*, 1998). Our data differ from both, estimating a long reproductive period beginning in late summer and ending in early spring. Nevertheless, analyzing the original data from both publications indicates that discrepant results seem to be due to species plasticity in relation to the sampling site and environmental variability, not to the method of analysis.

Tab. 3. Reproductive season and core reproductive period for dominant fish species in the Patos Lagoon basin, according to published data. Symbols indicate the references for species.

Species	Reproductive Season	Core Reproduction	Source
Astyanax fasciatus	Sept. to Jan.		Nomura (1975)
Cyphocharax voga	*Sept. to Apr. ‡ early winter to late spring	*‡Sept./Oct.	*Hartz, Barbieri (1994); ‡Schifino *et al.* (1998)
Hoplias malabaricus	*Sept. to Dec. ‡Annual	Nov.	*Barbieri (1989); ‡Araújo-Lima, Bittencourt (2001)
Oligosarcus jenynsii	* July to Oct. ‡May to Oct.	*July to Oct. ‡July/Aug.	*Fialho *et al.* (1998); ‡Nunes *et al.* (2004)
Oligosarcus robustus	May to Aug.	July/Aug.	Nunes *et al.* (2004)
Hoplosternum littorale	*Oct. to Jan. ‡Dec. to Mar.	‡Feb./Mar.	*Mesones *et al.* (1998); ‡Sá-Oliveira, Chellappa (2002)
Loricariichthys anus	Nov. to Mar.	Nov.	Bruschi Junior *et al.* (1997)
Parapimelodus nigribarbis	-	-	-
Trachelyopterus lucenai	*Nov. to Feb. ‡Oct. to Mar.	*Dec./Jan. ‡Nov./Dec.	*Becker (2001); ‡Maia *et al.* (2013)
Pachyurus bonariensis	*Spring ‡Oct. to Feb.	‡Nov. to Jan.	*Flores, Hirt (2002); ‡Lagemann, Fialho (2014)

On the other hand, considering the results for most of the species, the published reproductive periods derived from morphological interpretation are usually shorter, but with marked overlap with the estimated months with reproduction (using the G_{20} cut-off), or the core reproductive months (using the G_{30} cut-off). So, a new question arises: is a longer estimate of the reproductive period a problem? This depends on the objective. According to our proposal, if a month is set as reproductive, at least one female was caught that was already maturing for reproduction, or that had spawned recently. The problem is not about whether reproduction alone, but how much reproduction is occurring. The proposed criterion for the core reproductive period (G_{30} cut-off value) identifies a period when at least 50% of the females (50% above median) are showing relatively high values of GSI (30% of the maximum), i.e., close to spawning or that had just spawned. This seems very reasonable, although still artificial. However, considering a win-or-lose balance, the proposal of a well-delimited threshold makes comparisons over space and time much more objective, as it does not depend on the subjective interpretation of maturity scales. Defining the limits of a reproductive cycle could be based on a mathematical consensus, analogous to L_{50} for size at first maturity, and is a good starting point for standardization.

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References

Araújo-Lima CARM, Bittencourt MM. A reprodução e o início de vida de *Hoplias malabaricus* na Amazônia Central. Acta Amaz. 2001; 31(4):693-97.

Barbieri G. Dinâmica da reprodução e crescimento de *Hoplias malabaricus* (Bloch, 1794) na represa de Monjolinho, São Carlos/SP. Rev Bras Zool. 1989; 6(2):225-33.

Becker FG. Observations on the reproduction, sex ratio and size composition of *Trachelyopterus lucenai* (Teleostei, Auchenipteridae) in lake Guaíba, RS, Brazil. Biociências. 2001; 9(2):85-96.

Bertaco VA, Ferrer J, Carvalho FR, Malabarba LR. Inventory of the freshwater fishes from a densely collected area in South America: a case study of the current knowledge of Neotropical fish diversity. Zootaxa. 2016; 4138(3):401-40.

Bruschi Junior W, Peret AC, Verani JR, Fialho CB. Crescimento de *Loricariichthys anus* (Valenciennes, 1840) na Lagoa Emboaba, Osório, RS. Bol Inst Pesca. 1997; 24:65-71.

Ceni G, Fontoura NF, Cabral HN. The freshwater artisanal fishery of Patos Lagoon. J Fish Biol. 2016; 89(1):337-54.

CPC (Climate Prediction Center Internet Team). Climate Prediction Center: cold & warm episodes by season [Internet]. Maryland: NOAA & National Weather Service. 2017. [updated in September 22, 2017]; available from: http://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php

Fialho CB, Schifino LC, Verani JR. Biologia reprodutiva de *Oligosarcus jenynsii* (Güther) (Characiformes, Characidae) da Lagoa das Custódias, Tramandaí, Rio Grande do Sul, Brasil. Rev Bras Zool. 1998; 15(3):775-82.

Flores SA, Hirt LM. Ciclo reproductivo y fecundidad de *Pachyurus bonariensis* (Steindachner, 1879), Pisces, Scianidae. Bol Inst Pesca. 2002; 28(1):25-31.

Fontoura NF, Braun AS, Milani PCC. Estimating size at first maturity (L_{50}) from Gonadossomatic Index (GSI) data. Neotrop Ichthyol. 2009; 7(2):217-22.

Fontoura NF, Vieira JP, Becker FG, Rodrigues LR, Malabarba LR, Schulz UH, Möller OO, Garcia AM, Vilella FS. Aspects of fish conservation in the upper Patos Lagoon basin. J Fish Biol. 2016; 89(1):315-36.

Hartz SM, Barbieri G. Dinâmica da reprodução de *Cyphocharax voga* (Hensel, 1869) da lagoa Emboaba, RS, Brasil (Characiformes, Curimatidae). Rev Bras Biol. 1994; 54(3):459-68.

Kjerve B. Comparative oceanography of coastal lagoons. Esturine Variability. 1986; 1986:63-81.

Lagemann GI, Fialho CB. Biologia reprodutiva de *Pachyurus bonariensis* (Perciformes, Sciaenidae) na fase pré-represamento do arroio Taquarembó, Sul do Brasil. Iheringia, Sér Zool. 2014; 104(2):216-22.

Maia R, Artioli LGS, Hartz SM. Diet and reproductive dynamics of *Trachelyopterus lucenai* (Siluriformes: Auchenipteridae) in subtropical coastal lagoons in southern Brazil. Zoologia. 2013; 30(3):255-65.

Malabarba LR. Histórico sistemático e lista comentada das espécies de peixes de água doce do sistema da Laguna dos Patos, Rio Grande do Sul, Brasil. Com Mus Ciênc Tecn PUCRS, Sér Zool. 1989; 2(8):107-79.

Marques CS, Braun AS, Fontoura NF. Estimativa de tamanho de primeira maturação a partir de dados de IGS: *Oligosarcus jenynsii, Oligosarcus robustus, Hoplias malabaricus, Cyphocharax voga, Astyanax fasciatus* (Characiformes), *Parapimelodus nigribarbis, Pimelodus maculatus, Trachelyopterus lucenai, Hoplosternum littorale, Loricariichthys anus* (Siluriformes) e *Pachyurus bonariensis* (Perciformes) no Lago Guaíba e Laguna dos Patos, RS. Biociências. 2007; 15(2):230-56.

Mesones RV, Nieva L, Gonzo G. Ciclo sexual y organización histológica de las gonadas de *Hoplosternum littorale* (Pisces, Siluriformes, Callichthyidae) del río Bermejo, Salta, Argentina. Bol Soc Biol Concepc, 1998; 69:211-20.

Milani PCC, Fontoura NF. Diagnóstico da pesca artesanal na lagoa do Casamento, sistema nordeste da laguna dos Patos: uma proposta de manejo. Biociências. 2007; 15(1):82-125.

Nomura H. Fecundidade, maturação sexual e índice gônado-somático de lambaris do gênero *Astyanax* Baird & Girard, 1854 (Osteichthyes, Characidae), relacionados com fatores ambientais. Rev Bras Biol. 1975; 35(4):775-98.

Nunes DM, Pellanda M, Hartz SM. Dinâmica reprodutiva de *Oligosarcus jenynsii* e *O. robustus* na lagoa Fortaleza, Rio Grande do Sul, Brasil. Iheringia, Sér Zool. 2004; 94(1):5-11.

Reis RE, Kullander SO, Ferraris CJ, Jr., organizers. Check list of the freshwater fishes of South and Central America. Porto Alegre: Edipucrs; 2003.

Sá-Oliveira JC, Chellappa S. Fecundidade e tipo de desova do tamuatá, *Hoplosternum littorale* Hancock (Osteichthyes, Siluriformes) no Rio Curiaú, Macapá, Amapá. Rev Bras Zool. 2002; 19(4):1053-56.

Schemmel E, Friedlander AM, Andrade P, Keakealani K, Castro LM, Wiggins C, Wilcox BA, Yasutake Y, Kittinger JN. The codevelopment of coastal fisheries monitoring methods to support local management. Ecol Soc. 2016; 21(4):34[16p.]. https://doi.org/10.5751/ES-08818-210434

Schifino LC, Fialho CB, Verani JR. Reproductive aspects of *Cyphocharax voga* (Hensel) from Custódias Lagoon, Rio Grande do Sul, Brazil (Characiformes, Curimatidae). Rev Bras Zool. 1998; 15(3):767-73.

Villwock JA. Aspectos da sedimentação da região nordeste da Lagoa dos Patos: Lagoa do Casamento e Saco do Cocuruto. Pesquisas em Geociências. 1978; 11(1):193-223.

Wootton RJ, Smith C. Reproductive biology of teleost fishes. Oxford: John Wiley & Sons; 2015.

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