Neotropical Ichthyology, 10(4):847-854, 2012

Reproductive strategies of two Curimatidae species in a Mogi Guaçu impoundment, upper Paraná River basin, São Paulo, Brazil

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Considering that reproduction studies are fundamental to understand the life cycle of organisms, this study aimed to investigate the reproductive strategies of saguirus *Cyphocharax nagelii* and *Steindachnerina insculpta* in a dam of Mogi Guaçu River, (SP). Specimens were collected between August 2005 and July 2006 using gillnets with mesh sizes ranging from 1.5 to 5.0 cm between adjacent knots. Reproductive dynamics, body condition, reproductive period, spawning type and fecundity were analyzed in both species. The body condition reflected the reproductive dynamics, and differed significantly between the wet and dry seasons. During the dry season, the feeding activity and the accumulation of fat in the visceral cavity were higher. The reproductive period of both species covered the months between August and February, with peaks in October and December, and the distribution of the diameters of oocytes allowed to classify them as total spawners. The fecundity increased with weight and length, reaching 28,800 and 27,906 oocytes per female for *C. nagelii* and *S. insculpta*, respectively, whereas the amplitude of oocyte diameters varied between 50-1025 µm for the former and 75-975 µm for the later species. Taking into account that species with different reproductive strategies also respond differently to environmental impacts, our findings provide important information to subsidize management plans for these curimatid species.

Considerando que o conhecimento da reprodução é fundamental para a compreensão do ciclo de vida dos organismos, este trabalho buscou avaliar as estratégias reprodutivas dos saguirus *Cyphocharax nagelii* e *Steindachnerina insculpta* em um represamento no rio Mogi Guaçu (SP). Os exemplares foram coletados entre agosto de 2005 e julho de 2006 com redes-de-espera (com tamanhos de malha variando entre 1,5 e 5,0 cm entre nós adjacentes). A dinâmica reprodutiva, a condição corporal, o período reprodutivo, o tipo de desova e a fecundidade foram os parâmetros reprodutivos analisados nas duas espécies. A condição corpórea refletiu a dinâmica reprodutiva, sendo significativamente diferente entre os períodos seco e chuvoso. Durante o período seco, a atividade alimentar e o acúmulo de gordura na cavidade visceral foram maiores. O período reprodutivo das duas espécies abrangeu os meses entre agosto e fevereiro, com picos em outubro e dezembro, e a distribuição dos diâmetros de ovócitos permitiu classificá-las como desovadoras totais. A fecundidade aumentou com o peso e com o comprimento, atingindo em média 28.800 e 27.906 ovócitos por fêmea, para *C. nagelii* e *S. insculpta*, respectivamente, ao passo que a amplitude de diâmetros ovocitários variou de 50 a 1.025 µm para a primeira e de 75 a 975 µm para a segunda espécie. Considerando que espécies com estratégias reprodutivas diferentes também respondem de modos diferentes aos impactos ambientais, as informações obtidas são importantes subsídios para nortear medidas de manejo para essas espécies de curimatídeos.

Key words: *Cyphocharax*, Fecundity, Spawning, *Steindachnerina*, Toothless characin.

Introduction

The ichthyofauna in the upper Paraná River basin in Brazil is very diversified (Langeani *et al.*, 2007) and has been suffering a series of human impacts, such as dam construction, which is present in almost all of major rivers of São Paulo (SP) state, in southeastern Brazil (Agostinho *et al.*, 2007). The damming of rivers transforms the aquatic environment (Castro & Arcifa, 1987; Nilsson *et al*., 2005), which favors some fish species, but has deleterious effects in others. Species that are able to adapt to the lentic environment created by the dam can be benefited by the change, becoming abundant or even dominant in these environments, such as fishes of Curimatidae family (Castro & Arcifa, 1987; Barbieri, 1995; Loureiro-Crippa & Hahn, 2006; Novakowski *et al.*, 2007; Gonçalves & Braga, 2008).

Members of Curimatidae family (order Characiformes) are popularly known as saguirus, birus or manjubas, and comprises eight genera and 97 species widely distributed on Neotropical region (South America and Southeast Central America). The two studied species herein, *Cyphocharax*

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nagelii (Steindachner, 1881) and *Steindachnerina insculpta* (Fernández-Yépez, 1948), occurs in upper Paraná River basin (Brazil) (Vari, 2003). Curimatids have adaptations to iliophagous feeding habit (*i.e.* teeth are absent in adults, species have long intestines and many gill rakers) to provide greater efficiency in the use of the fine particulate organic matter present in sediment (Bowen, 1983; Vari, 1989; Silva *et al.*, 2005). Most curimatids are small-sized and fusiform-bodied (Vari, 2003). Detritivorous fishes play an important role in environments they inhabit, accelerating the recycling of organic matter (Bowen, 1983; Sazima & Caramaschi, 1989; Fugi *et al.*, 1996; Giora & Fialho, 2003). Besides its ecological importance, these species are an important fishery resource in certain regions, such as Amazon (Araujo-Lima *et al.*, 1986), and also represents an important food resource to piscivorous fish (Novakowski *et al.*, 2007). Regarding reproduction, curimatids may have long reproductive periods and parceled spawning with peaks occurring in warmer and wet months (Romagosa *et al.*, 1984; Barbieri, 1995; Holzbach *et al.*, 2005; Ribeiro *et al.*, 2007). However, some species perform short reproductive migrations (Godoy, 1975) and have total spawning (Alvarenga *et al.*, 2006).

Reproduction studies are important to understand the life cycle of fishes, mainly in order to establish management policies of fishery resources and species conservation (Vazzoler & Menezes, 1992; Casimiro *et al*., 2011). In this context, this study aimed to investigate the reproductive strategies of two curimatids, *Cyphocharax nagelii* and *Steindachnerina insculpta*, in the reservoir of a Small Hydro Power (SHP) of Mogi Guaçu River (SP), upper Paraná River basin.

Material and Methods

Study area

The SHP Mogi Guaçu (22°21'S 46°51'W) is located in Mogi Guaçu municipality, São Paulo state. This reservoir has 5.73 km^2 of area, extension of 8 km and 32.89 x 10^6 m^3 of volume to supply water to neighboring municipalities and to generate approximately 7.2 MW of electricity. Other characteristics are: barrage with extension of 150 m, water fall with 7 m, two installed turbines, four flood gates and a fish ladder with 21 step-tanks for fish passage (Brandimarte *et al.*, 2005; AES-Tietê, 2011). At the sampled site, bottom is muddy or sand-muddy, maximum depth is around 5 m and the border vegetation is dominated by sugar cane and pastures (Gonçalves & Braga, 2008). The analysis of temperature and rainfall in the studied area (average of 30 years, 1971 to 2005, data from meteorological station D4-100 located at Campininha farm) allowed the determination of dry (April to September) and wet (October to March) periods.

Sampling methods and data processing

Samples were taken between August 2005 and July 2006, comprising six samples (August, October and December 2005; February, April and June 2006). Sampling effort was standardized using gillnets with 40 m in length and with mesh sizes of 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 cm between adjacent knots, which were set in the afternoon and removed in the morning of the following day. Fishes were fixed in formalin 10% and kept in alcohol solution 70%. Voucher specimens were stored in the fish collection of Department of Zoology and Botany, IBILCE/UNESP, Brazil (*C. nagelii* DZSJRP 13267; *S. insculpta* DZSJRP 13266-68).

A total of 524 individuals of *C. nagelii* and 250 individuals of *S. insculpta* were analyzed, comprising 774 specimens. In the laboratory, the following data were obtained of each specimen: sex, standard length (mm) and total weight (g). Stomachs were visually classified according to its repletion: 1 (empty), 2 (partially full) and 3 (full). Visceral cavity was visually analyzed: 1 (no fat), 2 (partially with fat) and 3 (full). Mature ovaries were visually defined considering color, transparency, vascularization of gonads, oocytes appearance and visualization. Then, ovaries were weighted and kept in Gilson's solution until fecundity analysis (Vazzoler, 1996).

Data analysis

Reproductive dynamics. Reproductive dynamics was analyzed to each species by variations of stomach repletion and fat accumulation on visceral cavity. The chi-square test was used in order to verify the dependence of these variables between dry and wet periods (Zar, 2010).

The gonadosomatic relationship (*GSR*) was obtained: $GRS = W_O/W_T * 100$, where W_O is the ovarian weight (g), and W_T is the total weight (g) of the fish (Vazzoler, 1996). The reproductive period was determined analyzing the frequency of mature females bimonthly and *GSR* mean values. In order to verify the correspondence of mature females bimonthly, a chi-square test was applied (Zar, 2010).

Body condition. An analysis of covariance was performed to evaluate differences in weight (dependent variable) between species, periods (dry and wet) and gender (factors), using the standard length as covariate (Zar, 2010), following the model: $log(W_{ij}) = \mu + S_i + \beta_i (log\, L_{ij} - log\, L_i) + \varepsilon_{ij}$, where W_{ij} is the total weight of the individual *j* in sex i (male or female), μ is the population average, S_i is the effect of sex *i*, L_{ij} is the standard length of individual *j* in sex *i*, L_i is the average standard length of sex *i* and ε_{ij} is the random error associated to individual *j* in sex *i*. The objective was to determine whether, for the same length, the total weight varied between species, periods (dry and wet) or gender. Body-length difference between gender was verified by Kolmogorov-Smirnov test (Lilliefors test was used to check the normality of data and indicated a non-normal distribution). The level of significance was $\alpha = 5\%$ in all analyses (Zar, 2010).

Fecundity and spawning type. To evaluate fecundity, 121 and 47 pairs of mature ovaries of *C. nagelii* and *S. insculpta* were analyzed, respectively, using the volumetric method (Vazzoler, 1996 modified).The dissociated oocytes were placed in an Erlenmeyer flask with a constant volume of 70% alcohol (S). After shaking the flask, a sample of 2 ml (*s*) was taken with a

Stempel pipette. The counting of vitellogenic oocytes (perinucleolar oocytes were not considered) was performed under a stereomicroscope (40x magnification). The total number of oocytes (N) was estimated assuming: $N = (S * n)/s$, in which S is the volume of solution containing the oocytes in suspension, n is the number of vitellogenic oocytes, and s is the volume of a sample with 2 ml from each pair of ovaries analyzed. A t-test was performed to assess differences in fecundity between species (Zar, 2010). Subsequently, 100 oocytes were randomly selected and measured with an ocular micrometer (10x magnification). A Mann-Whitney test was performed to assess differences in oocytes diameters between species. For each gonad in which mature oocytes were counted and measured, a frequency distribution graph of oocytes diameters was constructed. These charts were compared and those with the same modal diameter were grouped. The frequency distribution of oocytes diameters of ovaries was graphically analyzed to determine the spawning type (total or multiple).

The population fecundity (Vazzoler, 1981) was estimated by correlating the fecundity with total weight and also with standard length, according to the expression: $F = A + BX$, where F is fecundity, X is the explanatory variables (weight or length), A the numerical value of the intercept, and B is the slope, estimated by the method of least squares after logarithmic transformation of the data (Sparre & Venema, 1997).

Results

Reprodutive dynamics

Significant differences were found on stomach repletion and fat accumulation degrees between dry and wet seasons to *C. nagelii* ($\chi^2 = 11.08$, df = 2, $p < 0.05$; $\chi^2 = 265.12$, df = 2, $p < 0.05$, respectively) and *S. insculpta* ($\chi^2 = 34.37$, df = 2, $p < 0.05$; $\chi^2 = 148.4$, df = 2, $p < 0.05$, respectively). Both species had higher frequency of full stomachs during the dry period (Fig. 1). Fat acumulation was low during the wet period, suggesting that fat accumulated during the period of highest feeding activity (dry season) was consumed in the reproductive season (Fig. 2).

Despite differences in the GRS peaks, *C. nagelii* showed a larger reproductive period covering both dry (August) and wet seasons (October and December) ($\chi^2 = 83.4$, df = 5, $p < 0.0001$), whereas *S. insculpta* concentrated its reproductive activity in wet season (October and December) ($\chi^2 = 261.1$, df = 5, $p < 0.0001$) (Fig. 3). The maximum GRS values obtained was 23.8% and 25.5%, to *C. nagelii* and *S. insculpta*, respectively, and the bimonthly variation of GRS for each species are presented in Fig. 3.

Body condition

The body condition was assessed to identify differences in the relationship between weight and length of species (Table 1). There was no significant interaction between species and the covariate length, which indicate that length-weight relationship is the same to *C. nagelii* and *S. insculpta* ($p = 0.592$), but body condition was significantly different between dry and wet periods ($p < 0.05$). There was no significant difference in the length-weight relationship between genders (Table 2), although females were larger than males in *C. nagelii* ($\text{mean}_{(\text{females})} = 106.2 \pm 14.3$ mm sd, $\text{mean}_{(\text{males})} = 102.4 \pm 14.1$ mm sd, D = 0.1251, $p < 0.05$) and in *S. insculpta* ($\text{mean}_{(\text{females})} = 101.4 \pm 10.6$ mm sd, $\text{mean}_{(\text{males})} = 101 \pm 10.6$ mm sd, D = 0.1850, $p < 0.05$).

Fecundity and spawning type

Fecundity was estimated from the analysis of mature ovaries of *C. nagelii* and *S. insculpta*, and ranged from 3,935 to 43,659 oocytes (mean = 17,560 ± 9,106.32 sd), and from 10,964 to 42,786 oocytes (mean = 26,815 ± 8,333.47 sd) (t = -4.64, df = 166, $p < 0.0001$), for each species, respectively.

The amplitude of oocytes diameters ranged from 50 to 1,075 µm to *C. nagelii* and from 75 to 975 µm to *S. insculpta* (U = 590, $p < 0{,}01$). In the oocyte's distribution of *C. nagelii* (Fig. 4) it is possible to note a continuous gradient of development (Fig. 4). Group I show two modes: one mode is

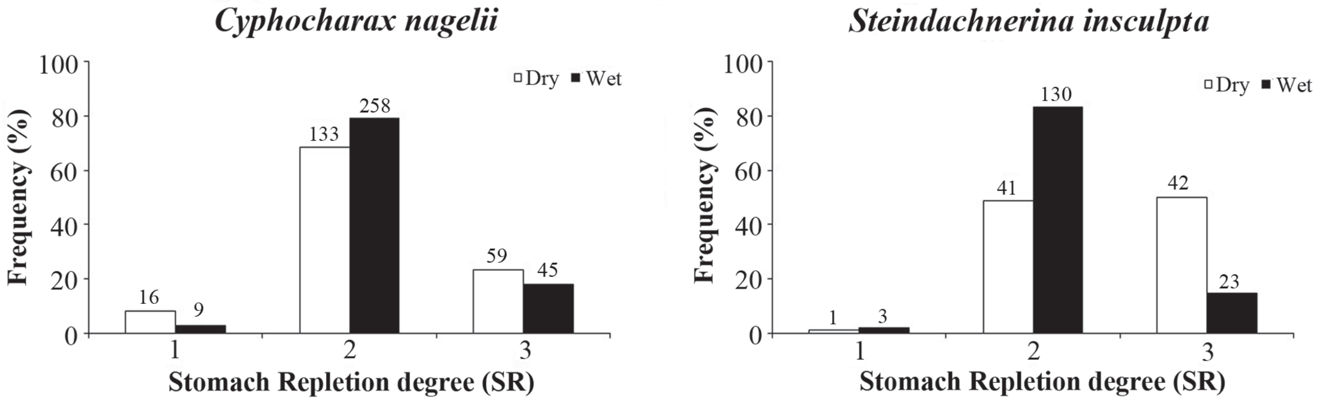

Fig. 1. Stomach repletion degree (SR) of Curimatidae species during dry and wet seasons. 1: empty stomachs, 2: partially filled stomachs, and 3: full stomachs. Values above bars represent the absolute number of stomach repletion degrees obtained in each period.

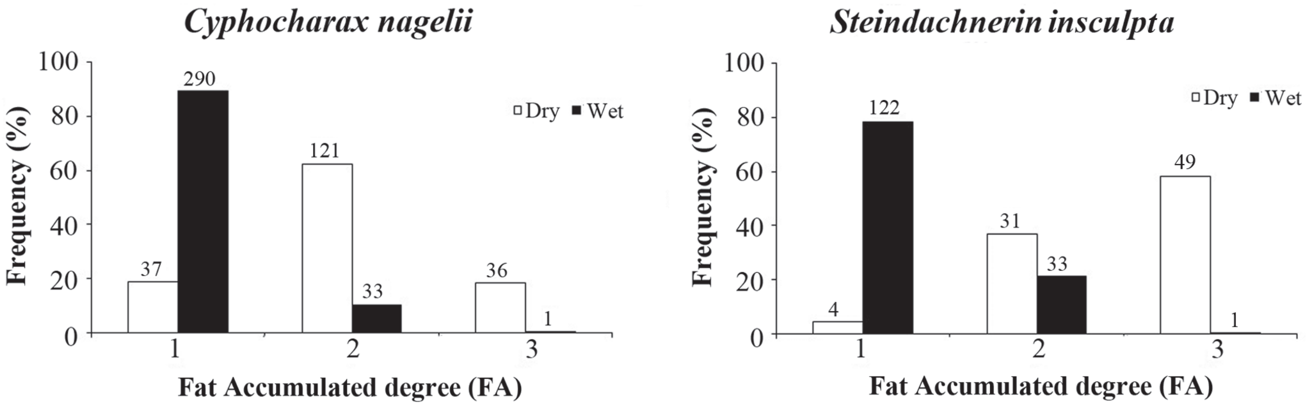

Fig. 2. Fat accumulated degree (FA) of Curimatidae species during dry and wet seasons. 1: no fat, 2: visceral cavity partially filled with fat, and 3: visceral cavity completely filled with fat. Values above bars represent the absolute numbers of fat accumulated degrees obtained in each period.

represented by oocytes with smaller diameters, and the other mode represented by oocytes with larger diameter. The modal oocyte diameter gradually increases from the first to the third group of distribution. *Cyphocharax nagelii*'s GSR follows this trend, increasing steadily from group I to group III (Table 3). *Steindachnerina insculpta* presented the same pattern of oocytes distribution, similar to group III of *C. nagelii*, although the smaller oocytes were less conspicuous (Fig.5).

Fecundity (F) increased with weight (W) and length (L) in *C. nagelii* (F = 10616 + 287.42W and F = -14383 + 326.06L), and in *S. insculpta* (F = -6083.7 + 656.9W and F = -40603.4 + 641.3L) (Fig. 6).

Discussion

Fish are able to adapt to environments in which biotic and abiotic conditions can vary widely, allocating energy by feeding that, in turn, is converted to offspring, increasing their reproductive success (Vazzoler, 1996). In general, fat accumulation occurs in the dry period, when the foraging activity is higher and the gonads are resting, whereas fat levels decrease later, coinciding with the maturation of the gonads and subsequent spawning (Alvarenga *et al.*, 2006; Braga *et al.*, 2008; Carmassi *et al.*, 2008; Giora & Fialho, 2003; Ribeiro *et al.*, 2007).

In the studied area, the body condition of *C. nagelii* and *S. insculpta* was not different either between species or between gender, but there were differences between dry and wet seasons. These results indicate that males and females of both species probably use the environmental resources in a similar way. The body condition differences between dry and wet seasons for both species corresponded to their reproductive dynamics, which indicated that feeding activity and fat accumulation were more intense during the dry season. The decrease of feeding activity observed during the wet

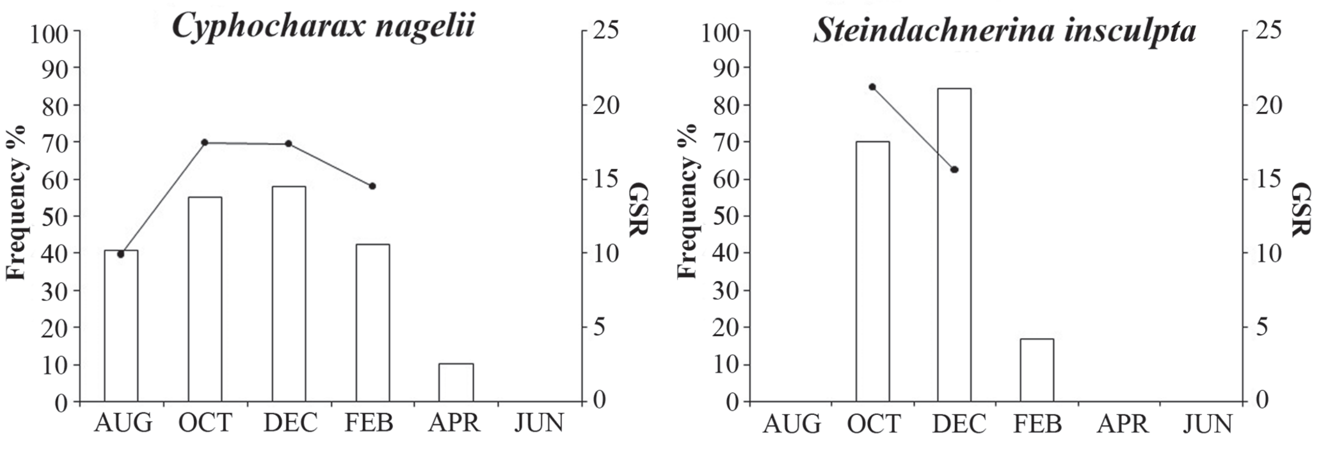

Fig. 3. Proportion of mature females (%) (columns) and values of gonadossomatic relationship (GSR) (lines) of both studied Curimatidae species between August 2005 and June 2006. Dry period: April-August; wet period: October-February.

Table 1. Covariance analysis for the natural logarithm (Ln) of total weight (TW - dependent variable) in relation to the following factors: standard length (SL - covariate), species (*Cyphocharax nagelii* and *Steindachnerina insculpta* levels), and periods (dry and wet levels) (n = 695; $r^2 = 0.93$).

Sources of variation	Sum of Squares	df	Mean Square	F	P
LnSL	50.823	1	50.823	4855.286	<0.001
Species	0.003	1	0.003	0.241	0.624
Periods	0.052	1	0.052	4.956	0.026
LnSL*Species	0.003	1	0.003	0.288	0.592
LnSL*Periods	0.054	1	0.054	5.172	**0.023**
Species*Periods	0.002	1	0.002	0.189	0.664
LnSL*Species*Periods	0.002	1	0.002	0.197	0.657
Residual	7.191	687	0.010		

season for both species may be due to compression of internal organs caused by the gonads growth (Slotte, 1999), or due to rainfall that makes difficult the sedimentation of organic matter in the water (Teixeira & Gurgel, 2004), and could compromise feeding of curimatid species.

The GSR index indicates the degree of ovarian development, therefore, more developed ovaries present higher GSR values. This relationship between oocytes development and GSR supports the ovarian dynamics due to the accumulation of yolk in oocytes (West, 1990). According to the bimonthly frequency of mature females and GSR values, the reproductive period of the two studied species was concentrated in the wet season, which has been also reported to other curimatids (Braga, 1990; Carmassi *et al.*, 2008). Moreover, Godoy (1975) observed *C. nagelii* and *S. insculpta* performing massive reproductive migrations in Cachoeira de Emas (located downstream the studied area herein), with spawning occurring between November and January.

The ovaries have a seasonal cycle of oocytes development, associated with the vegetative functions of fish (Nikolsky, 1963; Nikolskii, 1969). Inside the ovaries, the germinal epithelium is in constant cellular activity producing oocytes, which results in the presence of oocytes at different stages of development within the same ovary (Wallace & Selman, 1981). The ovaries examined in this study were considered mature, but oocytes were at different stages of development (less developed and

Table 2. Covariance analysis for the natural logarithm (Ln) of total weight (TW - dependent variable) in relation to standard length (SL - covariate) and gender (males and females) factors for *Cyphocharax nagelii* and *Steindachnerina insculpta*.

Sources of variation	*Cyphocharax nagelii* (n = 477, $r^2 = 0.95$)			*Steindachnerina insculpta* (n = 218, $r^2 = 0.91$)		
	df	F	P	df	F	P
LnSL	1	7456.831	<0.001	1	1724.173	<0.001
Gender	1	0.753	0.386	1	0.366	0.546
Gender * LnSL	1	1.010	0.315	1	0.518	0.473
Residual	473			214		

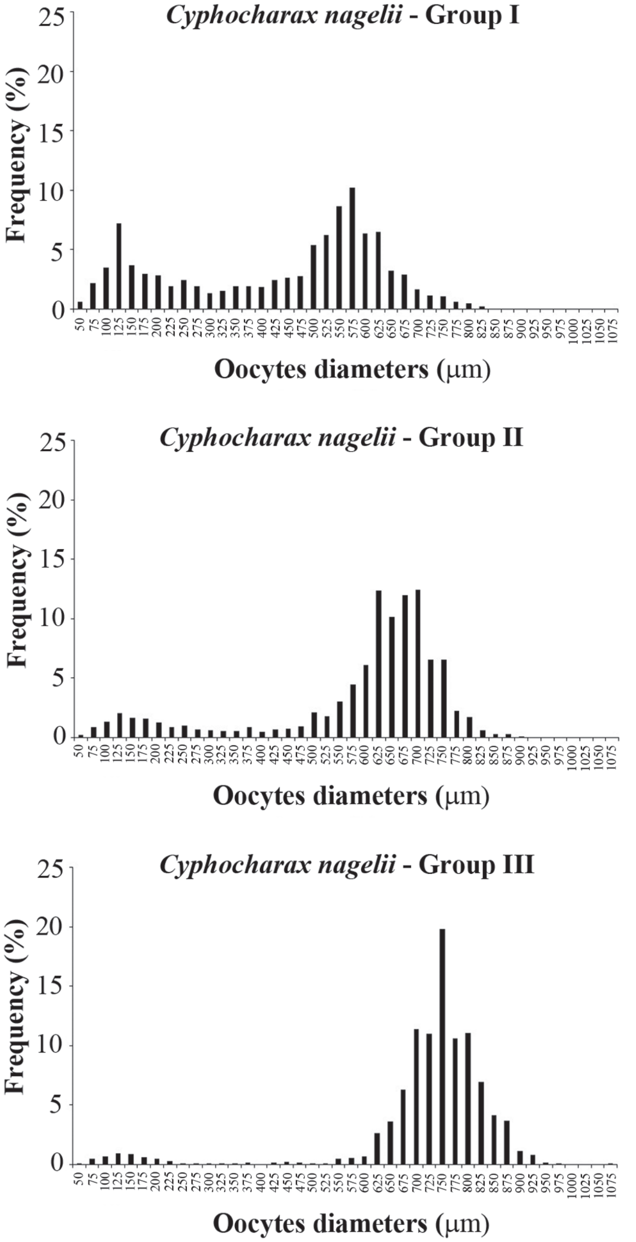

Fig. 4. Frequency distribution of oocytes diameters of *Cyphocharax nagelii* according to the developmental groups.

Table 3. Number of mature females analyzed (N), mean fecundity (and range), and mean gonadosomatic relationship (GSR) to Curimatidae species.

Species	Groups	N	Fecundity	GSR
Cyphocharax nagelii	I	16	18,000 (8,550 - 35,775)	8.27
	II	78	20,112 (4,800 - 44,175)	13.07
	III	27	25,488 (10,800 - 42,825)	18.22
Steindachnerina insculpta		47	26,815 (10,964 - 42,786)	17.5

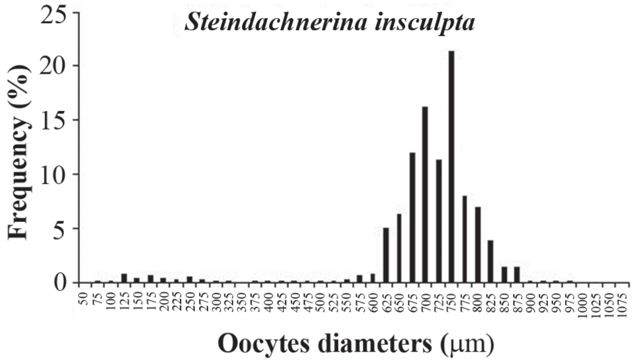

Fig. 5. Frequency distribution of oocytes diameters of *Steindachnerina insculpta*.

more developed). The less developed oocytes (non-vitellogenic) occurred in smaller proportion, when compared to mature oocytes or in the process of maturation, and were not considered. The spawning type is determined by the interaction between the dynamics of oocyte development, the frequency of spawning within a breeding season and the frequency of this events during his life (Araujo, 2009). Vazzoler (1996) described the dynamics of oocytes development as follow: (i) synchronous in one group (ii) synchronous in two groups, and (iii) synchronous in more than two groups. In the second case, in each reproduction period, two batches of oocytes are evident within the ovaries: the reserve stock and the batch of oocytes that will mature and be eliminated during spawning. In this study, oocyte development groups of *C. nagelii* showed a steady increase in the modal diameter, with a consequent disappearance of the smaller batch of oocytes (more evident in group I), leaving only one batch of oocytes in group III. Romagosa *et al*. (1984), analyzing the curimatid *Curimata gilberti* (= *Cyphocharax gilberti*), showed that oocytes with diameters up to 200 µm compose the oocytes group that is coming out of the lot stock, and does not participate in spawning. In the present work, the smaller batch of oocytes present in groups I and II are located precisely in this size range. The continuous increase in the GSR from group I to group III also indicates further development of the ovaries from group I to group III, since the GSR is indicative of the stage of maturity. In *S. insculpta*, only one group was formed, with characteristics very similar to group III of *C. nagelii*, including GSR values. According to these findings, we concluded that *C. nagelii* and *S. insculpta* presented one group of oocytes (not considering the batch stock). If the batch stock had been considered in our analysis, both species should share the same type of synchronous development in two groups, as described by Vazzoler (1996). As total spawners are species that mature and spawn only one batch of mature oocytes (*i.e.* ignoring the batch stock) per spawning season and multiple spawners release its oocytes in more than one batch during the spawning season (*e.g.* Godinho *et al*. 2010), we concluded that *C. nagelii* and *S. insculpta* are total spawners in the studied area.

West (1990) stated that the type of oocytes development is not necessarily an immutable characteristic of each species. Thus, the spawning type presented by a species reflects the

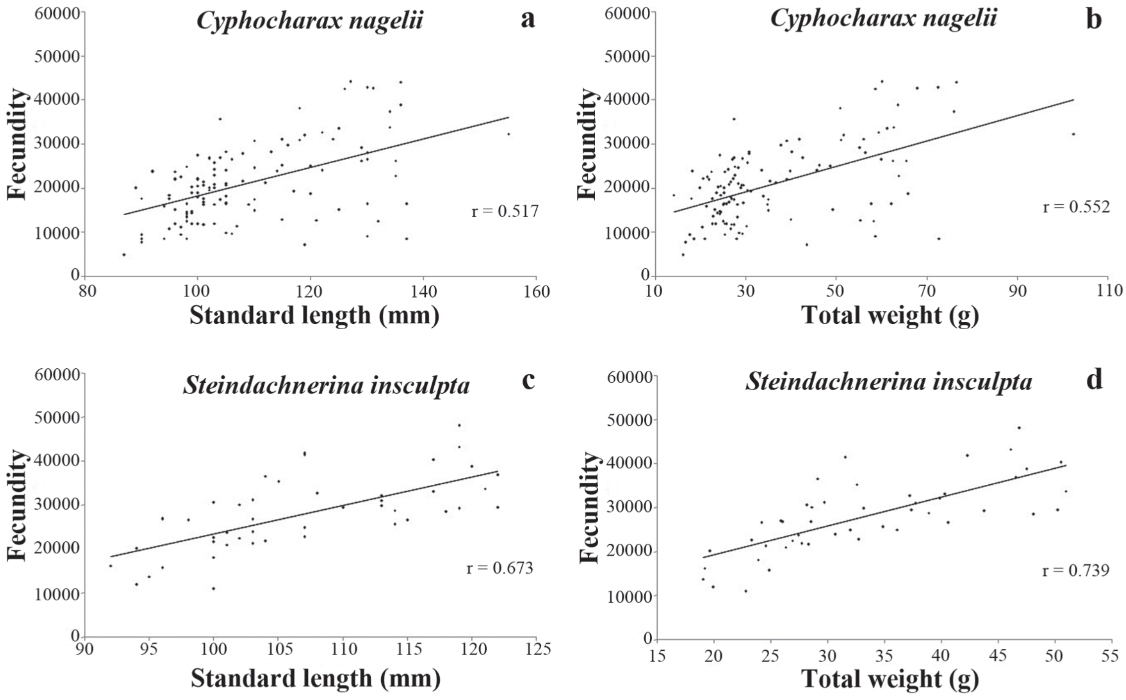

Fig. 6. Fecundity-standard length (mm) and fecundity-total weight (g) relationships to both studied Curimatidae species.

local characteristics of the environment it inhabit. For example, other studies with Curimatidae species indicated that they can be multiple spawners (Romagosa *et al*., 1984; Hartz *et al*., 1994; Barbieri, 1995; Holzbach *et al*., 2005; Ribeiro *et al*., 2007; Petesse *et al*., 2007) or total spawners (Alvarenga *et al*., 2006; Carmassi *et al*., 2008), but all these studies agreed that spawning peaks occurs during the months of highest rainfall and temperature, similar to our findings.

The high fecundity found in this study correspond to those reported in literature for other species of the family Curimatidae, which can vary from 4,380 to 100,130 oocytes (Nomura & Taveira, 1979; Romagosa *et al.*, 1984; Hartz *et al*., 1994; Schifino *et al*., 1998; Sampaio & Sato, 2007), as well as the oocyte diameters, that ranges from 427 to 4,789 µm in high Paraná River basin fishes (Vazzoler, 1996). For many authors, fecundity and oocyte diameters do not necessarily depend on fish size, but on reproductive tactics (Vazzoler, 1996; Araujo, 2009; Casimiro *et al*., 2011). Fecundity and oocyte diameters are reproductive traits that have many variations in inter- and intraspecific levels, depending on the food availability, how many times the fish spawned, temperature and altitude (Nikolsky, 1963; Hartz *et al*., 1999). Moreover, the fertility is inversely proportional to the parental care (Lagler *et al*. 1977), which corresponds to the results obtained here, since Curimatidae species do not perform parental care (Vazzoler, 1996).

There was a correlation between fecundity and length and between fecundity and weight to both Curimatidae species in the present study, which is common for several species (Martins-Queiroz *et al*., 2008; Agostinho & Júlio Jr, 1999). The positive correlations between fecundity and length or weight in fishes are evidences that larger females are more fecund, because the fecundity depends mainly on the size of coelomic cavity to accommodate the mature ovaries (Wootton, 1992), since the oocytes size does not increase with the size of the female.

Overall, our findings correspond to those available in the literature for species of Curimatidae family. Although the reproduction is an aspect of the life cycle more studied in fish, information on some reproductive tactics obtained here (e.g. spawning and fecundity) is still scarce (Potts & Wotton, 1984). Thus, besides filling a gap in knowledge regarding the reproduction of these species, our results may also be useful as a basis for comparison in future studies. Moreover, considering that fishes with different reproductive strategies respond in different ways to environmental impacts (Vazzoler & Menezes, 1992), our findings can be helpful to guide protection and management plans for these curimatid species.

Acknowledgments

The authors are grateful to Francisco Langeani for fishes identification, IBAMA for collection permits (02027.000991/2005-71), CAPES for dissertation fellowship granted to the second author, FAPESP (2009/52478-6) for grants received by the first author, and Haru Ushisawa Yamamoto for reviewing the English. This paper is part of graduation course of Alexandre Peressin concluded at UNESP (Rio Claro/SP).

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Submitted August 5, 2011
Accepted September 18, 2012