Seasonal changes in the gonadossomatic index, allometric condition factor and sex ratio of an auchenipterid catfish from eastern Amazonia

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The aim of the present study was to evaluate the seasonal pattern of the gonadosomatic index (GSI), condition factor (K), and sex ratio in the catfish *Auchenipterichthys longimanus* (Siluriformes: Auchenipteridae) as an approach to identify its reproductive period. A total of 589 *A. longimanus* specimens (251 males and 338 females) were captured in the rivers of the Caxiuanã National Forest, in the Brazilian state of Pará, between July, 2008 and July, 2009. Among the male specimens, 171 were classified as adults and 80 as juveniles, while there were 249 adults and 89 juvenile females. Using a sinusoidal equation, analysis of the GSI revealed a reproductive asynchrony between the genders, with males attaining their highest GSI values in January, while females peaked in March. For males, the sinusoidal regression for GSI values was significant only when used the complete data set (P=0.001), wears no trend was identified for bimonthly means (P=0.136). For females, by contrast, significant values were obtained for both the complete data set (P=0.012) and bimonthly GSI means (P=0.026). For the condition factor, the sinusoidal equation returned significant seasonal variation in both raw data (P=0.02) and with mean values (P=0.00) for males, but only with raw data for females (P=0.04), which appears to reflect variation in the energy budget between genders. With regard to the sex ratio, more reproductive females were captured than males in January and March, 2009, which suggests a pattern of segregation related to the reproductive process. These parameters are fundamental to the assessment, protection, and management of natural fish stocks, as well as providing guidelines for the development of conservation strategies.

O objetivo deste estudo foi avaliar alterações sazonais no índice gonadossomático (IGS%), fator de condição (K) e proporção sexual, a fim de determinar o período de atividade reprodutiva do bagre *Auchenipterichthys longimanus* (Siluriformes: Auchenipteridae), a partir da análise de exemplares coletados em igarapés da Floresta Nacional de Caxiuanã, estado do Pará, Brasil. Através de coletas bimestrais entre julho de 2008 e julho de 2009, foram capturados 589 exemplares de *A. longimanus*, sendo 251 machos e 338 fêmeas. Dentre os machos, 171 exemplares foram classificados como adultos e 80 foram jovens, e dentre as fêmeas, 249 eram adultas e 89 jovens. Por meio do estabelecimento de uma equação senoidal, a análise do IGS% evidenciou uma assincronia reprodutiva entre os sexos, pois os machos obtiveram maiores valores de IGS% em janeiro e as fêmeas apresentaram seu pico em março. Para os valores de IGS% de machos, a equação senoide mostrou-se significante somente para os valores brutos (P=0,001), sendo não identificada uma tendência com os valores médios do IGS% (P=0,136). Para as fêmeas, os valores de significância da equação senoide para o IGS% foram obtidos tanto para os dados brutos (P=0,012) quanto para os dados médios (P=0,026). Para o Fator de Condição, a equação senoide demonstrou variação nos valores brutos e médios de machos adultos (P=0,02 e P=0,00, respectivamente) e nos valores brutos de fêmeas (P=0,04), refletindo diferenças nos padrões de investimento energético entre os sexos. Em relação à proporção sexual, foi observada uma maior frequência de capturas de fêmeas reprodutivas em relação aos machos nos meses de Janeiro e Março de 2009, sugerindo um padrão de segregação sexual com fins reprodutivos. Esses parâmetros são fundamentais na avaliação, conservação e manejo dos estoques naturais de peixes, assim como para subsidiar estratégias e procedimentos para a preservação e conservação da ictiofauna.

Key words: *Auchenipterichthys longimanus*, Hydrological seasons, Reproduction, Siluriformes.

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Introduction

The reproductive cycle of fish inhabiting rivers with inundated riparian areas (várzea or igapó) are strongly influenced by the seasonal flood dynamics. As recognize by many authors (*e.g.* Goulding, 1980), the floodplain provides feeding sites, nursery areas and protection from predators for many fish species. According to the flood pulse concept, proposed by Junk *et al.* (1989), the annual floodplain inundation triggers blooms of primary and secondary production, and the fish species in these complex systems have reproductive ecologies adapted to exploit this pulse of production (Bailly *et al.*, 2008).

The understanding of these seasonal patterns provides an important baseline for the description of the fish biology and their role in aquatic ecosystems (Vazzoler, 1996). When direct inspection of gonadal maturation is not available, reproductive studies are frequently based on quantitative indices, as gonadosomatic index values (GSI) and condition factor (K), which help to define reproductive cycles and possible variation in the physiological condition of species during the course of its lifespan (Kreiner *et al.*, 2001; Braga, 2005).

The analysis of gonadosomatic index values (GSI), which provide a measure of gonad size relative to body weight (Wootton, 1991), can provide a quantitative assessment of the degree of gonadal development, the breeding season, and the reproductive cycle (Gutiérrez-Estrada *et al.*, 2000). The condition factor (K) is another commonly-used index in the study of fish biology, which provides information on the physiological state of these animals, based on the assumption that individuals of a given body length are in better condition when their mass is greater (Jones *et al.*, 1999; Anene, 2005). The condition factor depends on the coefficient of allometry (*b*) of the weight-length equation, which reflects the growth pattern of a species (Le Cren, 1951).

The inspection of the seasonal variation of the condition factor is also being used as a complementary parameter aiming to describe natural cycles in reproduction and feeding ecology (Braga, 1986; Lizama & Ambrósio, 2000; Anene, 2005). However, the condition factor can suffer from any change related to the growth cycle of fish, a pattern already identified for perciforms (Bervian *et al.*, 2006) and characiforms (Fontoura *et al.*, 2010). Therefore, it would be appropriate to analyze such quantitative indices considering different size classes, since the energy budget of an individual can changes during ontogeny (Swain & Foote, 1999).

Within teleost, several recent studies have demonstrated the important role played by seasonal floods on theses reproductive parameters both in temperate (Zeug & Winemiller, 2007, 2008) and tropical rivers (Agostinho *et al.*, 2004; Bailly *et al.*, 2008). Nevertheless, the available studies on the biology and ecology of Auchenipteridae catfishes focus on few species (Araújo *et al.*, 1999; Silva & Viana, 2003; Bailly *et al.*, 2008) and insufficient information are available for the driftwood catfish *Auchenipterichthys longimanus* (Günther, 1864).

Thus, the aim of the present study is to describe aspects of the reproductive cycle of the catfish *A. longimanus* by inspecting the seasonal cycles of gonadal maturity, variation in the gonadosomatic index, condition factor and sex ratio. Considering that fish species can modify the growth pattern during ontogeny, this paper aims also to evaluate changes in the condition factor concerning ontogenetic development.

Material and Methods

Samples were collected bimonthly between July, 2008 and July, 2009, in rivers located within the Caixuanã National Forest (1º45'27.5"S 51º27'33.2"W; Anapu River Basin), which are situated in the municipalities of Melgaço and Portel in the Brazilian state of Pará (Fig. 1). The sampling area is described as a blackwater river of a lowland amazon rainforest, being influenced both by the tide and by the seasonal oscillation of the Anapu River (Hida *et al.*, 1999). However, both flows have very low amplitude and annual fluctuation do not exceed one meter as a general average (Hida *et al.*, 1999). Typical flow cycle presents four hydrological periods (Fig. 2): drawdown (June-August), dry (September-November), filling (December-February) and flood (March-May).

Specimens of *A. longimanus* were collected using 100 m-long gillnets with a height of 1.5 m, and meshes of 30 mm and 60 mm. The nets were set for six hours per day at sites within the flooded forest sites selected with the guidance of a local fisherman. Following collection, the specimens were sexed, measured (total length in cm), and weighed (body weight and gonad weight, in grams).

Also following collection, the stages of gonadal maturation were determined through macroscopic characteristics of the
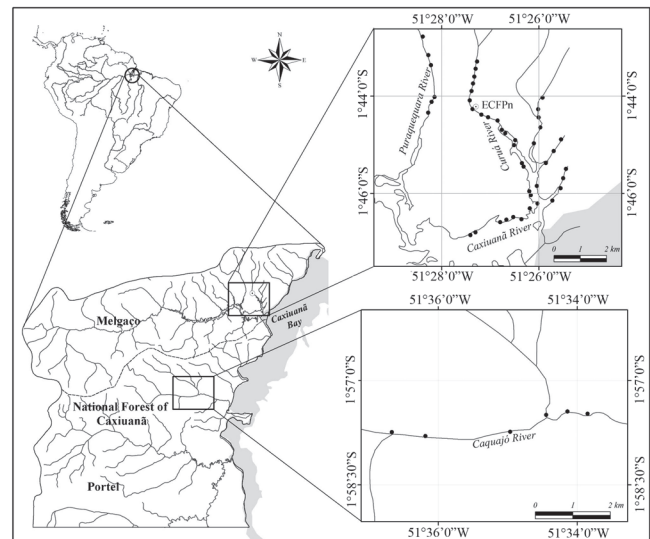

Fig. 1. Sampling area in the National Forest of Caxiuanã, Pará State, showing the rivers where the fish were collected. Curuá River - Ferreira Penna Research Station (ECFPn), Caxiuanã River; Puraquequara River and Caquajó River. Some black spots represent more than one collection site.

gonads following the general description proposed by Vazzoler (1981): stage I (immature); gonads of reduced size, translucent, located adjacent to the vertebral column; stage II (maturing); gonads occupying a third of the abdominal cavity with well-developed capillary network; stage III (mature); gonads turgid, occupying the majority of the abdominal cavity (in the females, the oocytes are visible to the naked eye, while in the males, the testes are whitish); and stage IV (at rest or empty); gonad completely flaccid with a hemorrhagic aspect.

Then they were fixed in 10% formalin for approximately 48 hours before being conserved in 70% ethanol. The specimens were subsequently deposited in the ichthyological collection of the Museu Paraense Emílio Goeldi (MPEG) in Belém, Pará State, registered under the numbers MPEG 15257-15259, MPEG 15260-15264, MPEG 15432-15442, MPEG 15498-15507, MPEG 15544-15555, MPEG 15844-15860, and MPEG 16221-16231.

The breeding season was identified by using two different approaches: (1) the seasonal variation concerning relative frequencies of ovarian and testis classified according to the maturation stage; and (2) through the analysis of the seasonal variation in the gonadosomatic index (GSI) (Vazzoler, 1996; Becker *et al.*, 2008; Carvalho *et al.*, 2009; Santos *et al.*, 2010).

The gonadosomatic index (GSI), defined as the percentage of the fish's total weight represented by the gonads (Vazzoler, 1996), was analyzed for each individual through the formula **G**SI=Wg/Wt*100; where Wg is the weight of the gonads (g); and W_t is the total weight of the individual (g). Considering that only maturing/mature/spent individuals are related with reproductive events, the GSI analysis included only adult individuals. It was applied the Spearman correlation for the breeding parameter (GSI) and the regional flood pulse, aiming identify possible relationships between riverine water level and reproductive activity.

The allometric condition factor (K) was analyzed for adults and juveniles separately, being estimated through the formula: $K=Wt/Lt^b$; where Lt is the total length (cm); and b is the coefficient of allometry of the weight-length relationship. Estimates of the size at first maturity and the allometric coefficients (b) for the weight/length relationship for *A. longimanus* follows Freitas (2010): males and females were considered adults with total lengths over 12.5 and 13.0 cm respectively; b values were 2.88 for juvenile males, 3.18 for adult males, 3.22 for juvenile females, and 2.95 for adult females.

For both parameters, GSI and the K , a sinusoidal equation (SE) was applied, following the approach of Fontoura & Agostinho (1996) for the description of the seasonal effect of temperature on the growth of fish. The advantage of the use of time series instead of categorical ANOVA to compare reproductive parameters along the year is derived from the gain in terms of sensitivity due to the inclusion of additional information derived form a continuous process instead of independent categories. Also, regression models derived from sine functions allows the numerical description of the phenomena, and the direct comparison of time lags between distinct cycles. In this case, the sinusoidal function was
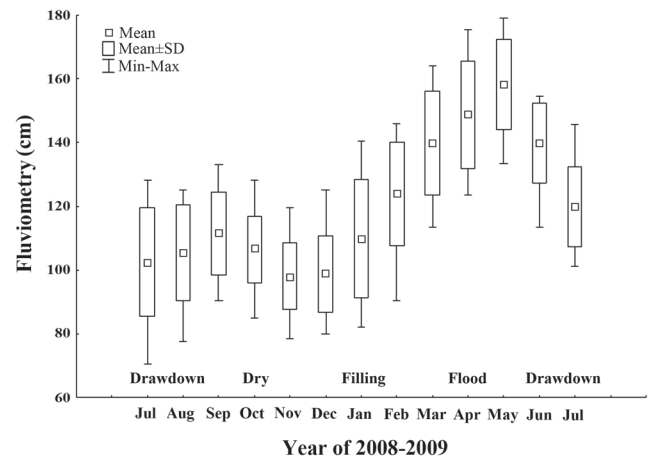

Fig. 2. Seasonal variation of the water level in the Caxiuanã National Forest. Data from the fluviometric station of Caiçara, Agência Nacional das Águas (ANA), from July 2008 to July 2009.

applied to the description of the annual variation of the indices (GSI and K), using the formula:

GSI or K = M + A * $cos(2\pi/Frq * (t - f))$,

where M is the mean annual GSI or K; A is the amplitude of variation of GSI or K values; Frq is the unit of time in which the events were measured (here, Frq=12, considering that time is measured on a monthly scale); t is a numerical scale of time (*i.e.*, January=1, February=2, March=3… December=12) and *f* is the time difference between the baseline (zero) and the time with the maximum value of GSI or K. This latter parameter represents the month of the year with bigger estimated values for GSI or K.

The parameters of the sinusoidal function were estimated by using the Solver routine of the Microsoft Office Excel® 2007 (the *Loss* function using the least squares). The coefficient of determination (R^2) is calculated as the ratio between the residual sum of squares and total sum of squares (Zar, 1999). The *P* values were obtained by the test of significance for the coefficient of determination, through the F statistic (Zar, 1999). Finally, the sex ratios recorded during the study were analyzed using the Chi-square test (χ^2), with a 5% significance level and null hypothesis of equal numbers of males and females through the study period.

Results

A total of 589 *A. longimanus* specimens were collected, of which 251 were males and 338 females. Of the males, 171 were classified as adults and 80 as juveniles, whereas the females consisted of 249 adults and 89 juveniles.

By analyzing the seasonal distribution of males and females in each maturation stages (Fig. 3), it was found fish in reproductive activity (stages II, III and IV) throughout the year. However, the more advanced reproductive stages (III and IV) suggest the mainly breeding during the flood period (March and May) for both genders, although the males
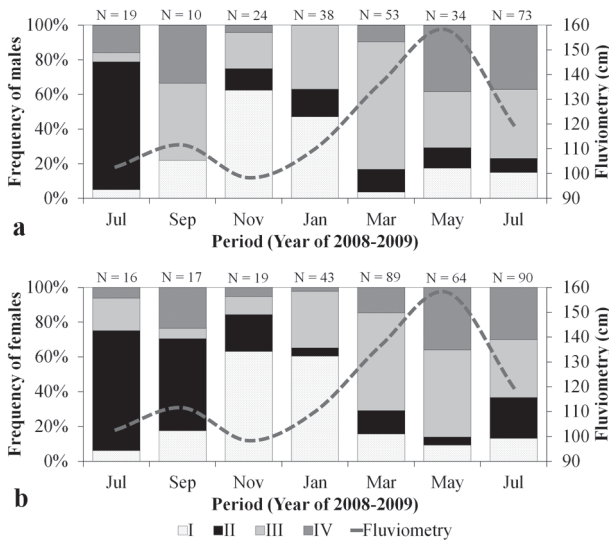
Fig. 3. Relative frequency (%) of gonadal maturation stages of male (**a**) and female (**b**) of *Auchenipterichthys longimanus* collected from July 2008 to July 2009 in the Caxiuanã National Forest. Four gonadal maturation stages were identified: (I) Imature, (II) Maturing, (III) Mature and (IV) Spent. N = number of specimens analyzed.

presented a high percentage of advanced reproductive stages also in September, during the dry season.

The mean GSI for adult males was 2.53 (SD=2.2; min=0.03; max=9.75; mean stage I= 0.20, stage II=1.68, stage III=4.05, stage IV=1.38), and 2.72 for females (SD=2.91; min=0.08; max=18.76, mean stage I= 0.30, stage II=0.99, stage III=4.82, stage IV=0.48). Differences between both genders in the monthly variation in the GSI are presented in Fig. 4, and the parameters used in the sinusoidal equation and their respective significance values are given in Table 1. Based on the sinusoidal equation adjusted for the gonadosomatic index (GSI), males presented the highest GSI values in January, 2009 ($f \approx 1$; filling period), while the females peaked in March, 2009 ($f \approx 3$; flood period). In the males, the sinusoidal regression was significant only for complete data set (P=0.001), wears no trend was identified for bimonthly means (P=0.136). In the females, by contrast, significant values were obtained for both the raw data (P=0.012) and the monthly means (P=0.026). The Spearman correlation also showed significance relationship between riverine water level and reproductive activity based on the GSI bimonthly variation (p<0.05 for both genders).

Seasonal variation in the condition factor (K) is presented in Figs. 5 and 6 for males and females, respectively. Data concerning of the adjustment of the parameters of the sinusoidal
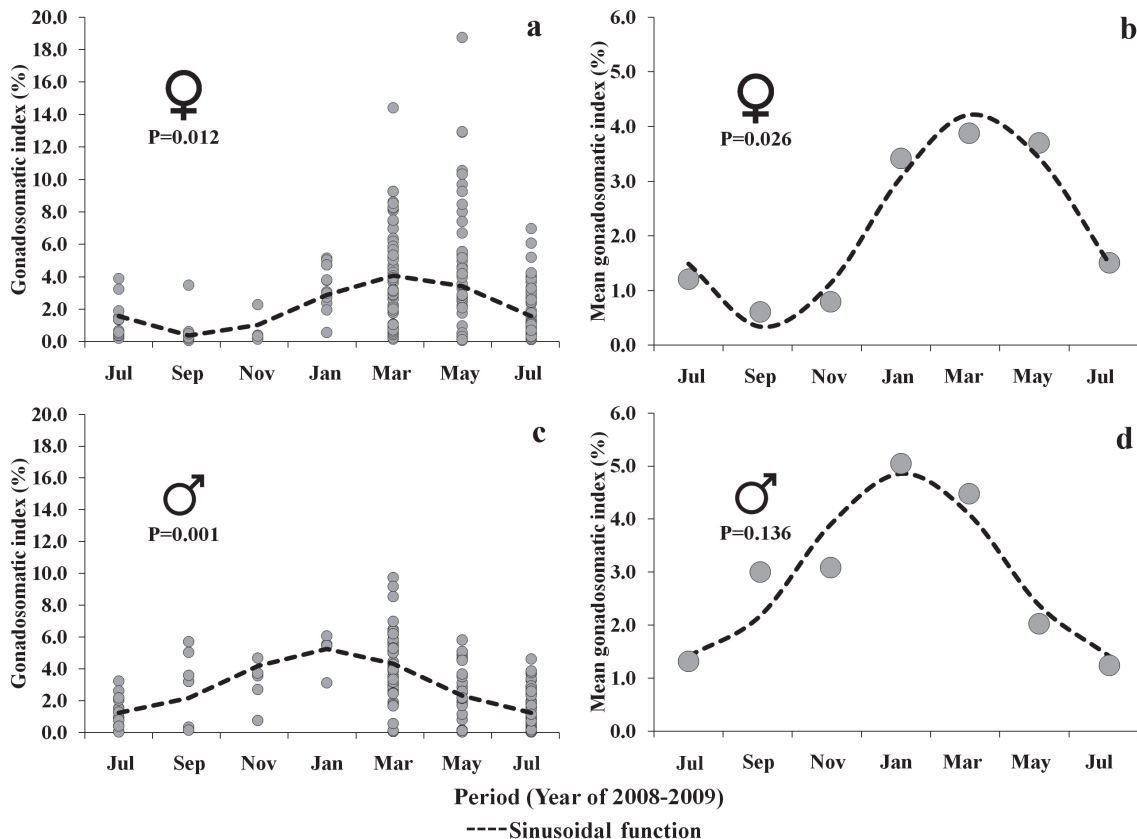

Fig. 4. Bimonthly variation in the raw data (**a**,**c**) and mean values (**b**, **d**) of the gonadosomatic index (GSI) for female (**a**, **b**) and male (**c**, **d**) of the catfish *Auchenipterichthys longimanus* collected from July 2008 to July 2009 in the Caxiuanã National Forest.

Table 1. Parameters of the sinusoidal equation for the variation of raw data and bimonthly means in the GSI of the catfish *Auchenipterichthys longimanus* collected from July 2008 to July 2009 in the Caxiuanã National Forest.

	Parameters	Males		Females	
		Raw	Mean	Raw	Mean
Gonadosomatic Index (GSI)	Amplitude (A)	2.010	1.726	1.872	1.948
	Mean (M)	3.247	3.136	2.231	2.278
	Phase (*f*)	1.079	1.132	3.322	3.205
	R²	0.407	0.872	0.171	0.954
	P value	0.001	0.136	0.012	0.026

equation and the significance values are presented in Table 2.

For the condition factor (K), the sinusoidal equation for adult males was significant for both the raw data (P=0.027) and the mean values (P=0.000). For both equations, the "phase" parameter (*f*) was approximately 7, which represents month July (filling season). In juvenile males, variation in K was not significant during the study period for either the raw data (P=0.110) or the mean values (P=0.684).

In the adult females, variation in the sinusoidal equation for K was significant for the raw data (P=0.040), but not for mean values (P=0.586). Once again, the value of the phase (*f*) for the raw data was close to 7 (July; filling season). As in

the juvenile males, variation in K in juvenile females was not significant for either the raw data (P=0.086) or mean values (P=0.688).

For males the condition factor was highest in the juveniles (K=0.017; Kmin=0.011; Kmax=0.038) in comparison with the adults (K=0.009; Kmin=0.006; Kmax=0.014), due to the difference in the coefficient of allometry (b), which was 2.88 in the juveniles, and 3.18 in the adults. The situation is just the opposite in females, in which the highest K values were recorded for adults (K=0.018; Kmin=0.010; Kmax=0.026) in comparison with juveniles (K=0.008; Kmin=0.005; Kmax=0.014). As for males, these observed K values of females reflects also differences in the allometric coefficients for adult and juvenile (b=3.22 for juveniles, and 2.95 for adults).

Considering the number of captured animals, there was an overall bias towards females (χ^2=12.85; P=0.000; Table 3), and the number of females exceeded that of males in March (P=0.003) and May (P=0.002), months of increased GSI values for females (Fig. 2a-2b).

Discussion

By analyzing both the frequency distributions of gonadal maturation stages and the seasonal variation of the GSI, we
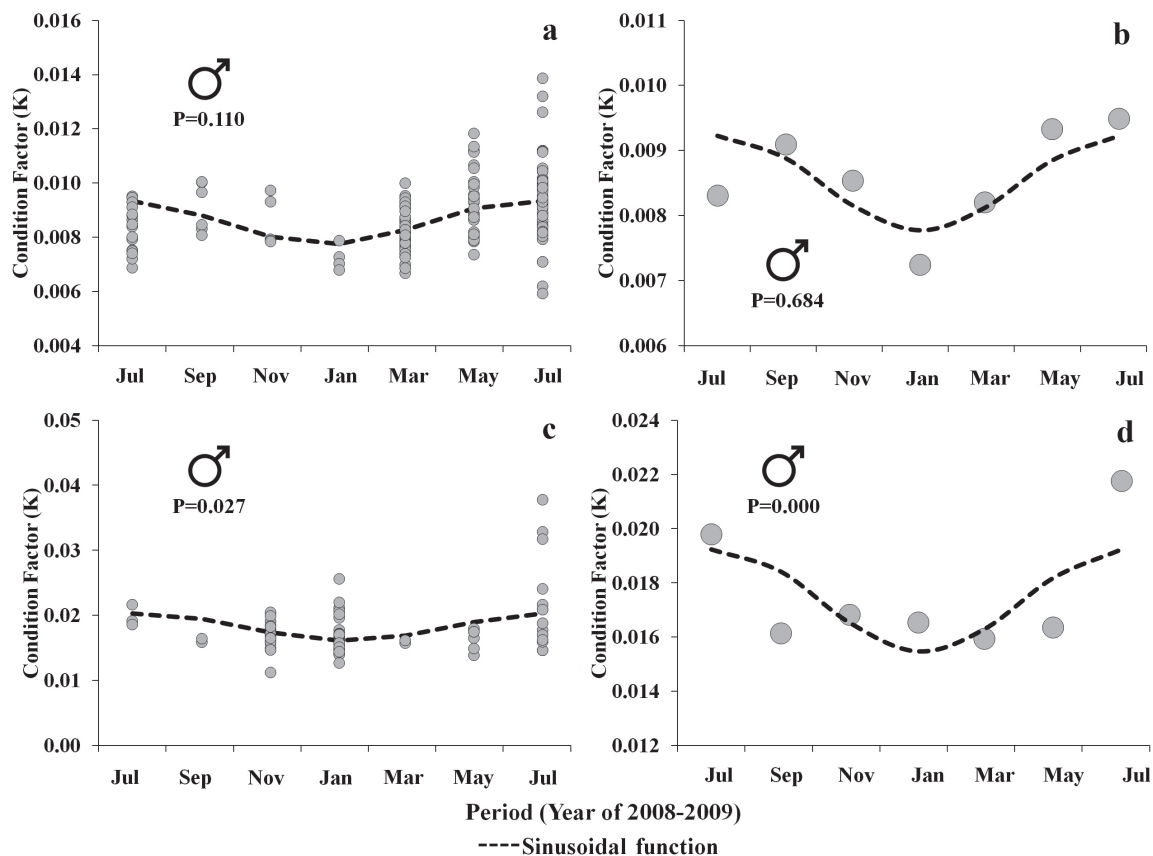

Fig. 5. Bimonthly variation in the raw data (**a**,**c**) and mean values (**b**, **d**) for the condition factor (K) in juvenile (**a**, **b**) and adult (**c**, **d**) male of the *Auchenipterichthys longimanus* collected from July 2008 to July 2009 in the Caxiuanã National Forest.

Fig. 6. Bimonthly variation in the raw data (**a**,**c**) and mean values (**b**, **d**) for the condition factor (K) in juvenile (**a**, **b**) and adult (**c**, **d**) female of the *Auchenipterichthys longimanus* collected from July 2008 to July 2009 in the Caxiuanã National Forest.
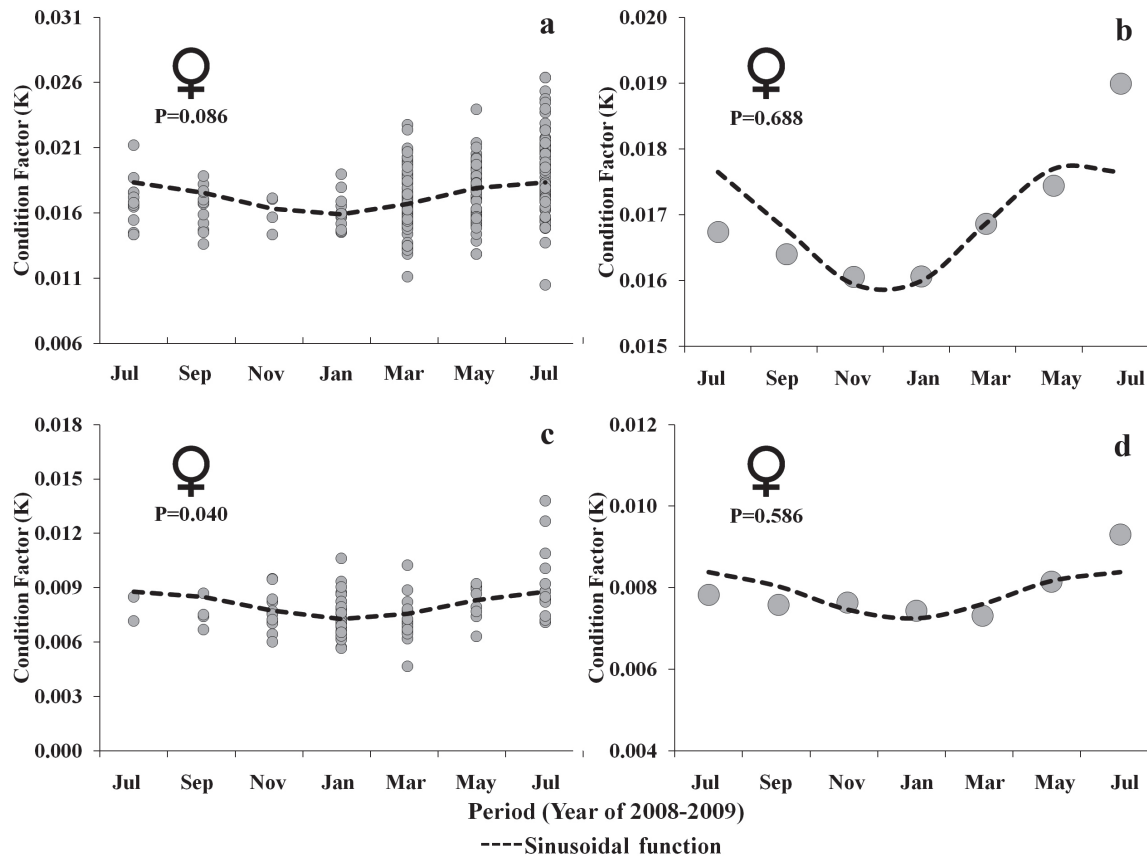
suggest an extended spawning period for *A. longimanus* population in rivers of the Caxiuanã National Forest, a pattern similar to the already described for other auchenipterids (Araújo *et al.*, 1999; Bailly *et al.*, 2008). The reproductive period was identified with greater intensity during the flood period, in which the flooded forest forms a rich environment for fish (Goulding, 1980), suggesting the flood pulse as the primary environmental factor influencing fish recruitment (Zeug & Winemiller, 2008).

In auchenipterids, *Trachycorystes galeatus* (=*Trachelyopterus galeatus*) presented the same pattern of asynchrony between males and females (Silva & Viana, 2003). A similar condition was already recorded for other fish species as *Piaractus mesopotamicus* (Characiformes: Characidae) (Costa & Mateus, 2009)*, Cathorops spixii* (Siluriformes: Ariidae) (Fávaro *et al.*, 2005) and a number of marine species (Murua & Saborido-Rey, 2003).

Despite the observed asynchrony in the highest values of the gonadosomatic index (GSI) between genders - January for males and March for females - some females matured earlier than average. In this respect, it would be advantageous for a male to have early gonadal maturation, in order to be apt when the first females are ready for copulation, thus increasing their chances of success during

a given breeding season (Fontoura *et al.*, 2010).

Particularly for auchenipterid catfish, this asynchrony in GSI values may be reflecting the reproductive strategy of many species in this family. Burgess (1989) identified that the actual fertilization takes place only at the time of the egg deposition, without any males assistance, by using a storage of sperm (Melo *et al.*, 2011), which the females could retain for up to four months (varying upon the species). Hence, the more accurate term ''internal insemination'' had been used recently instead ''internal fertilization'' (Burns *et al.*, 2002; Mazzoldi *et al.*, 2007; Parreira *et al.*, 2009). This insemination strategy may also allow the temporal gap between mating and spawning (Pusey & Stewart, 1989; Azevedo *et al.*, 2000), as suggested for *A. longimanus* in this study (based on sex ratio; discussion below).

Concerning the condition factor, the difference in the allometric coefficient between juveniles and adults males (b=3.22 for juveniles, and 2.95 for adults) suggest a increased energy budget for adults males, probably related to the beginning of reproductive activities. Also, K values for males were highest in the period immediately before and after the month of spawning (January, 2009). In this month, a larger number of mature males with relatively smaller values of K were captured. In females, by contrast, the relationship

Table 2. Parameters of the sinusoidal equation for the variation of raw and bimonthly means in the condition factor (K) in *Auchenipterichthys longimanus* collected from July 2008 to July 2009 in the Caxiuanã National Forest.

	Parameters	Male				Female			
		Juvenile		Adult		Juvenile		Adult	
		Raw	Mean	Raw	Mean	Raw	Mean	Raw	Mea
Allometric condition factor (K)	Amplitude (A)	0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.00
	Mean (M)	0.018	0.017	0.009	0.009	0.008	0.008	0.017	0.01
	Phase (*f*)	7.275	7.140	6.642	7.055	7.255	6.742	6.669	5.90
	R²	0.158	0.455	0.164	0.996	0.163	0.451	0.096	0.55
	P value	0.110	0.684	0.027	0.000	0.086	0.688	0.040	0.58

Table 3. Sex ratio for the catfish *Auchenipterichthys longimanus* collected in the Caxiuanã National Forest, Pará State, between July 2008 and July 2009.

Sex	2008			2009				
	Jul	Sep	Nov	Jan	Mar	May	Jul	Total
Male	19	9	24	38	54	34	73	251
Female	16	17	19	43	89	64	90	338
Chi-square (χ^2)	0.257	2.46	0.581	0.309	8.566	9.184	1.773	12.85
P value	0.612	0.117	0.446	0.579	0.003	0.002	0.183	0.000

between the breeding season and the condition factor was more discreet. Even so, it is possible to verify an inverse relationship between K and GSI, given that the highest mean value of K was recorded in July (K=0.018; phase ≈ 7), when the females presented the lowest GSI values. These results also suggest that the reduction in K values reflects the energetic costs of the reproductive process for both males and females.

Although both the gonadosomatic index and the condition factor are applied for evaluation of the reproductive cycle in fish, values of K may also reflect any change in the feeding ecology of individual specimens (Braga, 1986; Kreiner *et al.*, 2001; Gomiero & Braga, 2003; Anene, 2005). For both genders we observed that the changing pattern of the condition factor was inversely related to the GSI, which may be related to investments of the body's reserves into gonadal development (Braga, 1986) or energetically-demanding reproductive strategies such as courtship and copulation (Peres & Renesto, 2007).

Despite its relatively limited usefulness for the description of reproductive cycles in *A. longimanus*, the condition factor is relevant to inspect possible changes in the pattern of energy investment during individual life histories. For this reason, Goulart & Verani (1992) suggested the use of the condition factor as an efficient indicator of seasonal variation in aspects of feeding ecology (e.g. diet change, energy absorption).

Silva & Viana (2003) reported that *Trachycorystes galeatus* (= *Trachelyopterus galeatus*) presents a higher condition factor during the wet season in northeastern Brazil, when the water level spread over surrounding areas, providing a wider range of refuges and feeding sites. By contrast, Bailly *et al.* (2008) found that the gonadal development of three auchenipterid fishes (*Auchenipterus osteomystax*, *A. nigripinis*, and *Parauchenipterus galeatus* (= *Trachelyopterus galeatus*) were not affected by the hydrological cycle in the Brazilian Pantanal wetlands. These results emphasize that auchenipterid species can show different strategies in relation to the reproductive site and natural environmental cycle, and more studies on these species are in need to allow the understanding of the group framework.

With regard to the sex ratio, the present study recorded a predominance of females for *A. longimanus*. According to Nikolsky (1963) sex ratio deviations may be related to genetic

factors, differences regarding habitat occupation, differential mortality rates, and even biased sampling by the fishing equipment (Barbieri, 1992; Hojo *et al.*, 2004). A change in the sex ratio may happen not only among populations of a given species, but also within the same population during different periods (Nikolsky, 1963). In the present study, the predominance of females was especially marked during the spawning period (January through March, 2009), which suggests a pattern of sexual segregation during the reproductive process.

Concluding, the present study concerning aspects of the reproductive biology of *A. longimanus* in the blackriver system of the Caxiuanã National Forest identified an extended spawning period, but with increased reproductive activity related to the flooding cycle. Differences in the condition factor (K) were also recorded, which could be reflecting variation in the energy budget between genders and size classes related to the attainment of maturity. An identified asynchrony between males and females in the reproductive process may be related to the species' internal fertilization strategy, with females retaining sperm prior to spending.

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