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Age and growth of the endemic Xingu River stingray *Potamotrygon leopoldi* validated using fluorescent dyes

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Between 2003 and 2005, vertebrae of 151 Xingu River *Potamotrygon leopoldi* (Potamotrygonidae) (75 males and 76 females) were analysed to derive a growth curve for this species. The disc width (W_D) was significantly different between sexes, with females measuring 149–700 mm W_D and males 109–500 mm W_D. The average percentage error for vertebrae readings of the whole sample was 2·7%. The marginal increment ratio (R_{MI}) showed an increasing trend with the highest value in November, decreasing from December on. The majority of vertebrae displaying R_{MI} zero, occurred in September, but the annual periodicity of ring deposition throughout the year was not conclusive. Tetracycline (TCN) injected specimens were held in captivity for 13 months and displayed a fluorescent mark in vertebrae confirming a yearly periodicity of band pair formation with the translucent ring deposited in September–October. The Akaike information criterion (AIC) showed that, among the seven models considered, the best fit was obtained for the von Bertalanffy modified with W_0 (where $W_0 = W_D$ at birth) for both sexes. Growth parameters for females were: $W_0 = 149$ mm; $W_\infty = 763{\cdot}06$ mm; $k = 0{\cdot}12$ year^{-1}, whereas for males: $W_0 = 109$ mm; $W_\infty = 536{\cdot}4$ and $k = 0{\cdot}22$ year^{-1}. Maximal ages were 7·2 years in males and 14·3 years in females. The species shows sexual dimorphism expressed in the growth pattern, size at maturity, longevity and asymptotic sizes. Concern for sustainability is raised due to the construction of the Belo Monte Hydroelectric Power Plant (2015 and 2016) in the State of Pará causing changes to the habitat of this species, which is endemic to the Xingu River and two of its tributaries.

Key words: anthropogenic effects; Brazilian Amazon; *Potamotrygon leopoldi*; vertebrae; Xingu River.

INTRODUCTION

The Xingu River, Brazil is a 2500 km tributary of the Amazon River in Brazil, which shelters the Xingu River stingray *Potamotrygon leopoldi* Castex & Castello 1970, a valuable species in the international ornamental trade due to its attractive colour patterns. The species belongs to the Potamotrygonidae, a family which is found in the

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The authors made an equal contribution of this work.

Neotropical region and is the only family comprising exclusively freshwater species (Compagno & Cook, 1995).

The construction and operation of the Belo Monte hydroelectric power plant in 2015 to 2016 brought significant changes to the habitat of this species, which is endemic to the Xingu River and two of its tributaries, the Iriri and Curuá rivers (Rosa, 1985; Charvet-Almeida, 2006). The limited distribution of the species renders it vulnerable to decline due to habitat degradation (Charvet-Almeida *et al.,* 2002; Araújo *et al.,* 2004).

Regarding fisheries, as per Charvet-Almeida *et al.* (2002), stingrays comprise 1% of the total ornamental fish exports and *P. leopoldi*, together with another five Potamotrygonidae species, make up 67% of all freshwater stingrays exported from Manaus, Amazonas state (Charvet-Almeida, 2006).

Published information on the Xingu River stingray is scant, with age and growth studies referring to their marine counterparts of the genera *Dasyatis* Rafinesque 1810 (Cowley, 1997; Ismen, 2003), *Himantura* Müller & Henle 1837 (Tanaka & Ohnishi, 1998), *Urolophus* Müller & Henle 1837 (Babel, 1967; White *et al.,* 2001) and *Urotrygon* Gill 1863 (Santander Neto, 2015), considered potential ancestors of freshwater stingrays. Overall, females attain larger disc width (W_D) than males; the former are mature at 43–46 cm W_D and the latter at 34–37 cm W_D (Charvet-Almeida, 2006). *Potamotrygon leopoldi* exhibits trophodermic-matrotrophic viviparity; the gestation period lasts *c.* 5–6 months and births occur over 4–5 months (Charvet-Almeida, 2006). The reproductive cycle is strongly influenced by the alternating of wet and dry seasons, which triggers changes in the river flow from <1000 to $>20\,000\ \mathrm{m^3\ s^{-1}}$ (dos Santos *et al.,* 2016).

Habitat changes caused by the Belo Monte power plant affected the area called Volta Grande do Xingu, made up of great waterfalls following uneven terrain of ≤85 m over 160 km. A reduction of more than 80% of the water flow of the river took place, which consequently worsened the water quality in the preferential site of the *P. leopoldi*.

The exploitation for ornamental or other purposes in the context of limited life-history and population data, precludes an accurate assessment of the consequences of captures that are needed to ensure the species survival. Information on aspects of population dynamics such as age and growth are crucial for fishery management, but are still not available. Owing to insufficient knowledge, the species was categorized both in IUCN (Charvet-Almeida *et al.,* 2009) and in national assessments (ICMBio, 2014) as Data Deficient, preventing conservation measures being drawn up to the present date.

In view of the risk factors that include habitat changes and fishing, the goal of the current study, on the basis of samples collected prior to the environmental changes, was to provide estimates of age and growth required for age-based methods used for management of the species. Thus, counts of band pairs from their vertebrae were validated for periodicity through the use of tetracycline fluorescent dyes and growth parameters were calculated by a statistical fit to seven different growth functions.

MATERIALS AND METHODS

The site of collection corresponds to the middle portion of the Xingu River, a stretch from the confluence with the Iriri River, upstream from the city of Altamira, through the locality known as Kaitucá, which is located downstream from this city just before the great waterfalls. This portion of the river totals 145 km, between 03° 49′ S; 52° 38′ W and 03° 34′ S; 51° 52′ W, encompassing six points of collection: three upstream and three downstream from Altamira:

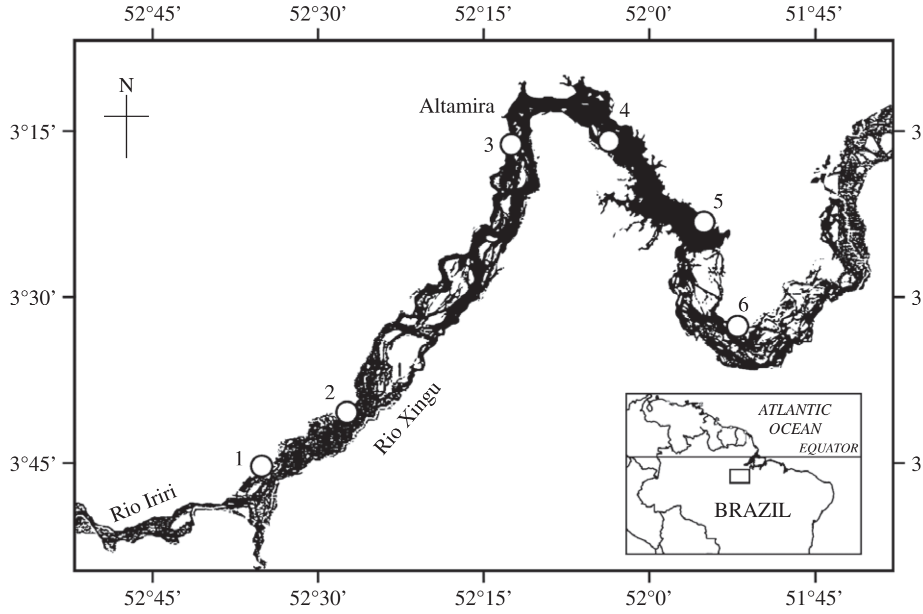

FIG. 1. Sampling locations (○) for *Potamotrygon leopoldi* along the Xingu River, Brazil: 1, Iriri; 2, Porção; 3, Curicas; 4, Cotovelo; 5, Arroz Cru; 6, Kaitucá.

Iriri, Porção, Curicas, Cotovelo, Arroz Cru and Kaitucá (Fig. 1). Data collection was carried out before the construction and operation of the Belo Monte power plant, from March 2003 to December 2005, during the wet (February–March), the dry (July–September) and the transition seasons. Specimens were captured using four longlines equipped with 50 hooks, sized from 6 (the smallest) to 3 (the largest). Other gear was also used to catch the sizes not attained with the longlines, such as dip-nets, hand-lines and cast nets.

The sex of each specimen was noted and the disc widths (W_{D}, mm) were measured. After the posterior synarcual plaque (from the third vertebra) a sequence of five vertebrae was removed, fixed and stored in ethyl alcohol (70 and 90% water solution, respectively); the excessive tissue was then taken out, exposing the cleaned vertebral corpus. A single vertebra of each specimen was isolated, dried and embedded in polyester resin. One to three sections were taken with a low speed metallographic saw (Isomet Buehler; www.buehler.co.uk) equipped with a diamond cutting disc. Longitudinal sections through the centre of the vertebrae were taken with a thickness of 0·1–0·2 mm (Cailliet *et al.,* 1983).

A growth band pair consisted of a translucent (narrow) ring and an opaque (wide) ring (Casselman, 1983; Cailliet & Goldman, 2004) (Fig. 2), counted and measured using a compound microscope provided with a micrometric eyepiece of ×10 magnification (1 μm unit = 1 mm), on a black background and with reflected light. The distances from the focus of the vertebrae to the outer margin of each translucent ring and to the margin of the vertebrae (radius) were noted. Overall, three readings were carried out without the knowledge of individual size. Assessed ages were compared and ages that differed were re-assessed simultaneously by both readers for agreement (Carlson & Baremore, 2005).

The relationship between the radius of the vertebra (V_{R}) and W_{D} was calculated by means of a linear regression for each sex; parameters of regressions were tested by ANCOVA.

Considering the date of collection and results of the readings, the average percentage of error (*%E*) (Beamish & Fournier, 1981) was estimated (R^{-1}) by: $\%\mathrm{E} = R^{-1}\left[\sum_{i=1}^{R}\left(\left|X_{ij}-\overline{X}_j\right|\overline{X}_j^{-1}\right)\right]^{-1}$ 100 in which R is the number of readings; X_{ij} is the mean age of *j*th at the *i*th reading and $\overline{X}_j$ is the mean age calculated for the *j*th reading.

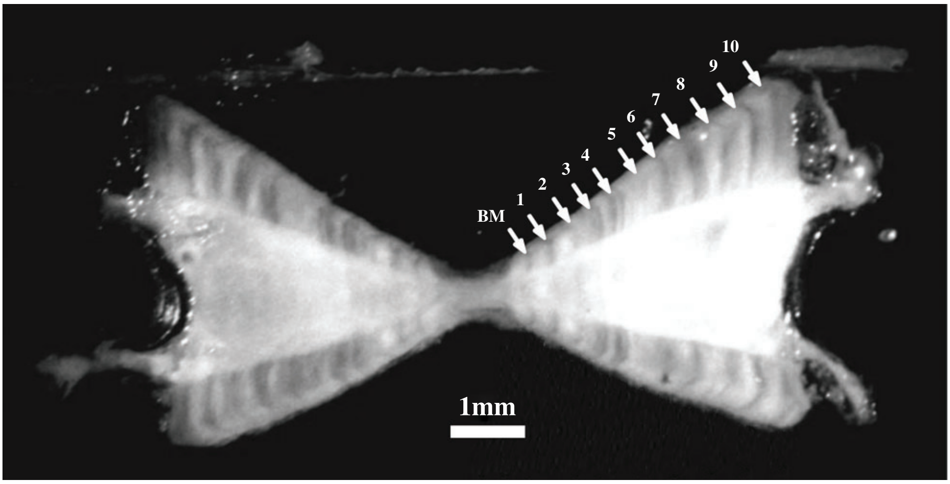

FIG. 2. Vertebral section (female, 576 mm disc width) of *Potamotrygon leopoldi*, estimated to be 10 years of age, from the Xingu River. BM, the birth mark; 1–10 correspond to one opaque and one translucent growth-band pair .

One attempt to validate the deposition frequency of growth marks on vertebrae was the relative marginal increment ratio (R_{MI}) (Panfili & Morales-Nin, 2002), analysed monthly: $R_{MI} = (V_R - R_n)(R_n - R_{n-1})^{-1}$, where V_R is the vertebral radius, R_n is the last complete band and R_{n-1} is the penultimate band. Monthly variation of opaque rings ($R_{MI} > 0{\cdot}3$), translucent rings ($R_{MI} > 0$ to $0{\cdot}3$) and rings beginning to form ($R_{MI} = 0$) per month was assessed. An ANOVA among months was performed to observe differences in the R_{MI} values.

Additionally, four *P. leopoldi* captured using previously described methods, were placed individually in plastic bags containing enough water and oxygen to be transported by plane from the city of Altamira to the laboratory in Recife. The plastic bags were placed in styrofoam boxes to protect the stingrays from temperature variations. This procedure allowed the validation of the time of ring deposition to be carried out through tetracycline (TCN) labelling (Holden & Vince, 1973; Geffen, 1992). The stingrays were injected in August 2009 with TCN (35 mg kg^{-1}) in the coelomic cavity and held in tanks for 13 months. The tanks had 3000 l capacity with a sub-gravel filtration system; water temperature was from 26 to 27.4° C with an average photoperiod of 12 h day^{-1}. The captive *P. leopoldi* were fed live shrimp (*Macrobrachium* spp.) *ad libitum* twice daily. Water quality variables were kept constant and monitored weekly.

Vertebrae were removed in August 2010 from the TCN labelled stingrays, cleaned and stored dry in the dark. Sections were examined using reflected and transmitted ultraviolet light to fluoresce the TCN mark (Olympus U-MF2 tetracycline filter was used with excitation filter: 387/11 nm; dichroic filter: 405 nm; barrier filter: 525/50 nm; www.getolympus.com), making them visible under a fluorescence light microscope (OPTON; www.zeiss.com). Photographs were taken under reflected light. The distance between the TCN mark and the edge was measured and the number of rings between the fluorescent mark and the edge was determined by observing the section under an external light source together with the ultraviolet light of the compound microscope.

Using the multi-model inferences approach, seven models were adjusted to pairs of relative observed ages (relative to the anniversary date) and W_D data: the von Bertalanffy (1938) growth function (VBGM) (g_1); a modified form of the VBGM (g_2) fitted to ensure that the curve passed through the mean back-calculated disc width at birth (W_0); the generalized VBGM (g_3); the two-phase growth model (TPGM, g_4) (Araya & Cubillos, 2006); the logistic model (g_5) (all in Katsanevakis, 2006); the Gompertz model (g_6), (Schnute, 1981); and finally the Richards model (g_7) (Richards, 1959): g_1, $W_t = W_\infty \times \left[1 - e^{-k \times (t - t_0)}\right] + \varepsilon$;

g_2, $W_t = W_0 + (W_\infty - W_0) \times \lfloor 1 - e^{-k_1 \times t} \rfloor + \varepsilon$; g_3, $W_t = W_\infty \times \left[1 - e^{-k_2 \times (t - t_1)}\right]^p + \varepsilon$; g_4, $W_t = W_\infty \times \lfloor 1 - e^{-k_6 \times A_t \times (t - t_3)} \rfloor + \varepsilon$; g_5, $W_t = W_\infty \times \left[1 + e^{-k_3 \times (t - t_2)}\right]^{-1} + \varepsilon$; g_6, $W_t = W_\infty \times e^{\left[-a \times e^{(-k_4 \times t)}\right]} + \varepsilon$; g_7, $W_t = (W_\infty) \times \left\{\left[1 + e^{(-k_5 \times t + b)}\right]^m\right\}^{-1} + \varepsilon$; where the parameter W_t is the predicted disc width at age t; W_∞ is the mean asymptotic W_D; k is the relative growth coefficient parameter; t_0 is the age when disc width is theoretically zero, W_0 is the W_D at birth; interpretation of k_1 and t_1 are similar to those of k and t_0 respectively; k_2 is also a growth coefficient while t_2 is the inflection point of the sigmoidal curve; A_t is a factor that modifies k when age is increased ($A_t = 1 - \{(h) \times [(t - t_h)^2 + 1]^{-1}\}$); t_h is the age at which the transition between the two phases occurs; h determines the magnitude of the maximum difference in length at age between the VBGM and TPGM at point t_h; ε is the error (residual plots) for each growth model. Parameters of these models were estimated using the Solver function of the Excel programme (www.solver.com). In the same manner, the likelihood tools and bootstrap iterations of the PopTools programme (Hood, 2006) were used to generate confidence intervals of each parameter.

The Akaike information criterion (AIC, Akaike, 1974) was used to determine which model was the best. Differences in AIC values ($\Delta_i = \text{AIC}_{\text{c,I}} - \text{AIC}_{\text{c,min}}$) and the weight of evidence (w_i) (Burnham & Anderson, 2002) in favour of each model were computed to select the most suitable models. After identifying the most plausible growth model based on the AIC, comparisons of growth curves by sex were performed based on the likelihood test (Kimura, 1980) and corroborated by Cerrato (1990). The AIC values were estimated for sexes separately and combined.

Longevity (t_{max}) was estimated using the Fabens (1965) equation: $t_{\text{max}} = \{5 \times [\ln(2)]\} \times k^{-1}$, where t_{max} represents the age and k the VBGM growth constant. Finally, the age composition of the sample, supposedly reflecting the population, was estimated using the age-length key proposed by Bartoo & Parker (1983) for the entire sample. Throughout the text all statistical inferences were made at a significance level of 0·05.

RESULTS

A sample of 75 male and 76 female *P. leopoldi* was collected with significant differences in size compositions by sex (Student's *t*-test, $t = 1{\cdot}98$, d.f. = 149, $P < 0{\cdot}01$), in which females measured 149 to 700 mm W_D (modal sizes 450–600 mm W_D, mean size of 489·6 ± 113·5 mm W_D) and males measured between 109 and 500 mm W_D (modal sizes 400–450 mm W_D and mean size of 385·6 ± 80·9 mm W_D) (Fig. 3).

There were significant differences by sex in the relationship between vertebral radius and disc width (ANCOVA, $F = 1069{\cdot}93$, d.f. = 1, $P < 0{\cdot}01$; Fig. 4). The number of bands (excluding birth marks) counted along the corpus calcareum ranged from 1 to 14 for females and 1 to 8 for males, with $\%E$ values between 0·0 and 4·8%, respectively. Considering the total sample, a $\%E = 2{\cdot}7\%$ was estimated.

In regard to the marginal increment ratio, despite no specimens collected in January, May, July and August, the mean R_{MI} presented an increasing trend with the highest value in November, decreasing from December onward. R_{MI} values were almost constant from February to June, not displaying significant differences ($P > 0{\cdot}05$). Also, for the entire period, monthly differences in R_{MI} were not significant [ANOVA, $P < 0{\cdot}05$; Fig. 5(a)]. Moreover, taking into account the frequency of R_{MI} distribution values of 0, >0 to 0·3 and > 0·3, the highest number of vertebrae displaying R_{MI} zero, meaning the translucent ring at the border, occurred in September, suggesting an annual periodicity of ring deposition [Fig. 5(b)].

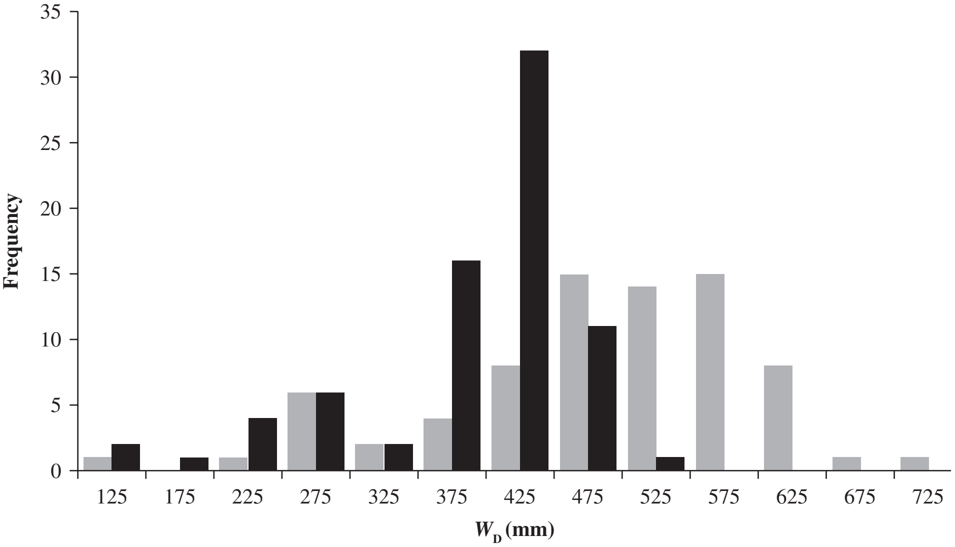

FIG. 3. Disc width (W_D)-frequency distributions by sex (■, males; ▨, females) for *Potamotrygon leopoldi* from the Xingu River.

Because marginal increment analysis was not completely convincing, four individuals (two females, 410 and 503 mm W_D, and two males, 356 and 434 mm W_D) were held in captivity for 13 months. All specimens TCN injected displayed a fluorescent mark on their vertebrae in August 2010. The *P. leopoldi* were 3 to 7 years of age and the resulting periodicity of the band pair was once a year. The relative and observed positions of the opaque and translucent rings determined by the TCN labelling and the types of edges are shown in Fig. 6(a), (b). The formation of the translucent ring was estimated to occur between September and October [Fig. 6(c)].

Because the highest R_{MI} values occurred from September onward as well as the formation of the translucent ring shown by TCN marks, ring deposition was found to take place from that month on, having 1 September considered as the anniversary date.

The model exhibiting the best fit was the modified von Bertalanffy, with W_0 (g_2) for both sexes, followed by the traditional VBGM for females and the TPGM and VBGM models for males, which were ranked the second and third respectively (Table I), according to the lowest AIC values (Akaike, 1974); the $\Delta i < 2$ and highest w_i, in both sexes accounting for ≥70% of the overall Akaike weight. For both sexes all other models (g_3, g_4, g_5, g_6 and g_7 for females and g_3, g_5, g_6, g_7 for males) received low statistical support and were not found suitable. Since there were significant differences in growth between the sexes and the modified VBGM was the best model describing growth, parameters for females were: $W_0 = 149$ mm; $W_\infty = 763{\cdot}06$ mm; $k = 0{\cdot}12$ year^{-1}, whereas for males they were: $W_0 = 109$ mm; $W_\infty = 536{\cdot}4$ mm and $k = 0{\cdot}22$ year^{-1} (Fig. 7).

The likelihood ratio test for the modified VBGM (chosen as the best model according to the AIC) revealed significant differences in growth between sexes ($\chi^2 = 14{\cdot}02$, d.f. = 1, $P < 0{\cdot}05$), which led to separate growth curves for each sex (Fig. 7). The estimated AIC values were 1528·35 for separate sexes and

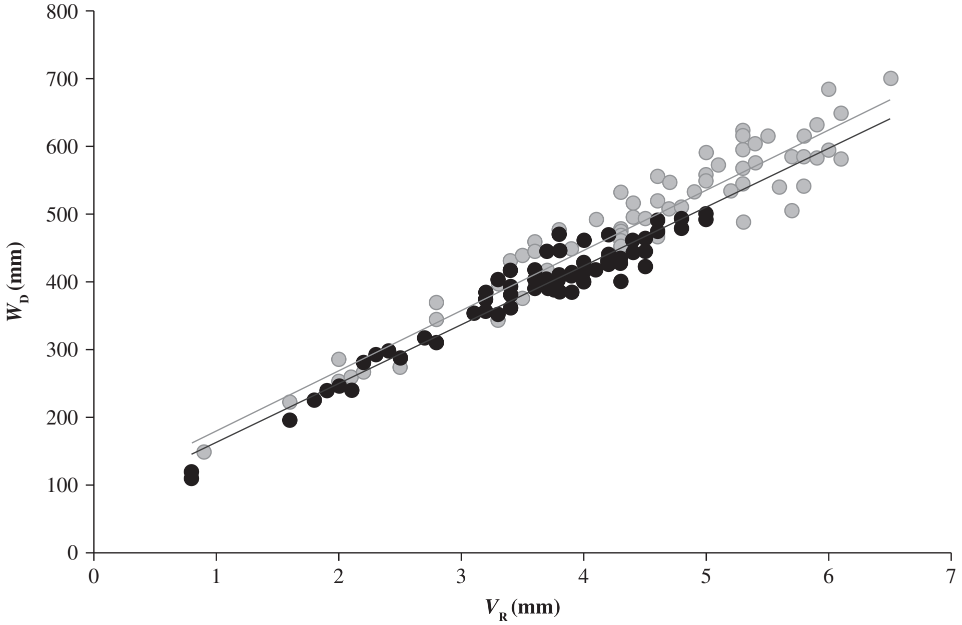

FIG. 4. Relationship between vertebral radius (V_R) and disc width (W_D) by sex (●, ——, male $y = 86.5x + 77·6$, $r^2 = 0·93$; ●, ——, female $y = 88.8x + 91·9$, $r^2 = 0·91$).

1542·41 for sexes combined, with a $\Delta_i > 2$, demonstrating that the models are dissimilar.

Sizes of males and females in the entire sample corresponded to 0^+ and 7·2 years (median age 4 years) in the former, while in the latter, ages were from 0^+ to 14·3 years (median age 8 years) (Fig. 8). According to Charvet-Almeida (2006), females reach maturity from 431 to 460 mm W_D and males became mature from 341 to 370 mm W_D, which corresponded to the ages at maturity estimated by inverted VBGM modified of 5·0–5·7 years and 3·5–4·2 years, respectively. Ultimately, 65·3% of males and 52·6% of females were immature, based from the calculated age at maturity (Fig.8). The estimated longevity by Fabens (1965) is 28·1 years for females and 15·3 years for males.

DISCUSSION

The highly endemic *P. leopoldi* is imperilled due to habitat changes and fishing that favour the capture of juveniles, better prized in trade (R. Glémet, unpubl. data). The study sample reflects the prevalence of individuals caught in shallow waters, where some size classes were misrepresented. Males were more vulnerable to fishing gear than females, perhaps due to their segregation behaviour displayed by some classes, an aspect that contributed to some extent to the differential growth by sex (Cailliet, 1990; Natanson & Kohler, 1996). Such a fishing pattern has consequences for the sustainability of the population, as risk of recruitment declines is increased.

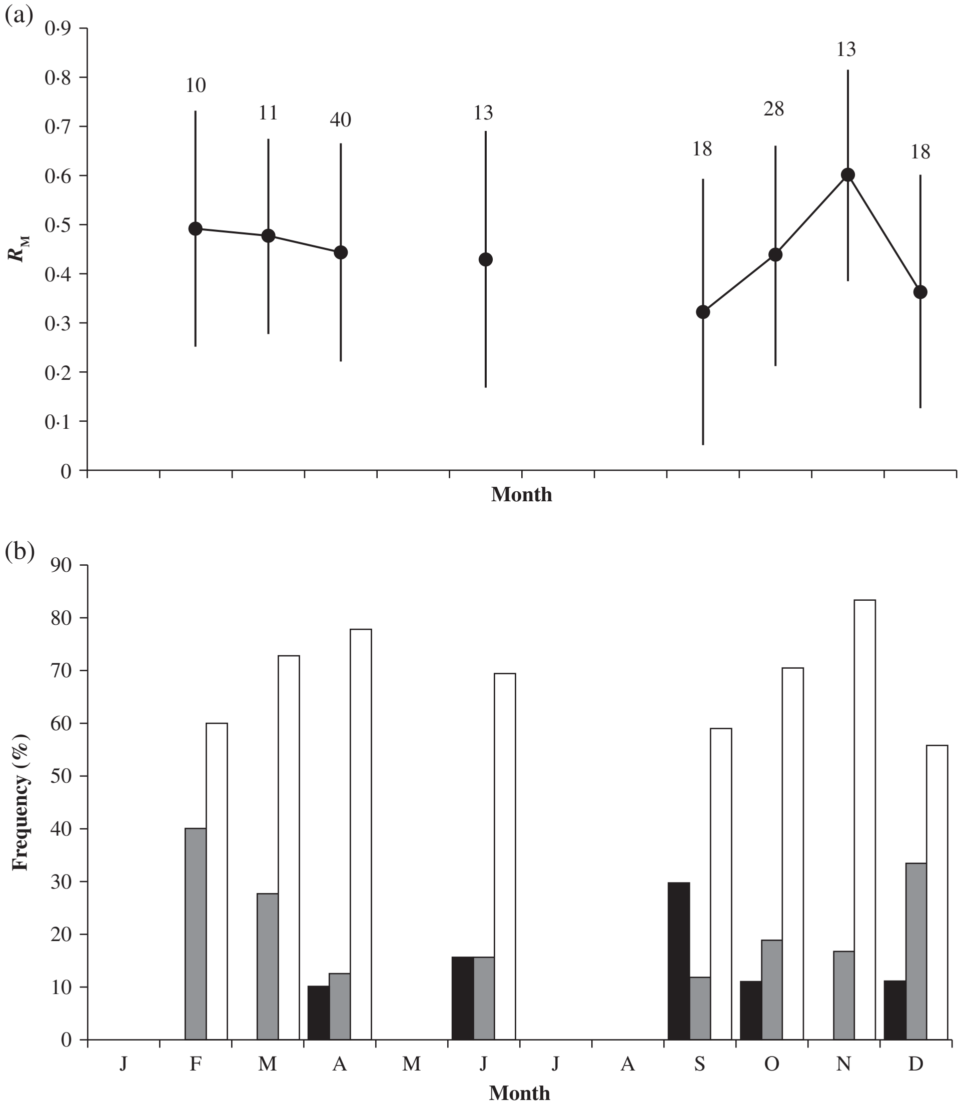

FIG. 5. (a) Monthly mean (●, ± S.D.) relative marginal increments (R_{MI}) by month on vertebrae for *Potamotrygon leopoldi* from the Xingu River and (b) frequency distribution of R_{MI} values by month (■, $R_{MI} = 0$; ▨, $R_{MI} = 0–0·3$; □, $R_{MI} > 0·3$).

Referring to precision of readings, the vertebrae from *P. leopoldi* proved highly reliable for age estimations as evidenced by the low average percentage of error (*%E*). Although the vertebrae of older females were more prone to reading errors due to the overlapping of growth band pairs, the overall rate of reading errors for the entire sample of $\%E = 2·7\%$, was well below the limit proposed by Campana (2001). For other species of the same suborder as *Dasyatis chrysonota* (Smith 1828) (Cowley, 1997), *Hypanus dipterurus* (Jordan & Gilbert 1880) (Smith *et al.*, 2007) and *Urobatis halleri*

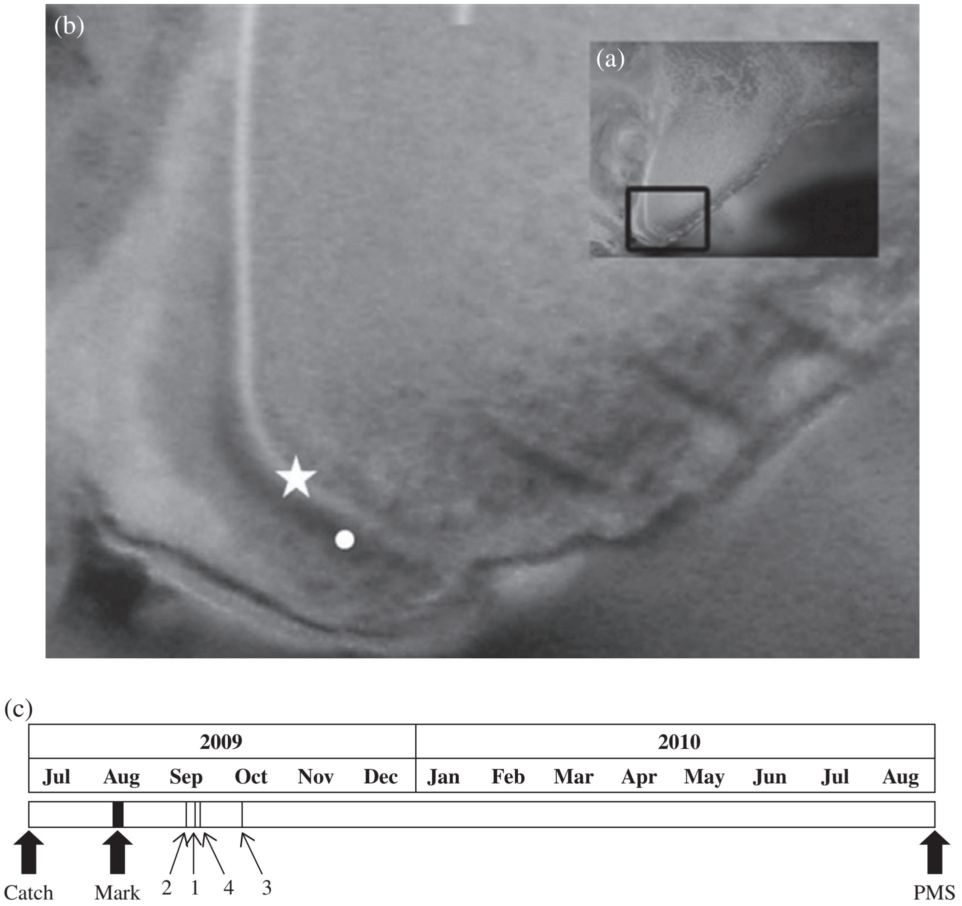

FIG. 6. (a) Transverse vertebral section of *Potamotrygon leopoldi* from the Xingu River and (b) detail with tetracycline (TCN) fluorescent mark (☆) and last translucent ring formed (○). (c) Diagrammatic representation of the relative positions of the real and estimated date of capture (Catch), TCN marking (Mark), the estimated time of TCN fluorescent mark deposition of four individuals (1–4)and *post mortem* sampling (PMS).

(Cooper 1863) (Hale & Lowe, 2008), a high readability of vertebrae was also demonstrated.

Distinct vertebral diameter gave rise to differences in V_R to W_D relationship (ANCOVA, $P < 0{\cdot}05$) implying that the analyses had to be conducted separately by sex as was also obtained for *Paratrygon aiereba* (Müller & Henle 1841) from the Rio Negro basin, Brazil (Araujo, 2011) and have been commonly found in other elasmobranchs analysed by Natanson & Kohler (1996); Schwartz (1983); Yudin & Cailliet (1990) and Lessa *et al.* (2004). The distinct growth pattern is in accordance with the varying biological features, such as for *D. chrysonota* (Cowley, 1997), *H. dipterurus* (Smith *et al.,* 2007), *Dasyatis pastinaca* (L. 1758) (Yeldan *et al.,* 2008) and *Hemitrygon fluviorum* (Ogilby 1908) (Pierce & Bennett, 2010).

TABLE I. Parameter values generated for seven growth models from the adjustment of the relative ages and disk widths (± C.I.) for *Potamotrygon leopoldi* with the AIC, differences (Δ_i) and weights (w_i) for each model

Sex	Model	W_∞ (mm)	k_n (year^{-1})	t_n (years)	AIC	Δ_i	w_i (%)
Females	g_1: VBGM	753·38 (675·05 ± 920·26)	0·128 (0·080 ± 0·176)	−1·641 (−2·849 ± −0·878)	774·68	1·93	20·82
	g_2: Modified. VBGM	763·07 (687·62 ± 870·64)	0·123 (0·095 ± 0·157)	–	772·75	0·00	54·70
	g_3: General. VBGM	795·20 (648·50 ± 12 028·28)	0·101 (<0·001 ± 0·249)	−1·077 (−33·495 ± −0·117)	776·56	3·81	8·13
	g_4: Logistic	651·40 (620·97 ± 685·83)	0·308 (0·248 ± 0·378)	2·920 (1·241 ± 5·453)	779·20	6·45	2·17
	g_5: Gompertz	681·59 (631·05 ± 765·07)	0·220 (0·163 ± 0·274)	–	776·50	3·75	8·37
	g_6: Richards	650·08 (613·11 ± 705·78)	0·101 (0·090 ± 0·117)	–	781·21	8·46	0·80
	g_7: TPGM	789·66 (703·25 ± 1077·23)	0·115 (0·061 ± 0·160)	−1·691 (−0·451 ± 0·005)	777·53	4·78	5·02
Males	g_1: VBGM	532·37 (497·17 ± 586·84)	0·234 (0·177 ± 0·289)	−0·939 (−1·456 ± −0·607)	684·97	1·92	16·57
	g_2: Mod. VBGM	536·40 (503·24 ± 576·83)	0·227 (0·192 ± 0·268)	–	683·07	0·00	43·21
	g_3: Gen. VBGM	582·26 (494·04 ± 6560·90)	0·151 (<0·001 ± 0·359)	−0·412 (−4·467 ± <0·001)	686·16	3·10	9·16
	g_4: Logistic	477·69 (459·76 ± 497·69)	0·510 (0·423 ± 0·609)	1·707 (1·040 ± 2·342)	695·06	12·01	0·11
	g_5: Gompertz	493·97 (469·77 ± 524·84)	0·378 (0·308 ± 0·451)	–	689·48	6·43	1·74
	g_6: Richards	477·69 (458·81 ± 503·76)	0·154 (0·143 ± 0·171)	–	697·06	14·01	0·04
	g_7: TPGM	584·65 (521·06 ± 729·74)	0·200 (0·130 ± 0·265)	−1·007 (−1·604 ± −0·628)	683·84	0·79	29·18

VBGM, von Bertalanffy growth model; TPGM, two-phase growth model; W_∞, disc width at infinite age; k_n, annual growth rate; Other parameters; females: disc width at birth (W_0) = 149 mm (g_2), $p = 0{\cdot}784$ (g_3), $a = 1{\cdot}390$ (g_5), $b = 0{\cdot}294$ (g_6), $m = 3{\cdot}070$ (g_6), $h = -0{\cdot}093$ (g_7), $t_h = 3{\cdot}991$ (g_7); males: $W_0 = 109$ mm (g_2), $p = 0{\cdot}653$ (g_3), $a = 1{\cdot}372$ (g_5), $b = 0{\cdot}263$ (g_6), $m = 3{\cdot}308$ (g_6), $h = 0{\cdot}133$ (g_7), $t_h = 6{\cdot}993$ (g_7).

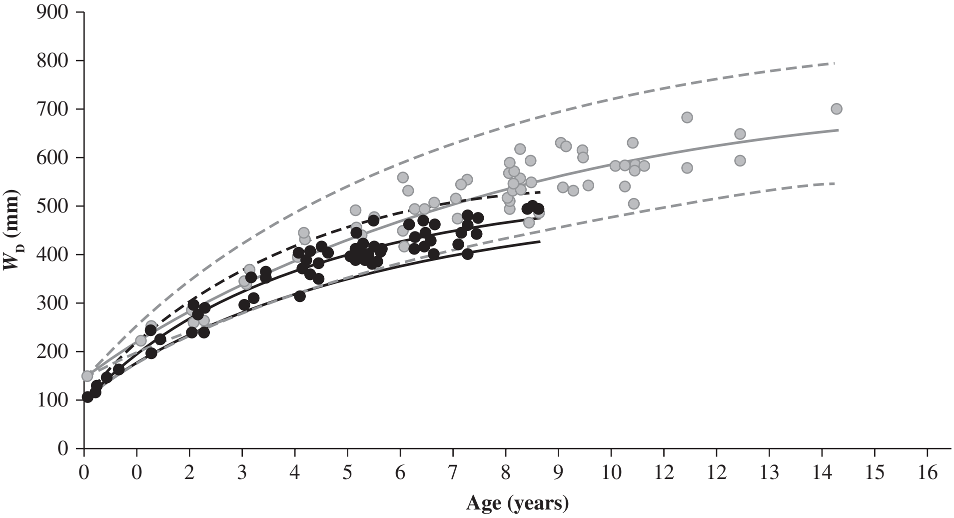

FIG. 7. Modified von Bertalanffy growth curves from adjusted data of the relative ages and disc widths (W_D) of *Potamotrygon leopoldi* from the Xingu River. ____ ± – ------ C.I., male; ____, ± – ------ C.I female.

In the present study, multi-model inferences, using several models provided AIC values of around the same magnitude. Thus, the modified VBGM and the three-parameter VBGM were the best models for females, whereas the modified VBGM, TPGM and the traditional VBGM provided $\Delta i < 2$ for males, which made them the best models for this species (Katsanevakis, 2006). Moreover, the AIC weight (w_i), used to quantify the plausibility of each model (Katsanevakis & Maravelias, 2008), revealed that the modified VBGM got the highest weight (w_i) among all models in both sexes (Akaike, 1974;

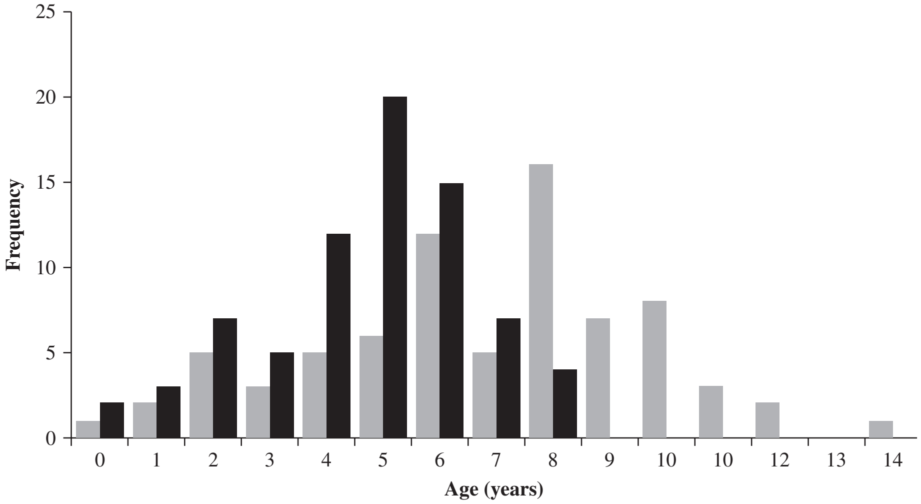

FIG. 8. Age-frequency distribution by sex (■, males; ■, females) of *Potamotrygon leopoldi* from the Xingu River. structure.

Burnham & Anderson, 2002) and was elected the best model in the present study. The modified VBGM is reputed for providing good fits, facilitating verification whether or not the W_0 supplied to the model is a realistic value (Cailliet *et al.,* 2006).

Regarding the reliability of the W_∞, the largest female collected in the area measured 700 mm W_D (present study) and the largest male was 500 mm W_D. Thus, the W_∞ generated by fitting the modified VBGM extrapolates the maximal observed size by only 7·6 and 7·2%, respectively, which is plausible according to Campana (2001). When considering the k value, the best model led to $k = 0{\cdot}123$ (0·095–0·157) in females and 0·227 (0·192–0·268) in males, characterizing a species which grows moderately (Musick, 1999: Frisk *et al.,* 2001).

The annual deposition of one band pair is a standard feature for batoids (Cailliet & Goldman, 2004; Jacobsen & Bennett, 2010), a pattern also displayed by the studied species and by the freshwater stingray *P. aiereba* (Araújo, 2011). In the current study however, efforts to validate ring formation using R_{MI} and the vertebral edge were not completely convincing, as significant differences throughout the observed period were not found. Thus, we considered marginal increment analyses not conclusive, providing low support to annual periodicity and depicting only a weak tendency for periods of low and high means during the months when the new band would be forming (Lessa *et al.,* 2004).

On the contrary, the TCN validation of growth ring periodicity, to our knowledge used here for the first time in freshwater stingrays, clearly confirmed the pattern of deposition of one opaque and one translucent ring per year. The formation of the opaque band occurred between the winter (wet) and early summer (dry) and the translucent band was observed in the summer (from September onwards). The completion of the band pair in September may be related to the reproductive season (Charvet-Almeida, 2006) taking place prior to the formation of the translucent ring.

From results obtained in the current study, dimorphism is an outstanding feature generating differences by sexes: in the maturation pattern (males of 4–5 years and females of 6–7 years); in the growth pattern (distinct growth curves); in asymptotic size (50 mm W_D in males and 70 mm W_D in females); in longevity between sexes (males 15·3 years and females 28·1 years). Together, these constitute an expression of a dimorphic pattern (Stamps, 1993), described to varying extents in species from the same group as *D. chrysonota* (Cowley, 1997), *Gymnura micrura* (Bloch & Schneider 1801) (Yokota & Lessa, 2006), *Urotrygon microphthalmum* Delsman 1941 (Santander Neto, 2015) and *Hypanus guttatus* (Bloch & Schneider 1801) (Thorson, 1983; Yokota & Lessa, 2007).

Ultimately, considering the limited area of occupation, the endemic *P. leopoldi* deserves conservation attention once the effects of environmental changes and fishing have been thoroughly assessed, taking into account the present much-needed age-based assessment.

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