

Original article

## Reproductive biology of the Brazilian blind electric ray *Benthobatis krefftii* (Chondrichthyes: Narcinidae)

Mariana F. Martins<sup>1,2,3</sup> and Otto B. F. Gadig<sup>2,3</sup>

This study provides information on the reproductive biology of the Brazilian blind electric ray *Benthobatis krefftii*, endemic to southern and southeastern Brazil. Individuals were caught by bottom trawl carried out in 2003 and 2007, at 492-501 m depth off the São Paulo State continental slope. A total of 152 females (115-299 mm) and 144 males (91-243 mm) were sampled. Maturity was first observed at 177 and 162 mm, with total length at 50% maturity of 191 and 176 mm in females and males respectively. Uterine fecundity ranged from 1-3 and was not related to female total length. Size at birth estimated from the largest near-term observed embryos and smallest free-swimming ray was 91-100 mm. The low fecundity observed is typical of deepwater elasmobranch species, as well as late maturity in comparison with coastal species. The relatively large size-at-birth suggests that this species invests more in length of each embryo than in litter size, increasing the offspring's survival chance. In this context, these parameters highlight the vulnerability of this and other deepwater species to non-natural death, mostly caused by deep-sea fisheries.

**Keywords:** Deepwater, Fecundity, Size at birth, Size at maturity, Torpediniformes.

Este estudo apresenta informações sobre a biologia reprodutiva da raia elétrica cega brasileira *Benthobatis krefftii*, endêmica do sul e sudeste brasileiros. Os indivíduos foram capturados com arrasto de fundo, em 2003 e 2007, a 492-501 m de profundidade no talude continental do Estado de São Paulo. Um total de 152 fêmeas (115-299 mm) e 144 machos (91-243 mm) foram capturados. A maturidade foi observada em fêmeas de 177 mm e machos de 162 mm, com o tamanho onde 50% dos indivíduos encontra-se maduros calculado em 191 mm (fêmeas) e 176 (machos). O tamanho da prole foi de 1-3 embriões e não foi relacionado ao comprimento materno. O tamanho ao nascer foi estimado a partir do tamanho do maior embrião à termo e o menor neonato e foi de 91-100 mm. A baixa fecundidade observada é típica de elasmobrânquios de profundidade, bem como a maturidade tardia, comparados com espécies costeiras. O grande tamanho ao nascer sugere que essa espécie investe no tamanho de cada embrião em vez do número de embriões produzidos, aumentando a chance de sobrevivência da prole. Neste contexto, estes parâmetros ressaltam a vulnerabilidade desta e outras espécies de elasmobrânquios a mortes ocasionadas pela pesca em maiores profundidades.

**Palavras-chave:** Fecundidade, Profundidade, Tamanho ao nascer, Tamanho de primeira maturidade, Torpediniformes.

### Introduction

Elasmobranchs are generally considered as a k-strategy group, being vulnerable to non-natural mortality due to their slow growth, late maturity, low fecundity and long gestation periods, which result in lower rates of population increase and, consequently, susceptibility to overexploitation (Dulvy *et al.*, 2000; Stevens *et al.*, 2000). Deepwater species, compared to coastal and pelagic ones, are even more susceptible since they have slower growth and attain maturity later (IUCN SSC Shark Specialist Group, 2007; García *et al.*, 2008). Therefore, deepwater

elasmobranch populations are less productive than coastal species and, subsequently, less resilient (Simpfendorfer, Kyne, 2009).

During the last decades, fisheries have increased efforts in deepwater marine habitats, mostly due to overexploitation of near-shore resources (Roberts, 2002). Deepwater habitats, however, are more susceptible to collapse, and should be considered areas for conservation (Morato *et al.*, 2006). Understanding life history patterns of a species is a powerful tool for its management and conservation once this information is needed to measure the impact of fisheries on elasmobranch populations. In this context,

<sup>1</sup>Instituto de Ciências Biológicas, Universidade Federal do Rio Grande-FURG, Av. Itália Km 8, Rio Grande, RS, Brazil. marianadafmartins@gmail.com, <https://orcid.org/0000-0002-5835-3023> (corresponding author)

<sup>2</sup>Laboratório de Pesquisa de Elasmobrânquios, Instituto de Biociências, Campus do Litoral Paulista-UNESP, Praça Infante Dom Henrique s/nº, 11380-972 São Vicente, São Paulo SP, Brazil. gadig@clp.unesp.br, <https://orcid.org/0000-0002-8109-5085>

<sup>3</sup>Instituto de Biociências, Campus de Rio Claro-UNESP, Av. 24 A 1515, 13506-900 Rio Claro, SP, Brazil.

reproductive parameters such as size at maturity, fecundity and reproductive cycle are especially necessary for assessing population status (Walker, 2005).

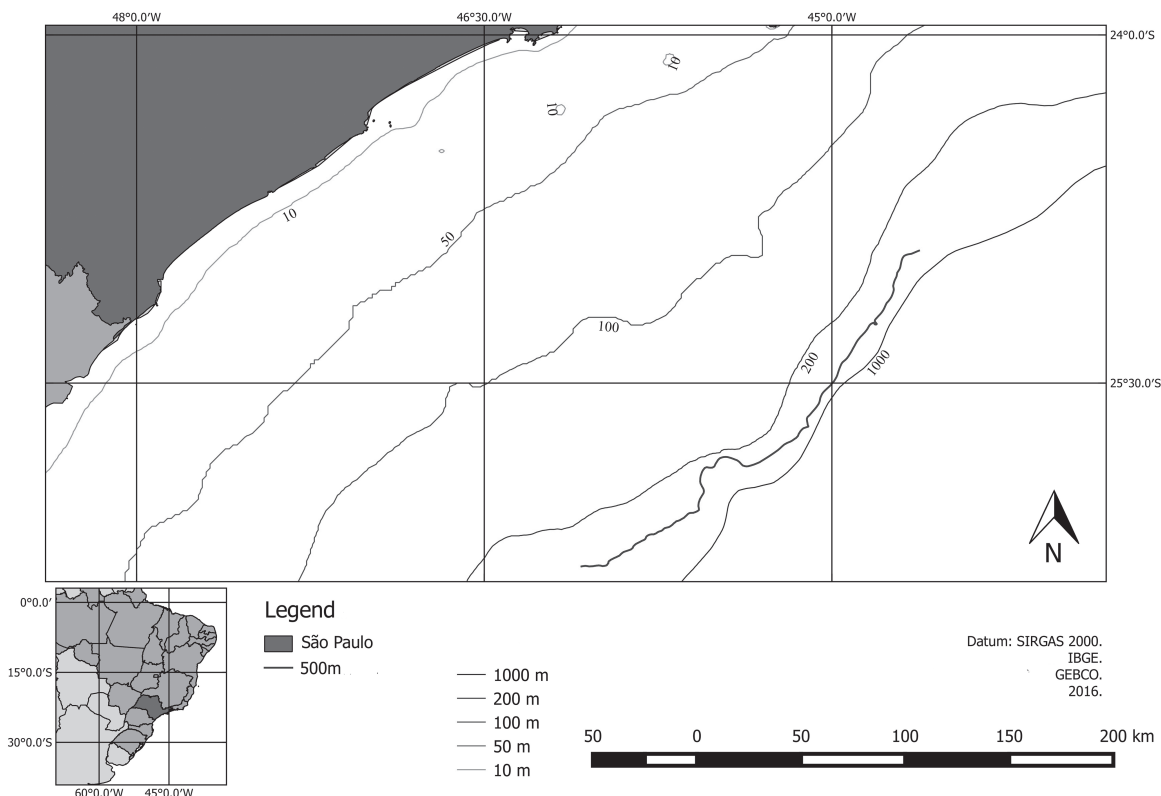
Electric rays (Order Torpediniformes) represent the third most diverse group among batoids, with about 70 species described (Weigmann, 2016). Numbfishes (Family Narcinidae) are small to moderate size benthic rays occurring in almost all marine regions, inhabiting continental and insular shelves, as well as slopes (e.g. the deepwater genus *Benthobatis*, which can be found in depths around 1000 m) (Carvalho, 1999; McEachran, Carvalho, 2002).

The Brazilian blind electric ray *Benthobatis kreffti* Rincón, Stehmann & Vooren, 2001 has a restricted distribution, occurring in the Southwest Atlantic and in southern and southeastern Brazil continental slopes, at 400-600 m depth. This species was studied previously by Rincon (1997, as *Benthobatis* sp.), and, posteriorly described as a new species by the same author (Rincon *et al.*, 2001). Biological aspects of feeding and reproduction were described for specimens from southern Brazil (Rincon, 1997) and additional specimens were recorded along the Southeast and South Brazilian continental slopes (Bernardes *et al.*, 2005). In southern populations, size at maturity was estimated in 150 mm and uterine fecundity was estimated in two embryos/female (Rincon *et al.*, 2001). Despite those information, biological data of individuals from the northern portion of its range remains unknown.

Previous described life-history characteristics of *B. kreffti* and the ones presented herein highlight the need to carefully consider this species in management and conservation measures, especially due to its categorization by the IUCN (Rincon, 2004). In this context, considering that deepwater fisheries have increased in the past decades in Brazil (Perez *et al.*, 2003; 2009) and that *B. kreffti* is incidentally captured by squid trawlers (Rincon *et al.*, 2017), impacts of these fisheries in *B. kreffti* populations might be expected. Therefore, the aim of the present study was to analyze the reproductive biology of *B. kreffti* off São Paulo coast, southwestern Atlantic, including its sexual development, size at maturity, fecundity and size at birth.

## Material and Methods

**Study area.** Individuals were captured in São Paulo State continental slope, Southeast Brazil (Southwest Atlantic), from Cananéia to Santos cities (25°44'-25°45'S /45°09'-45°11'W and 26°09'-26°10'S /45°45'-45°47'W; Fig. 1), in four samples (three in July/August 2003 and one in December 2007) from three different sample stations during a research cruise carried out by the São Paulo State University (UNESP) aboard the Brazilian Government Research Vessel “Soloncy Moura”. The fishing gear was a bottom trawl, operating between 492-500 m depth. All samples were fixed in buffered formalin 4% and preserved in ethanol 70%.



**Fig. 1.** Studied area where individuals of *Benthobatis kreffti* were captured off São Paulo State continental slope in 2003 and 2007. Dark grey color represents São Paulo State, in the left-lower square and central square represents São Paulo State continental slope. Thicker grey line indicates the depth where samples were taken (500 m).

**Biological data recorded at laboratory.** Fish total length ( $L_T$ , to the nearest 1.0 mm), gonad mass ( $M_O$  for females and  $M_T$  for males, to the nearest 0.001 g), liver mass ( $M_L$ , to the nearest 0.001 g) and sex were recorded. Sex identification was based on the presence of claspers. For females, uteri width ( $W_U$ , to the nearest 0.01 mm), number of ovarian follicles ( $N_F$ ) and diameter of the largest ovarian follicle ( $D_F$ , to the nearest 0.01 mm) were recorded. Clasper inner length ( $L_C$ , to the nearest 0.01 mm), clasper gland length ( $L_{CG}$ , to the nearest 0.01 mm) and diameter of largest testicular lobule ( $D_L$ , to the nearest 0.01 mm) were recorded for males.

A maturity scale for viviparous elasmobranchs was adapted for use with this species (ICES, 2013). Because Narcinidae batoids do not present macroscopically visible oviducal glands (Prasad, 1945) and due to visualization difficulties of some reproductive organs fixed in formalin and preserved in ethanol 70% (e.g. vascularization of the uteri and vitellogenic activity in ovarian follicles), maturity stages were condensed and simplified. Maturity stages herein considered were: a) immature, developing and capable to reproduce, for both sexes; and; b) early pregnancy; late pregnancy and post-partum stages for females only. Neonates were considered those individuals with a yolk-sac scar in their abdomen.

**Data analysis.** Sexual development through maturity stages was analysed by plotting data for sexual characteristics as dependent variables, with  $L_T$  as the independent variable. Size at maturity was calculated by fitting binomial data (immature/mature) to the logistic equation:  $Y = [1 + e^{-(a+bX)}]^{-1}$ , where  $Y$  is the proportion of mature individuals per length class  $X$  and  $a$  and  $b$  are parameters of the equation. Mean size at maturity, which is the size where 50% of the individuals are mature, was calculated by the ratio:  $L_{T50} = -a/b$  (Mollet *et al.*, 2000) for both sexes. Ovarian fecundity was estimated by counting the number of ovarian follicles with vitellogenic activity (*i.e.* vitellogenic follicles) in both ovaries (Fitz, Daiber, 1963; Capapé, Quignard, 1975) and uterine fecundity was calculated based on the number of uterine eggs or embryos in both uteri. Uterine fecundity was represented by the total number of developing embryos or uterine eggs, while litter size was represented by number of near-term embryos. No embryos were observed outside the uteri and abortion events were not observed in the field. In addition, cloacal conditions such as distensions were not observed as well. For this reason, size at birth was estimated based on observations of the smallest free-swimming individuals (neonate) and largest near-term embryos observed.

Male and female gonad symmetry was analyzed with Wilcoxon's paired test, with the null hypothesis being that no difference between mass in left and right structure exists (Zar, 2010). All statistics were carried out using R 3.1 software ([www.r-project.org](http://www.r-project.org)) and a significance level of 5% was considered. Descriptive statistics for total length in each maturity stages were represented by (mean  $\pm$  standard deviation and  $n$  represents the sample size).

## Results

**Female sexual development.** A total of 152 females were analyzed in this study. Immature females ranged from 115 to 184 mm  $L_T$  ( $150.0 \pm 20.2$ ;  $n=18$ ) and presented two small symmetric ovaries ( $W=2130.5$ ;  $n=89$ ;  $p=0.600$ ) lacking macroscopically visible ovarian follicles. Two thread-like oviducts were observed, and uteri were not differentiated from other oviduct portions. Liver mass presented a slow potential growth pattern at this stage.

During the developing maturity stage, females presented developing ovaries, and a few small ovarian follicles could be observed from 167 mm onward. Uteri were slightly enlarged but virtually not differentiated from the oviduct. A continuous liver mass increase was observed at this stage, following the same potential pattern previously described. Total length ranged from 146 to 215 mm ( $181.1 \pm 18.1$ ;  $n=25$ ) at this stage.

The smallest mature female was classified as capable to reproduce and was 177 mm  $L_T$  and maximum  $L_T$  recorded for females at this stage was 270 mm ( $220.4 \pm 25.4$ ;  $n=26$ ). Uteri were large and clearly differentiated from the oviduct. Both ovaries were functional and had large vitellogenic follicles. At this stage, ovaries attained their maximum mass, with an abrupt increase right after first maturity was observed.

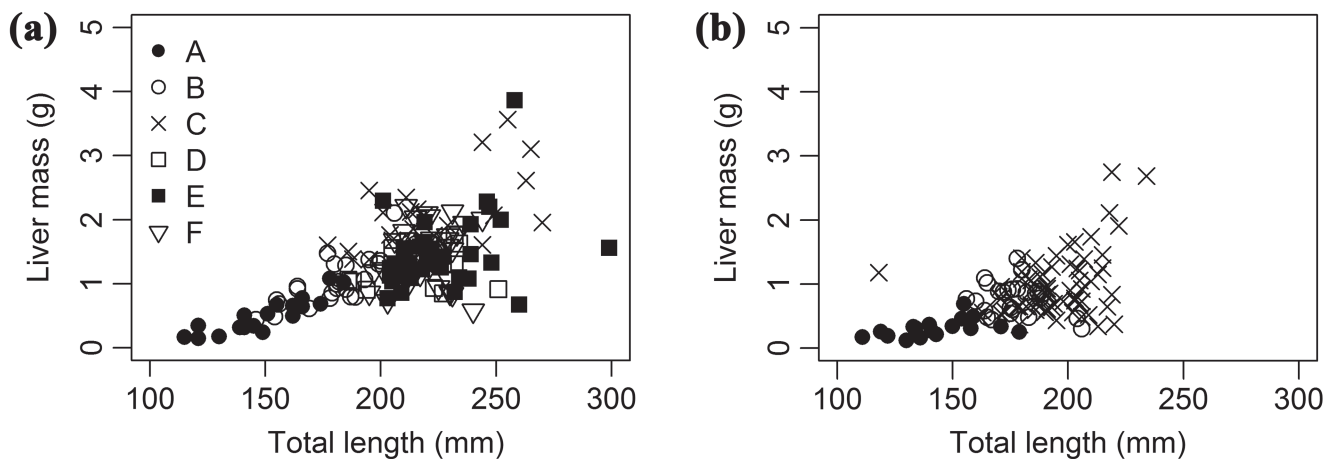
Vitellogenic follicles could still be observed in pregnant females, but were small in size and number. Both uteri were functional and early and late pregnancy stages were characterized by the presence of uterine eggs or embryos, respectively. Total length ranged from 186 to 251 mm ( $216.3 \pm 19.2$ ;  $n=14$ ) in early pregnancy females and from 201 to 299 mm ( $226.2 \pm 21.6$ ;  $n=33$ ) in late pregnancy ones. Liver mass was high for all maturity stages, especially in capable to reproduce females.

In observed post-partum females, uteri were large, flaccid, and some vitellogenic follicles could be observed in both ovaries. Females at this stage had  $L_T$  of 195-244 mm ( $220 \pm 12.4$ ;  $n=32$ ). Female reproductive structures are provided in (S1 - Available only as online supplementary file accessed with the online version of the article at <http://www.scielo.br/ni>) and descriptive statistics and the relation between total length and the sexual variables regarding the observed structures are available in Tab. 1 and Fig. 2a and Fig. 3.

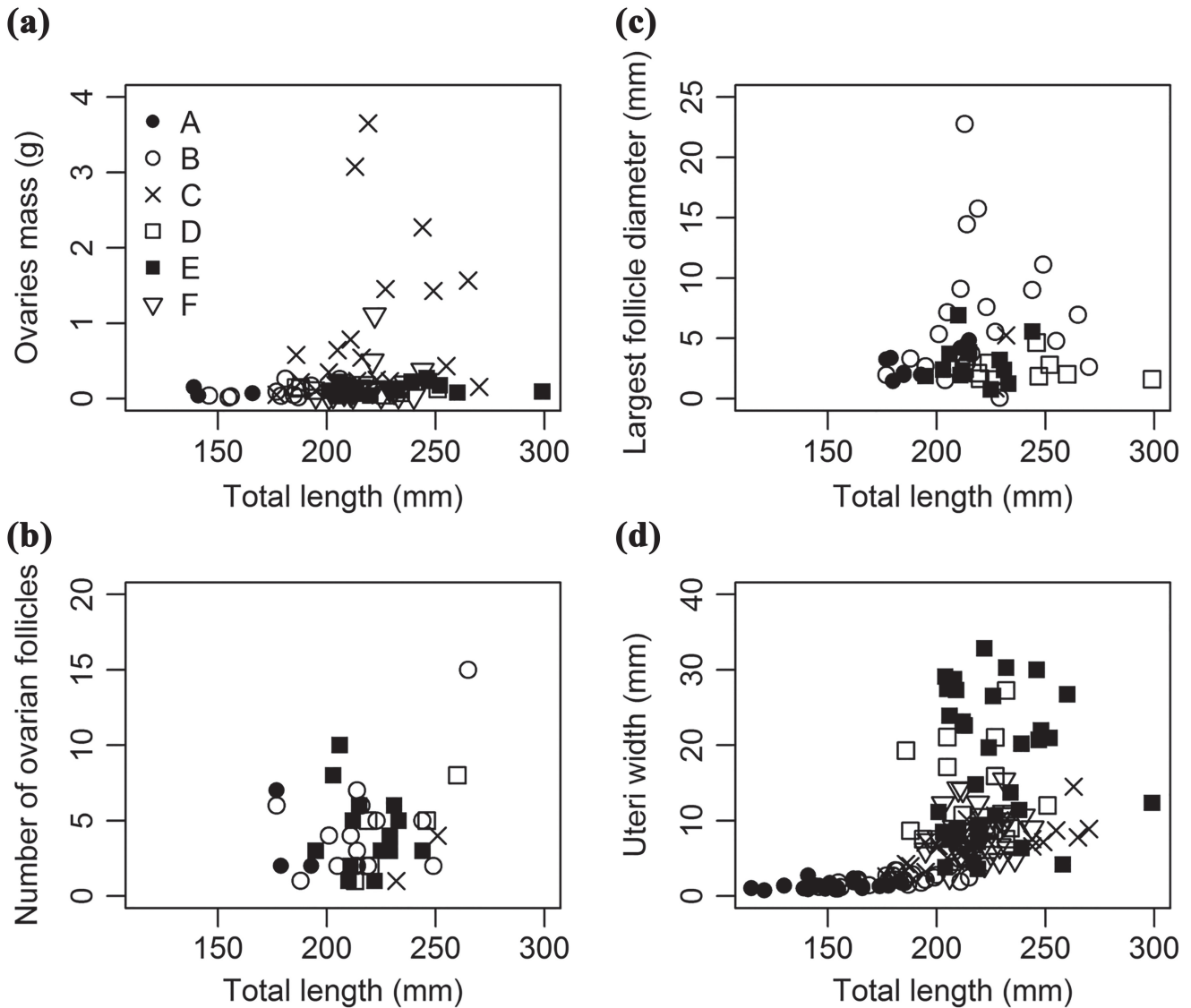
**Male sexual development.** A total of 114 males were captured. Only one neonate male was observed in this study and was 91 mm  $L_T$ . Dissection was not carried out in this individual, though. Immature males ranged from 91 to 179 mm  $L_T$  ( $144.7 \pm 17.7$ ;  $n=16$ ) and had small, flexible and non-calcified claspers. Clasper glands were hardly differentiated from the adjacent muscular tissue and ducts were straight and thread-like. A three-phase sigmoid growth pattern was observed for  $L_C$  and  $L_{CG}$  and immature males had a slow growth pattern. Both testicles were undeveloped and testicles mass increased slowly. Small testicular lobules could be observed in a few individuals at this stage.

**Tab. 1.** Descriptive statistics of the sexual variables: liver mass ( $M_L$ , g), ovaries mass ( $M_O$ , g), diameter of the largest ovarian follicle ( $D_F$ , mm), number of ovarian follicles ( $N_F$ ) and uteri width ( $W_U$ , mm) for females of *Benthobatis krefftii*. Min: minimum value recorded, Max=maximum value recorded,  $n$ : sample size and SD: standard deviation.

Variable	Maturity Stage	$n$	Min	Max	Mean	SD
$M_L$	Immature	18	0.147	1.078	0.505	0.278
	Developing	25	0.438	2.103	1.047	0.383
	Capable to reproduce	26	1.363	3.562	2.013	0.579
	Early pregnancy	14	0.850	1.765	1.274	0.321
	Late pregnancy	33	0.675	3.866	1.472	0.598
	Post-partum	33	0.581	2.210	1.441	0.462
$M_O$	Immature	3	0.042	0.152	0.0881	0.0574
	Developing	13	0.015	0.265	0.1022	0.0890
	Capable to reproduce	21	0.051	3.652	0.8724	1.0212
	Early pregnancy	8	0.044	0.149	0.1056	0.0352
	Late pregnancy	16	0.031	0.271	0.1254	0.0788
	Post-partum	26	0.025	1.111	0.1645	0.2225
$D_F$	Immature	0				
	Developing	8	1.45	4.83	2.887	1.208
	Capable to reproduce	21	0.07	22.77	6.805	5.495
	Early pregnancy	2	0.87	5.25	3.060	3.097
	Late pregnancy	9	1.60	4.65	2.457	0.952
	Post-partum	12	0.76	6.90	3.013	1.775
$N_F$	Immature	0				
	Developing	3	2	7	3.7	2.9
	Capable to reproduce	15	1	15	4.4	3.5
	Early pregnancy	2	1	4	2.5	2.1
	Late pregnancy	5	1	8	4.2	2.8
	Post-partum	14	1	10	4.3	2.6
$W_U$	Immature	17	0.73	2.70	1.373	0.534
	Developing	25	1.00	3.92	2.202	0.721
	Capable to reproduce	26	2.60	14.50	6.917	2.612
	Early pregnancy	14	7.55	27.25	13.900	6.344
	Late pregnancy	33	3.60	32.85	17.260	9.336
	Post-partum	33	2.60	15.41	8.498	3.069



**Fig. 2.** Relationship between total length and liver mass for **a.** females and **b.** males of *Benthobatis krefftii* from São Paulo State continental slope, Brazil. Maturity stages considered were: (A) immature, (B) developing, (C) capable to reproduce (females and males), and (D) early pregnancy, (E) late pregnancy and (F) post-partum (females only).



**Fig. 3.** Relationship between total length and **a.** ovaries mass; **b.** number of vitellogenic follicles; **c.** largest follicle diameter and **d.** uteri width of females of *Benthobatis krefftii* from São Paulo State continental slope, Brazil. Maturity stages considered were: (A) immature, (B) developing, (C) capable to reproduce, (D) early pregnancy, (E) late pregnancy, and (F) post-partum.

Total length ranged from 156 to 206 mm ( $176.8 \pm 12.3$ ;  $n=25$ ) in developing males. At this stage, claspers were still non-calcified, but testicular lobules, and coiled ducts were observed in those individuals. Clasper glands could be distinguishable from the adjacent muscular tissue and a rapid growth of these structures, as well as claspers was observed at this stage. Testicles and liver mass increased abruptly at this stage, as well as  $L_c$  and  $L_{CG}$ .

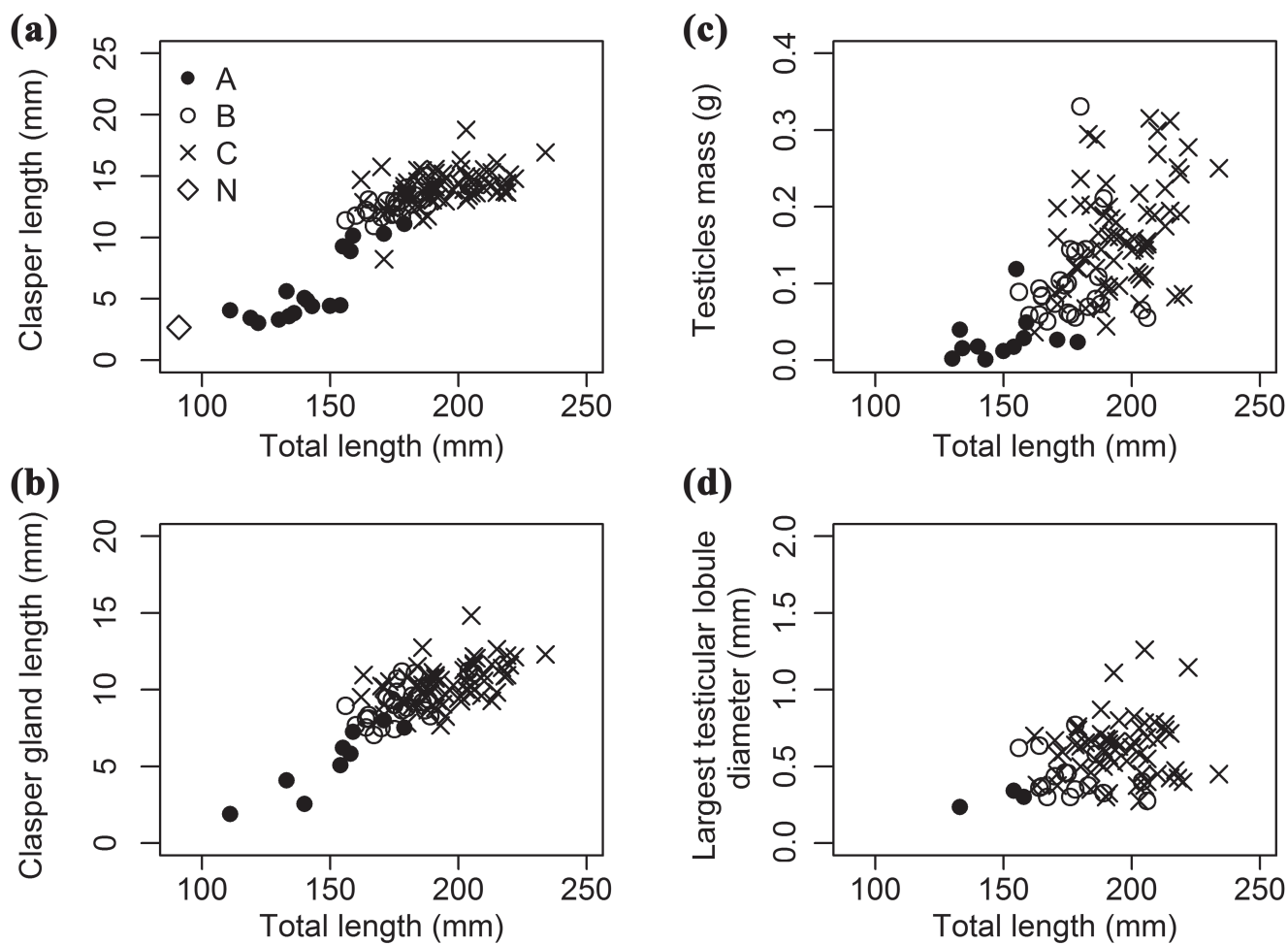
Capable to reproduce males were observed from 162 mm to 234 mm  $L_T$  ( $195.0 \pm 15.6$ ;  $n=68$ ), and had fully calcified claspers, enlarged clasper glands and fully coiled ducts. Difficulties in observing sperm within the ducts were attributed to fixation. Claspers and clasper glands showed a slow growth, representing the plateau of the sigmoid pattern observed for  $L_c$  and  $L_{CG}$  growth. An abrupt mass increase in

testicles was observed at this stage and enlarged testicular lobules were observed in both testicles. However, testicles were asymmetric ( $W=387.5$ ;  $n=107$ ;  $p=0.003$ ). Males reproductive structures are provided in (S2 - Available only as online supplementary file accessed with the online version of the article at <http://www.scielo.br/ni>) and descriptive statistics and the relation between total length and the sexual variables regarding the observed structures are available in Tab. 2 and Fig. 2b and Fig. 4.

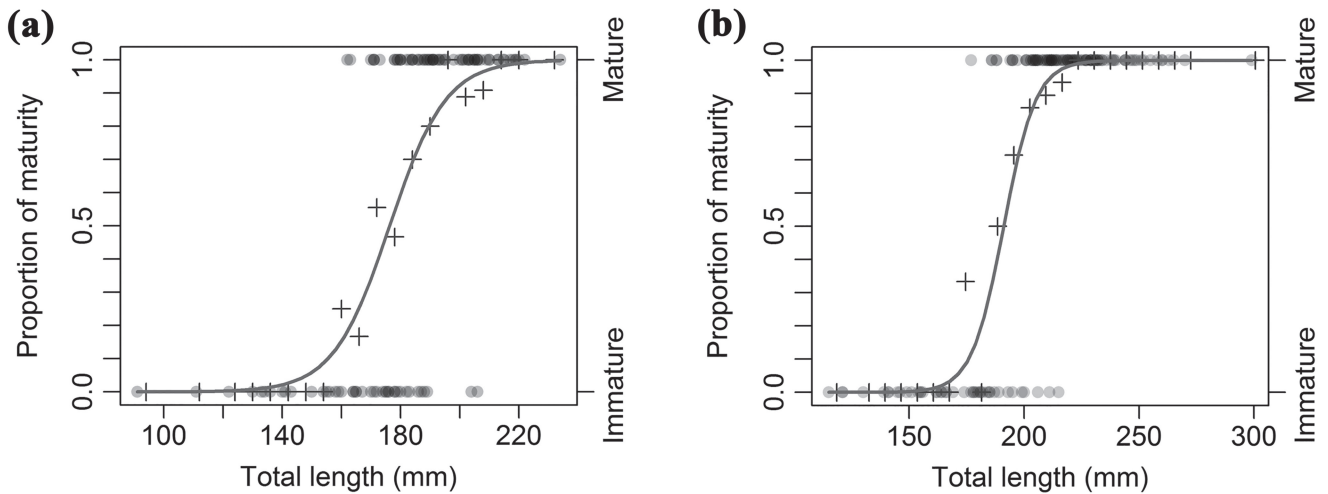
**Size at maturity.** Size at maturity ( $L_{T50}$ ) was 191.1 mm  $L_T$  (95% c.i.: 186.3-195.4) for females, representing 63% of maximum total length recorded (Fig. 5a). Size at maturity ( $L_{T50}$ ) was 176.3 mm  $L_T$  for males (95% c.i.: 170.4-181.4) and represented 75.3% of the maximum total length recorded (Fig. 5b).

**Tab. 2.** Descriptive statistics of the sexual variables: liver mass ( $M_L$ , g), clasper length ( $L_C$ , mm), clasper gland length ( $L_{CG}$ , mm), testicles mass ( $M_T$ , g), and diameter of the largest testicular lobule ( $D_L$ , mm) for males of *Benthobatis krefftii*. Min: minimum value recorded, Max=maximum value recorded,  $n$ : sample size and SD: standard deviation.

Variable	Maturity Stage	$n$	Min	Max	Mean	SD
$M_L$	Immature	17	0.119	0.688	0.3082	0.1400
	Developing	25	0.301	1.400	0.7791	0.2593
	Capable to reproduce	69	0.340	2.750	0.9830	0.4835
$L_C$	Neonate	1	2.69	2.69	2.685	0.000
	Immature	17	3.03	11.10	5.861	2.818
	Developing	25	10.92	15.00	12.370	1.003
$L_{CG}$	Capable to reproduce	68	8.23	18.77	14.080	1.425
	Immature	9	1.90	8.00	5.376	2.174
	Developing	25	7.05	11.22	9.045	1.257
$M_T$	Capable to reproduce	68	7.67	14.84	10.380	1.286
	Immature	12	0.001	0.014	0.0313	0.0314
	Developing	25	0.051	0.331	0.0998	0.0680
$D_L$	Capable to reproduce	67	0.036	0.315	0.1627	0.0675
	Immature	3	0.235	0.340	0.2917	0.0530
	Developing	16	0.275	0.770	0.4381	0.1425
	Capable to reproduce	61	0.275	1.260	0.6095	0.1944



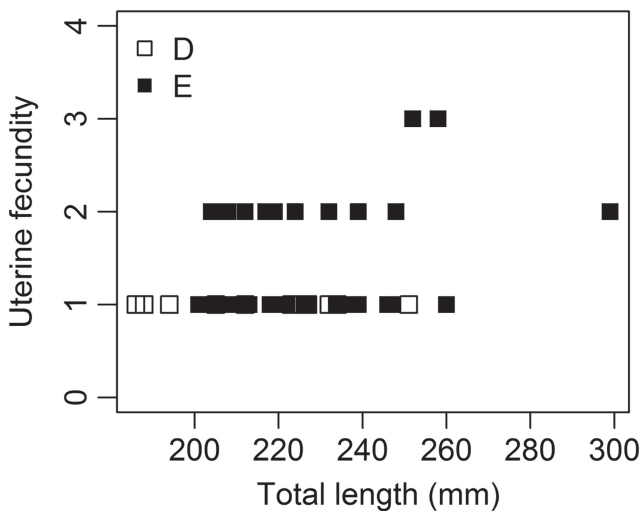
**Fig. 4.** Relationship between total length and **a.** clasper length; **b.** clasper gland length; **c.** testicles mass, and **d.** largest testicular lobule diameter of males of *Benthobatis krefftii* from São Paulo State continental slope, Brazil. Maturity stages considered were: (N) neonate, (A) immature, (B) developing, and (C) capable to reproduce.



**Fig. 5.** Mean size at maturity for **a.** males and **b.** females of *Benthobatis krefftii* from São Paulo State continental slope, Brazil.

**Fecundity.** Capable to reproduce females presented mean ovarian fecundity of 4.4 vitellogenic follicles per female (S.D.=3.5;  $n=15$ ), ranging from one to 15 follicles. Uterine fecundity ranged from one to three embryos or eggs per female. Uterine eggs were covered by a thin membrane which was absent in late pregnancy females and were observed in 14 females and just one in each female. Embryos were observed in 33 females and both uteri were functional. Twelve females had uterine fecundity  $\geq 2$ , with 58.3% presenting embryos in both uteri and 41.7% with two embryos in one uterus.

No evident relationship between uterine fecundity and total length was observed, although smaller females presented only one embryo and larger females presented two or three embryos (Fig. 6). In addition, a high overlap of females bearing one and two embryos was observed between 204 and 248 mm  $L_T$  and maximum fecundity (three embryos/female) was observed in females of 252 and 258 mm  $L_T$ .



**Fig. 6.** Relationship between total length and fecundity for *Benthobatis krefftii* from São Paulo State continental slope, Brazil, considering (D) early pregnancy and (E) late pregnancy females.

**Size at birth.** Embryos had total length ranging from 14 to 100 mm  $L_T$  ( $73.0 \pm 22.4$ ,  $n=37$ ). Near-term embryos were 91-100 mm  $L_T$ , with female ones being 91-96 mm  $L_T$  ( $n=4$ ) and males presenting  $L_T$  of 95 mm ( $n=1$ ). One male neonate of 91  $L_T$  was observed.

## Discussion

Reproductive knowledge is essential for management and conservation plans (Walker, 2005). Yet, information about the reproductive biology of Brazilian deepwater elasmobranchs is scarce due to the lack of individuals discharged and also low commercial value of this group. *Benthobatis krefftii* is the only species of this genus occurring in the Southwest Atlantic, being relevant for the local ichthyofauna. Since little biological information is available for this species, no predictions can be provided under an overfishing scenario. This, added to its limited geographic distribution, indicates a potential vulnerability of this species.

Sexual development started with follicle growth in ovaries, followed by uteri enlargement in females, around 170 mm  $L_T$ , and maturity onset at 177 mm  $L_T$ . Maturity was first observed at 162 mm  $L_T$  in males, with clasper gland and lobules enlargement, followed by an increase in testes mass. *Benthobatis krefftii* seems to have an abrupt transition from developing to capable to reproduce stages, within a limited total length range, as observed for other deepwater elasmobranchs species (Rigby *et al.*, 2016). Since deepwater fishes tend to present slower metabolisms (Koslow, 1996) resulting in slower growth and late maturity, is reasonable to suggest that this abrupt transition reflects the habitat where this species occurs.

Rincon (1997) observed only one functional ovary in specimens of *B. krefftii* from southern Brazil. Torpediniformes present, however, usually present two functional ovaries, as observed in this study and for *Torpedo torpedo* (Linnaeus, 1758) (Capapé *et al.*, 2000),

*Tetronarce californica* (Ayres, 1855) (Neer, Cailliet, 2001), *Narcine bancroftii* (Griffith & Smith, 1834) (Moreno *et al.*, 2010) and *Narcine entemedor* Jordan & Starck, 1895 (Burgos-Vázquez *et al.*, 2017). Differences in reproductive functionality have been reported between species for the genus *Squatina* (Vooren, Da Silva, 1991), however, differences in the number of gonads between populations was never reported for elasmobranchs. Further studies are needed to understand if there is an intraspecific variation in gonad functionality between southern and southeastern Brazilian populations of *B. krefftii*.

Gonad mass increased abruptly as a function of  $L_T$  at larger developing individuals, suggesting that reproductive investment occurs in a short period before maturation and continues increasing, slowly, in mature individuals. Rapid gonad growth may reflect a reproductive strategy related to metabolism and slow growth of deepwater species due to low temperatures of deeper waters (Rigby *et al.*, 2016). Capable to reproduce females had the heaviest ovaries, followed by post-partum ones. During early pregnancy, a rapid decrease in  $M_O$  was observed, followed by a recovery in late pregnancy and post-partum stages, suggesting that mating does not immediately follows parturition. The same pattern was observed for *Tetronarce nobiliana* (Bonaparte, 1835), with females presenting distended uteri and vitellogenic activity (Capapé *et al.*, 2006) and for *Narcine brasiliensis* (Olfers, 1831) (Villavicencio-Garayazar, 1993). Capapé *et al.* (2001), however, found gravid females of *Torpedo mackayana* Metzelaar, 1919 with degenerating oocytes.

Liver mass rapidly decreased during early-pregnancy, and increased for post-partum females, achieving its maximum at capable to reproduce females. Vitellogenic activity starts, especially, in late-pregnancy and post-partum stages but at capable to reproduce stages, females have larger livers than in any other stage, being synchronous with ovaries mass increase. Such a synchronous growth between liver and gonad mass is expected once yolk plays an important role during the whole embryo development for *B. krefftii* (Hamlett *et al.*, 2005).

Clasper length presented a sigmoid growth pattern, with a slow growth, followed by a rapid growth and finally a slow growth, when individuals reach maturity, similar to other elasmobranchs (Parsons, 1983; Costa *et al.*, 2005; Oddone *et al.*, 2008; Ebert *et al.*, 2008). In clasper gland length, the same sigmoid growth pattern was observed and, considering the secretory function of the clasper gland related to copulatory activity (Piercy *et al.*, 2006), a synchronous growth is expected for both structures.

Size at maturity was different between sexes, with females attaining maturity at larger lengths than males (191.1 mm in females and 176.3 mm in males). Sexual differences in  $L_{T50}$  are common among elasmobranchs, including Narcinidae family (Moreno *et al.*, 2010; Rolim *et al.*, 2015) and this delay in maturity may reflect the reproductive cost of females, such as vitellogenesis and

embryo nutrition, for example (Cortés, 2000). In addition, attaining larger sizes may represent a reproductive advantage to females regarding embryo development and growth within the uterus.

Specimens from southern Brazil mature, in general, at 150 mm for both sexes (Rincon *et al.*, 2001), whereas in this study, maturity was first observed at 177 mm and 162 mm for females and males, respectively. Such discrepancies may be a result of different methods for maturity estimates or, in fact, an evidence of two distinct populations, as observed for *Mustelus manazo* Bleeker, 1855 by Yamaguchi *et al.* (2000). In this case, molecular analyses would be necessary to elucidate such questions.

*Benthobatis krefftii* attains maturity at 63% and 73% of total length for females and males respectively, suggesting a late maturation. Although age-estimation studies have not been yet carried out for this species, it is known that late maturation is associated with long life-span (Roberts, 2002). In addition, elasmobranchs present a relative late maturation (Cortés, 2000), with several species maturing at >60% of the total body length (Mejía-Falla *et al.*, 2012; Clarke *et al.*, 2014). Late maturity, added to a possible longevity of this species make them vulnerable to non-natural deaths (Cortés, 2000), highlighting the need of studies concerning biology parameters for eventual management and conservation plans.

Litter size recorded for *Benthobatis krefftii* was low in comparison with other Torpediniformes (Capapé *et al.*, 2006; Moreno *et al.*, 2010; Rolim *et al.*, 2015). El Kamel-Moutalibi *et al.* (2011) found 1-13 embryos/female in *Torpedo torpedo*, whilst Burgos-Vázquez *et al.* (2017) observed 1-24 embryos in *Narcine entemedor*. Such differences in fecundity among Torpediniformes might suggest that low fecundity is more related to depth than to phylogenetic relationships. Litter size in this study was also different than the one observed for *B. krefftii* in southern Brazil (Rincon, 1997). The low fecundity observed for *B. krefftii* and other deepwater species, highlights that deepwater Chondrichthyes are less fecund than coastal species and might be even more vulnerable to fishing mortality (García *et al.*, 2008).

Differences in ovarian fecundity and number of uterine eggs or embryos were observed for *B. krefftii*, as already reported for several elasmobranchs. Consalvo *et al.* (2007) observed 2-22 ovarian follicles but 4-19 embryos in *T. torpedo*, whereas, for *T. marmorata*, ovarian follicles ranged from one to 35 and litter size was 3-16. These differences are probably a result of reabsorption events once not all follicles are ovulated and might undergo atresia (Conrath, 2005).

Near-term embryos (*i.e.* fully developed embryos without yolk-sac) were observed from 90 to 100 mm  $L_T$  and one neonate was observed with 91 mm  $L_T$ . Therefore, birth may occur when embryos attain 90-100 mm total length. *Benthobatis krefftii* invests in size of each embryo, rather than litter size. Therefore, despite its low fecundity, neonates are 1/3 of the mother size and might have better



chances of survival. *Narcine brasiliensis* size at birth was estimated at 110-112 mm  $L_T$ , while females attain 840 mm  $L_T$  (Villavicencio-Garayzar, 1993). Other Torpediniformes also present smaller relative size at birth, with term embryos being approximately 20% of maximum total length recorded (Capapé *et al.*, 2001; Capapé *et al.*, 2006). Such strategy of producing large neonates as observed in this study may not be a characteristic in common among Torpediniformes, but specifically of this species due to its habitat.

In conclusion, *B. krefftii* presents a relative low fecundity, but large size at birth. This strategy seems to be advantageous in a non-explored environment. However, under circumstances of deep-sea exploration, these reproductive aspects may affect recruitment success, making this species vulnerable. In addition, after attaining maturity, females seem to, rapidly, contribute to the population increase, since a short period of body growth is observed in the mean  $L_T$  between capable to reproduce and pregnant females. Reproductive investment in gonad increase occurs right before maturity is reached, indicating that this species may invest in somatic growth during most of the immature and developing stages.

This study provides information about the reproductive biology of the rare and unknown southeastern benthic elasmobranch species, *B. krefftii*, from the southeastern Brazilian slope. Life history differences were observed between the southern and southeastern populations highlighting the need to consider these populations differently for management and conservation purposes. Several studies show that populations present a wide variation in life-history patterns, including size at maturity (Yamaguchi *et al.*, 2000; Capapé *et al.*, 2006; Farrel *et al.*, 2010) and that could be the reason of such differences in *B. krefftii* studies. Additional biological studies, considering larger samples and including molecular approach are needed to understand the population structure.

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