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(ZOOLOGIA)

**Estrutura populacional e biologia reprodutiva da
raia-elétrica-cega, *Benthobatis krefftii*
(Chondrichthyes, Narcinidae) no talude continental
do estado de São Paulo**

Mariana da Fontoura Martins

Dissertação apresentada ao Instituto de
Biociências do Campus de Rio Claro,
Universidade Estadual Paulista, como parte dos
requisitos para obtenção do título de Mestre em
Ciências Biológicas (Zoologia)

Rio Claro (SP),

Maior de 2017

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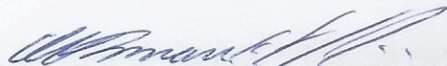
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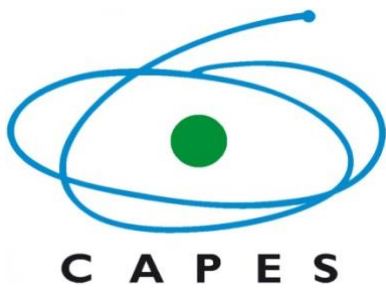
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DEDICATÓRIA

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RESUMO

A raia elétrica cega *Benthobatis krefftii* é endêmica do Atlântico Sul Ocidental e apresenta distribuição restrita desde o sul até o sudeste Brasileiros, ocorrendo em profundidades entre 400 e 600 m. Apesar de seu extremo endemismo, sua biologia é virtualmente desconhecida, especialmente no que diz respeito à porção norte de sua distribuição. O objetivo deste trabalho foi estudar a estrutura populacional e biologia reprodutiva desta espécie, no talude continental do estado de São Paulo. Os indivíduos foram capturados em julho e agosto de 2003 e dezembro de 2007 no cruzeiro científico Soloncy Moura, o qual operou em 492-501 m de profundidade entre os municípios de Santos e Cananéia. Quanto à composição de captura, *B. krefftii* foi o condricte mais capturado, seguido por *Gurgesiella dorsalifera*. Os demais foram *Hydrolagus matallanasi*, *Dipturus* sp. e *Torpedo*, sp. Quanto à estrutura em tamanho, fêmeas diferem de machos por apresentarem maiores comprimentos (máximo de 299 mm em fêmeas e 256 mm em machos). A razão sexual total foi diferente de 1:1, bem como para indivíduos adultos. Nos outros estágios de maturidade considerados (imaturo e em desenvolvimento), não houve diferença significativa. A relação comprimento-peso e o tamanho onde 50% da população encontra-se maduro (L_{T50}) também foram diferentes entre os sexos, em favor das fêmeas. Fêmeas apresentaram L_{T50} de 191 mm enquanto machos maturam em 176 mm. A fecundidade uterina foi de três embriões por fêmea, com embriões nascendo entre 90 e 100 mm de comprimento (cerca de 1/3 do máximo comprimento registrado para a espécie).

Palavras-chave: estrutura em tamanho, fecundidade, maturidade, razão sexual, relação comprimento-peso, talude continental

ABSTRACT

The blind electric ray *Benthobatis krefftii* is endemic to the South West Atlantic and has a restricted distribution, from south and southeast Brazil, occurring in 400-600 m depth. Despite its endemism, its biology is virtually unknown, especially concerning the north extreme of its distribution. For this reason, the aim of this work was to study the population structure and reproductive biology of *B. krefftii* off São Paulo State continental slope. Individuals were captured in July and August of 2003 and December 2007 during the scientific cruise Soloncy Moura, operating in 492-501 m depth between Santos and Cananéia municipalities. *Benthobatis krefftii* was the most captured chondrichthyan, followed by *Gurgesiella dorsalifera*. Other chondrichthyan captured were: *Hydrolagus matalanasi*, *Dipturus* sp and *Torpedo* sp.. Concerning size structure, females were larger than males (females: 299 mm; males: 256 mm total length). Total sex ratio was different from 1:1, as well as for adult individuals. At other maturity stages considered (immature and developing), no differences were observed. The length-weight relationship and size at 50 % maturity (L_{T50}) were also different between sexes, biased toward females. Female's L_{T50} was 191 mm, while in males, L_{T50} was 176 mm total length. Uterine fecundity was three embryos/ female, with size at birth estimated in 90-100 mm total length (about 1/3 of the total length recorded for this species)

Key-words: continental slope, fecundity, length-weight relationship, maturity, sex-ratio, size structure

INTRODUÇÃO GERAL

Diversidade de Chondrichthyes

A Classe Chondrichthyes representa os vertebrados gnatostomados vivos mais antigos (Wourms, 1977), sendo composta por duas Subclasses: Holocephali, que compreende as quimeras, e Elasmobranchii, composta pelos tubarões e raias (Aschliman

et al., 2012). Em comparação com os demais vertebrados, este é um grupo com baixa riqueza de espécies, apresentando cerca de 1188 descritas (Weigmann, 2016). Ainda assim, seus representantes se destacam ecologicamente, sobretudo nos ambientes marinhos, onde usualmente desempenham importante papel no funcionamento de ecossistemas, atuando, principalmente, como predadores apicais e mesopredadores em diferentes nichos (Stevens *et al.*, 2000). No Brasil, são conhecidas cerca de 165 espécies Chondrichthyes marinhos, compreendendo 12 ordens (Rosa & Gadig, 2004).

A ordem Torpediniformes, composta pelas raias elétricas, é o terceiro maior grupo em termos de riqueza de espécies, com cerca de 70 espécies de quatro famílias (Weigmann, 2016). No entanto, em águas brasileiras, apenas duas famílias (Torpedinidae e Narcinidae) e oito espécies foram até o momento registradas (Rosa & Gadig, 2014). Caracterizam-se, entre outros atributos morfológicos, pela presença de órgãos produtores de eletricidade, dispostos dorso-ventralmente na área branquial do disco, normalmente riniformes. Exibem hábitos bentônicos, desde áreas costeiras até taludes continentais, onde vivem em descanso ou baixa motilidade, muitas vezes semi enterradas no substrato (McEachran & Carvalho, 2002).

A família Narcinidae é composta por espécies de pequeno a médio porte e distribuição global (Carvalho *et al.*, 1999; McEachran & Carvalho, 2002). São vivíparas lecitotróficas e apresentam uma peculiaridade no que diz respeito ao seu aparelho reprodutor: a ausência de glândula oviducal macroscopicamente visível (Prasad, 1945). Esta estrutura é responsável, principalmente, pelo armazenamento de esperma e produção dos envoltórios embrionários, além de ser o sítio onde ocorre a fertilização (Hamlett *et al.*, 2005). São conhecidos quatro gêneros e pouco mais de 30 espécies (Weigmann, 2016), sendo que seis espécies foram registradas no Brasil até o presente (Rosa & Gadig, 2014).

O gênero *Benthobatis* Alcock, 1898 se distribui de maneira dispersa em águas frias de profundidade desde regiões tropicais até sub-temperadas. São conhecidas quatro espécies: *B. marcida* Bean & Weed, 1909, do Atlântico Norte ocidental; *B. moresbyi* Alcock, 1898, do Índico ocidental; *B. yangi* Carvalho, Compagno & Ebert, 2003, do Pacífico Norte ocidental; e *B. krefftii* Rincon, Stehmann & Vooren, 2001, do Atlântico Sul ocidental. O gênero é virtualmente desconhecido quanto à muitos aspectos da sua biologia e história natural, sendo que os poucos estudos publicados disponíveis consideram aspectos da sua sistemática e taxonomia (Carvalho, 1999; Rincon *et al.*, 2001; Carvalho *et al.*, 2003).

O sistema reprodutor feminino de *Benthobatis marcida* foi descrito por Daiber (1959), com base no exame de apenas um exemplar, medindo 435 mm de comprimento total. De acordo com esse estudo, o sistema reprodutor feminino é composto por ovários e ovidutos pares. A glândula oviductal não foi observada neste estudo. Posteriormente, na descrição da espécie *B. krefftii*, Rincón *et al.* (2001) forneceu alguns dados biológicos sobre a mesma, tais como tamanho, estimativa de maturidade sexual e fecundidade.

A raia elétrica cega brasileira, *Bentobatis krefftii* (Figura 1), tem ocorrência conhecida até o momento, restrita no Brasil, entre os estados de São Paulo e Rio Grande do Sul (Rincon, 2004), habitando o ambiente bentônico do talude continental entre profundidades de 400 a 600 m (Rincon, 1997; Bernardes *et al.*, 2005). Aspectos da biologia desta espécie foram abordados com base em 63 indivíduos coletados no talude continental do Rio Grande do Sul, considerando aspectos reprodutivos, alimentação e taxonomia (Rincon, 1997). Nesse estudo acima citado, os tamanhos máximos registrados para machos e fêmeas foram de 200 e 268 mm, respectivamente, e o tamanho de primeira maturidade foi estimado em 150 mm. A fecundidade uterina foi estimada em dois embriões e não foram observadas gônadas pares, baseado em um número amostral de 63

indivíduos. A ausência de uma gônada diverge do observado para *B. marcida* (Daiber, 1959), no entanto, ambos os trabalhos foram realizados baseados em um baixo número amostral, reforçando a necessidade de mais estudos a respeito deste grupo. Bernardes *et al.*, (2005) reporta indivíduos de até 296 mm de comprimento total baseado em uma amostra de 31 espécimes coletados no sudeste e sul do Brasil. No entanto, a localidade onde o maior indivíduo foi capturado não é informada.

Atualmente, *B. krefftii* é classificada como “Vulnerável” pela Lista Vermelha de Espécies Ameaçadas da União Internacional para a Conservação da Natureza (IUCN) (Rincon, 2004). A distribuição extremamente restrita e conseqüente vulnerabilidade da espécie, acrescida da falta de informações a respeito da mesma, bem como de outras espécies do gênero, destaca a importância do conhecimento da sua biologia.

Reprodução em Chondrichthyes

O estudo da reprodução em Chondrichthyes iniciou-se com observações feitas por Aristóteles. Descrição de cápsulas ovíferas, sistema reprodutor e a classificação dos modos de reprodução em oviparidade e viviparidade são algumas das contribuições feitas pelo mesmo (Wourms & Demski, 1993). Os Chondrichthyes representam o primeiro grupo na linhagem de vertebrados a apresentar ou estabilizar condições de fertilização interna, viviparidade e mecanismos placentários para nutrição embrionária, sendo o seu estudo de grande interesse (Wourms, 1977). O desenvolvimento de estruturas copulatórias foi, primeiramente, observado em placodermes (Longet *et al.*, 2014). No entanto, no grupo dos Chondrichthyes, tornou-se chave para o sucesso evolutivo do grupo. A fertilização interna permitiu uma otimização da reprodução neste grupo, assegurando que o investimento em gametogênese seja mais eficiente (Engel & Callard, 2005).

O sucesso evolutivo deste grupo pode ser atribuído, em parte, pela fertilização interna e os diversos modos de reprodução que se desenvolveram. Diversos modos de oviparidade e, principalmente, viviparidade são observados na classe Chondrichthyes (Wourms, 1977). Atualmente, o critério de classificação baseado em local do desenvolvimento e nutrição embrionária apresenta dois modos de oviparidade e seis modos de viviparidade (Musick & Ellis, 2005), sendo a oviparidade exclusivamente lecitotrófica e a viviparidade lecitotrófica ou matrotrófica.

A lecitotrofia é o modo de nutrição embrionário onde o embrião nutre-se do vitelo produzido via vitelogenina, secretada no fígado materno, e armazenado no saco de vitelo (Hamlett *et al.*, 2005). Neste modo, existe nenhum ou muito pouco aporte energético materno durante o desenvolvimento embrionário, de forma que há uma perda de peso seco do embrião e seu saco de vitelo entre o início e final do desenvolvimento embrionário. Na matrotrofia, no entanto, a nutrição embrionária ocorre via vitelo provido inicialmente pela fêmea, mas diferentes formas de aporte energético materno ocorrem (oofagia, histotrofia ou placentotrofia) (Angelini & Ghiara, 1984).

Na oviparidade, o desenvolvimento embrionário ocorre no ambiente externo, no interior de estruturas rígidas complexas chamadas de cápsulas ovíferas e o modo de nutrição é lecitotrófico, ou seja, os embriões nutrem-se, exclusivamente de vitelo contido no saco de vitelo (Wourms, 1977; Hamlett *et al.*, 2005b). A viviparidade, no entanto, é caracterizada pela retenção dos embriões no oviduto materno até o nascimento, os quais podem nutrir-se apenas de vitelo contido no saco de vitelo ou receber aporte materno ao longo do desenvolvimento.

A viviparidade surgiu repetidas vezes na evolução de vertebrados e seu surgimento foi concomitante com o desenvolvimento de diferentes modos de nutrição embrionária e,

consequentemente, especializações do útero (Wourms, 1981). A viviparidade lecitotrófica é o modo mais frequente dentro de Chondrichthyes, ocorrendo na maioria das ordens de Elasmobranchii, exceto em Lamniformes e Heterodontiformes (Hamlett *et al.*, 2005). Neste modo, o embrião desenvolve-se no oviduto materno, mas nutre-se apenas do vitelo contido no saco vitelínico (Wourms, 1977; Wyffels, 2009).

O estudo da reprodução, em uma abordagem quantitativa, é considerado uma ferramenta importante para a avaliação do status das populações. Especificamente, razão sexual ao nascer, fecundidade e ogivas de maturidade são três componentes fundamentais relacionados à reprodução para essa avaliação (Walker, 2005). Além disso, o tamanho de primeira maturidade, por exemplo, é usado como indicador do mínimo tamanho de captura permitido (Lucifora *et al.* 1999). A estimativa de fecundidade, por sua vez, é, frequentemente, correlacionada com a capacidade de recuperação de uma população, embora seja reconhecido que ela não é suficiente para essa recuperação (Hutchings *et al.* 2012).

Vulnerabilidade e conservação de Chondrichthyes

Chondrichthyes são considerados particularmente vulneráveis à exploração pesqueira, em função das suas características de história de vida, como crescimento lento, maturação tardia e baixa fecundidade (Dulvy *et al.*, 2000; Stevens *et al.*, 2000). Essas características resultam em baixas taxas de reprodução e potencial de incremento da população, culminando com a baixa resistência à mortalidade causada pela pesca (Hoenig & Gruber, 1990, Walker & Hislop, 1998). Não obstante, este grupo tem se tornado alvo direto da indústria pesqueira, principalmente em função do declínio de algumas populações de teleósteos e também do alto valor de suas nadadeiras, fígado e até mesmo

brânquias (Clarke *et al.*, 2006), levando diversas espécies ao declínio e até extinção local (Brander, 1981).

Espécies que habitam altas profundidades são consideradas ainda mais susceptíveis à exploração pesqueira uma vez que, de maneira geral, são menos produtivas do que espécies costeiras (Simpfendorfer & Kyne, 2009). Estudos mostram que tais espécies são entre 1,6 e 2,9 vezes menos resilientes que outros elasmobrânquios (Stevens *et al.*, 2000, García *et al.*, 2008), portanto mais vulneráveis a eventuais pressões por pesca. Somado a isso, habitats de maior profundidade vem sendo explorados gradativamente em função da depleção de estoques costeiros (Morato *et al.*, 2006), tornando-se fator preocupante para o equilíbrio dessas populações.

A ausência de informações sobre a história de vida e outros parâmetros biológicos espécie-específicos torna os Chondrichthyes ainda mais vulneráveis, uma vez que essa ausência de dados dificulta a determinação do status de conservação das espécies, bem como a elaboração de planos de manejo e conservação (Dulvy *et al.* 2003). Além disso, padrões de história de vida, bem como as respostas à exploração pesqueira podem variar entre espécies e populações de uma mesma espécie, ao longo de sua distribuição geográfica (Ebert *et al.*, 2008; Clarke *et al.*, 2014).

Estruturação da dissertação

O presente trabalho apresenta resultados obtidos sobre a estrutura populacional e biologia reprodutiva da raia elétrica cega brasileira *B. krefftii*, baseado em indivíduos coletados no talude continental do Estado de São Paulo. A amostra utilizada representa a maior coleção de indivíduos do gênero *Benthobatis*, disponível até hoje (Figura 2). Inicialmente, 341 indivíduos foram coletados nos cruzeiros científicos dos anos de 2003 e 2007 a bordo do N/Pq Soloncy Moura (Figura 3). Destes indivíduos, uma subamostra

foi doada, outros foram utilizados em trabalhos de iniciação científica desenvolvidos no Laboratório de Pesquisa de Elasmobrânquios da Universidade Estadual Paulista “Júlio de Mesquita Filho”, sob orientação do Prof. Dr. Otto Bismarck Fazzano Gadig.

Os resultados apresentados no primeiro capítulo desta dissertação são provenientes de uma amostra de 284 indivíduos coletados em três arrastos de fundo feitos em Julho e Agosto de 2003. O motivo pelo qual as amostras de 2007 não estão incluídas neste primeiro capítulo é a ausência de dados coletados de indivíduos frescos (comprimento/peso) no ano de 2007. Já o segundo capítulo apresenta dados obtidos de indivíduos coletados em Julho e Agosto de 2003 e Dezembro de 2007 (266 indivíduos). Todos os dados biométricos utilizados no segundo capítulo foram obtidos de exemplares fixados em formalina 4% e posteriormente armazenados em álcool 70%. A disparidade no número amostral utilizado em cada capítulo ocorreu em função de, no capítulo II, somente indivíduos inteiros (não processados anteriormente) terem sido considerados.

Referências

Angelini, F.; Ghiara, G. (1984). Reproductive modes and strategies in vertebrate evolution. *Bolletino de Zoologia* **51**, 121–203.

Aschliman, N. C.; Claeson, K. M.; McEachran, J. D. (2012). Phylogeny of Batoidea. In: Carrier, J. C.; Musick, J. A.; Heithaus, M. R. (eds). *Biology of Sharks and Their Relatives*, pp. 57–96. Boca Raton: CRC Press.

Bernardes, R. A.; Figueiredo, J. L.; Rodrigues, A. R.; Fischer, L.G.; Vooren, C. M.; Haimovici, M. & Rossi-Wongtschowski, C. L. D. B. (2005). Peixes da zona econômica

exclusiva da região sudeste-sul do Brasil: Levantamento com armadilhas, pargueiras e rede de arrasto de fundo. São Paulo, Edusp. 295 p.

Brander, K.(1981). Disappearance of common skate *Raia batis* from Irish Sea. *Nature* **290**, 48–49.

Carvalho, M. R.; Compagno, L. V.; Last, P. R. (1999). Order Torpediniformes, Narcinidae. In: Carpenter, K. E.; Niem V. H. *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 3. Batoid fishes, chimaeras and bony fishes part 1 (Elopidae to Linophrynidae)*, pp. 1397-2068. Rome: FAO.

Carvalho, M. R.; Compagno, L. V.; Ebert, D. A. (2003). *Benthobatis yangi*, a new species of blind electric ray from Taiwan (Condriichthyes: Torpediniformes: Narcinidae). *Bulletin of Marine Sciences* **72**(3), 923-939.

Clarke, S. C., McAllister, M.K., Milner-Gulland, E.J., Kirkwood, G.P., Michielsens, C.G.J., Agnew, D.J., Pikitch, E.K., Nakano, H., Shivji, M.S. (2006). Global estimates of shark catches using trade records from commercial markets. *Ecology Letters* **9**(10), 1115-1126.

Clarke, S.C.; Espinoza, M.; Wehrtmann, I. S. (2014). Reproductive ecology of demersal elasmobranchs from a data-deficient fishery, Pacific of Costa Rica, Central America. *Fisheries Research* **157**, 96-105.

Daiber, F. C. (1959). An Observation on the Deep-Sea Electric Ray. *Copeia* **1945**(1), 74.

Dulvy, N. K.; Metcalfe, J. D.; Glanville, J.; Pawson, M. G.; Reynolds, J. D. (2000). Fishery Stability, Local Extinctions, and Shifts in Community Structure in Skates. *Conservation Biology* **14**, 283-293.

Dulvy, N. K.; Sadovy, Y.; Reynolds, J. D. (2003). Extinction vulnerability in marine populations. *Fish and Fisheries* **4**, 25-64.

Ebert, D.; Compagno, L. V.; Cowley, P. D. (2008). Aspects of the reproductive biology of skates (Chondrichthyes: Rajiformes: Rajoidei) from southern Africa. *ICES Journal of Marine Sciences* **65**, 81-102.

Engel, K.B.; Callard, G.V. (2005). The Testis and Spermatogenesis. In: Hamlett, W.C. (ed.) *Reproductive biology and phylogeny of chondrichthyes: sharks, rays and chimaeras*, pp. 171-200. Endfield, USA: Science Publishers

Garcia, V. B.; Lucifora, L. O.; Myers, R. A. (2008). The importance of habitat and life history to extinction risk in sharks, skates, rays and chimaeras. *Proceedings of the Royal Society B* **275**, 83-89.

Hamlett, W. C.; Knight, D. P.; Pereira, F. T. V.; Steele, J.; Sever, D. M. (2005a). Oviducal glands in Chondrichthyes. In: Hamlett, W.C. (ed.) *Reproductive biology and phylogeny of chondrichthyes: sharks, rays and chimaeras*, Vol. 3. Endfield, USA: Science Publishers: 395-434.

Hamlett, W. C., Kormanik, G., Storrie, M., Stevens, B., Walker, T.I. (2005b). Chondrichthyan parity, lecithotrophy and matrotrophy. In: Hamlett, W.C. (ed.) *Reproductive biology and phylogeny of chondrichthyes: sharks, rays and chimaeras*, Vol. 3. Endfield, USA: Science Publishers: 395-434.

Hoening, J. M.; Gruber, S. H. (1990). Life-History Patterns in the Elasmobranchs: Implications for Fisheries Management. In: Pratt, H. L.; Gruber, S. H.; Taniuchi, T. (eds.). *Elasmobranchs as living resources: advances in the biology, ecology, systematics, and the status of the fisheries. Technical Report NMFS 90*, pp 1-16. Washington: National Oceanic and Atmospheric Administration.

Hutchings, J. A.; Meyers, R. A.; Garcia, V. B.; Lucifora, L. O.; Kuparinen, A. (2012) Life-history correlates of extinction risk and recovery potential. *Ecological Application* **22**(4), 1061-1067.

Long, J. A., Mark-Kurik, E., Johanson, Z., Lee, M. S. Y., Young, G. C., Min, Z., Ahlberg, P. E., Newman, M., Jones, R., Blaauwen, J., Choo, B., Trinajstić, K. (2014). Copulation in antiarchplacoderms and the origin of gnathostome internal fertilization. *Nature* **517**, 196–199.

Lucifora, L. O.; Valero, J. L.; Garcia, V. B. (1999). Length at maturity of the greeneyespurdog shark, *Squalus mitsukurii* (Elasmobranchii: Squalidae), from the SW Atlantic, with comparisons with other regions. *Marine Freshwater Research, Australia* **50**, 629-632.

McEachran, J. D.; Carvalho, M. R. (2002). Batoid fishes. In *The Living Marine Resources of the Western Central Atlantic. FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists, Vol. 5* (Carpenter, K., ed.), pp. 508–589. Rome: FAO.

Morato, T.; Watson, R.; Pitcher, T. J.; Pauly, D. (2006). Fishing down the deep. *Fish and Fisheries* **7**, 24–34.

Musick, J. A.; Ellis, J. K. (2005). Reproductive Evolution of Chondrichthyes. In: Hamlett, W. C (ed.). *Reproductive biology and phylogeny of chondrichthyes: sharks, rays and chimaeras*, pp. 45-79. Endfield, USA: Science Publishers

Prasad, R. R. (1945). The structure, phylogenetic significance, and function of the nidamental glands of some elasmobranchs of the Madras coast. *Proceedings of the National Institute of Science India* **11**, 282-303.

Rincon, G. F. (1997). Taxonomia, alimentação e reprodução raia elétrica *Benthobatis* sp. (Torpediniformes: Narcinidae) no sul do Brasil. Pós-Graduação em Oceanografia Biológica. Fundação Universidade do Rio Grande.

Rincon, G.; Stehmann, M. F. W.; Vooren, C. M. (2001). Results of the research cruises of the FRV 'Walter Herwig' to South America. LXXIV. *Benthobatis krefftii* n. sp. (Chondrichthyes, Torpediniformes, Narcinidae), a new deep-water electric ray from off South Brazil and the third species of the genus. *Archive of Fishery Marine Research* **49**(1), 45-60.

Rincon, G. (2004). *Benthobatis krefftii*. The IUCN Red List of Threatened Species. Version 2014.3.<www.iucnredlist.org>. Downloaded on 18 May 2015.

Rosa, R., Gadig, O. B. F. (2014). Conhecimento da diversidade dos Chondrichthyes marinhos no Brasil: a contribuição de José Lima de Figueiredo. *Arquivos de Zoologia* **45**, 89-104.

Simpfendorfer, C.;Kyne, P. M. (2009). Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environmental Conservation* **6**, 97-103. doi: 10.1017/S0376892909990191

Stevens, J. D.; Bonfil R.; Dulvy, N. K.; Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Sciences* **57**, 476-494.

Walker, P. A. (2005). Reproduction in fisheries science. In: Hamlett, W. C. (ed.). *Reproductive Biology and Phylogeny of Chondrichthyes: sharks, rays and chimaeras*, pp. 81–127. Enfield: Science Publishers.

Walker, P. A.; Hislop, J. R. G. (1998). Sensitive skates or resilient rays? Spatial and temporal shifts in ray species composition in the central and north-western North Sea between 1930 and the present day. *ICES Journal of Marine Sciences* **55**, 797-808.

Weigmann, S. (2016). Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. *Journal of Fish Biology* **88**, 837-1037.

Wourms, J. P. (1977) Reproduction and Development in Chondrichthyan Fishes. *American Zoologist* **17**(2), 379-410.

Wourms, J. P.; Demski, L. S. (1993). The reproduction and development of sharks, skates, rays and ratfishes: introduction, history, overview, and future prospects. *Environmental Biology of Fishes* **38**, 7-21.

Wourms, J. P. (1981). Viviparity: the maternal-fetal relationship in fishes. *American Zoologist* **21**, 473-515.

Wyffels, J. T. (2009). Embryonic development of chondrichthyan fishes – A review. In: Kunz, Y., Kapoor, B. G., Luer, C. A. (eds). *Development of Non-Teleost Fishes*, pp. 1-103. Enfield: Science Publishers Inc.

Figuras



Figura 1. Exemplar fêmea de *Benthobatis krefftii* coletado no talude continental do estado de São Paulo



Figura 2. Coleção de *Benthobatis krefftii* coletadas no talude continental do estado de São Paulo em profundidades de 492 a 501 m, a qual foi utilizada no presente estudo.



Figura 3. Espécimes de *Benthobatis krefftii* (esquerda) a bordo do cruzeiro científico Soloncy Moura, o qual operou no talude continental do estado de São Paulo em profundidades de 492 a 501 m. Créditos: Teodoro Vaske Júnior.

OBJETIVOS

Objetivos Gerais

Estudar aspectos da estrutura populacional e biologia reprodutiva da raia elétrica cega *Benthobatis krefftii* no talude continental do estado de São Paulo.

Objetivos específicos

Capítulo I

- Determinar a estrutura em tamanho
- Determinar a razão sexual na área estudada
- Calcular a relação comprimento-peso para machos e fêmeas

Capítulo II

- Analisar o desenvolvimento gonadal e de estruturas acessórias
- Calcular o tamanho onde 50% da população encontra-se madura (L_{T50})
- Estimar as fecundidades ovariana e uterina
- Estimar o tamanho ao nascer

CAPÍTULO I

Catch and size composition, sex ratio and length-weight relationship of the rare blind electric ray, *Benthobatis krefftii* Rincon, Stehmann & Vooren, 2001 (Chondrichthyes, Narcinidae)

Catch and size composition, sex ratio and length-weight relationship of the rare blind electric ray *Benthobatis krefftii* Rincon, Stehmann & Vooren, 2001 (Chondrichthyes, Narcinidae)

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The following study reports information on catch and size composition, sex ratio and presence of sexual dimorphism for the length-weight relationship for the Brazilian electric ray *Benthobatis krefftii*. Individuals (156 females; 128 males) were captured along São Paulo State continental slope, at 492-500 m depth. Length composition and respective sexual differences were analyzed and tested, as well as total and per maturity stage sex ratio. Length-weight relationships were fitted and sexual differences were tested. Total length ranged from 118 to 299 mm for females and from 96 to 256 mm for males. Length structure was different between sexes, with females achieving larger length classes. Total sex ratio was also biased toward females, as well as for the adult sample, while in immature and developing individuals, sex ratio was 1:1. Finally, total length-total weight relationship was sexually different, with females being larger and heavier than males. Larger and heavier females are commonly observed among elasmobranchs and may be related to different reproductive strategies, where females attain larger sizes in able to become more prolific. Sex ratio biased toward females indicates a possible sexual segregation, especially concerning mature individuals.

Key-words: continental slope, electric ray, population structure, SW Atlantic,

Introduction

Deep-sea elasmobranchs are those who live restricted or most of their lifecycle in depths >200 m, which includes continental or insular slopes, abyssal plains and oceanic seamounts (Kyne & Simpfendorfer, 2007). Such animals present lower recovery potential

due to their low fecundity rates, compared to coastal elasmobranchs (Simpfendorfer & Kyne, 2009; Dulvy *et al.*, 2014). Habitat and reproductive modes influence the extinction risk of elasmobranchs and deep-water ones are 58% even more vulnerable than coastal species (García *et al.*, 2008). Despite these evidences, little is known about biology and habitat use of deep-sea elasmobranchs, remarkably the batoids (skates and rays).

Electric rays (Order Torpediniformes) are benthic batoids of small to medium-size, attaining <180 cm total length and present paired electrogenic organs in both sides of the branchial region of the disc (McEachran & Carvalho, 2002). Numbfishes (family Narcinidae) have a broad geographical distribution, occurring mostly in the continental shelves, but also some species inhabiting deep-water environments, in tropical to temperate latitudes (Carvalho *et al.*, 1999).

The genus *Benthobatis* was described in 19th century by Alcock (1898) and is represented by small-size, blind deep-sea batoids whose biology is poorly known. Four species represent this genus: *B. marcida* Bean & Weed, 1909, from the western North Atlantic; *B. moresbyi* Alcock, 1898, Indian Ocean; *B. yangi* Carvalho, Compagno & Ebert, 2003, western equatorial Pacific; and *B. krefftii* Rincon, Stehmann & Vooren, 2001, western South Atlantic (Carvalho *et al.*, 2003).

Benthobatis krefftii, the Brazilian blind electric ray, is endemic to the Southwestern Atlantic, with a very restricted distribution known to date, from south to southeastern Brazilian slopes in depths of 400-600 m. This species is categorized as “Vulnerable” according to the International Union for Conservation of Nature and Natural Resources (IUCN) Redlist of Threatened Species, due to its possible low resilience to non-natural deaths and very restricted distribution (Rincon *et al.*, 2001; Rincon, 2004). Little is known about the biological aspects and population structure of this species among its

geographical distribution. Rincon (1997) provided information about feeding habitats, reproduction and taxonomy of this species in south Brazil, and Bini *et al.* (2015) described anatomy of the scapulocoracoid and gill arches. However, no information on biological aspects and life-history patterns is available for the southeastern individuals.

The remarkable lack of information about the species along its entire known distribution highlights the need for more detailed studies on its basic biological parameters, since elasmobranchs show distinct life-history patterns according to latitude suggesting different populations (Yamaguchi *et al.*, 2000; Lombardi-Carlson *et al.*, 2003). Therefore, the aim of the present study is to analyze the population structure of *B. krefftii* in São Paulo continental slope (southeastern Brazil), considering the catch, length and sex composition, and total length-total weight relationship, verifying sexual differences concerning to this features.

Material and Methods

Sampling

Individuals were caught by bottom trawl nets from July 31th to August 2nd 2003 in São Paulo State continental slope, southeastern Brazil, at three sampling stations located between Santos and Cananéia municipalities (25°44'S - 25°45' S /45°09' - 45°11' W; 26°04'S - 23°03' S / 45°37' - 45°36' W; 26°09'S - 26°10' S /45°45' - 45°47' W – Figure 1), by the Research vessel “Soloncy Moura”, in depths varying between 492-501 m.

Biological data recorded at laboratory

All individuals were sexed, based on the presence or absence of copulatory appendices (claspers). Total length (TL, mm), measured from the tip of the rostrum to the distal

extreme of the caudal fin; and total weight (TW, g) were recorded for each specimen. The maturity stages considered were adapted from the maturity scale proposed by Stehmann (2000) and a maturity scale proposed for the ICES (2013) for viviparous elasmobranchs. Since maturity scales highly consider aspects of the oviducal gland, a specific scale was proposed for this species due to the lack of macroscopic oviducal gland, already recorded for Narcinidae species (Prasad, 1945). In addition, vascularization of the uteri could not be observed due to the condition of the material. Three maturity stages (immature, developing and mature) were herein considered (Table I).

Data analysis

Male and female length-frequency distribution was determined based on total length classes (of 10 mm) and absolute frequency of individuals per length class. Differences in the total length-absolute frequency distributions between sexes were tested with Kolmogorov-Smirnov test and differences in total length were tested with Mann-Whitney test (Zar, 2009). Total length results were expressed as mean \pm standard deviation and n represents the sample size.

Sex ratio was tested with chi-squared test for the total sample and by maturity stage in able to detect sexual segregation.

Total length-total weight relationship were calculated for both sexes using the linearized formula $LogY=a+bLogX$, where Y is the total weight, x is the total length and a and b are parameters of this equation (King, 2011). The presence of sexual differences for the linear regressions were tested with analysis of co-variance, applied to log transformed data. All of the statistical testes considered a significance level of 5%.

Results

During samples, a total of 886 Kg of fishes (Teleostei and Chondrichthyes) were captured. Chondrichthyes represented 3.23 % (28.65 kg) of the total catch and were represented by *B.kreffti*, *Gurgesiella dorsalifera* McEachran & Compagno, 1980, *Hydrolagus matallanasi* Soto & Vooren, 2004, *Torpedo* sp. and *Dipturus* sp. *Benthobatis krefftii* was the most frequent species among samples (20.75 Kg), followed by *G. dorsalifera* (5.7 Kg) (Figure 2).

A total of 284 specimens of *B. krefftii* were captured with 55% females (n=156) and 45% males (n= 128). Thirty five immature (51% of females; 49% of males), 51 developing (51% of females; 49% of males) and 178 mature (60% of females; 50% of males) were recorded.

Total length ranged from 118 to 299 mm (214.4 ± 32.2) for females, and from 96 to 256 mm (183.1 ± 34.6) for males. Mean total length was significantly different between sexes ($U=4751.5$; $p<0.01$), with females being larger than males. Twenty one length classes with 10 mm interval were considered, varying from 90 to 300 mm and length frequency distributions (Figure 3) also differed between sexes ($D=0.452$; $p<0.05$). Females were more frequent between 210 and 260 mm length classes while males were more frequent in 170-220 mm length classes. A high frequency of larger individuals, was observed for males and females.

Sex ratio was 1:1.22 ($\chi^2=5.470$; d.f.=1; $p=0.0193$, n=264) for the entire sample, significantly different from the 1:1 expected ratio and biased toward females. In immature individuals, sex ratio was 1:1 ($\chi^2=0.0286$; d.f.=1; $p=0.866$, n=35), as well as in developing ones ($\chi^2=0.0196$; d.f.=1; $p=0.889$, n=51). Sex ratio was, however, biased toward adult females (1:1.52; $\chi^2=7.2809$; d.f.=1; $p=0.007$, n=178).

In males, TW ranged from 7.6 to 134.2 g (49.51 ± 19.80 , $n=123$) and from 11.9 to 168.8 g (79.81 ± 30.20 , $n=156$) in females. The relationship between TL and TW in females (Figure 4) was represented by the equation $\log TW = -10.197 + 2.704 TL$ ($n=156$, $r^2=0.894$) and by the equation $\log TW = -8.854 + 2.289 TL$ ($n=123$, $r^2=0.903$). Total length-total weight relationship was significantly different between sexes ($F=6.930$; $d.f.=2, 179$; $p=0.009$).

Discussion

Benthobatis krefftii has restricted geographical distribution, reported at momenta, only along the continental slope of the southern Brazilian coast, southwestern Atlantic, between 400 to 600 m depth (Rincon, 2004), as well as *G. dorsalifera* (Menni & Stehmann, 2000). This species feeds mainly on small teleostean fishes, copepods and crustaceans (Rincón *et al.*, 2008). Potential similarities in habitat use between this two species may be studied and the high frequency of *B. krefftii* in chondrichthyan captures highlights the importance of this species in the trophic net of this deep water ecosystem along the western South Atlantic continental slope.

Maximum total length reported in this study differs from the results found for south Brazilian specimens (Rincon, 1997) where females attained maximum TL of 268 mm and males had maximum body size in the length class of 200 mm. Bernardes *et al.* (2005) reported maximum of 296 mm TL, based on specimens from São Paulo to Rio Grande do Sul continental slopes, but does not present further data on the size distribution according latitudinal variation. In the same geographical region, intraspecific differences in maximum body size along its geographical distribution were observed for *Psammobatis extenta* (Garman, 1913) (Braccini & Chiaramonte, 2002; Martins *et al.*, 2005) and *Dipturus chilensis* (Guichenot, 1848) (Licandeo & Cerna, 2007). These variations

between latitudes are possibly a result of differences in oceanographic conditions which alter biological patterns such as maximum body size, maturity and fecundity (Lombardi-Carlson *et al.*, 2003) and this seems to be the case for *B. krefftii*. Total length reported was also different between sexes. Such sexual differences are common among elasmobranchs, especially in viviparous species. Ebert *et al.* (2008) proposed that oviparous small-size skate species should not present sexual differences in maximum TL. In yolk-sac viviparous species, however, differences are often reported. Females of *Narcine brasiliensis* (Olfers, 1831) attain larger TL than males, in southeastern Brazil (Rolim *et al.*, 2015) and the same was observed for *Rhinobatos rhinobatos* (Linnaeus, 1758) in eastern Mediterranean (Lteif *et al.*, 2016) and *Squatina aculeata* Cuvier, 1829 off Senegal and Tunisia (Capapé *et al.*, 2005). In an intraspecific level, sexual differences in maximum size may reflect differences in reproductive costs among sexes (Cortés, 2000), with body size being possibly related to embryo retention.

Sex ratio biased toward females, especially in the adult phase, indicates a possible sexual segregation for this species in São Paulo continental slope. In addition, the presence of immature and developing individuals suggests that sexual segregation may be related to maturity, with mature individuals segregating, but immature and developing co-inhabiting in the same area as mature females. Sexual segregation has been well documented in elasmobranchs (Carasson *et al.*, 1992; Rigby *et al.*, 2016) and is possibly related to different habitat preferences due to resource utilization and/or reproductive strategies (Klimley, 1897; Rodriguez-Cabello *et al.*, 2007). Variability in feeding ecology of elasmobranchs along their ontogeny may reduce intraspecific competition and enable mature females and younger individuals to use the same habitat, although studies on feeding ecology of this species are necessary to understand the reasons of sexual segregation for *B. krefftii*.

In this study, *B. krefftii* females are heavier, in relation to length, than males. Sexual differences for length-weight relationships have been observed in different elasmobranch species (Ebert, *et al.*, 2008; Silva-Júnior, 2011; Pasquino *et al.*, 2016). Such differences may be a result of pregnant females in the sample and also variation in stomach repletion levels (Dias *et al.*, 2014). However, it can also represent sexual differences in the life-history strategies, with females being heavier due to the reproductive energetic costs.

This study presents, for the first time, aspects of the population structure of *B. krefftii* in southeastern Brazilian continental slope. *Benthobatis krefftii* seems to present a clear sexual dimorphism in total length, with females being substantially larger than males and attaining heavier bodies, which may be related to reproduction success. Sexual segregation may occur in this species for this area. In addition, intraspecific variation along its geographical distribution, with clear differences in size between southeastern and southern individuals may indicate the presence of two distinct populations. Nevertheless, molecular tools may be useful to verify if should these individuals be regarded as different populations. Finally, the data herein presented is pivotal as conservation tools to allow the population management of this rare and virtually unknown deep water elasmobranch species, categorized as “Vulnerable” by the IUCN.

References

Bini, I. F.; Santos, C. M. H.; Gadig, O. B. F. 2015. Anatomical Description of Scapulocoracoid and Gill Arches of *Benthobatis krefftii*. *Universitas Scientium*, 20(3): 305-312. doi: 10.11144/Javeriana.SC20-3.adsg.

Braccini, J. M.; Chiaramonte, G. E. 2002. Reproductive biology of *Psammobatis extenta*. *Journal of Fish Biology*, 61: 272-288.

Bernardes, R.A.; Figueiredo, J.L.; Rodrigues, A.R.; Fischer, L.G.; Vooren, C.M.; Haimovici, M. & Rossi-Wongtschowski, C.L.D.B. (2005). *Peixes da zona econômica exclusiva da região sudeste-sul do Brasil: Levantamento com armadilhas, pargueiras e rede de arrasto de fundo*. São Paulo, Edusp. 295 p.

- Capapé, C.; Diatta, Y.; Seck, A. A.; Guélorget, O.; Ben Souissi, J.; Zaouali, J. 2005. Reproduction of the sawback angelshark *Squatina aculeata* (Chondrichthyes: Squatinidae) off Senegal and Tunisia. *Cybium*, 29(2): 147-157.
- Carassón, M.; Stefanescu, C.; Cartes, J. E. 1992. Diets and bathymetric distributions of two bathyal sharks of the Catalan deep sea (western Mediterranean). *Marine Ecology Progress Series*, 82: 21-30.
- Carvalho, M. R. 1999. A synopsis of the deep-sea genus *Benthobatis* Alcock, with a redescription of the type species *Benthobatis moresbyi* Alcock, 1898 (Chondrichthyes, Torpediniformes, Narcinidae). *Society France Ichthyology*, 231-255
- Carvalho, M. R.; Compagno, L. J. V.; Ebert, D. A. 2003. *Benthobatis yangi*, a new species of blind electric ray from Taiwan (Chondrichthyes, Torpediniformes, Narcinidae). *Bulletin of Marine Science*, 72(3): 923-939.
- Cortés, E. 2000. Life History Patterns and Correlations in Sharks. *Reviews in Fisheries Science*, 8(4): 299-344.
- Dias, J. F.; Wellington, S. F.; Schmidt, T. C. S. 2014. Length-weight relationship of 73 fish species caught in the southeastern inner continental shelf region of Brazil. *Latin American Journal of Aquatic Research*, 42(1): 127-136.
- Dulvy, N. K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., Carlson, J.K., Davidson, L.N.K., Fordham, S.V., Francis, M.P., Pollock, C.M., Simpfendorfer, C.A., Burgess, G.H., Carpenter, K.E., Compagno, L.J.V., Ebert, D.A., Gibson, C., Heupel, M.R., Livingstone, S.R., Sanciangco, J.C., Stevens, J.D., Valenti, S., White, W. T. 2014. Extinction risk and conservation of the world's sharks and rays. *eLife* 3, e00590

Ebert, D. A.; Compagno, L. J. V.; Cowley, P. D. 2008. Aspects of the reproductive biology of skates (Chondrichthyes: Rajiformes: Rajoidei) from southern Africa. *ICES Journal of Marine Science*, 65: 81–102.

García, V. B., Lucifora, L. O. & Myers, R. A. (2008). The importance of habitat and life history to extinction risk in sharks, skates, rays and chimaeras. *Proceedings of the Royal Society*, 275: 83–89. doi: 10.1098/rspb.2007.1295

ICES. 2013. Report of the workshop on Sexual Maturity Staging of Elasmobranchs605 (WKMSSEL), 11-14 December 2012, Lisbon, Portugal. ICES CM 2012/ACOM:59

King, M. 2011. Fisheries Biology, Assessment and Management. Oxford: Fishing News Books.

Klimley, A. P. 1987. The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. *Environmental Biology of Fishes*, 18: 27-40.

Kyne, P. M.; Simpfendorfer, C. A. 2007. A Collation and summarization of available data on deepwater Chondrichthyans: Biodiversity, life history and fisheries. *Biodiversity, Life History and Fisheries IUCN SSC Shark Specialist Group for the Marine Conservation Biology Institute*, 1-137.

Licandeo, R.; Cerna, F. T. 2007. Geographic variation in life-history traits of the endemic kite skate *Dipturus chilensis* (Batoidea: Rajidae), along its distribution in the fjords and channels of southern Chile. *Journal of Fish Biology*, 71: 421–440.

Lombardi-Carlson, L. A.; Cortés, E.; Parsons, G. R.; Manire, C. A. 2003. Latitudinal variation in life-history traits of bonnethead shark *Sphyrna tiburo* (Carcharhiniformes:

Sphyrnidae) from the eastern Gulf of Mexico. *Marine and Freshwater Research*, 54: 875-883.

Lteif, M.; Mouawad, R.; Khalaf, G.; Lenfant, P.; Verdoit-Jarraya, M. 2016. Population biology of an endangered species: the common guitarfish *Rhinobatos rhinobatos* in Lebanese marine waters of the eastern Mediterranean Sea. *Journal of Fish Biology*, 88: 1441-1459.

McEachran, J. D. & Carvalho, M. R. 2002. Batoid fishes. In *The Living Marine Resources of the Western Central Atlantic. FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists*, Vol. 5 (Carpenter, K., ed.), pp. 508–589. Rome: FAO.

Martins, I. A.; Martins, C. L.; Leme, M. H. A. 2005. Biological parameters and population structure of *Psammodontus oblongus* in Ubatuba region, north coast of the State of São Paulo, Brazil. *Journal of Marine Biology Association U.K*, 85, 1113-1118

Menni, R. C.; Stehmann, M. F. W. 2000. Distribution, environment and biology of batoid fishes off Argentina, Uruguay and Brazil. A review. *Revista del Museo Argentino de Ciencias Naturales*, 2(1):69-109.

Motta, F. S.; Gadig, O. B. F.; Namora, R. C.; Braga, F. M. S. 2005. Size and sex compositions, length–weight relationship, and occurrence of the Brazilian sharpnose shark, *Rhizoprionodon lalandii*, caught by artisanal fishery from southeastern Brazil. *Fisheries Research*, 74: 116-126.

Pasquino, A. F.; Martins, M. F.; Gadig, O. B. F. 2016. Length–weight relationship of *Rhinobatos horkelii* Müller & Henle, 1841 and *Zapteryx brevirostris* (Müller & Henle, 1841) off Brazil, southwestern Atlantic Ocean. *Journal of Applied Ichthyology*, 1-2.

- Rigby, C. L.; White, W. T.; Smart, J. J.; Simpfendorfer, C. A. 2016. Life histories of two deep-water Australian endemic elasmobranchs: Argus skate *Dipturus polyommata* and eastern spotted gummy shark *Mustelus walker*. *Journal of Fish Biology*, 88: 1149-1174.
- Rincon, G. F. 1997. Taxonomia, alimentação e reprodução raia elétrica *Benthobatis* sp. (Torpediniformes: Narcinidae) no sul do Brasil. Pós-Graduação em Oceanografia Biológica. Fundação Universidade do Rio Grande.
- Rincon, G. F.; Stehmann, M. F. W.; Vooren, C. M. 2001. Results of the research cruises of FRV 'Walther Hewig' to South America. LXXIV. *Benthobatis krefftii* n. sp. (Chondrichthyes, Torpediniformes, Narcinidae), a new deep-water electric ray from off South Brazil and the third species of the genus. *Archive of Fishery and Marine Research*, 49(1): 45-60
- Rincon, G. 2004. *Benthobatis krefftii*. The IUCN Red List of Threatened Species 2004: e.T44577A10921737. <http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T44577A10921737.en>. Downloaded on 17 November 2016.
- Rincon, G.; Vaske Junior, T.; Voren, C. M. 2008. Stomach contents and notes on the reproduction of the Onefin Skate *Gurgesiella dorsalifera* (Chondrichthyes: Rajidae) off Southern Brazil. *Neotropical Ichthyology*, 6(4):689-692
- Rodriguez-Cabello, C.; Sánchez, F.; Olaso, I. 2007. Distribution patterns and sexual segregations of *Scyliorhinus canicula* (L.) in the Cantabrian Sea. *Journal of Fish Biology*, 70: 1568-1586.
- Rolim, F. A.; Caltabellotta, F. P.; Rotundo, M. M.; Vaske-Júnior, T. 2015. Sexual dimorphism based on body proportions and ontogenetic changes in the Brazilian electric

ray *Narcine brasiliensis* (von Olfers, 1831) (Chondrichthyes: Narcinidae). *African Journal of Marine Sciences*, 37(2): 167-176.

Silva-Junior, L. C.; Andrade, A. C.; Vianna, M. 2011. Length-weight relationships for elasmobranchs from southeastern Brazil. *Journal of Applied Ichthyology*, 27: 1408-1410.

Simpfendorfer, C. & Kyne, P. M. 2009. Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environmental Conservation* 6: 97–103. doi: 10.1017/S0376892909990191

Stehmman, M. 2002. Proposal of a maturity stages scale for oviparous and viviparous cartilaginous fishes (Pisces, Chondrichthyes). *Archive of Fishery and Marine Research*, 50 (1): 23-48

Yamaguchi, A.; Taniuchi, T.; Shimizu, M. 2000. Geographic variations in reproductive parameters of the starspotted dogfish, *Mustelus manazo*, from five localities in Japan and in Taiwan. *Environmental Biology of Fishes*, 57 (2): 221-233. doi: 10.1023/A:1007558324902.

Zar, J. H. (2010). *Biostatistical Analyses*. 5th edition. New Jersey: Prentice Hall.

Tables

Table I. Maturity scale adapted from Stehmann (2002) and ICES (2013) for males and females of *Benthobatis krefftii* captured in São Paulo State continental slope. Mat. Stage= Maturity stages

Mat. Stage	Females	Males
Immature	Ovaries without macroscopic follicles Oviduct with no distinction of the uterus	Uncalcified claspers Clasper gland white in color but very attached to adjacent muscular tissue Testicles without macroscopic lobules
Developing	Ovaries may present white of translucent follicles Filamentous oviduct with little distinction of the uterus	Uncalcified claspers Clasper gland yellow and may not be observed through skin Small lobules may be observed in testicles
Mature	Ovaries full of large yellow follicles Uterus clearly distinguishable from the oviduct	Calcified claspers Clasper gland easily separated from adjacent muscular tissue and observed through skin Testicles with the presence of testicular lobules

Figures

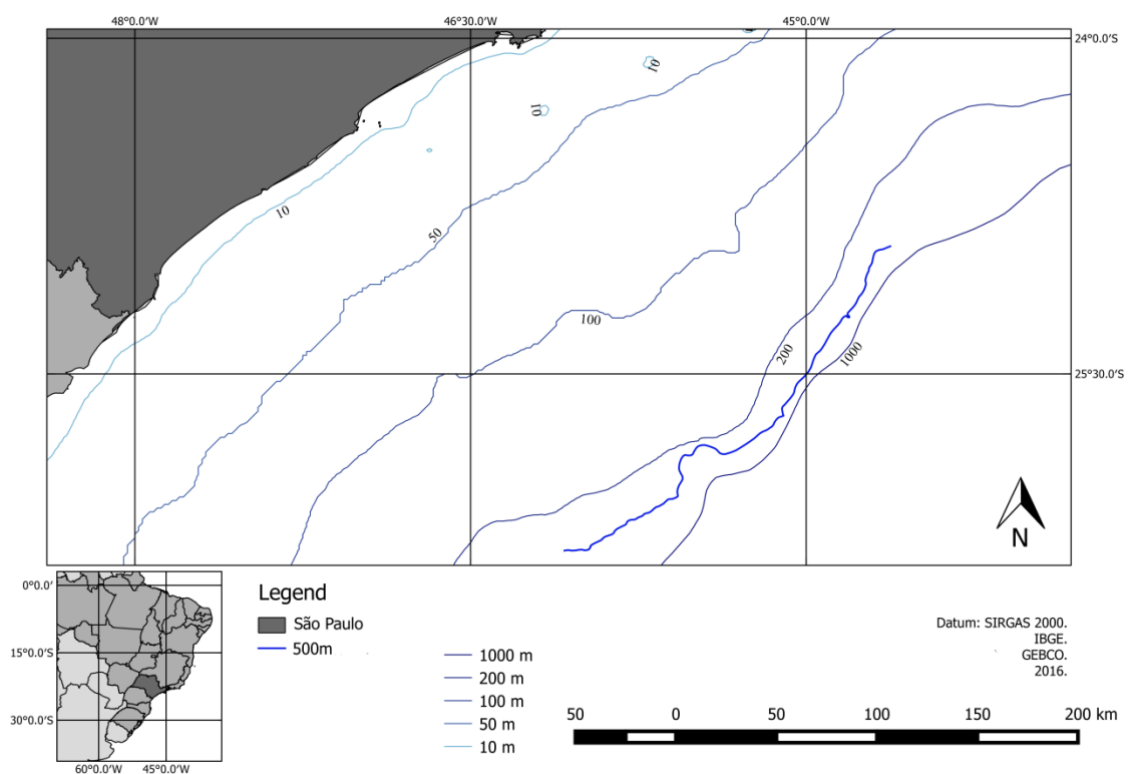


Figure 1. Map of the study area. Dark grey color represents São Paulo State, in the left-lower square and central square represents São Paulo State continental slope. Thicker blue line indicates the depth where samples were taken (500 m).

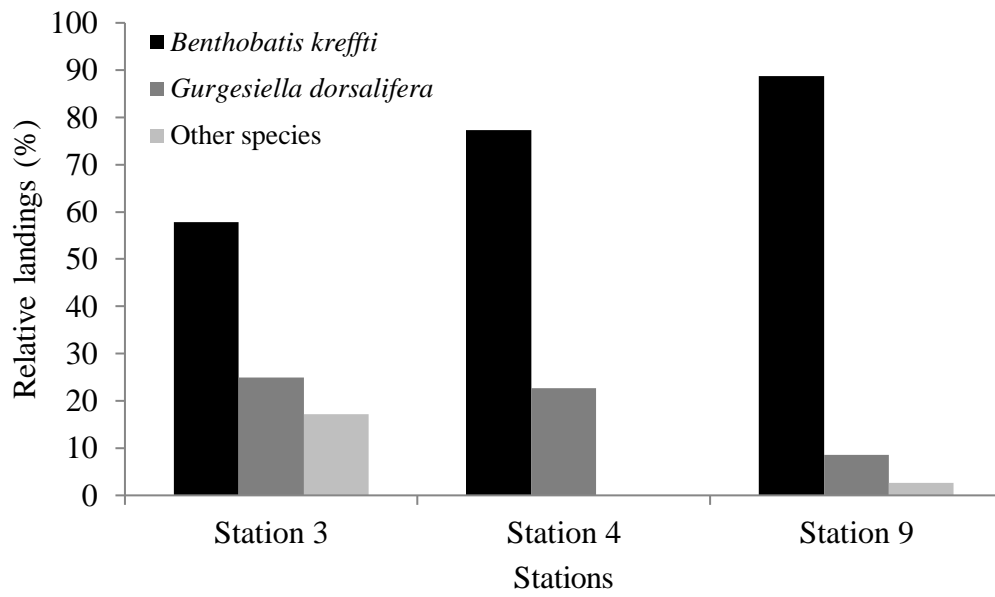


Figure 2. Catch composition of Chondrichthyes during July-August 2003 in the São Paulo State continental slope, considering landings relative to the Chondrichthyes landings and the stations sampled.

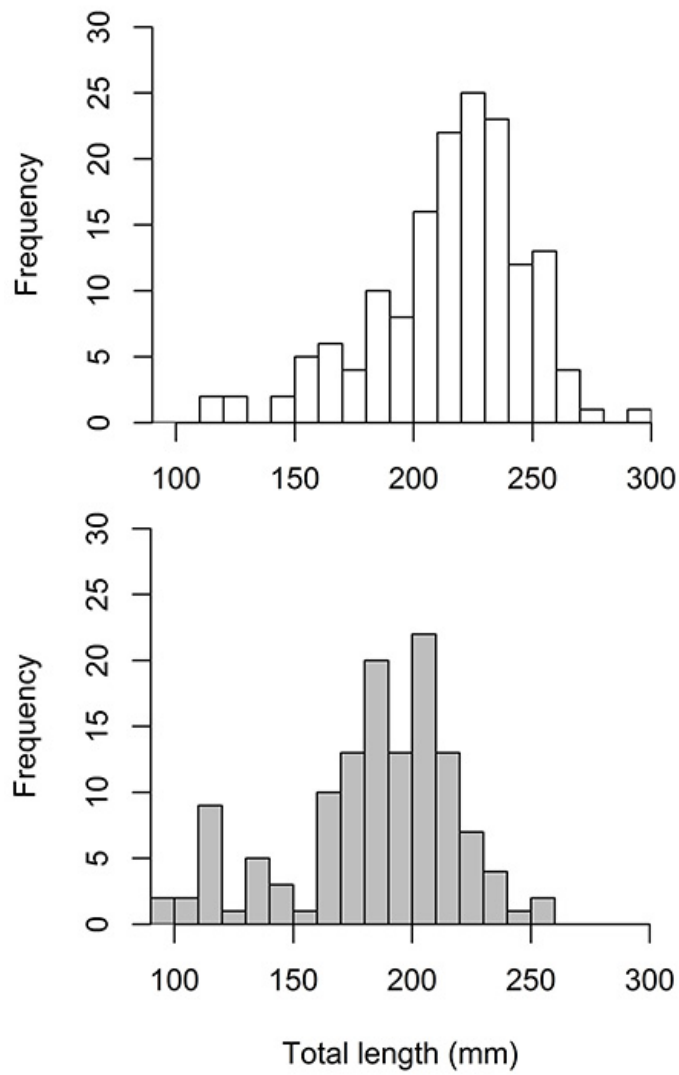


Figure 3. Length-frequency distribution for females (white) and males (grey) of *Benthobatis krefftii* with 10 mm length classes.

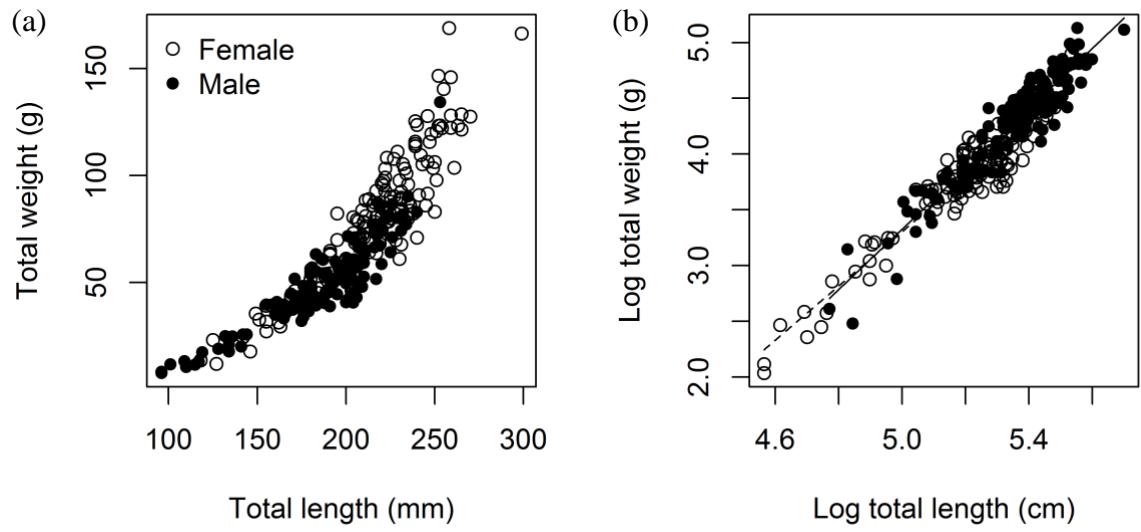


Figure 4. Length-weight relationship for females (F) and males (M) of *Benthobatis krefftii* with (a) raw data and (b) log-transformed total length and total weight data.

CAPÍTULO II

**Reproductive biology of the rare blind electric ray,
Benthobatis krefftii Rincon, Stehmann & Vooren, 2001
(Chondrichthyes, Narcinidae)**

**Reproductive biology of the rare blind electric ray *Benthobatis krefftii* Rincon,
Stehmann and Vooren, 2001 (Chondrichthyes, Narcinidae)**

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This study provides information about the reproductive biology of *Benthobatis krefftii*, a rare batoid elasmobranch, from the southeastern Brazilian continental slope. Individuals were caught by bottom trawl carried out in 2003 and 2007, in depths from 492 to 501 m. Size and sexual variables were recorded for each male and female. 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 in females and males respectively, with total length at 50% maturity of 191 and 176 mm for both sexes. Females mature at a larger size than males and this may represent different reproductive investment between sexes. Uterine fecundity was relatively low (1-3 eggs or embryos/female) and size at birth was estimated at 91-100 mm. The low fecundity observed was already expected since *B. krefftii* is a deep-water elasmobranch species and those usually present lower fecundity, as well as late maturity in comparison with costal species. Despite this fact, the large neonate size suggests that this species invests more in length of each embryo than in offspring size.

Key-words: size at birth, size at maturity, sexual development, yolk-sac viviparity

Introduction

Elasmobranchs are generally regarded as a k-strategy group, with life history patterns such as slow growth, late maturity, long gestation periods and low fecundity. Those patterns place them at a susceptible situation when submitted to non-natural deaths (*e. g.* overexploitation by fisheries) (Dulvy *et al.* 2000; Stevens *et al.* 2000). Such vulnerability increases even more in deep-water elasmobranch species, which have slower growth and attain maturity later than coastal ones (Garcia *et al.* 2008; Kyne and Simpfendorfer 2007). Due to those characteristics, deep-water elasmobranch populations are less productive than coastal species and, consequently, less resilient, remarkably when facing non natural pressures on its population (Simpfendorfer and Kyne 2009).

During the last decades, fisheries have increasing and expanded its efforts to deep-water marine habitats. Those areas, however, are more susceptible to collapse, and should be regarded as the future areas for conservation (Morato *et al.* 2006). Understanding life history patterns in a specie-specific approach is a powerful tool for management and conservation measures concerning fisheries effect on elasmobranch populations. The knowledge of reproductive aspects such as maturity, fecundity and reproductive cycle are, specially, necessary for assessing the population status (Walker 2005).

Electric rays (Order Torpediniformes) represent the third largest group among batoids, with about 70 known extant species (Weigmann 2016). Numbfishes (Family Narcinidae) are small to moderate size benthic elasmobranchs occurring in almost all marine regions, inhabiting continental and insular shelves, as well as slopes, especially concerning the deep-water genus *Benthobatis*, which can be found in depths around 1000 m) (Carvalho *et al.* 1999; McEachran and Carvalho 2002). Reproductive mode in this group involves yolk-sac viviparity, with embryos nurished by yolk from the yolk-sac (Musick and Ellis 2005).

The Brazilian blind electric ray *Benthobatis krefftii* Rincón, Stehmann and Vooren, 2001 is a deep-water batoid, inhabiting between 400-600 m depth and with a known restricted geographic distribution in the western South Atlantic, between São Paulo (north) to Rio Grande do Sul (south) States, southern Brazil. This species was previously studied by Rincon (1997, as *Benthobatis* sp.), and, posteriorly described as a new species by the same author (Rincon *et al.* 2000). These authors presented some biological aspects of feeding and reproduction based on a relative small sample (63 individuals). During the following years, additional specimens were recorded along the Southeast and South Brazilian continental slopes (Bernardes *et al.* 2005), but no further biological aspects were exhaustively studied and the available information on this species remains scarce.

Elasmobranchs show a wide variation in life-history patterns among distinct populations. Thus, the lack of consistent biological knowledge about *B. krefftii* along its entire known distribution is evident, which highlights the need of studies concerning this enigmatic deep-water small elasmobranch. At this point of view, the aim of the present study is to analyse the reproductive biology of *B. krefftii*, concerning to its size at maturity, size at birth and fecundity, as well as analysing the sexual development.

Material and Methods

Study area

Individuals were captured in São Paulo State coast, Southeast continental slope of Brazil (western South Atlantic), from Cananéia to Santos municipalities (about 25°44'S - 25°45' S /45°09' - 45°11' W and 26°09'S - 26°10' S /45°45' - 45°47' W –Fig. 1), in four samples (three in July/August 2003 and one in December 2007) and three different sample stations during a research cruise carried out by the São Paulo State University (UNESP) aboard of 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 formalin 4% and preserved in ethanol 70%.

Biological data recorded at laboratory

All individuals had total length (L_T , mm), gonad mass (M_O for females and M_T for males, g), liver mass (M_L , g) and sex recorded based on the presence of male copulatory organ (clasper). For females, uteri width (W_U , mm); number of ovarian follicles (N_F) and diameter of the largest ovarian follicle (D_F , mm) were recorded. Clasper inner length (L_C , mm), clasper gland length (L_{CG} , mm) and diameter of largest testicular lobule (D_L , mm) were recorded for males.

A maturity scale for viviparous elasmobranchs proposed by ICES (2013) was adapted to this species, since Narcinidae batoids do not present macroscopically visible oviducal glands (Prasad 1945) and due to the difficulties to visualization 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 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 (Table 1). Neonates were considered those individuals with yolk sac scar in their abdomen.

Data analysis

Sexual development through maturity stages was analysed by plotting all sexual variables as dependent, with L_T being the independent variable. Size at maturity was calculated 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 model. 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).

Ovarian fecundity was estimated by counting the number of ovarian follicles with vitellogenic activity in both ovaries (Fitzz and Daiber 1963; Capapé and Quignard 1975). Estimation of uterine fecundity was based on number of uterine eggs or embryos in both uteri. No extrauterine embryo was caught during the samples and abortion events were carefully checked still in the field. Size at birth was estimated based on observation of the smallest free swimming individual classified (neonate) and near term embryos observed.

Testicles and ovaries symmetries were analysed with Wilcoxon's Paired test, with null hypothesis being that no difference between mass in left and right structure exists (Zar 2010). All statistics were carried out at R 3.1 software (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

A total of 152 females and 114 males were analysed. Total length was 115-184 mm (150.0 ± 20.2 ; $n=18$) in immature females; 146-215 mm (181.1 ± 18.1 ; $n=25$) in developing ones; and 177-270 mm (220.4 ± 25.4 ; $n=26$) in capable to reproduce. Early and late pregnancy individuals had 186-251 mm (216.3 ± 19.2 ; $n=14$) and 201-299 mm (226.2 ± 21.6 ; $n=33$) L_T , respectively; and post-partum, 195-244 mm (220 ± 12.4 ; $n=32$).

Immature males had 91-179 mm L_T (144.7 ± 17.7 ; $n=16$), developing ones had 156-206 mm L_T (176.8 ± 12.3 ; $n=25$), and capable to reproduce males ranged from 162 to 234 mm L_T (195.0 ± 15.6 ; $n=68$).

Female sexual development

Liver mass ranged from 0.147 to 3.866 g. Immature females had M_L of 0.147-1.078 g, developing ones had 0.438-2.103 g and capable to reproduce ones, 1.362-3.562 g. In early and late pregnancy females, M_L ranged from 0.850 to 1.765 g and 0.675 to 3.866 g, respectively; and in post-partum females, from 0.581 to 2.210 g [Fig. 2(a), Table 2].

Females presented both functional and symmetric ovaries ($W=2130.5$; $n=89$; $p=0.600$), which increased in mass in two different growth phases [Fig. 3(a)]. In smaller individuals, ovaries grew slowly and, at 177 mm L_T , an abrupt growth was observed with a high overlap between developing females and capable to reproduce individuals. Late-pregnancy and post-partum females presented similar M_O . Ovaries mass ranged from 0.042 to 0.152 g in immature females. Developing females had M_O of 0.015-0.256 g and few follicles with vitellogenic activity were present. Ovaries mass ranged from 0.051 to 3.652 g in capable to reproduce; 0.044 to 0.149 g in early pregnancy; 0.031 to 0.272 g in late pregnancy females and from 0.025 to 1.111 g in post-partum females.

Ovarian follicles presenting vitellogenic activity were first observed in a few developing females, larger than 169 mm L_T and presenting mean D_F of 2.887 mm. Vitellogenic follicles mean diameter was 6.805 mm in specimens capable to reproduce; 3.060 mm in early pregnancy; 2.457 mm in late pregnancy and 3.013 mm in post-partum females [Fig. 3(c), Table 2].

Larger developing females presented N_F from two to 7 per female, while capable to reproduce presented 1-15 follicles, representing the maximum number of follicles recorded. Early and late pregnancy females had 1-4 and 1-8 follicles/female, respectively, and those in post-partum had 1-10 [Fig. 3(b), Table 2].

Uteri width had a small variation between immature and developing females and also between capable to reproduce and post-partum females. Immature stage presented a W_U from 0.73 to 2.70 mm, and developing females had 1.00-3.92 mm W_U . In capable to reproduce females, W_U was 2.6-14.5 mm; in early and late pregnancy females, W_U was 7.25-27.25 and 3.6-32.85 mm, respectively; and W_U ranged from 2.6 to 15.4 mm in post-partum females [Fig. 3(d), Table 2].

Male sexual development

Males presented M_L ranging from 0.119 to 2.750 g. Immature individuals had M_L of 0.119-0.688 g, developing males had 0.301-1.400 g M_L and capable to reproduce, 0.340-2.750 g M_L [Fig. 2(b), Table 3].

In neonates, L_C was 2.69 mm, while in immature, L_C ranged from 3.03 to 11.10 and in developing males, from 10.92 to 15.00 mm. Fully calcified clasper length ranged from 8.23 to 18.77 mm. Clasper length grew in a three-phase sigmoid-like pattern [Fig. 4(a)]. A slow growth was observed for neonate and immature individuals, followed by a rapid growth at 179 mm L_T which characterize the second phase. At this point, a high overlap of immature, developing and capable to reproduce individuals could be observed. The smallest capable to reproduce male had 8.23 mm L_C and was 162 mm L_T , and from 220 mm L_T onward, all males had calcified claspers.

Clasper gland length showed a similar sigmoid growth pattern [Fig. 4(b)]. From 163 to 176 mm L_T , a high overlap was observed for the three stages of maturity herein adopted and from 202 mm L_T (L_{CG} of 11.28 mm) onward, all males were capable to reproduce. In immature males, L_{CG} ranged from 1.90 to 8.00 mm. In developing specimens, L_{CG} was 7.05-11.22 mm. Capable to reproduce males had clasper glands from 7.67 to 14.84 mm L_{CG} .

Testicles were asymmetric ($W=387.5$; $n=107$; $p=0.003$), presenting a lightly potential-like growth pattern, considering L_T [Fig. 4(c)]. Immature males had M_T of 0.001-0.014 g and in developing individuals, testicles ranged from 0.051 to 0.331 g M_T . Testicular lobules started to appear from 164 mm L_T onward, in developing males and reached their maximum in capable to reproduce ones, with M_T ranging from 0.036 to 0.315 g in this group (Table 3).

The presence of testicular lobules was observed in most of large immature, developing males and in capable to reproduce ones. Immature males presented few small lobules ($D_L=0.235-0.340$ mm), while developing ones had D_L of 0.275-0.770 mm. Capable to reproduce ones had testicles full of lobules which ranged from 0.275 to 1.260 mm [Fig. 4(d)].

Size at maturity

Capable to reproduce females were first observed at 177 mm L_T . Size at maturity (L_{T50}) was calculated in 191.1 mm L_T (95% *c.i.*:186.3-195.4) in these individuals, representing 63% of maximum total length recorded [Fig. 5(a)].

The smallest mature male was 162 mm L_T , showing a large (8.23 mm L_C) calcified clasper, swollen and distinguishable clasper glands and evident and large testicular lobules. Size at maturity (L_{T50}) was 176.3 mm L_T and represented 75.3% of the maximum total length for males [Fig. 5(b)].

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 and egg capsules were present only in uterine eggs. Uterine eggs were present 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 ≥ 2 , with 58.3% presenting embryos in both uteri and 41.7% with two embryos in one uteri.

No evident relationship between fecundity and total length was observed, although smaller females presented uterine fecundity of one embryo and larger females presented uterine fecundity of two or three embryos (Fig. 6). In addition, a high overlap of fecundity of 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 .

Size at birth

Length of the embryos ranged from 14 to 100 mm L_T (73.0 ± 22.4 , $n=37$). Near-term embryos measured from 91 to 100 mm L_T , with females 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

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 onset was observed at 162 mm L_T in males, with clasper gland and lobules enlargement, followed by testicles mass increase were the main structures observed during maturing phase. *Benthobatis krefftii*, as a wide range of other elasmobranch species (Oddone *et al.* 2007; Rocha and Gadig 2013), seems to have a abrupt transition from developing to capable to reproduce stages, in a short length range, which could reflect an adaptive response to the deep-water habitat where this species occur, as proposed for other deep-water species (Rigby *et al.* 2016).

Symmetric ovaries were observed during the present study. Rincon (1997), however, found only one functional ovary in specimens of southern Brazil. Regarding this, differences in functionality may occur, as observed for the genus *Squatina* (Vooren and Da Silva 1991). Asymmetry observed between populations was, however, never reported and may be a result of smaller sample sizes. Further studies are highly recommended to prove if there is an intraspecific variation in gonad functionality between southern and southeastern populations.

Gonad mass had an abrupt increase, related to L_T growth at developing phase, 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 effects of cold water in metabolism and slow growth of deep-sea species (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. 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* (Villavicencio-Garayazar 1993). Capapé *et al.* (2001), however, found gravid females of *Torpedo mackayana* Metzelaar, 1919 with degenerating oocytes, possibly representing a resting phase of the ovarian cycle.

In addition, liver rapidly decreases during early-pregnancy, and increases in mass in post-partum stage, achieving its maximum at capable to reproduce females. This synchronous growth suggests that vitellogenic activity starts, especially, in late-pregnancy and post-partum stages. Such a synchronous growth between liver and gonad mass is expected once in *B. krefftii* yolk plays an important role during the whole embryo development (Hamlett *et al.* 2005).

Testicular lobules were present in all maturity stages considered, yet diameter of testicular lobule had its maximum at capable to reproduce males, with 39% of the diameter being increased from developing to capable to reproduce maturity stages. In females, ovarian follicles attained their maximum diameter in capable to reproduce individuals. These follicles were, probably, those about to be ovulated, since *B. krefftii* does not present large follicles in pregnancy or post-partum maturity stages, as observed in other Torpediniformes (Capapé *et al.* 2001; Capapé *et al.* 2006).

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. This has been widely documented for elasmobranchs (Parsons 1983; Costa *et al.* 2005; Oddone *et al.* 2008; Ebert *et al.* 2008). In clasper gland length, such a sigmoid growth pattern was also observed and, concerning the additional secretory function of clasper gland related to copulatory activity (Piercy *et al.* 2006), a synchronous growth could be expected for both structures.

Size at maturity was different between sexes, with females attaining maturity at larger sizes 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, which are able to properly ensure embryo development.

Males attained maturity at 73 % of maximum total length recorded, while females mature at 63%. Late maturity is a known characteristic of elasmobranchs, which make them vulnerable to non-natural deaths (Cortés 2000). *Benthobatis krefftii* grows >60% to

attain maturity, as most elasmobranchs (Mejía-Falla *et al.* 2012; Clarke *et al.* 2014), and this relative late maturity may lead this species to vulnerability.

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 and L_{T50} observed in this study was 170 for males and 191 for females. Such discrepancies may be a result of different methods for maturity estimates or, in fact, an evidence of two distinct populations, as observed by Yamaguchi *et al.* (2000). In this case, molecular analyses would be necessary to elucidate such questions.

Fecundity recorded for *Benthobatis krefftii* was very low in comparison with other Torpediniformes (Capapé *et al.* 2006; Moreno *et al.* 2010; Rolim *et al.* 2015). Uterine fecundity estimated in this study was also different than the one observed for *B. krefftii* in southern Brazil (Rincon 1997). Ovarian fecundity was higher than uterine fecundity, which is already expected since some ovarian follicles may undergo atresia or be reabsorbed during the reproductive cycle (Conrath 2005). The low fecundity observed for *B. krefftii* highlights that deep-water Chondrichthyes are less fecund than coastal species and might be even more vulnerable to fishing mortality (Garcia *et al.* 2008).

Term embryos were observed from 90 to 100 m L_T and one neonate was observed with 91 mm L_T . Therefore, birth may occur when embryos attain from 90 to 100 mm total length. In this study, size at birth is relatively large, with embryos attaining 1/3 of the maximum total length recorded. *Narcine brasiliensis* size at birth is estimated in 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 with Torpediniformes, but specifically of this species due to its habitat.

In conclusion, *B. krefftii* has a reproductive strategy that consists of low fecundity still large term embryos are produced. 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 most of the immature and developing stages.

This study provides information about the reproductive biology of the rare and unknown deep water benthic elasmobranch species, *B. krefftii*, for the south eastern Brazilian slope. Some differences could be observed between the study carried out in south Brazil and the present study, highlighting the need to consider these populations differently for management or 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. Even so, more adequate further biological studies, considering larger samples and molecular approach are needed to solve this subject.

References

Bernardes RA, Figueiredo JL., Rodrigues AR., Fischer LG, Vooren CM, Haimovici, M Rossi-Wongtschowski CLDB (2005) Peixes da zona econômica exclusiva da região

sudeste-sul do Brasil: Levantamento com armadilhas, pargueiras e rede de arrasto de fundo. Edusp, São Paulo.

Capapé C, Quignard JP (1975) Essai d'évaluation de la fécondité chez les sélaciens ovipares: cas de *Raja miraletus* Linné, 1758 et de *R. radula* Delaroche, 1809 des côtes tunisiennes. Arch Inst Pasteur de Tunis 52:263–276.

Capapé CC, Seck AA, Diatta Y, Diop M (2001) Observations on the reproductive biology of *Torpedo (Tetronarce) mackayana* (Torpedinidae), from the coast of Senegal (Eastern tropical Atlantic). *Cybium* 25 (1):95–99.

Capapé CC, Guélorget O, Vergne Y, Quignard JP, Ben Amor MM, Brandäi MN (2006) Biological observations on the black torpedo, *Torpedo nobiliana* Bonaparte 1835 (Chondrichthyes: Torpedinidae), from two Mediterranean areas. Ann Ser Hist Nat 16 (1):19–28.

Clarke TM, Espinoza M, Wehrtmann IS (2014) Reproductive ecology of demersal elasmobranchs from a data-deficient fishery, Pacific of Costa Rica, Central America. Fish Res 157:96–105. doi:10.1016/j.fishres.2014.04.003

Cortés E (2000). Life history patterns and correlations in sharks. Rev Fish Sci 8:299–344. doi:10.1080/10408340308951115

Conrath CI (2005) Reproductive biology. In: Musick JA, Bonfil R (eds.) Management and techniques for elasmobranch fishes, FAO, Rome, pp 103–126.

Costa ME, Erzini K, Borges TC (2005) Reproductive biology of the black mouth catshark, *Galeus melastomus* (Chondrichthyes: Scyliorhinidae) of the south coast of Portugal. J Mar Biol Assoc U K 85:1173–1183.

Dulvy NK, Metcalfe JD, Ganville J, Pawson MG, Reynolds JD (2000) Fishery stability, local extinctions, and shifts in community structure in skates. *Conserv Biol* 14 (1):283–293. doi:10.1046/j.1523-1739.2000.98540.x

Farrel ED, Marinani S, Clarke MD (2010) Reproductive biology of the starry smooth-hound shark *Mustelus asterias*: geographic variation and implications for sustainable exploitation. *J Fish Biol* 77:1505–1525. doi:10.1111/j.1095-8649.2010.02771.x.

Fitz Jr ES, Daiber FC (1963) An introduction to the biology of *Raja eglanteria* Bosc 1800 and *Raja erinacea* Mitchill, 1825 as they occur in Delaware Bay. *Bull Bing Ocean Coll* 18:69–97.

Garcia VB, Lucifora LO, Myers RA (2008) The importance of habitat and life history to extinction risk in sharks, skates, rays and chimaeras. *Proc R Soc Lond B Biol Sci* 275:83–89. doi:10.1098/rspb.2007.1295

Hamlett WC, Kormanik G, Storrie M, Stevens B, Walker TI (2005) Chondrichthyan Parity, Lecithotrophy and Matrotrophy. In: Hamlett WC (ed) *Phylogeny and reproductive biology of Chondrichthyes*. Science Publishers, Enfield, pp 81–128.

Kyne PM, Simpfendorfer CA (2007) A Collation and summarization of available data on deepwater Chondrichthyans: Biodiversity, life history and fisheries. Biodiversity, Life History and Fisheries IUCN SSC Shark Specialist Group for the Marine Conservation Biology Institute 1–137.

McEachran JD, Carvalho MR (2002) Batoid fishes. In: Carpenter K (ed) *The Living Marine Resources of the Western Central Atlantic*. FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists. FAO, Rome, pp 508–589.

Mejia-Falla PA, Navia AF, Cortés E (2012) Reproductive variables of *Urotrygon rogersi* (Batoidea: Urotrygonidae): a species with a triannual reproductive cycle in the eastern tropical Pacific Ocean. *J Fish Biol* 80:1246-1266. doi: 10.1111/j.1095-8649.2012.03237.x.

Mollet HF, Cliff G, Pratt Jr HL, Stevens JD (2000) Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. *Fish Bull* 98(2):299–318.

Morato T, Watson R, Pitcher TJ, Pauly D (2006) Fishing down the deep. *Fish Fish Ser* 7:24–34.

Musick JA, Ellis JK. (2005) Reproductive Evolution of Chondrichthyes. In: Hamlett WC (ed) *Reproductive biology and phylogeny of chondrichthyes: sharks, rays and chimaeras*. Science Publishers, Endfield, pp 45–79.

Oddone MC, Norbis W, Mancini P, Amorim AF (2008) Sexual development and reproductive cycle of the Eyespot skate *Atlantoraja cyclophora* (Regan, 1903) (Chondrichthyes: Rajidae: Arhynchobatinae), in southeastern Brazil. *Act Adri* 49 (1):73–87.

Parsons GR (1983) The reproductive biology of the atlantic sharpnose shark *Rhizoprionodon terranova* (Richardson). *Fish Bull* 81(1):61–73.

Piercy AN, Gelsleichter J, Snelson FF (2006) Morphological changes in the clasper gland of the atlantic stingray, *Dasyatis sabina*, associated with the seasonal reproductive cycle. *J Morph* 267:109–114. doi: 10.1002/jmor.

Rigby CL, White WT, Smart JJ, Simpfendorfer CA (2016) Life histories of two deep-water Australian endemic elasmobranchs: Argus skate *Dipturus polyommata* and eastern spotted gummy shark *Mustelus walkeri*. *J Fish Biol* 88(3):1149–1174. doi:10.1111/jfb.12891

Rincon G (1997) Taxonomia, alimentação e reprodução da raia elétrica *Benthobatis krefftii* (Torpediniformes: Narcinidae) no sul do Brasil. Dissertation. Federal University of Rio Grande.

Rincon G, Stehmann M, Vooren CM (2001) Results of the research cruises of FRV 'Walther Herwig' to South America. LXXIV. *Benthobatis krefftii* n.sp. (Chondrichthyes, Rajiformes, Narcinidae), a new deep-water electric ray from off South Brazil and the third species of the genus. *Arch Fish Mar Res* 49(1):45–60.

Rocha F, Gadig OBF (2013) Reproductive biology of the guitarfish *Rhinobatos percellens* (Chondrichthyes, Rhinobatidae) from the São Paulo Coast, Brazil, western South Atlantic Ocean. *J Fish Biol* 82:306–317. doi:10.1111/j.1095-8649.2012.03493.x

Rolim FA, Rotundo MM, Vaske-Júnior T (2015) Notes on the reproductive biology of the Brazilian electric ray *Narcine brasiliensis* (Elasmobranchii: Narcinidae). *J Fish Biol* 89(1):1105–1111. doi:10.1111/jfb.12778

Simpfendorfer C, Kyne PM (2009) Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environ Conserv* 6:97–103. doi:10.1017/S0376892909990191

Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES J Mar Sci* 57(3):476–494. doi: 10.1006/jmsc.2000.0724

Villavicencio-Garayzar CJ (1993) Observaciones sobre la biología reproductiva de *Narcine brasiliensis* (Olfers) (Pisces: Narcinidae), em Bahía Almejas, B.C.S., México. Rev Inves Cien 4(1):95–98.

Vooren CM, Da Silva KG (1991) On the taxonomy of the angel sharks from southern Brazil, with the description of *Squatina occulta* sp. n. Rev Bras Zool 51(3):589–602.

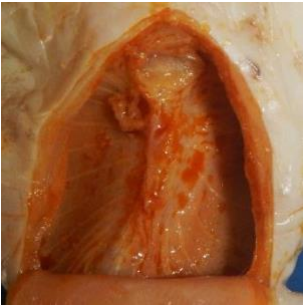
Walker TI (2005) Reproduction in Fisheries Science. In: Hamlett WC (eds) Phylogeny and reproductive biology of Chondrichthyes,. Science Publishers: Enfield pp 81–128.

Yamaguchi A, Taniuchi T, Shimizu M (2000) Geographic variations in reproductive parameters of the starspotted dogfish, *Mustelus manazo*, from five localities in Japan and in Taiwan. Environ Biol Fishes 57(2):221–233. doi: 10.1023/A:1007558324902

Zar JH (2010) Biostatistical Analyses.5th edition. Prentice Hall: New Jersey.

Tables

Table 1. Maturity scale adapted from Ponz-Louro (1995) and ICES (2013) for *Benthobatis krefftii*, with description of each maturity stage per sex and illustrations.

Maturity Stage	Description	Image
<i>Females</i>		
Immature A. Immature	Ovaries without macroscopic visible follicles and threadlike oviduct	

B. Developing

Ovaries may present small macroscopic follicles and threadlike oviduct begins to distinguish from the uterus



Mature

C. Capable to reproduce

Ovaries with large macroscopic follicles; uteri enlarged and distinguished from oviduct



D. Early pregnancy

Uteri with uterine eggs present



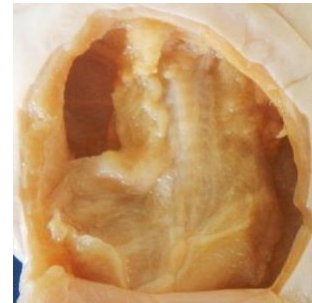
E. Late pregnancy

Uteri with macroscopic embryos present



F. Post-partum

Ovaries with few small ovarian follicles; enlarged and flaccid uteri.



Males


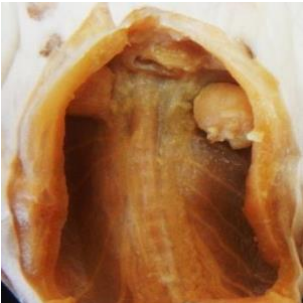
Immature	A. Immature	Uncalcified and small claspers; small and little distinguished clasper glands and shrunken testicles, with any of few small testicular lobules. Filiform ducts.	Not available
	B. Developing	Uncalcified clasper, small but distinguishable clasper gland and testicles with different size testicular lobules. Ducts starting to coil, especially in the posterior part.	
Mature	C. Capable to reproduce	Calcified claspers, enlarged clasper glands; and testicles totally covered by testicular lobules. Ducts tightly coiled.	

Table I. 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

Table II. 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), 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
	Capable to reproduce	68	8.23	18.77	14.080	1.425
L_{CG}	Immature	9	1.90	8.00	5.376	2.174
	Developing	25	7.05	11.22	9.045	1.257
	Capable to reproduce	68	7.67	14.84	10.380	1.286

M_T	Immature	12	0.001	0.014	0.0313	0.0314
	Developing	25	0.051	0.331	0.0998	0.0680
	Capable to reproduce	67	0.036	0.315	0.1627	0.0675
D_L	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

Figures

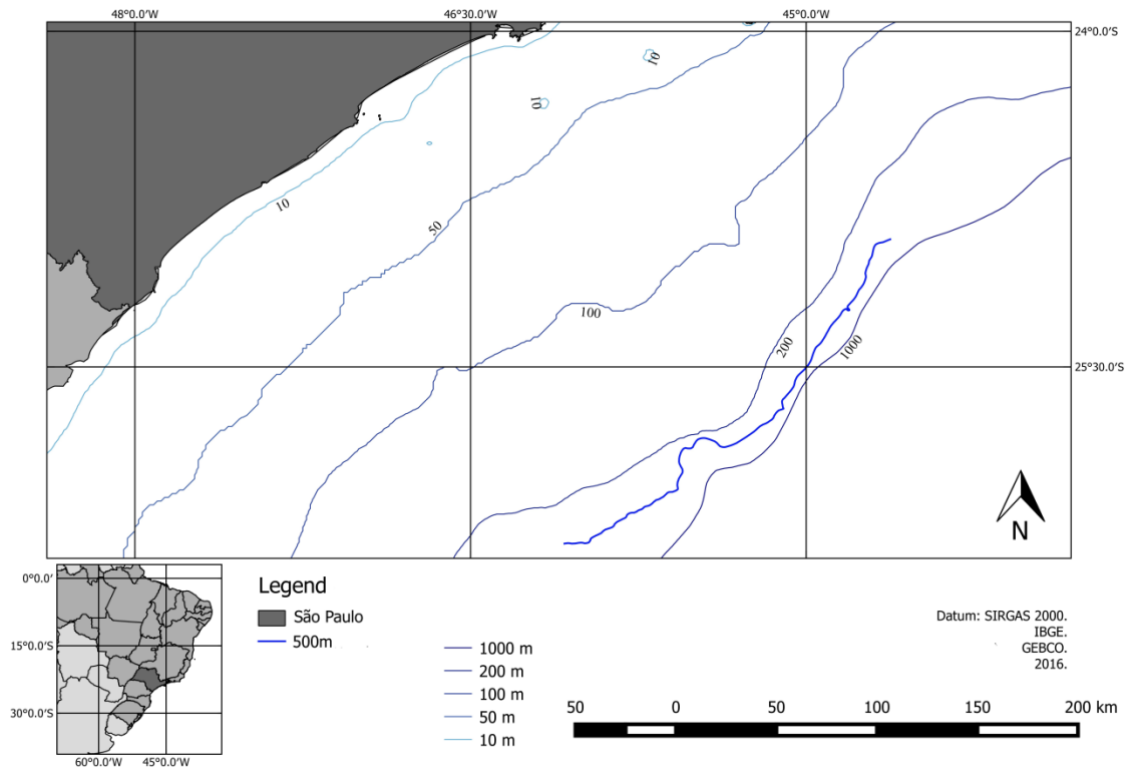


Figure 1. Studied area. Dark grey color represents São Paulo State, in the left-lower square and central square represents São Paulo State continental slope. Thicker blue line indicates the depth where samples were taken (500 m).

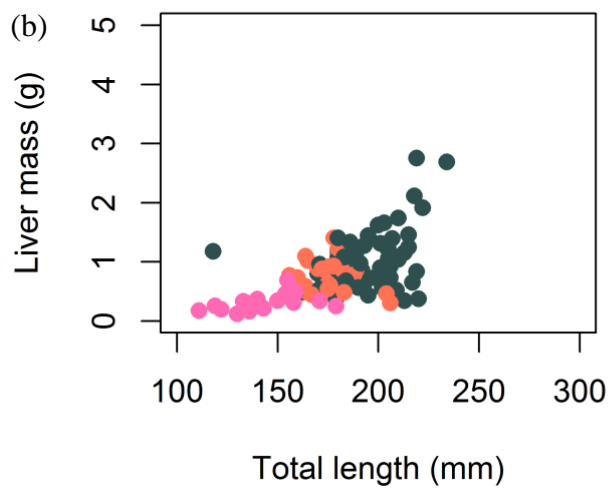
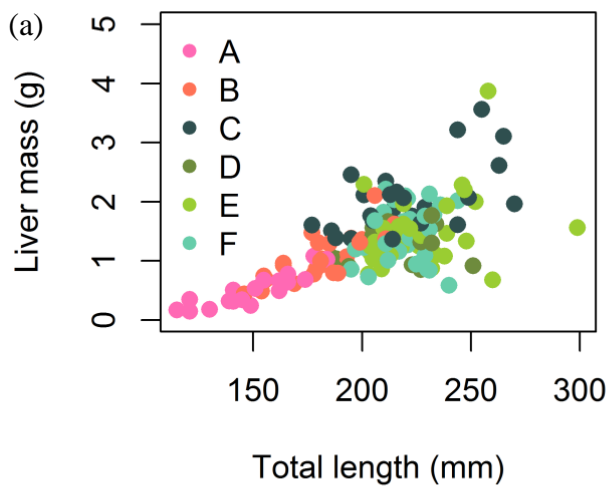


Figure 2. Relationship between total length and liver mass for (a) females and (b) males of *Benthobatis krefftii* from south eastern Brazilian continental slope, considering the maturity stages: (A) immature, (B) developing, (C) capable to reproduce (females and males), and (D) early pregnancy, (E) late pregnancy and (F) post-partum (females only).

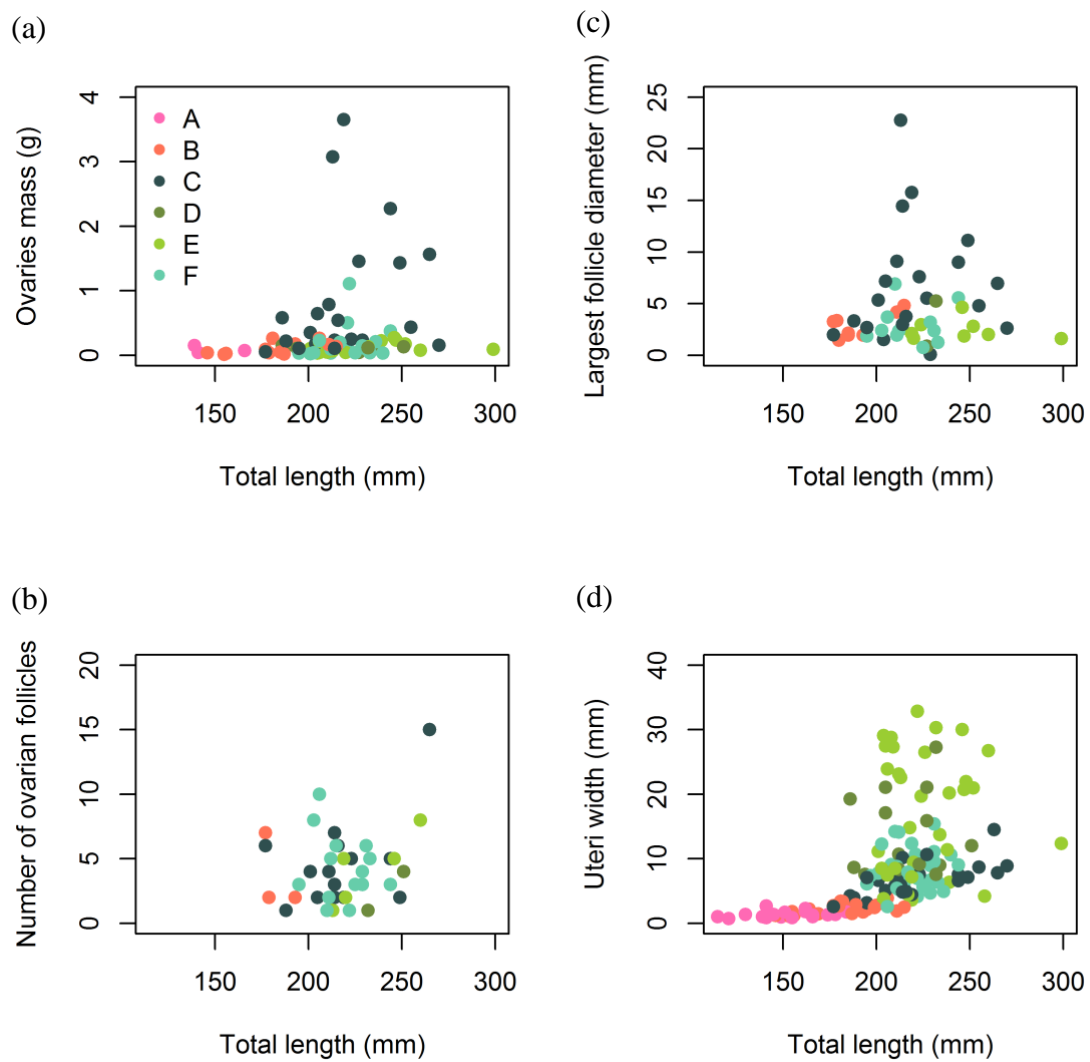


Figure 3. Sexual development of females of *Benthobatis krefftii* from south eastern Brazilian continental slope, considering the maturity stages considered: (A) immature, (B) developing, (C) capable to reproduce, (D) early pregnancy, (E) late pregnancy and (F) post-partum.

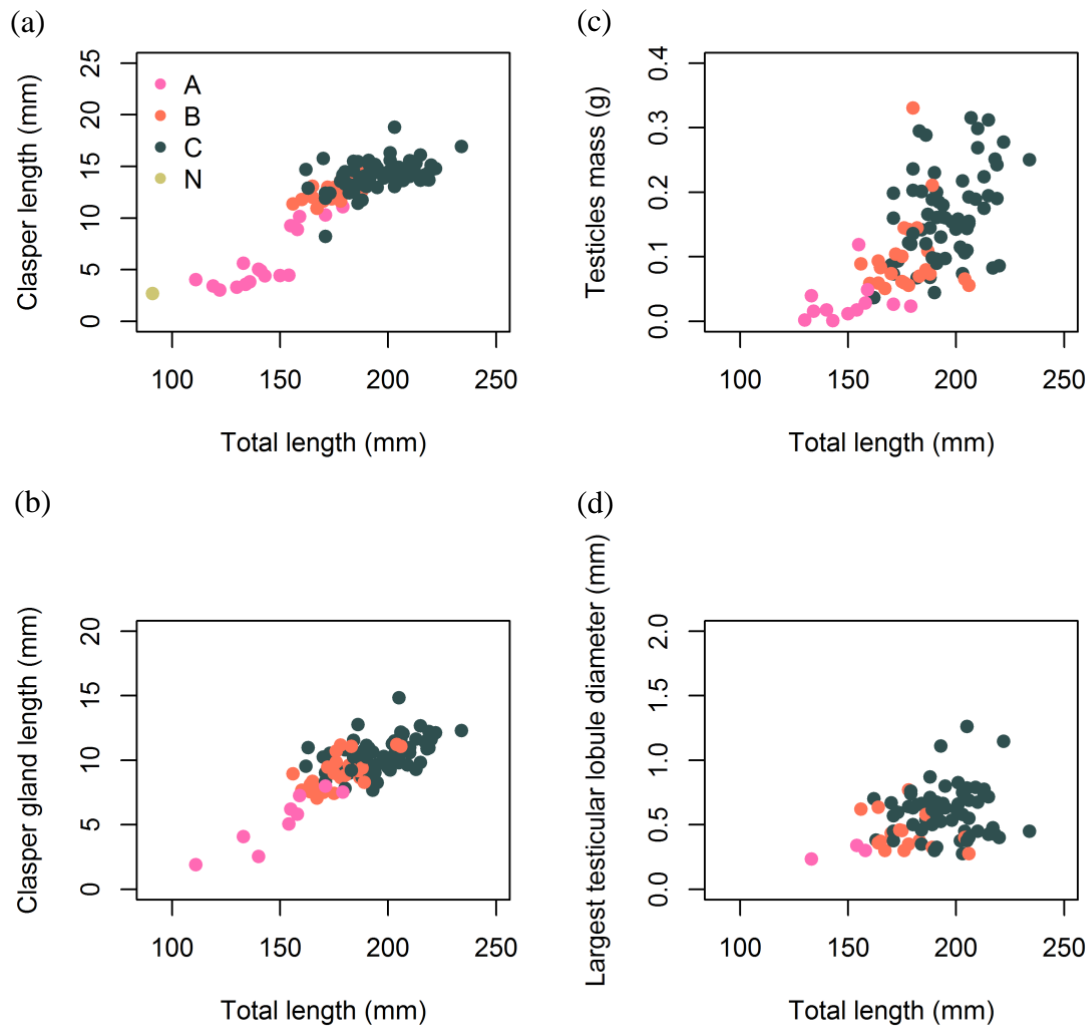


Figure 4. Sexual development of males of *Benthobatis krefftii* from south eastern Brazilian continental slope, considering the maturity stages considered: (N) neonate, (A) immature, (B) developing and (C) capable to reproduce.

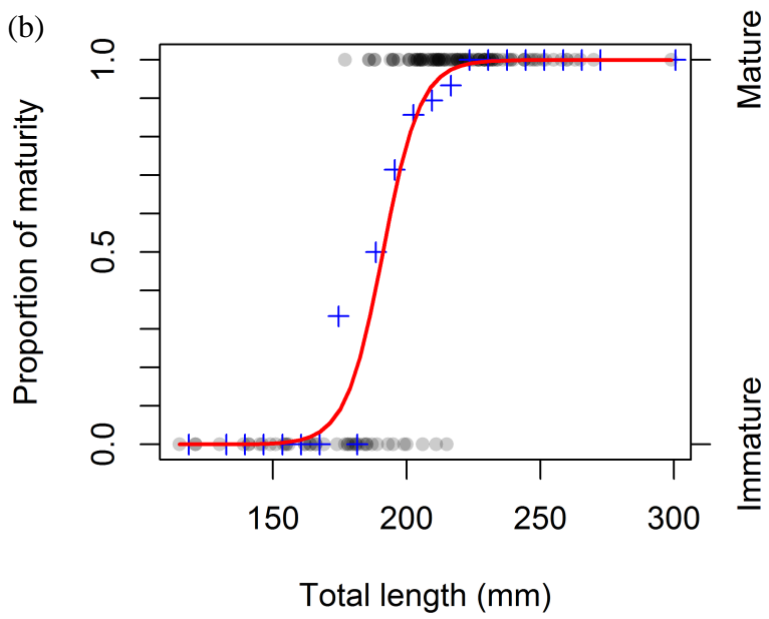
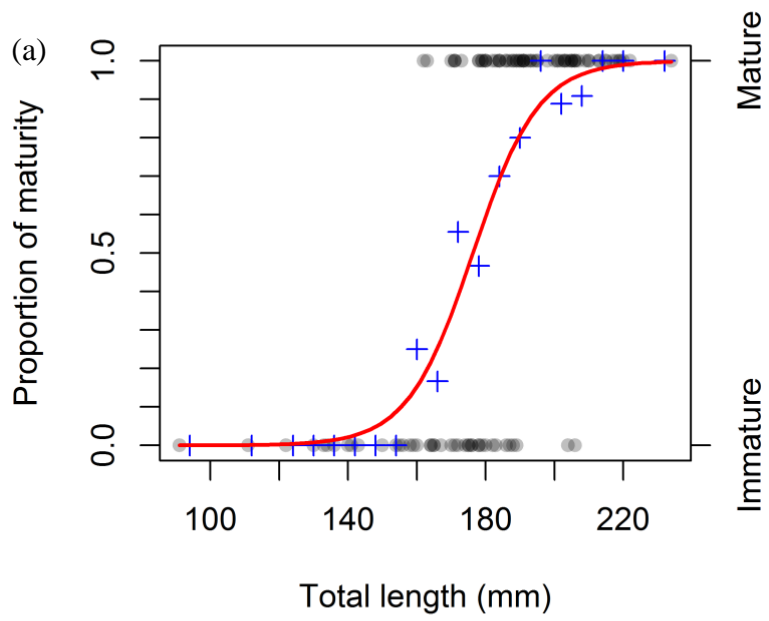


Figure 5. Mean size at maturity for (a) females and (b) males of *Benthobatis krefftii* from southeastern Brazil.

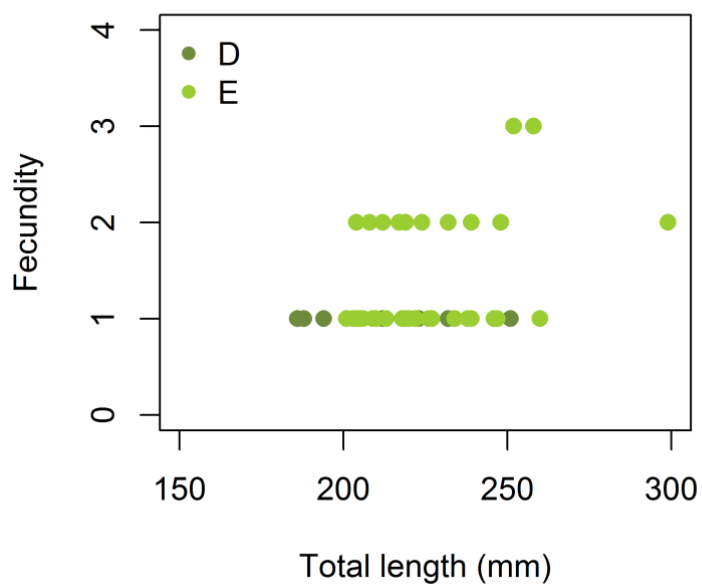


Figure 6. Relationship between total length and fecundity for *Benthobatis krefftii* from south eastern Brazil, considering (D) early pregnancy and (E) late pregnancy females.

CONSIDERAÇÕES FINAIS

Esta dissertação teve como objetivo inicial estudar a estrutura populacional e biologia reprodutiva da raia elétrica cega *Benthobatis krefftii*, na região do talude continental do estado de São Paulo. Ao longo do estudo, diferenças entre o único estudo de biologia disponível para esta espécie e o presente foram evidenciadas.

Diferenças no tamanho de maturidade, fecundidade e até mesmo anatomia do sistema reprodutor foram encontradas comparando os resultados do presente estudo com o estudo disponível para espécimes do sul do Brasil. Estas diferenças devem ser consideradas e estudos comprovando a existência de duas populações são encorajados.

A espécie em questão foi descrita no corrente século e, até o presente, o único trabalho abordando aspectos da biologia básica da mesma e, de maneira geral, do gênero, é apresentado em formato de dissertação, escrita em português e de acesso restrito. A necessidade de conhecimento sobre esta espécie é evidente, no momento em que a mesma apresenta uma série de características que a tornam ainda mais vulnerável.

Neste estudo, foi observado que *B. krefftii* apresenta fecundidade extremamente baixa (1 a 3 embriões) se comparada com outras espécies de elasmobrânquios. Esta baixa fecundidade certamente corrobora a baixa fecundidade esperada para elasmobrânquios habitando profundidades maiores que 200 m. De maneira geral, se exposta à uma mortalidade por pesca, por exemplo, esta espécie não será capaz de se recuperar. Este fato, somado ao endemismo e distribuição restrita da espécie, a tornam extremamente vulnerável à extinção, frente alguma perturbação.

Machos e fêmeas, de maneira geral, atingem a maturidade com grandes tamanhos corporais (cerca de 60, 70 % o tamanho máximo registrado), sugerindo que esta espécie

apresenta uma maturação tardia. No entanto, estudos de idade de crescimento e, conseqüentemente, idade de maturidade, elucidariam melhor esta questão.

A estratégia reprodutiva de *B. krefftii* pode ser determinada como um investimento no tamanho dos embriões, sobrepondo o investimento em tamanho da prole. Ou seja, há a produção de poucos, porém grandes embriões, os quais podem nascer atingindo cerca de 1/3 do tamanho máximo registrado para a espécie. De maneira geral, elasmobrânquios vivíparos lecitotróficos, como *B. krefftii*, não apresentam embriões tão grandes. Desta forma, estudos a respeito do modo de nutrição embrionário desta espécie são necessários para compreender o mecanismo de nutrição e alguma possível nutrição matrotrofica.

Por fim, o presente estudo forneceu informações importantes sob o ponto de vista biológico desta espécie rara, de distribuição restrita e extremo endemismo. Os resultados deste estudo auxiliarão de maneira significativa no entendimento da biologia de espécies do gênero *Benthobatis*, bem como no entendimento da biologia de raias habitantes de profundidades, das quais pouco se conhece.