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Original article

Use of a nursery area by cownose rays (Rhinopteridae) in southeastern Brazil

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Using non-lethal methods for data collection of individuals and participatory monitoring by fishermen, we provide the first empirical evidence of the use of a nursery area by neonate and young-of-the-year cownose rays *Rhinoptera bonasus* and *R. brasiliensis* in southeastern Brazil. Two methods were used to collect data: (1) information provided by fishermen (reports, pictures, and videos) and (2) field sampling by researchers. A total of 746 cownose rays were captured; 113 have been identified as *R. bonasus*, 15 as *R. brasiliensis*, and 618 were reported by fishermen and could not be identified to the species. Records of newborns were made only in late spring and summer in 2015, 2016, and 2017, which suggests an annual reproductive cycle, with birth in late spring, extending to summer. A repeated use of this area by *R. bonasus* suggests that it is potentially important to the reproduction of this species. However, *R. brasiliensis* requires more studies. Small increases in mortality, resulting from increased fishing or other anthropogenic stressors, can have a disproportionately large effect on population viability. Thus, management of areas used during critical stages of the life cycle of rays is crucial to their conservation.

Keywords: Early life-history, Neonates, Newborn, Non-lethal, Young of the year.

Usando métodos não-letais para levantamento de dados dos indivíduos e o monitoramento participativo dos pescadores fornecemos a primeira evidência empírica do uso de uma área de berçário por neonatos e jovens do ano de raias Ticonha *Rhinoptera bonasus* e *R. brasiliensis* no sudeste do Brasil. Dois métodos foram utilizados para coletar dados: (1) informações fornecidas pelos pescadores (relatórios, fotos e vídeos) e (2) amostragem de campo pelos pesquisadores. Um total de 746 raias Ticonha foram capturadas; 113 foram identificadas como *R. bonasus*, 15 como *R. brasiliensis* e 618 foram registrados pelos pescadores e não puderam ser identificadas em nível específico. Os registros de recém-nascidos foram feitos apenas no final da primavera e no verão de 2015, 2016 e 2017, o que sugere um ciclo reprodutivo anual, com nascimento no final da primavera, se estendendo até o verão. O uso repetido desta área por *R. bonasus* sugere sua potencial importância para a reprodução destas espécies. No entanto, *R. brasiliensis* requer mais estudos. Pequenos aumentos na mortalidade, resultantes do aumento da pesca ou outros estressores antropogênicos, podem ter um efeito desproporcionalmente grande na viabilidade populacional. Assim, o gerenciamento das áreas usadas durante os estágios críticos do ciclo de vida das raias é crucial para sua conservação.

Palavras-chave: Início da história da vida, Jovens do ano, Não letal, Neonatos, Recém nascido.

Introduction

Due to their peculiar biological characteristics, such as late sexual maturity, long gestation period, and low reproductive potential, batoids (skates and rays) are highly susceptible to anthropogenic stressors (*e.g.* by overexploitation and persistent organic pollutants) (Gelsleichter *et al*., 2006; Dulvy *et al*., 2014; Sawyna *et al*., 2016). Therefore, the identification of critical areas involved in the life cycle of these fish is essential for their management and conservation (Heithaus, 2007; Tavares *et al*., 2016). Nursery areas of elasmobranchs are usually located in shallow estuarine or coastal areas, where there is protection against predators and abundant food, which enables permanence in the first few months or years of their life (Springer, 1967; Castro, 1993; Heupel *et al*., 2007). Although nursery areas of sharks have been well-studied (Gadig *et al*., 2002; Heithaus, 2007; Bornatowski, 2008; Kinney, Simpfendorfer, 2009), little is known about the nursery areas of batoids (Cerutti-Pereyra *et al*., 2004; Yokota, Lessa, 2006; Araújo *et al*., 2016).

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Cownose rays (Chondrichthyes; Rhinopteridae) are benthopelagic, with large schools often reported in estuarine and coastal regions, grouped by size and sex (Smith, Merriner, 1987; Schwartz, 1990; Fisher *et al*., 2013; Ajemian, Powers, 2016). They occur in temperate and tropical regions, migrate, and breed in seasonal cycles (Grusha, 2005; Fisher *et al*., 2013; Ajemian, Powers, 2016). Although most of the biological knowledge about cownose rays comes from northern populations of *Rhinoptera bonasus* (Mitchill, 1815) (Neer, Thompson, 2005; Fisher, 2010; Fisher *et al*., 2013; Ajemian, Powers, 2016), other congeneric species share its life history strategy (McEachran, Carvalho, 2002; Domingues *et al*., 2009; Rangel *et al*., 2017).

When adults, cownose rays use different areas for breeding (in summer) and feeding (in winter). Mating occurs in an annual (Fig. 1) (Fisher, 2010; Ajemian, Powers, 2016) or biennial cycle (Pérez-Jiménez, 2011), and pregnant females access the nursery areas to give birth. Some of these critical areas are known for a few populations in the northern hemisphere (Neer, Thompson, 2005; Collins *et al*., 2008; Fisher *et al*., 2013; Ajemian, Powers, 2016). However, knowledge about the biology of *R. bonasus* and *R. brasiliensis* Müller, 1836 in the southern hemisphere is poor, with occasional reports of potential nursery areas (Domingues *et al*., 2009).

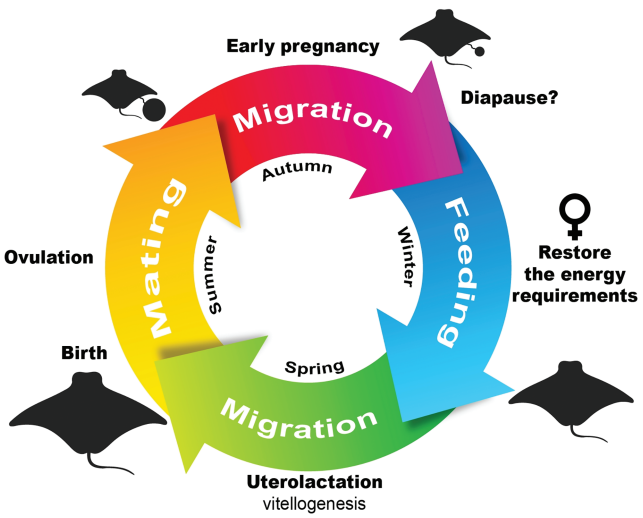

Fig. 1. Diagram of the reproductive cycle of *Rhinoptera bonasus*, based on data from the present study and Smith, Merriner (1987), Blaylock (1993), Fisher (2010), Goodman *et al.* (2010), Fisher *et al.* (2013), and Ajemian, Powers (2016).

Rhinoptera bonasus occurs from southern New England (USA) to southern Brazil, including the Gulf of Mexico (Bigelow, Schroeder, 1953; McEachran, Carvalho, 2002). It is currently classified as "Near Threatened" on the International Union for the Conservation of Nature (IUCN) Red List assessment (Barker, 2006), and is listed in Appendix II of the list of endangered species of wildlife in the State of São Paulo, as a species that requires care in fishery management for its conservation (ALESP, 2014). *Rhinoptera brasiliensis* is classified as "Endangered" on the IUCN Red List and as "Critically Endangered" on the Brazilian national assessment (ICMBio, 2016), and shows a more restricted distribution, occurring in the Gulf of Mexico, Colombia (Caribbean coast), and from Venezuela to southern Brazil (McEachran, Carvalho, 2002; Grijalba-Bendeck *et al*., 2007; Lasso-Alcalá *et al*., 2009). Basic studies on life history aspects are urgently required in Brazil (Vooren, Lamónaca, 2004). A few studies were carried out only at genus level (*Rhinoptera* spp.), making difficult of raising what has already been achieved in monitoring and reports of occurrence of these populations in Brazil (Cavalcante *et al*., 1997; Vooren *et al*., 2005; Costa, Chaves, 2006).

The recovery of impacted elasmobranchs populations requires attention in all age classes (Kinney, Simpfendorfer, 2009). Factors such as recruitment, survival of the parental stock and protection of nursery areas, are important for the stability of populations (Camhi *et al*., 1998; Dulvy *et al*., 2014). Despite the concern about the conservation of elasmobranchs, knowledge of nursery areas is still poor (Yokota, Lessa, 2006; Tavares *et al*., 2016). Thus, the goal of the present study was to assess the potential use of a nursery area by the cownose rays *R. bonasus* and *R. brasiliensis* during early stages of life. We highlight the bycatch in small-scale artisanal fisheries on the central coast of the state of São Paulo, southeastern Brazil, the use of a non-lethal methodology for data collection of individuals and participatory monitoring by fishermen for research and monitoring of neonates and young of the year (YOY). We discuss the implications of the reproductive strategy of these species, the importance of monitoring them in this region, and the limitations of assessing the neonatal period in cownose rays with the criteria proposed for elasmobranchs, as they have different reproductive strategies.

Materials and Methods

The rays were collected from November 2015 to May 2017 in Bertioga, Guaibe Sector, a Marine Protected Area located in the São Paulo coast, southeastern Brazil (23°49'35.02"S, 46°5'41.69"W) (Fig. 2). The specimens sampled were obtained from the bycatch of the beach seine fishing, using a 350 x 11 m fishing net, mesh-size of the 70 mm between knots in the wings and 80 mm in the bag, thrown at 400-600 m from the beach and the gathered by manual traction.

After fishing, two methods were used to collect data of individuals: (1) information provided by fishermen (reports, pictures, and videos) and (2) field sampling by researchers (Fig. 3). The information collected by fishermen through images and capture reports were: popular name of rays, number of individuals, date and local of capture. The rays were released immediately (approximately 5 minutes). Animals that died in the process were also analyzed and then kept by fishermen.

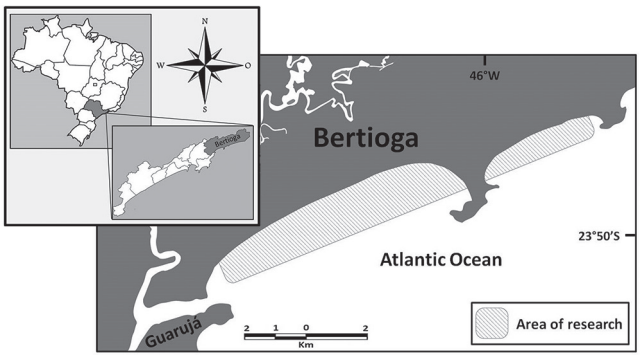

Fig. 2. Map of the location where the animals were caught in Bertioga, São Paulo, southeastern Brazil (23°49'35.02" S, 46°5'41.69" W).

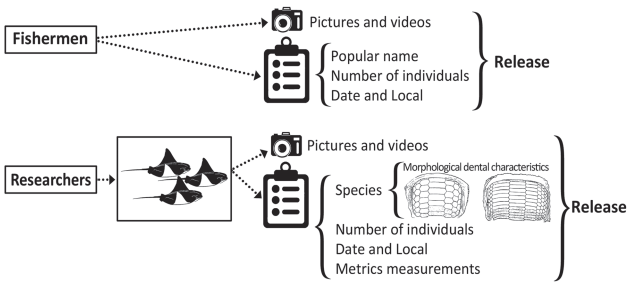

Fig. 3. Methods to collect data of individuals: (top) information provided by fishermen (reports, pictures, and videos) and (down) field sampling by researchers.

During the sampling by researchers, the animals were removed from the fishing net and stored in plastic containers (50L) filled with seawater (2 or 3 individuals per box) to reduce air exposure during the procedures of metric measurements (disc width-DW). Species were identified through morphological dental characteristics (counting the rows of teeth). *Rhinoptera brasiliensis* presents nine rows in the dental plates and *R. bonasus* presents seven rows of teeth (McEachran, Carvalho, 2002). The animals were released at the end of data collection (approximately 30 minutes).

For a preliminary analysis of possible periods of birth, the *R. bonasus* were classified as neonates (< 50 cm) or YOY (> 50 cm and < 70 cm), using the study of age and growth of Fisher (2010) and Fisher *et al.* (2013). For the *R. brasiliensis* the same classification was used, since the two species have similar size at birth. However, this is not a study about age and/or growth of *R. brasiliensis*.

The criteria proposed by Heupel *et al*. (2007) was used to define nursery area and all the three criteria seem to fit, at least to the region of Bertioga, state of São Paulo. (1) There is more YOY in this region than in surrounding regions (Domingues *et al*., 2009). (2) YOY tend to remain for long periods in place. In the present study, there were individuals with little more than a year (individual > 60 cm, with ~ 1 year old). (3) The habitat is repeatedly used over the years. Research permits were granted by SISBIO (ICMBIO/SISBIO # 48572-1) and the Animal Ethics Committee (CEUA; # 258/2016) of the Institute of Biosciences, University of São Paulo.

Results

A total of 746 cownose rays were captured: 113 were identified as *R. bonasus*, 15 as *R. brasiliensis,* and 618 were reported by fishermen and could not be identified to the species level. The largest number of animals recorded was obtained through pictures and videos made by fishermen (Figs. 4 a-c) and reports (as shown in the video **S1** - Available only as online supplementary file accessed with the online version of the article at http://www.scielo.br/ni). Data provided by fishermen is an effective tool in the monitoring of these rays at the study site, allowed to quantify the amount of animals captured and to estimate the life-stage (young or adult).

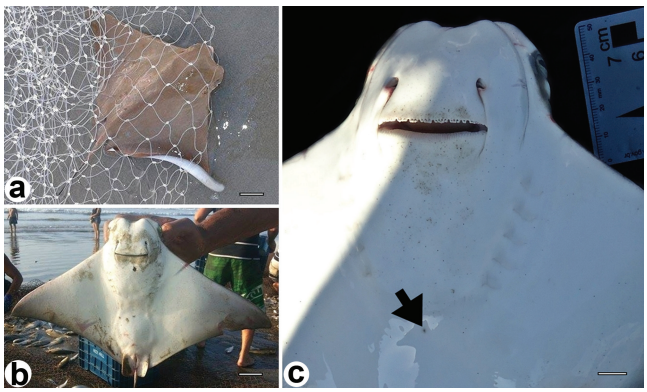

Fig. 4. a. *Rhinoptera* spp. YOY captured, still on the net. **b.** Rays being released by fishermen. **c.** Individual ray recorded with the scar of the umbilical cord (arrow). Scale bar: **a.** 3.5 cm; **b.** 3 cm; **c.** 1 cm.

The largest catches of neonates and YOY were made in late spring, throughout summer, and in early fall (Fig. 5). Large schools of 20-55 animals were captured between November and March inside the bay (Fig. 2). The largest school recorded comprised 55 individuals (including neonates and YOY), but the average number of animals in school was 34 ± 12.07. In these events, due to the large number of individuals captured, only a few were measured.

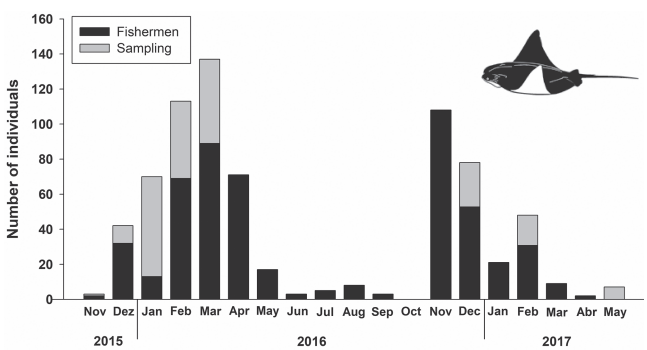

Fig. 5. Number of monthly catches of *Rhinoptera* spp. neonates and YOY in Bertioga, São Paulo, southeastern Brazil (November 2015 - May 2017).

Among the animals measured and identified (n = 128), *R. bonasus* was more frequent (n = 113) than *R. brasiliensis* (n = 15). In *R. bonasus,* the amplitude of DW was 31.5-75 cm, and in *R. brasiliensis*, 35.5 – 58.5 cm (Fig. 6a). Few individuals analyzed showed umbilical scars, *R. bonasus* (n = 5, 39.5 ± 3.12 cm DW) (Fig. 4c) and *R. brasiliensis* (n = 1, 45.5 cm DW). Smaller animals (35.8 ± 6.01 cm DW, n = 3) were recorded without any evidence of umbilical scars.

The smallest individuals captured (some with umbilical scars) were recorded in December (2015 and 2016) and February (2017) (Fig. 6b). The only adult individual was captured in February (2017), together with neonates. All rays captured were released immediately by fishermen and researchers, with very few reports of mortality (1.2 %) (n= 15), which occurred during night trawls and when a high number of teleost were caught, making difficult to locate rays during fish removal. Dead animals included nine *R. bonasus* (31.5 – 48 cm) and six *R. brasiliensis* (35.5 - 54 cm).

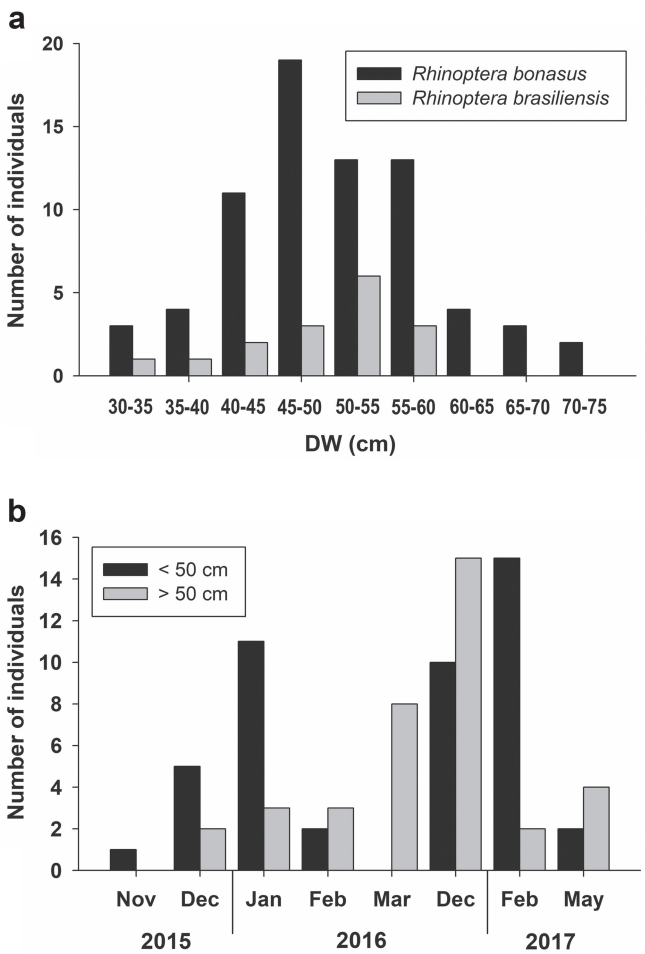

Fig. 6. a. Number of *Rhinoptera bonasus* and *R. brasiliensis* according to disc width (DW) and **b.** individuals < 50 cm and > 50 cm in the months sampled in Bertioga, São Paulo, southeastern Brazil (November 2015 – May 2017).

Discussion

This study is the first empirical evidence of the use of a nursery area by neonate and YOY cownose rays (*Rhinoptera bonasus* and *R. brasiliensis*) in southeastern Brazil. The reproductive cycle of *R. bonasus* and *R. brasiliensis* are poorly known in the study region. However, the record of newborns only during the late spring and summer in 2015, 2016, and 2017 suggests an annual cycle, with birth in late spring, extending during the summer (Fig. 5), confirming the criterion proposed by Heupel *et al.* (2007). The seasonality of catches seems to be related to the low frequency of fishing during late autumn and winter (from June to October), due to frequent changes in the temperature and oceanographic conditions in this period. It is also likely that pregnant females migrate to this region to mate and give birth (*i.e.*, Smith, Merriner, 1987; Fisher *et al*., 2013). Several studies on the migratory behavior of *R. bonasus* in the northern hemisphere confirmed the arrival of migratory parental rays in coastal and estuarine waters in mid-spring, where they remain throughout summer and migrate again in the middle of late autumn (Fig. 1) (Smith, Merriner, 1987; Schwartz, 1990; Blaylock, 1993; Neer, Thompson, 2005; Goodman *et al*., 2010; Ajemian, Powers, 2016). However, due to the capture of YOY throughout the year, animals may remain in the nursery area until the next migration season. They probably stay in nursery areas due to their low mobility at this age (Ajemian, Powers, 2016), to maximize growth and protection against predators (Fisher *et al*., 2013), and even because of their low energy stocks at this stage of life.

The presence of neonates and YOY were punctually recorded only in the State of Paraná, southern Brazil (n = 51; DW = 38.0-95.0 cm; Bornatowski *et al*., 2014) and in the State of Rio Grande do Norte, northeastern Brazil (n = 4; Yokota, Lessa, 2006; and n = 9; DW = 34.5-45; Lessa *et al*., 2015). There is capture data for *R. brasiliensis* in the state of Rio Grande do Sul, southern Brazil, during summer 1982-1985, with large schools composed of males with 78-91 cm (DW) and females with 77 to 102 cm (DW) (Vooren, Lamónaca, 2004). There are no records of the capture of newborns in Rio Grande do Sul, which suggests that birth and nursery area occur in some region further north (Vooren, Lamónaca, 2004). Pregnant females were occasionally captured in the Guarujá, state of São Paulo (Domingues *et al*., 2009), a region near the study area. Therefore, the present study is the first to record a large number of neonates and YOY of this species (n= 15).

Reports of newborns and embryos to term of *R. brasiliensis* suggest that the size at birth is 43 to 48 cm DW (Bigelow, Schroeder, 1953; Vooren, Lamónaca, 2004; Domingues *et al*., 2009). In the present study, individuals captured measured 35.5-58.5 cm (DW), representing neonates and YOY. The smallest newborn pup recorded

was captured in May, together with the information of a pregnant female in March and neonates in October (Domingues *et al*., 2009), and pregnant females in middle of the gestation in January, in Rio Grande do Sul State (Vooren, Lamónaca, 2004). Therefore, the time of birth and the reproductive cycle can be different from that observed in the present study for *R. bonasus*. However, more studies are required comprising the entire reproductive cycle of this species.

Some of the features observed in shark nursery areas can be applied to the present study, considering that these areas are characterized by both, the presence of pregnant females and the occurrence of newborns (Springer, 1967; Castro, 1993; Simpfendorfer, Milward, 1993). In sharks and other batoid species, the identification of neonates and YOY is based on the presence of an umbilical cord mark (Hussey *et al*., 2010; Belicka *et al*., 2012). However, in stingrays that exhibit histotrophic viviparity, the embryo consumes the yolk sac long before birth (Fisher, 2010). Thus, the scar is almost unnoticeable, which makes the identification difficult. In *Rhinoptera* spp., DW was used for classification into neonates and YOY, as only five rays show umbilical scars.

Rhinoptera bonasus shows ontogenetic segregation, with YOY and juveniles exploring coastal bays with fluvial influence or estuarine regions with low salinity, avoiding the competition with the pups of other age classes and protected from predators (Ajemian, Powers, 2016). Apparently, the same occur with *R. brasiliensis*. This information corroborates our findings because the region studied forms bays and undergoes fluvial influence. By capturing large groups of neonates and YOY, Fisher *et al*. (2013) and Ajemian, Powers (2016) noted that grouping could reduce the predatory effect at the beginning of life, bringing food advantage to these animals (Bedore *et al*., 2014; Rangel *et al*., 2017). Only ten juveniles (34 cm) and ten adults (images provided by fishermen) were recorded, which indicates either that they eventually come back or remain in more coastal nursery areas. However, further studies are required addressing other fishing gear with larger expanse to identify the local use of other age classes.

Rhinoptera bonasus and *R. brasiliensis* were routinely caught together, both neonates and YOY (Rangel *et al*., 2017). The common use of the area can bring advantages to newborns, reducing predation, but also disadvantages, such as possible competition for resources (Heupel *et al*., 2007). The sympatric and syntopic occurrence of *R. bonasus* and *R. brasiliensis* can increase the chances of survival and feeding success during the first few months or weeks of life (Rangel *et al*., 2017). Studies investigating the relationship between trophic relationship and habitat use of newborn pups of two species using stable isotopes and fatty acids (Belicka *et al*., 2012) can reveal how these species are sharing resources in the nursery area.

Due to the difficulty of managing populations of sharks and rays by traditional strategies used for fish, such as size limits (for age class) and fishing regulations and quotas (Heithaus, 2007), management of areas used during critical stages of the life cycle of these animals could help the conservation of these populations. The repeated use of the area by *R. bonasus* and *R. brasiliensis* suggests that it is important to the life history of these species. Small increases in mortality from increased fishing or other anthropogenic stressors can have a disproportionately large effect on the population viability (Walters, Martell, 2004), especially in critical stages of life history (Heupel, Simpfendorfer, 2002; Hussey *et al*., 2010).

Monitoring newborns and adults with the help of fishermen has been very efficient; it was possible to identify individuals to the genus level and the frequency of captures during the year. In addition, the immediate release of rays by fishermen proved to be a proper management, ensuring the survival of animals after the capture by beach seine. We recommend marking individuals released with plastic or transmitter for the assessment of the real post-released survival of these animals. Furthermore, as Bertioga is a region with high anthropogenic impact (Gonçalves *et al*., 2013), studies on contaminants and the impact of climate change are needed. On the one hand, the premature abandonment of newborns can hinder survival, exposing them to severe predation pressure (Springer, 1967) and energy challenges that they are not ready to deal with yet. On the other hand, staying too long in this region can result in competition the juveniles (> one year old) and newborns.

In conclusion, we identified the area as a nursery for both cownose ray species. Birth occurs in late spring and early summer. The method used for classifying neonate elasmobranchs is inaccurate for *Rhinoptera*, which have a matrotrophic (histotrophic) reproductive mode. However, more detailed analyses are necessary in a higher number of species to evaluate the umbilical cord scar and its effectiveness in the classification of the puppies. A new method should be developed for separating neonates from YOY. Additionally, inclusion of the pregnant females and analysis of embryos, as well as age and growth studies and biochemical analyses, such as stable isotopes will help to improve our understanding for correct classification of newborns. The beach seine method proved to have low-impact on rays in post-capture when prioritized the release soon after capture.

Acknowledgments

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