Defining the reproductive biology of two species of cownose ray, *Rhinoptera bonasus* and *Rhinop tera brasiliensis*, in the northern Gulf of Mexico

Definición de la biología reproductiva de dos especies de cownose ray, *Rhinoptera bonasus* y *Rhinoptera brasiliensis*, en el norte del Golfo de México

Définition de la biologie de la reproduction de deux espèces de raies cownose, *Rhinoptera bonasus* et *Rhinoptera brasiliensis*, dans le nord du Golfe du Mexique

ANNA K. MILLENDER[1, 2], JEREMY M. HIGGS[2], FRANK J. HERNANDEZ[1], and JILL M. HENDON[2]

[1] Division of Coastal Sciences, School of Ocean Science and Engineering, The University of Southern Mississippi, 703 East Beach Drive, Ocean Springs, MS, USA

[2] Center for Fisheries Research and Development, The University of Southern Mississippi, 703 East Beach Drive, Ocean Springs, MS, USA

anna.millender@usm.edu, j.higgs@usm.edu, frank.hernandez@usm.edu, jill.hendon@usm.edu

The Cownose Ray (*Rhinoptera bonasus*) is a highly mobile, coastal batoid that is found in the western North Atlantic Ocean from the northeastern United States to southeastern Brazil. The reproductive biology of *R. bonasus* has been assessed in Florida, Louisiana, and the Chesapeake Bay and size-at-maturity in the northern Gulf of Mexico has been documented to be 642 mm and 653 mm disc width for males and females, respectively. The Brazilian Cownose Ray (*Rhinoptera brasiliensis*) is a highly migratory, coastal batoid that has been considered endemic to 1,800 km of Brazil's coastline and has likely experienced greater than 80% population reduction due to overfishing (Carlson *et al.* 2020). Limited data exists for the reproductive biology of *R. brasiliensis* and to date only a single gravid female and associated neonate from Brazil have been documented in the scientific community.

Historically, *R. bonasus* was considered the only Rhinopterid residing in the northern Gulf of Mexico (nGOM); however, recent studies have confirmed the presence of *R. brasilisensis* throughout this region (Jones *et al.* 2017). These two cryptic species can only be externally distinguished by tooth series counts (7-8 for *R. bonasus*, 9-13 for *R. brasiliensis*). The presence of both species in the nGOM, brings into question whether the previous life history studies on *R. bonasus* (Neer and Thompson 2005) were species specific or if they inadvertently encompassed an assessment of both species. Neither Rhinopterid is managed by state or federal fisheries programs; however, the new interest in sport fishing for cownose and its frequent use as bait by recreational anglers and commercial fishers, has put more pressure on their popula-tions. In order to maintain these populations, it is vital that the life history of both species is fully investigated.

Cownose rays of both species were retained from long-term monitoring programs conducted across the nGOM from 2016 to 2021. The disc width (mm), body mass (g), and a full reproductive workup were conducted for each specimen. The testis length (mm), width (mm), and mass (g), and epididymis head width (mm) were collected for males. The oviducal width (mm), ovary mass (g), and maximum vitellogenic follicle diameter (mm) were collected for females. Maturity assessments were conducted for each specimen and rays were assigned a maturity index (0 = immature, 1 = mature). Males were deemed mature if 1) the claspers were calcified, 2) if the claspers rotated 180 degrees, 3) if the rhipidion was freely opening, and 4) if the seminal vesicles were curved. Females were deemed mature if 1) the oviduct was not embedded in the body wall, and 2) enlarged ovulatory follicles were present, or 3) if gravid. A binomial logistic regression was used to determine size at which 50% of the rays collected were mature. Gonadosomatic Index (GSI) was calculated using the equation: GSI = (gonad mass/(body mass-gonad mass))*100. The GSI equation was modified to remove embryo mass from the denominator if the female was gravid. If the female was gravid, the embryo disc width (mm) and embryo mass (g) were measured. Reproductive parameters were averaged by month for both species, by sex, to investigate reproductive seasonality. Embryonic disc widths were averaged by month, by species to investigate gestation duration and parturition date.

A total of 331 cownose rays were collected: 245 *R. bonasus* (139 males, 106 females; 346-930 mm disc width), 86 *R. brasiliensis* (55 males, 31 females; 364-1030 mm disc width). Size-at-maturity estimates for *R. bonasus* indicated that females mature at a smaller size than males (Table 1) and both male and female *R. bonasus* mature at a smaller size than *R. brasiliensis* (Table 1 and 2). Female *R. brasiliensis* mature at a larger size than *R. brasiliensis* males (Table 2). Results from the current study indicate that size-at-maturity estimates for male *R. bonasus* were larger and size-at-maturity estimates for female *R. bonasus* were smaller than those previously reported by Neer and Thompson (2005). This variability in life history estimates may be due to the sample sizes in each study or due to the differentiation of both species in the current study. Gonadal development is peaking in the Spring for both sexes (Table 1 and 2), indicating that mating likely occurs during May-June for both species. Averages of emb ryo disc width across months indicated an 11–12-month gestation period with parturition likely occurring in June and July for both species (Table 1 and 2). Examination of embryonic development indicates that *R. brasiliensis* attains a larger size prior to parturition than *R. bonasu*s.

Defining life history characteristics for these two species is the first step toward ensuring the future sustainability of these batoids. The findings of this study will be applicable to conducting species specific stock assessments by updating *R. bonasus* parameters and providing initial estimates of *R. brasiliensis*. Further understanding the range and life history parameters of *R. brasiliensis* will allow for the IUCN listing to be reassessed. To continue our research, age and growth will

be estimated for both species and age-at-maturity will be calculated for age-based stock assessments. Additionally, geographic variability will be investigated by species

KEYWORDS: Life history, Elasmobranch, Batoid

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Table 1. Reproductive parameters for male and female *Rhinoptera bonasus*

Reproductive Parameter	*R. bonasus* Males	*R. bonasus* Females
Size-at-maturity (50%)	672 mm disc width	625 mm disc width
Gonadosomatic Index	Peaks in March	Peaks in June
Testis Length (mm)	Peaks in March	n/a
Testis Width (mm)	Peaks in March	n/a
Epididymis Width (mm)	Peaks in May and June	n/a
Maximum Follicle Diameter (mm)	n/a	Peaks in June
Oviducal Width (mm)	n/a	Peaks in May
Parturition Period	n/a	June and July
Average Embryo Disc Width at Parturition	n/a	281 ± 5.0 mm

Table 2. Reproductive parameters for male and female *Rhinoptera brasiliensis*

Reproductive Parameter	*R. brasiliensis* Males	*R. brasiliensis* Females
Size-at-maturity (50%)	718 mm disc width	745 mm disc width
Gonadosomatic Index	Peaks in April	Peaks in May
Testis Length (mm)	Peaks in April	n/a
Testis Width (mm)	Peaks in May	n/a
Epididymis Width (mm)	Peaks in May	n/a
Maximum Follicle Diameter (mm)	n/a	Peaks in May
Oviducal Width (mm)	n/a	Peaks in April
Parturition Period	n/a	June and July
Average Embryo Disc Width at Parturition	n/a	412.5 ± 10.5 mm