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SPECIAL SECTION: ELASMOBRANCH LIFE HISTORY

Reproductive Biology of the Cownose Ray in the Charlotte Harbor Estuarine System, Florida

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Abstract

The Cownose Ray *Rhinoptera bonasus* is an abundant species in the western Atlantic Ocean, including the Gulf of Mexico, but its reproductive biology is not completely known because of the difficulty of obtaining year-round samples in large portions of its range where the species is migratory. To address this knowledge gap, Cownose Rays were studied in a subtropical estuarine system where rays are available year-round. Size at maturity and reproductive cyclicity were assessed using 140 females and 151 males. For females, the size at 50% maturity was 701 mm disk width (DW) based on ovary length and weight, maximum follicle diameter, and mating wounds and scars; the size at 100% maturity was 712 mm DW. For males, the size at 50% maturity was 681 mm DW based on clasper morphology, testis length and weight, and epididymis width; the size at 100% maturity was 712 mm DW. Overall testis size (length and weight) and mean testis lobe diameter peaked up to 2 months prior to ovary size and maximum follicle diameter, indicating that males were preparing to inseminate females during the entire parturition and mating period. Mating behaviors and fresh mating wounds were observed mostly between April and June. Ovulation peaked in May, and parturition occurred primarily in March and April after an 11–12 month, single-embryo gestation period, suggesting that females are synchronous in Charlotte Harbor. Size at birth was 202–383 mm DW. Concurrent vitellogenesis and gestation indicated a clearly defined annual reproductive cycle that may be completed within the estuary. Understanding reproductive life history in wide-ranging species such as the Cownose Ray is useful in the short term for making sound management decisions and for future comparison as the effects of global climate change are realized.

The current life history strategies and population dynamics of fishes reflect their evolutionary and zoogeographic histories. As a result, a wide array of life history strategies has evolved in fishes—the most diverse group of vertebrates. Population identification, including structure and connectivity, are important for a relatively complete understanding of the ecological interrelationships of a given species before its rangewide life history can be understood (e.g., latitudinal variation and migratory patterns) and effective management strategies can be implemented when required. These data are also needed as baselines for future comparison as fishes react to the effects of global climate change (Roessig et al. 2004).

Life history strategy directly affects how much mortality fishes can withstand before healthy populations decline to unsustainable levels and extend the time scale for recovery if overfishing occurs. In general, teleost fishes tend to be more *r*-selected (characterized by the production of many small offspring, early maturity, and the ability to recover from high levels of mortality) and elasmobranch fishes (rays and sharks) are *K*-selected (characterized by the production of comparatively few large young, later maturity, and difficulty recovering from high levels of mortality) (MacArthur and Wilson 1967; Hoenig and Gruber 1990). This difference causes populations of elasmobranchs that are fished or affected by disturbances (e.g., disease

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and red tides) to be reduced faster and replaced more slowly than those of the many exploited bony fishes that produce thousands of pelagic larvae (Stevens et al. 2000). Within each group, however, there are many inter- and intraspecific life history variations that make it important to consider each species individually (Cope 2006).

The life histories of many elasmobranch fishes are unknown or have to be assembled from different populations, sometimes on ocean-basin scales (Cope 2006). Even with the data-poor approach of extrapolating from multiple populations, of the more than 400 known species of sharks worldwide relatively complete reproductive life history data are available for only about 10 species (Parsons et al. 2008). This lack of knowledge limits our ability to assess the extent to which many species are declining due to numerous factors, including overfishing (Stevens et al. 2000). In the United States, nonprotected sharks are managed by the National Marine Fisheries Service according to life history and habitat use categories such as “large coastal species” and “small coastal species.” In the United States and elsewhere, most rays are not managed by state or federal governments, are usually not targeted by commercial and recreational fisheries, and if caught are rarely landed (Camhi et al. 1998). As a result, most rays, including the Cownose Ray *Rhinoptera bonasus* have garnered little attention from researchers and management agencies and the status of many of these species is unknown. Although not geographically extensive at present, Cownose Ray fisheries exist (Grusha 2005; Pérez-Jiménez 2011) and represent significant potential fishery resources, especially if other fish populations continue to decline. Nevertheless, if Cownose Ray fisheries expand, the species’ inter- and intrapopulation life history parameters will need to be understood to avoid overfishing. Specifically, good reproductive data will be essential to accurately assess pre- and postfishery stock status and prevent the unsustainable exploitation of these rays.

The Cownose Ray is found in coastal and estuarine waters of the western Atlantic Ocean from southern New England to Brazil, including the Gulf of Mexico (Bigelow and Schroeder 1953; Robins and Ray 1986). Large-scale movement data on these rays are scarce, but there could be at least two populations off North and Central America: one in the Atlantic that migrates in large schools between the northern portions of its range and at least northern Brazil, and another that is thought to migrate clockwise in the Gulf of Mexico (Schwartz 2003; Grusha 2005). These large-scale seasonal migrations have made it difficult to accurately assess reproductive seasonality and periodicity in this species. For many years, reproductive data were extrapolated across data gaps because of the difficulty in obtaining year-round samples in large portions of the species’ range where it is migratory (Smith and Merriner 1986; Neer and Thompson 2005; Pérez-Jiménez 2011). Thus, because Cownose Rays occur year-round in the subtropical Charlotte Harbor estuarine system in southwestern Florida (Collins et al. 2008), the objectives of this research were (1) to quantify the macroscopic aspects of female and male reproductive biology (e.g., size at maturity

and seasonality) and (2) to describe the species’ reproductive cycle.

METHODS

Sample Collection

The 56-km-long, 700-km² Charlotte Harbor system is one of the largest estuaries in Florida (Hammitt 1990). The climate is subtropical, with infrequent freezes and distinct wet (June–November) and dry (December–May) seasons that influence fish assemblages (Poulakis et al. 2003). Cownose Rays were collected during fisheries research and monitoring projects conducted throughout the estuary by the Florida Fish and Wildlife Conservation Commission between 2002 and 2012. The principal collecting gears were gill nets (45–183 m long, all with 152-mm stretched monofilament mesh) and center-bag haul seines (183 m long, with 38-mm stretched mesh). Details of these sampling techniques are given in Casey et al. (2007) and Poulakis et al. (2011). Following capture, all Cownose Rays were sexed and their maximum disk widths (DW; mm) were recorded. Disk width was defined as the stretched linear distance between the tips of the pectoral fins. For logistical and conservation reasons, no more than three individuals were typically retained each day for study based on gaps in the data, and any remaining rays were released alive. By the end of the study period, the goal was to dissect at least three juvenile males, three mature males, three juvenile females, and three mature females each month. The retained Cownose Rays were anesthetized in the field with MS-222 (tricaine methanesulfonate) and put on ice. Hydrologic data, including water temperature (°C), salinity (practical salinity units; psu), and dissolved oxygen (mg/L) were recorded at the surface at each sample site. Water depth (to the nearest 0.1 m) was also recorded at each site.

Cownose Rays were usually examined fresh in the field, though sometimes they were kept on ice for dissection later on the day of capture or, if logistics warranted, frozen for later examination. A series of standard morphological measurements were made (all linear measurements in millimeters, all weights in grams), and each individual was assessed for overall health (e.g., external and internal parasites and injuries). Particular attention was given to any potential mating-related bite marks or scarring, and photographs were taken of representative marks. This examination occurred for both males and females because males of other ray species have inflicted wounds on both sexes during courtship and mating (Kajiura et al. 2000). Because the research reported in this paper is part of a larger project on this species, fin clips (for genetic and stable isotope analyses), vertebral centra (for the determination of age and growth), and histological samples (for the examination of reproductive morphology) were taken from the Cownose Rays, including embryos, for future analysis (not included in this paper).

Recent data (e.g., Naylor et al. 2012; E. Hoffmayer, National Marine Fisheries Service, unpublished data) suggest the presence of other *Rhinoptera* species in the western North

Atlantic, including the Gulf of Mexico, but the summary of reproductive biology in this paper is thought to apply only to the Cownose Ray. Species identifications were based on preliminary mitochondrial DNA analyses (J. M. Quattro, University of South Carolina, unpublished data), which showed that other *Rhinoptera* species were found only rarely during the study (~1% of specimens); these individuals were removed from the data set. Studies on nuclear genes in these taxa are ongoing.

Reproductive Cycles

Females.—Females were considered mature if they were gravid or possessed vitellogenic follicles (>10 mm in diameter). The maximum length and maximum width of the left ovary (the only functional ovary; Smith and Merriner 1986), were measured, and then the ovary was separated from the epigonal organ and weighed. The diameter of the largest follicle was also measured. To separate the ovary from the lymphomyeloid epigonal organ, the ovary–epigonal organ complex was left at room temperature for 5–10 min until the epigonal organ liquefied and only the ovary remained. Ovaries were not weighed if they were preserved in the field for future histological analysis (not included in this paper). If the females were gravid, the embryos were sexed, measured (DW), and weighed following the same protocols as for free-swimming individuals, and the presence or absence of histotroph in the uterus was noted. Indicators of the developmental stage of the embryo (e.g., yolk sac diameter, disappearance of the yolk stalk, and the timing of calcification of the tail spine) were documented. Oocytes found in the uterus were assumed to have been fertilized. Ovary size (length and weight), maximum follicle diameter, the presence of mating wounds and scars, and embryo growth were used to assess size at maturity, mating season, gestation period, size at birth, and seasonal patterns during the female reproductive cycle. The presence of trophonemata was used to aid in determining the maturity of individuals if ovary morphology was not definitive; these structures were not useful as a seasonal reproductive characteristic, however, because trophonemata lengths varied depending on their location within the uterus.

Males.—Males were considered mature if they had calcified claspers and a freely opening clasper head or rhipidion (Clark and von Schmidt 1965; Smith and Merriner 1986). The right clasper of each Cownose Ray was measured from its junction with the pelvic fin to its distal end (Smith and Merriner 1986; Neer and Thompson 2005). The maximum length, width, and weight of the right testis were recorded after the testis had been separated from the epigonal tissue. To separate the testis from the lymphomyeloid epigonal organ, the testis–epigonal organ complex was left at room temperature for 5–10 min until the epigonal organ liquefied and only the testis remained. The left testis was preserved for future histological analysis (not included in this paper). In addition, due to testis morphology (Pratt 1988), the diameter and weight of 10 randomly selected testicular lobes from the central portion of the right testis were recorded for investigation of whether the lobes exhibited the same seasonal trends as the entire testis. Since testicular lobes

did not differentiate until maturity, lobe data were only collected on mature males. Clasper morphology, testis size (length and weight), and testis lobe size (diameter and weight) were used to assess size at maturity and seasonal patterns during the male reproductive cycle. The presence of distinct testicular lobes, maximum epididymis width (anterior end; ≥ 10 mm = mature), the presence of semen within the seminal vesicles (no attempt was made to quantify semen volume), and the shape of the anterior portion of the seminal vesicles (curved = mature) were used to aid in determining the maturity of individuals if clasper morphology was not definitive.

Data Analysis

Data from both sexes were analyzed to document the reproductive biology of Cownose Rays. To facilitate comparison with the results of other studies, a length–weight relationship ($W = aL^b$) was established and a logistic model ($Y = [1 + e^{-\{a + bx\}}]^{-1}$) was fitted to binomial maturity data (i.e., immature or mature) using least-squares nonlinear regression. Median disk width at maturity was determined as $-ab^{-1}$ (Mollet et al. 2000). Maturity ogives are not presented because the median disk widths at maturity were similar for both sexes, but the parameter estimates (a , b) are reported for each sex to aid in future stock assessments. To compare the size at maturity with that in studies that did not conduct this analysis, the disk width of the largest immature specimen encountered in this study was used as the threshold for maturity (> this size = 100% maturity). Reproductive variables (maximum follicle diameter, ovary weight, ovary length, clasper length, testis length, testis weight, testis lobe diameter, testis lobe weight, and embryo disk width) were examined by size and month to determine size at maturity for both sexes and the seasonality of the reproductive cycle (e.g., mating season and gestation period). The reproductive cycle (vitellogenesis and gestation) was summarized by plotting raw maximum follicle diameter data with embryo disk widths over the entire year using 1 May as day 0. Fertilized oocytes and early-developing embryos that did not have a disk to measure were assigned a disk width of 0.

RESULTS

From 2002 to 2012, 291 Cownose Rays (140 females and 151 males) were examined across all months to describe the reproductive biology of this species in the subtropical Charlotte Harbor estuarine system (Figure 1). The Cownose Rays in the study area rarely exceeded 800 mm DW (Figure 2). Females ranged from 345 to 840 mm DW (560.8–9,830 g) and males ranged from 314 to 800 mm DW (404.6–7,960 g). The length–weight relationships for females, gravid females, and males showed that gravid females were the heaviest individuals (Figure 2). Cownose Rays were observed and collected throughout the estuarine system, although they were taken most often in the mouths of the Myakka, Peace, and Caloosahatchee rivers—the three major freshwater inputs to the estuary. They were caught and observed alone, in small groups (5–10), and in large groups (hundreds). Sex and size segregation occurred

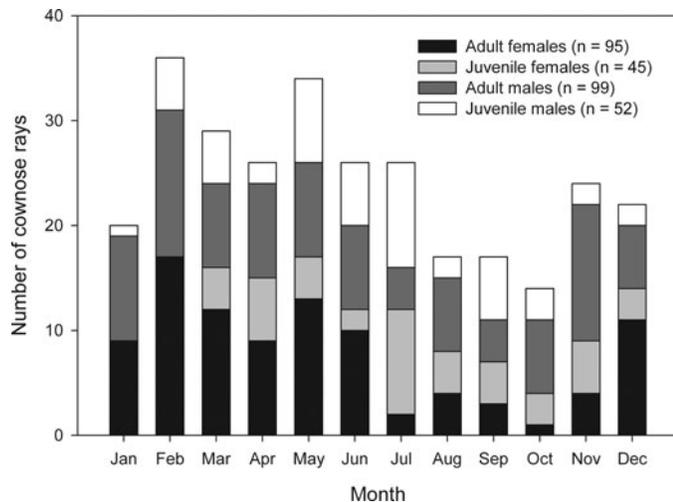


FIGURE 1. Monthly occurrence of Cownose Rays examined in the Charlotte Harbor estuarine system, by sex and maturity, 2002–2012.

but was not widespread; adults and juveniles of both sexes were commonly caught together. Cownose Rays were caught in water temperatures ranging from 14.1 to 34.3°C (mean = 25.2°C), salinities ranging from 1.0 to 35.9 psu (mean = 23.1 psu), dissolved oxygen levels ranging from 1.0 to 11.1 mg/L (mean = 7.4 mg/L), and water depths ranging from 0.3 to 4.0 m.

Mating

Mating behavior was observed in the estuary from early October to late June but primarily between April and June. During these events, solitary females (no claspers observed) were seen at the surface with the tips of their pectoral fins exposed to the air. Multiple Cownose Rays, presumably males, closely followed these females below the surface and eventually one would attempt to mate with her by biting the posterior margin of one of the pectoral fins. Females were sometimes observed swim-

ming quickly along the surface for several meters, presumably to avoid advancing males. Based on the presence of fertilized oocytes in the uterus as well as embryo development, ovulation and fertilization occurred mostly in May in Charlotte Harbor (see below) even though mating behaviors were observed from October to June.

Fresh margin abrasion and excision wounds (as defined by Kajiura et al. 2000 for Atlantic Stingrays *Dasyatis sabina*) were commonly observed on the posterior pectoral fin margins of mature females during the mating season (April–June) over the decade-long study period; these appeared reddish and were sometimes bleeding (Figure 3). Margin abrasions and excisions were observed only at specific, consistent locations on the pectoral fins of mature females. Males bit females on the posterior margin of either pectoral fin about 100 mm from its tip, leaving fresh bite marks and scarring that were approximately the same length as the width of the jaws of males (~60 mm), which contain molariform tooth plates. Margin abrasions were seen on the majority of mature females, were typically restricted to the 20- to 30-mm-wide edge of the disk margin, showed no distinct tooth tracks, and probably resulted from prolonged grasping of the fin margin during copulation. These abrasions were up to 110 mm long and up to 34 mm wide along the disk margin. Healing of severe and accumulated margin abrasions after the mating season sometimes resulted in the persistence of nonpigmented scars on the dorsal and ventral surfaces of the pectoral fins during the nonmating season (Figure 3a, b). Only one male (776 mm DW) had minor, but clear margin abrasions (48 × 12 mm) on the left pectoral fin where mating wounds and scars are typically seen on females. In addition to margin abrasions, at least 51 mature females had obvious excision wounds, where tissue was missing from their pectoral fins in the same region of the pectoral fin where margin abrasions occurred, presumably from more severe biting or mating activity over many years. Excisions typically affected a section of the disk margin 20–40 mm long and <10 mm wide, but some were more extensive (up to 80 mm long and 30 mm wide) and included splitting of the pectoral fin (Figure 3c–f). At least 18 females had multiple excisions (as many as three per pectoral fin).

Female Size at Maturity and Reproductive Seasonality

Only the ovary on the left side of the reproductive tract was functional. In juveniles, the ovary was oval and visible on the dorsal surface of the epigonal organ; follicles began to grow larger than 1 mm in diameter at about 500 mm DW (Figure 4). The largest follicles were recorded in individuals >700 mm DW (Figures 5, 6a). One follicle was often noticeably larger than the others, which corresponds to the normal brood size of one in this species (Figure 5); the largest follicle of the study was 40 mm in diameter and weighed 19.4 g. Two females had a 40-mm follicle: one (captured on 22 April 2009) was nongravid with fresh mating wounds and was probably about to ovulate; the other (captured on 26 April 2006) carried a 383-mm embryo, was probably about to give birth, and probably would have

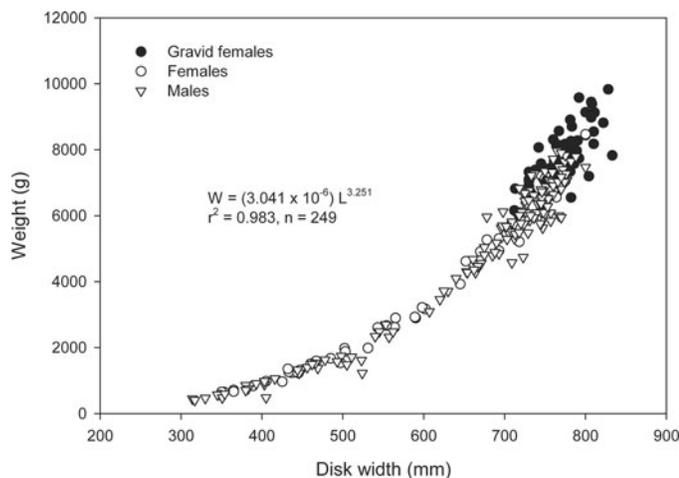


FIGURE 2. Length–weight relationship of Cownose Rays examined in the Charlotte Harbor estuarine system, 2002–2012.

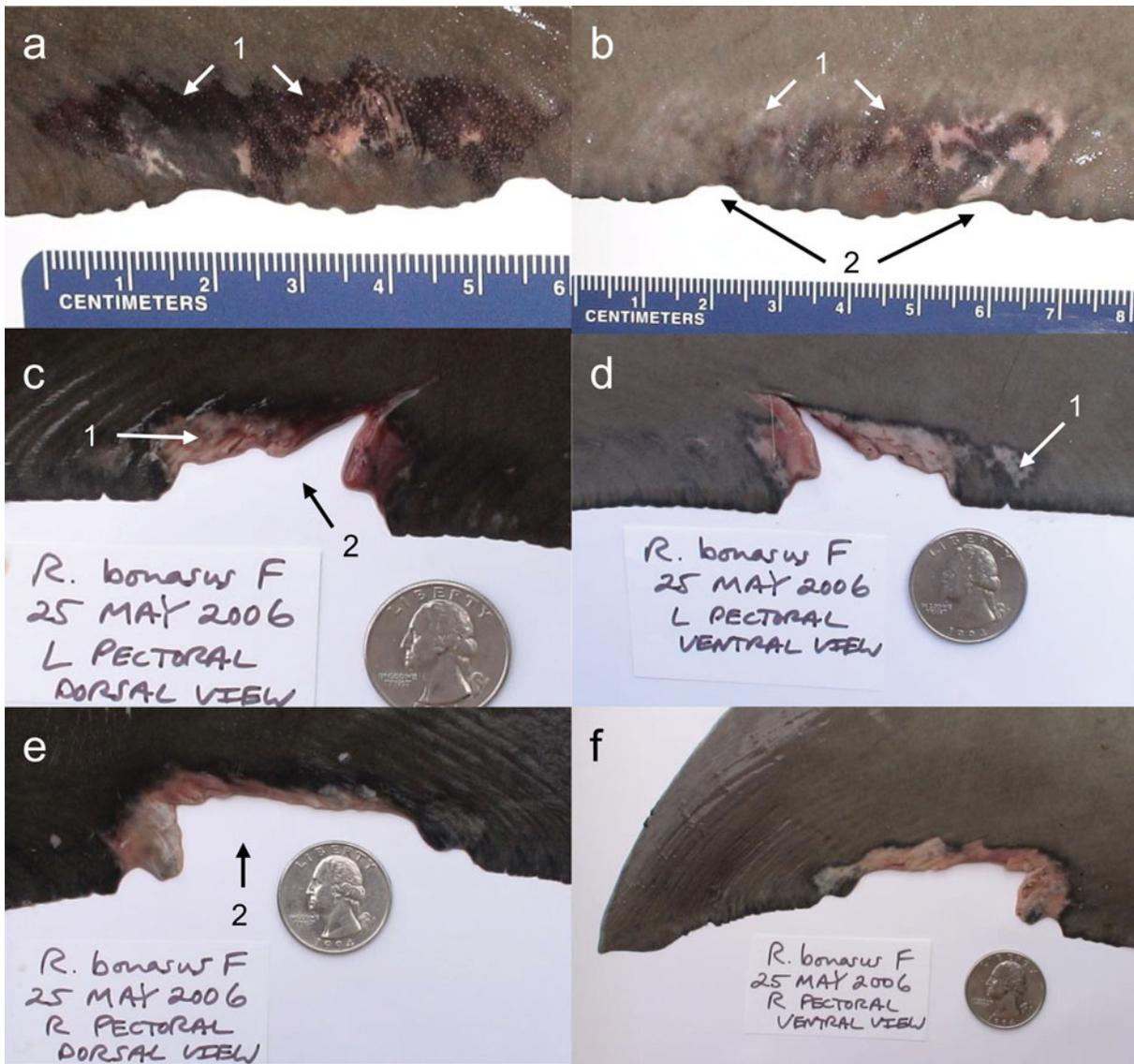


FIGURE 3. Bite wounds and scars on the posterior pectoral fin margins of female Cownose Rays. Panels (a) and (b) show dorsal and ventral views of healed scars on one individual during the nonmating season (1 = margin abrasions, 2 = excisions). Panels (c)–(f) show dorsal and ventral views of fresh wounds on one individual during the mating season. The males bite the females in a localized region about 100 mm from the tip of either pectoral fin (see panel f), leaving fresh bite marks over approximately the same length as the width of the jaws of males (~60 mm) during the mating season (April–June). Scarring results from wound healing from multiple mating events over time. The coin is 23 mm in diameter.

ovulated shortly thereafter. Ovary lengths and weights were also greatest in individuals >700 mm (Figure 6b, c). The size at 50% maturity was 701 mm DW ($a = -141.644$, $b = 0.202208$; $r^2 = 0.96$), and the size at 100% maturity was 712 mm DW. The smallest mature female was 700 mm DW, and the largest immature female was 711 mm DW.

Maximum follicle diameter and ovary weight were greatest between April and June (Figure 7). Ovary length was more variable. Based on embryo development and the presence of fertilized oocytes in the uterus, ovulation and fertilization occurred mostly in May. Nongravid, postpartum females were

rare because the majority of females appeared to become gravid soon after parturition. Only eight nongravid females were examined during the study (~8% of the mature females examined), and based on the large vitellogenic follicles in their ovaries, five were close to ovulation. Based on the rarity of nongravid females with follicles <10 mm in diameter ($n = 3$), most females appeared to reproduce each year.

Male Size at Maturity and Reproductive Seasonality

Both sides of the male reproductive tract were functional. In small juveniles (less than ~500 mm DW), a testis was visible



FIGURE 4. Immature ovary (1) on the dorsal surface of the epigonal organ (2) of a 564-mm (disk width) Cownose Ray. The fingertip is 15 mm wide.

as a long, thin, inconspicuous strip of tissue with >20 external papillae on the dorsal surface of the epigonal organ. In larger juveniles (>500 mm DW), the testis was more easily seen but was still small compared to the epigonal organ (Fig-

ure 8a). At about 500 mm DW, claspers (>10 mm) and testes (>40 mm) began to elongate (Figure 9a, b). Continued clasper development (elongation and calcification) and testis development (length and weight) were gradual until about 650 mm DW (Figure 9a–c). At that size, the claspers appeared to elongate more rapidly and completely calcify, the epididymides began to widen (≥ 10 mm; Figure 9d), the testes increased in size to the point that they dwarfed the epigonal organs during the mating season (Figure 8b), and semen was stored in the seminal vesicles, as in other elasmobranchs (Pratt and Tanaka 1994). Semen was found in the seminal vesicles of mature Cownose Rays almost year-round; September was the only month for which no semen was recorded. The smallest male with some semen in its seminal vesicles was 640 mm DW (15-mm uncalcified claspers; immature).

Two examples highlight the variability in the timing of maturation of the entire reproductive system and the benefits of using multiple characteristics to assess maturity in male Cownose Rays. On 27 September 2010, in a 661-mm DW immature male, the left testis had begun to develop before the right testis, including differentiation of testicular lobes—an asymmetry that was observed in maturing individuals (see more on testis development and morphology below). On 7 August 2009, a 720-mm DW mature male had 29-mm-long claspers that were not completely calcified; however, the rhipidion opened, there was semen in the seminal vesicles, the anterior portion of the seminal vesicles was curved (as was common in mature males; see Figure 8b), and the epididymis width was about the same as that of a 776-mm DW mature male caught on the same day. The 720-mm male would likely have been able to copulate during

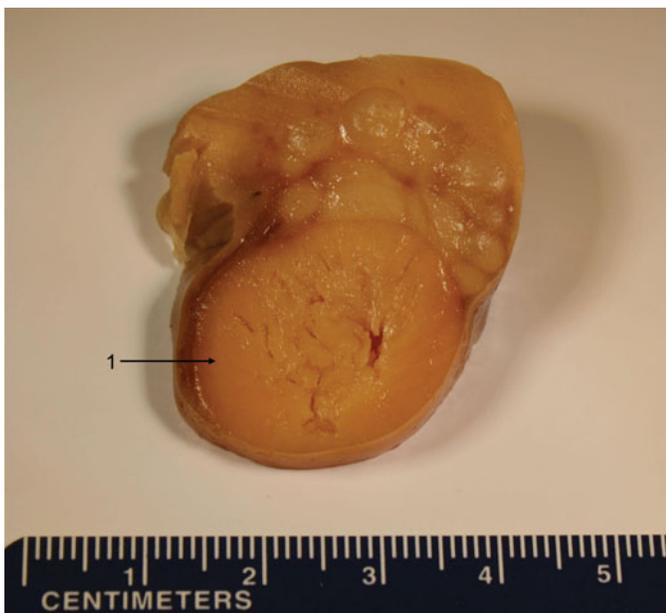


FIGURE 5. Cross-section through the ovary of an 807-mm (disk width) Cownose Ray captured on 11 March 2008. Note that one follicle (1) is noticeably larger than the others and that the brood size is typically one in this species. The entire ovary was immersed in JB-4 acrylic for several months to solidify the yolk and allow this photograph to be taken.

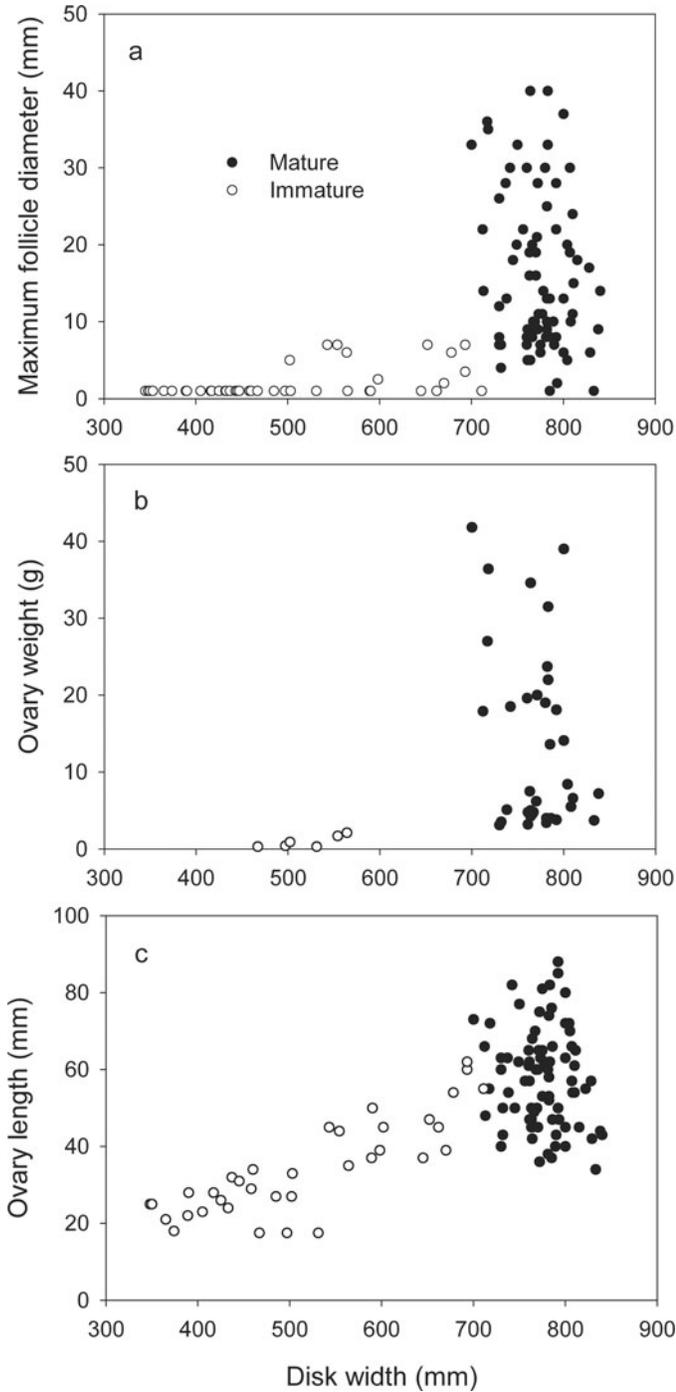


FIGURE 6. Disk width versus (a) maximum follicle diameter, (b) ovary weight, and (c) ovary length for Cownose Rays in the Charlotte Harbor estuarine system, 2002–2012.

the next mating season. The size at 50% maturity was 681 mm DW ($a = -174.416$, $b = 0.255978$; $r^2 = 0.90$), and the size at 100% maturity was 712 mm DW. The smallest mature male was 664 mm DW, and the largest immature male was 709 mm DW.

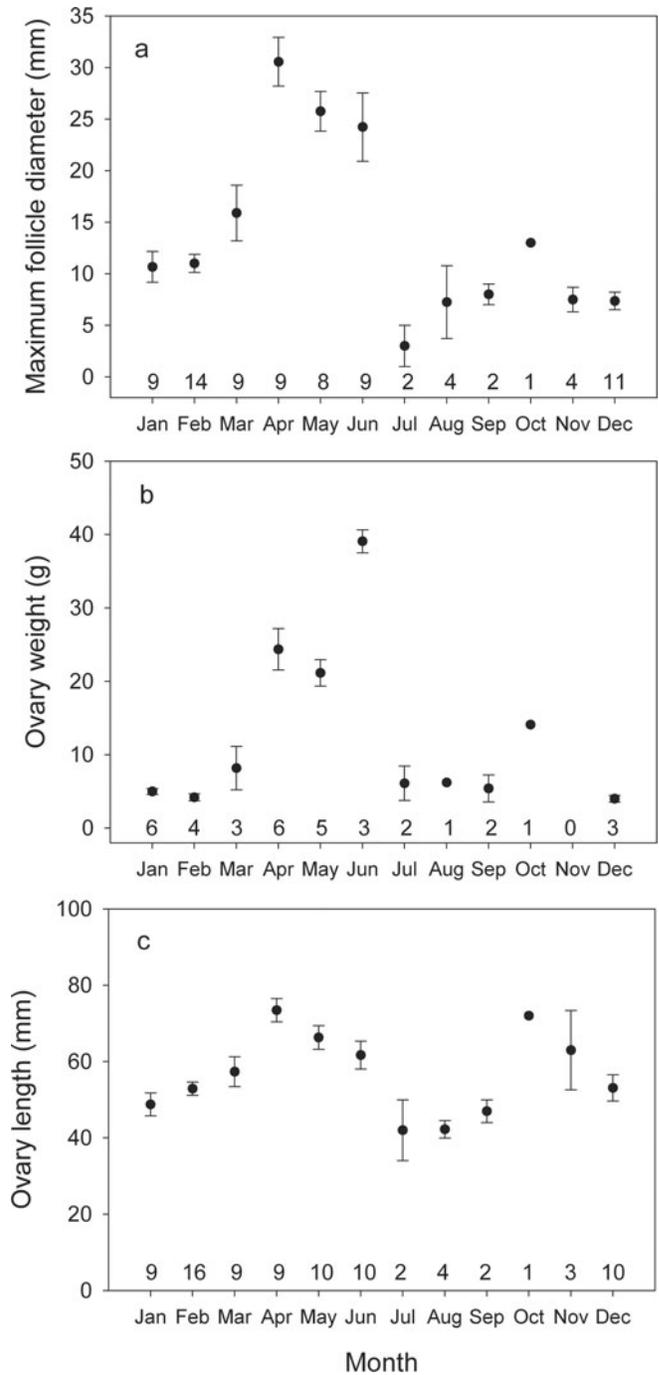


FIGURE 7. Monthly mean (a) maximum follicle diameters, (b) ovary weights, and (c) ovary lengths for mature Cownose Rays in the Charlotte Harbor estuarine system, 2002–2012; error bars = SEs. Ovary length was less useful than the other measurements in determining reproductive seasonality because follicle growth (vitellogenesis) increased the mass of the ovary more consistently than it did the length. Monthly sample sizes are included along the x-axis.

Testicular lobes were not macroscopically distinguishable in immature male Cownose Rays, but as the rays matured, a few spherical lobes began to differentiate until in mature specimens each testis was composed of distinct, disk-like lobes (Figure 8).

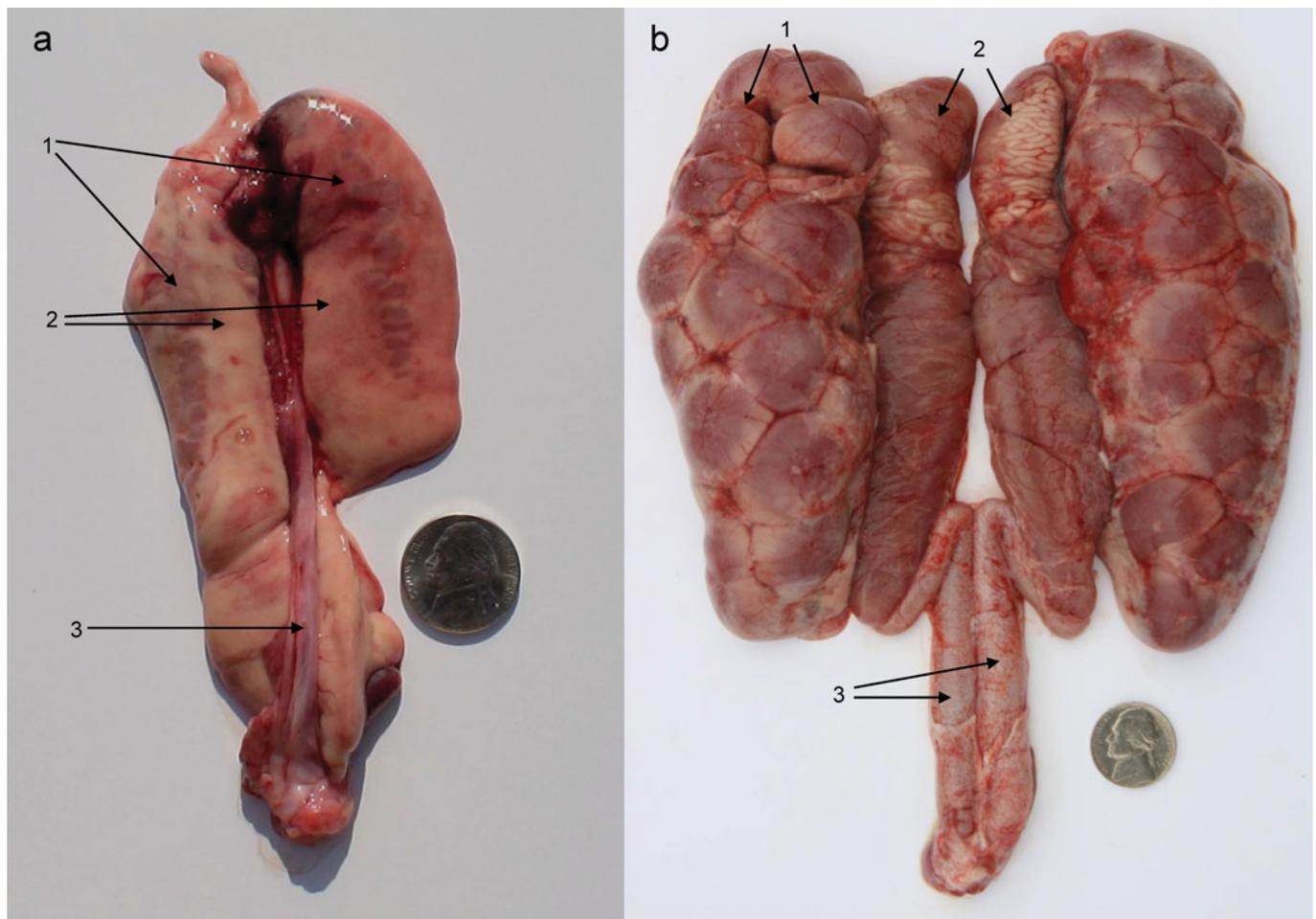


FIGURE 8. Panel (a) shows immature testes (1) on the dorsal surfaces of the epigonal organs (2) of a 545-mm (disk width) Cownose Ray. The reproductive tract, including the epididymides and the seminal vesicles (3) are just beginning to develop. Panel (b) shows a dorsal view of the reproductive tract of a 750-mm (disk width) mature male Cownose Ray captured on 3 April 2013. Note the exposed disk-like testicular lobes (1) that make up the testes. The reproductive tract, including the epididymides (2) and the seminal vesicles (3), are at their maximum sizes at that time of year. Note the curved shape of the anterior portion of the seminal vesicles in the mature specimen. The coin is 21 mm in diameter.

Smaller lobes were found at the ends of the mature testes. Once mature, the testes and their lobes changed size seasonally (Figure 10). Testis lengths and weights peaked between February and April (Figure 10a). Testis lobe diameter and weight mirrored overall testis length and weight and peaked in March and April (Figure 10b). The largest lobe diameter observed was 44 mm, and the heaviest lobe was 16.5 g. The total number of lobes in the right testis ranged from 16 to 28 (mean = 22).

Development, Brood Size, Modes of Nutrition, Gestation Period, Size at Birth, and Reproductive Cycle

In the Charlotte Harbor estuarine system, fertilization peaked in May, when fertilized oocytes were most often observed in the uterus ($n = 10$). However, ovulation can occur over at least a 4-month period because fertilized oocytes were also recorded on 4 February, 28 March, 26 April, and 10 June. Consequently,

even though most gravid females had only one embryo, embryos from multiple females captured on a given day sometimes had different disk widths. Only two females carried two embryos; one that carried a 99-mm DW embryo and a fertilized oocyte apparently ovulated twice about 1 month apart because the embryos were about 100 mm DW approximately 1 month after fertilization (Figure 11), and one female that carried a 117-mm DW embryo and a 128-mm DW embryo apparently ovulated twice over a period of several days because the embryos had about the same disk width.

Initially, the embryos were lecithotrophic; however, by about 150 mm DW (the end of July to mid-August), the yolk sacs had been consumed and the embryos had become histotrophic. At this point, trophonemata were secreting histotroph and this aplacental mode of nutrition continued until parturition. The largest embryos with a yolk sac had a DW of 133 mm (29 June; 19-mm-diameter yolk sac) and 143 mm (14 August;

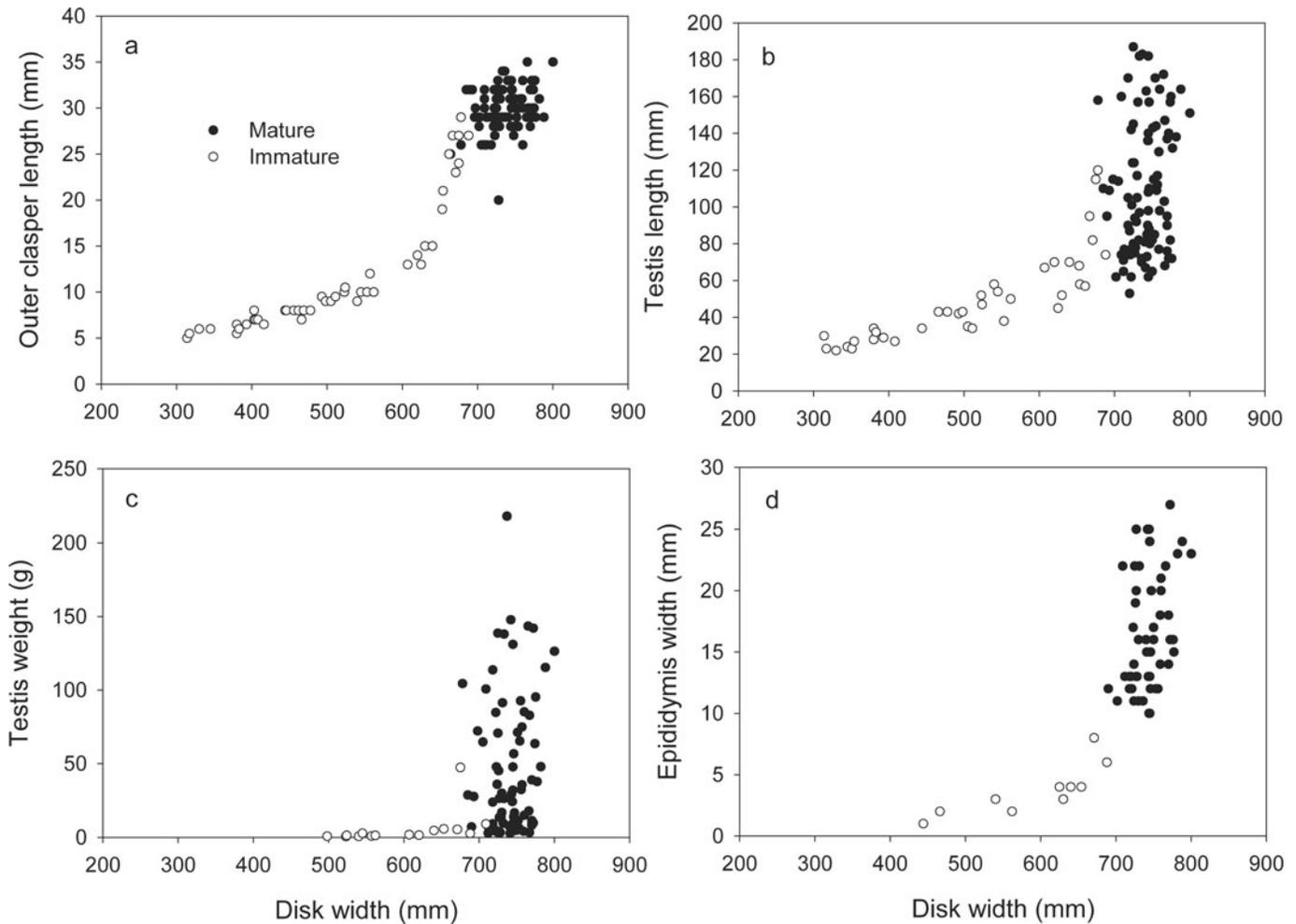


FIGURE 9. Disk width versus (a) outer clasper length, (b) right testis length, (c) right testis weight, and (d) epididymis width for Cownose Rays in the Charlotte Harbor estuarine system, 2002–2012. At about 650 mm, the claspers elongated and calcified, the testes increased in size to the point that they dwarfed the epigonal organs during the mating season, and the epididymides widened (see Figure 8).

5-mm-diameter yolk sac within the yolk stalk). On 26 July 2011, embryos of 117, 128, and 176 mm DW still had yolk stalks but did not have yolk sacs and histotroph was present in the uteri. The yolk stalk of the 176-mm embryo and another 182-mm embryo from 14 August 2007 were short and had been almost completely resorbed. The tail spines of all six of these embryos were calcified.

Most embryos reached >250 mm DW and >300 g before parturition (Figure 11). Pectoral fins were usually wrapped dorsally around the body, presumably to maximize growth within the uterus, and the embryos were typically oriented like the mother, with either dorsal or ventral side up. Parturition occurred mostly in March and April after an 11–12-month gestation period. The smallest free-swimming juveniles reported from the estuary were 202 and 225 mm DW and the largest embryo was 383 mm DW, so the size at birth is estimated at 202–383 mm DW. Twelve additional free-swimming rays <300 mm DW (230–291 mm) were found in the estuary, and 144 oth-

ers were smaller than the largest embryo (300–382 mm DW; Florida's Fisheries-Independent Monitoring program, 1990–2011, unpublished data). On 26 April 2006, the female that carried the 383-mm DW embryo was caught with another female that was carrying a fertilized oocyte. Vitellogenesis (follicle development) was concurrent with gestation, indicating an annual reproductive cycle, which is summarized in Figure 12.

DISCUSSION

Reproductive Biology

For many years, the gestation period and female reproductive cycle of Cownose Rays had to be extrapolated across data gaps because of the difficulty in obtaining samples year-round in large portions of the species' range where it is migratory (Smith and Merriner 1986; Neer and Thompson 2005). The year-round presence of Cownose Rays in this study allowed for a complete assessment of reproductive biology and confirms an annual

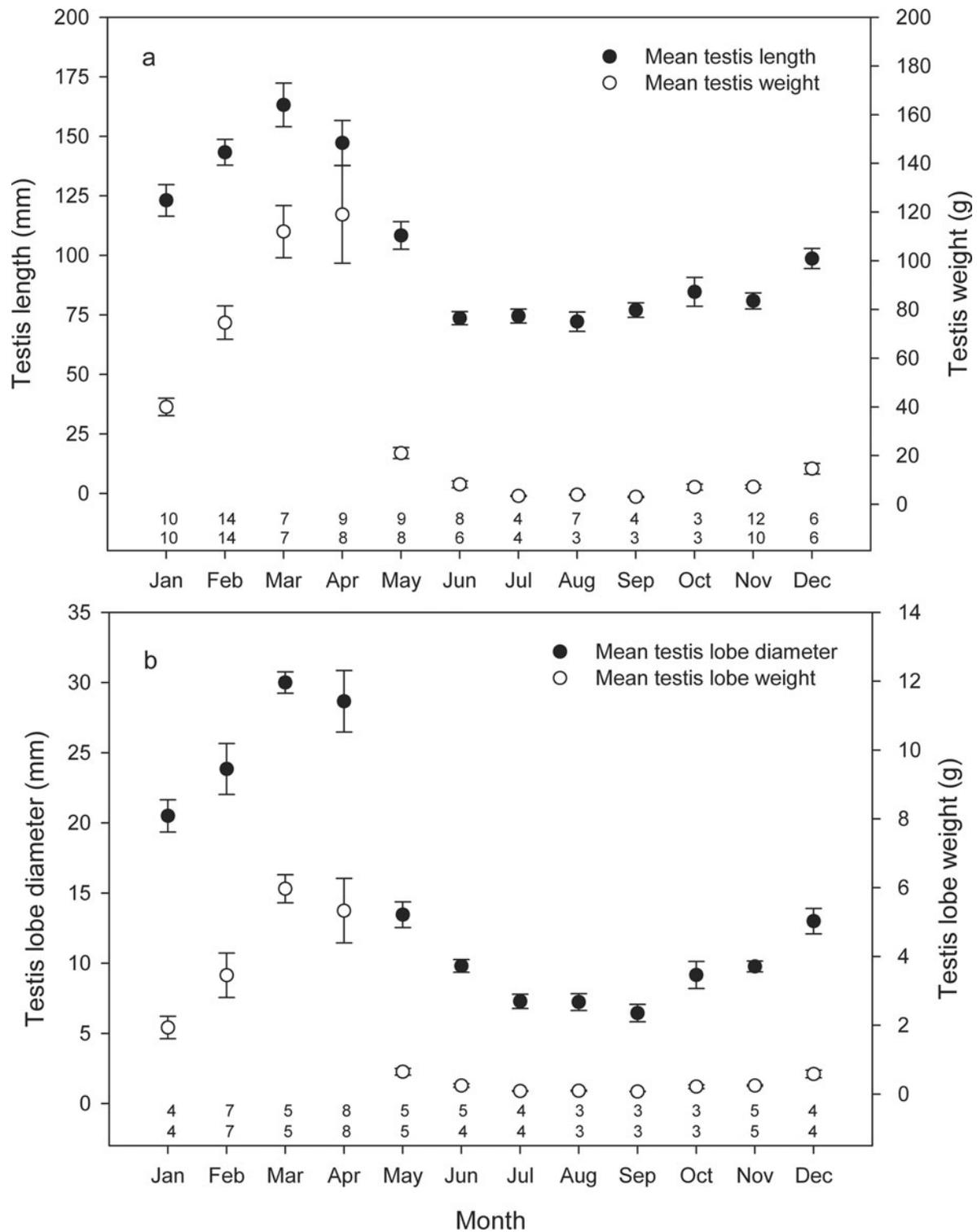


FIGURE 10. Monthly mean (a) right testis lengths and weights and (b) right testis lobe diameters and lobe weights for mature Cownose Rays in the Charlotte Harbor estuarine system, 2002–2012; error bars = SEs. Monthly sample sizes are included along the x-axes (in panel [a], the first row is for testis length, the second row is for testis weight; in panel [b], the first row is for lobe diameter, the second row is for lobe weight).

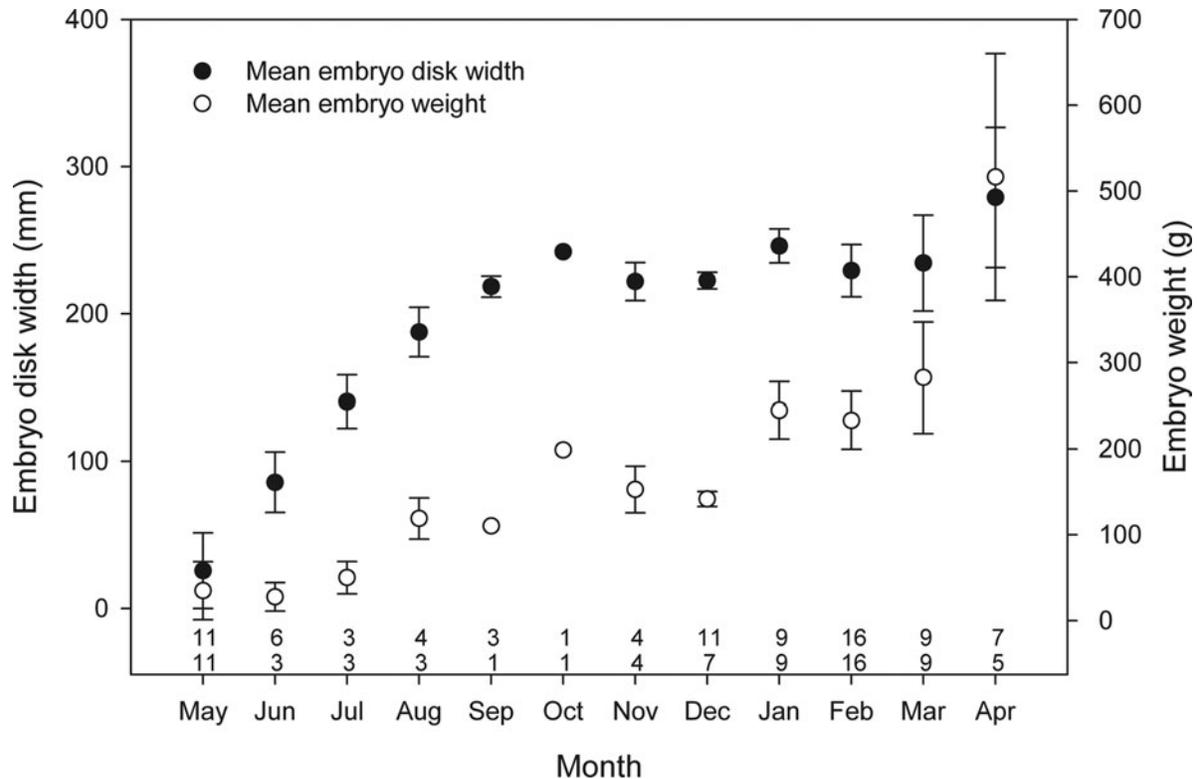


FIGURE 11. Monthly mean embryo disk widths and weights of Cownose Rays in the Charlotte Harbor estuarine system, 2002–2012; error bars = SEs. The smallest free-swimming juvenile caught during field sampling was 202 mm and the largest embryo was 383 mm, so the size at birth was estimated as 202–383 mm (disk width; see text for a discussion of the wide range in size at birth). Monthly sample sizes are included along the *x*-axis (the first row is for embryo disk width, the second row is for embryo weight).

reproductive cycle for this species (see Figure 12). Vitellogenesis and gestation were concurrent and, based on the rarity of nongravid females with follicles <10 mm in diameter ($n = 3$; ~3% of adult females), most females carried a single embryo each year. Other researchers have reported brood sizes of 2–6 (e.g., Smith 1907; Bigelow and Schroeder 1953), but studies focused on Cownose Rays have unanimously reported a typical brood size of one, or rarely two (Smith and Merriner 1986; Neer and Thompson 2005; Pérez-Jiménez 2011; this study). This study corroborates that gestation lasts 11–12 months as reported by Neer and Thompson (2005) in the northern Gulf of Mexico and suggested by Smith and Merriner (1986) for the mid-Atlantic region. As observed for other lower-latitude populations of elasmobranchs, parturition and mating were sometimes protracted (Castro 2009), but ovulation and fertilization peaked in May. Testis size (length and weight), including testicular lobe size (diameter and weight), peaked as much as 2 months earlier than follicle diameter and ovary weight, indicating that males were preparing to inseminate females, including nulliparous females (Castro 2009), during the entire peak parturition and mating periods (March–June; see Figures 7, 10). A biennial female reproductive cycle was reported in a study conducted in the southern Gulf of Mexico (Pérez-Jiménez 2011); that study,

however, was based on data collected from only half of the year, so it was necessary to interpret data across substantial time gaps. The asynchronous nature of female reproduction in tropical waters (Castro 2009), where stable environmental conditions and abundant food occur throughout the year, make it even more important to examine samples collected year-round. Although many of the results of the present study agree well with studies conducted in the northern Gulf of Mexico and mid-Atlantic (Smith and Merriner 1986; Neer and Thompson 2005), additional studies are needed to corroborate the annual reproductive frequency of Cownose Rays in more southerly portions of the species' range.

On the basis of size at 50% maturity, both sexes matured at smaller sizes in southwestern Florida than in the Chesapeake Bay and southern Gulf of Mexico portions of the species' range (Smith and Merriner 1986; Pérez-Jiménez 2011) but at larger sizes than in the northern Gulf of Mexico (Neer and Thompson 2005). However, it is important to consider differences in the methods used to assign maturity. Smith and Merriner (1986) used follicular development >10 mm diameter and trophonemata development to assess maturity in Cownose Rays from Chesapeake Bay. The reason why follicles 10 mm in diameter were used as the threshold was not discussed, but in

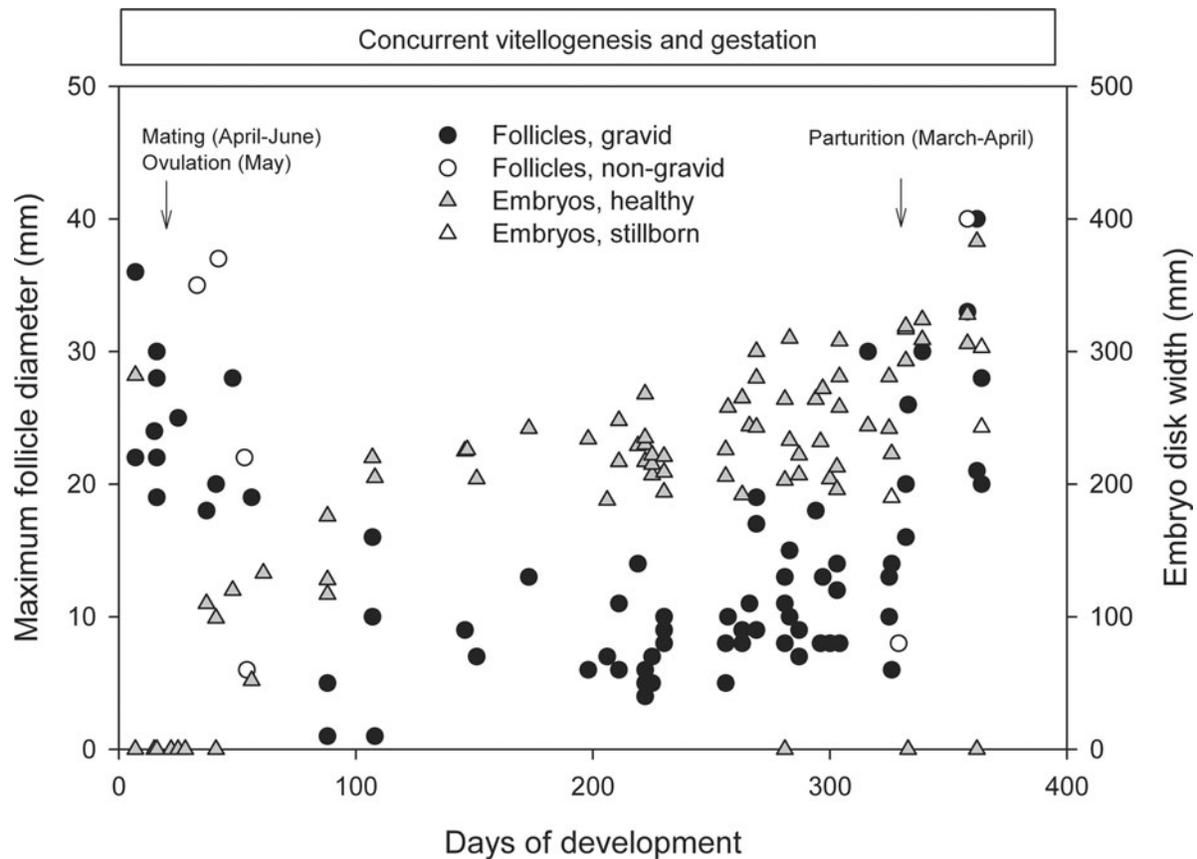


FIGURE 12. Summary of the annual reproductive cycle of Cownose Rays in the Charlotte Harbor estuarine system, 2002–2012. Day 0 corresponds to 1 May. Fertilized oocytes and early-developing embryos that did not have a disk to measure were assigned a disk width of 0.

the present study vitellogenesis had begun by the time follicles reached this size. Examining rays from the northern Gulf of Mexico, Neer and Thompson (2005) followed the criteria of Smith and Merriner (1986) but emphasized uterine width rather than trophonemata development to assess maturity. In most cases, these characteristics were probably sufficient to determine whether females were mature; however, nulliparous females might have been considered immature (or maturing) in those studies if the specimens were collected as the follicles were beginning to mature. In addition, the growth of follicles from the germinal epithelium sometimes results in mature follicles being located in the interior of the ovary, which may also lead to difficulty in assigning maturity status if only an exterior examination is used to locate the largest follicle (personal observation). Thus, ovary weight was helpful in assigning maturity because even though one follicle was often noticeably largest (see Figure 5), follicles throughout the ovary were always developing (personal observation). Ovary length was less useful in determining reproductive seasonality because follicle growth (vitellogenesis) increased the mass of the ovary more consistently than it did the length. Pérez-Jiménez (2011) considered some individuals with follicles up to 30 mm in diameter to be immature, which likely caused an overestimation of size

at maturity. For males, Smith and Merriner (1986) used only clasper calcification and development to assess maturity. Neer and Thompson (2005) used only clasper morphology and a qualitative assessment of vas deferens coiling (similar to epididymis width in this study) to assess maturity. These characteristics are useful in most cases, especially if only estimates of maturity are desired or reproduction is not the primary goal of a study.

In general, methods for quickly and accurately assigning maturity in elasmobranchs are less subjective for females than for males, and knowing how maturity is defined for a given species is important for fisheries assessments (Walker 2005, 2007). If accurate assignment of maturity is critical, the data from this study suggest that clasper morphology (length and calcification), epididymis width, and testis condition (length and weight), including distinct lobe formation, should be used as the primary characteristics for assessing maturity in male Cownose Rays. In females, maximum follicle diameter and ovary weight should be used to assess maturity. Examination of the freshest possible specimens is also recommended to aid reproductive studies because some characteristics (e.g., sperm production, lobe formation) become difficult to determine if the specimens have been frozen or kept on ice for long periods of time (days).

For this reason, examination of specimens or preservation of tissues is recommended immediately after death to avoid post-mortem change, especially if histological analyses are planned. If nonlethal techniques are desired, maturity could also be assessed by analyzing plasma steroid hormones (Sulikowski et al. 2007).

Excision bite wounds and scarring were more common in Cownose Rays than in Atlantic Stingrays (Kajiura et al. 2000). All of the excisions observed in Cownose Rays during this study were on the posterior margin of the pectoral fins of adult females ($n = 51$) where male rays typically grip females during courtship (e.g., Tricas 1980; Uchida et al. 1990), and 18 of these individuals had multiple excisions (up to three per pectoral fin). Kajiura et al. (2000) found only six excisions out of a total of 961 wounds and all were on males. In the present study, there was only one example of mating scars on a male, indicating that competition between males for females that results in injuries and random precopulatory biting were not as common in Cownose Rays as in Atlantic Stingrays, where dermal wounds were observed in both sexes over the entire 9-month mating season (Kajiura et al. 2000). These observations may be related in part to differences in habitat use between these species because Cownose Rays are normally found in the water column (where olfactory cues that originate at the cloaca would be easily detected by males, e.g., Johnson and Nelson 1978), whereas Atlantic Stingrays are often buried in soft sediments (where chemical cues might be masked). In addition, because multiple male Cownose Rays are known to follow and mate with a single female (Uchida et al. 1990; personal observation) and males bite females so consistently in such a localized area of the posterior disk margin, perhaps over the life span of adult females the disk margin in this localized region of the pectoral fins becomes less durable and more susceptible to excisions even though the dentition of males has molariform morphology. Qualitatively, seasonal dental sexual dimorphism was not observed in male Cownose Rays during the present study, as has been documented for male dasyatid rays (e.g., Kajiura and Tricas 1996); however, interspecific differences in dental and jaw morphology, bite force, and mating behavior likely play key roles in the type and extent of wounds and scarring that occur in rays.

Estimated size at birth (202–383 mm DW) was different in this study than in other studies of Cownose Rays (combined range = 323–440 mm DW; Smith and Merriner 1986; Neer and Thompson 2005; Pérez-Jiménez 2011). As in other studies, the upper limit of the size range at birth was determined by the largest embryo in this study; however, the lower limit of the size range at birth was determined with the aid of a 22-year, multigear, fisheries-independent monitoring data set from the study area. This data set contained 158 free-swimming Cownose Rays smaller than the largest embryo from the Charlotte Harbor estuarine system, and the smallest free-swimming individual was much smaller than previously reported (Florida's Fisheries-Independent Monitoring program, 1990–2011, un-

published data). It appears that embryos are capable of surviving if they are born after about 200 mm DW and 200 g, but most reach >250 mm DW and >300 g by parturition. The large size range at birth is due in part to the ~100-mm increase in mean embryo disk width and the >200-g increase in mean embryo weight in the last 1–2 months of gestation (March–April; see Figure 11). Further, the variability in embryo disk width after about 200 d of development (see Figure 12) may be due to a relationship between maternal size and embryo size because there is about a 150-mm DW and 2,000-g range in the size of adult females (see Figure 2). Collectively, these data suggest that the size range at birth for Cownose Rays is larger in other study areas also. In addition, Cownose Ray embryos were never observed being aborted due to capture stress during the fisheries surveys in Charlotte Harbor, as has occasionally been observed with the three *Dasyatis* ray species found in this estuary (Poulakis et al. 2004; personal observation).

Geographic Considerations

Cownose Rays can complete their reproductive cycle within the subtropical Charlotte Harbor estuarine system in southwestern Florida. Juveniles and adults of both sexes, including gravid females, can be found year-round in the system. This does not necessarily mean that individuals never leave the estuary, and more tagging studies are needed to determine whether these Cownose Rays are truly estuarine residents or whether some individuals also use coastal or offshore Gulf of Mexico habitats.

Geographic variation in intraspecific life history parameters has been shown for several wide-ranging elasmobranchs, including Cownose Rays (Smith and Merriner 1986; Lombardi-Carlson et al. 2003; Neer and Thompson 2005; Hoffmayer et al. 2010). These variations are important because they highlight the need to avoid biological and ecological generalization across large ranges (Cope 2006). For example, regional variation in growth rates can have direct implications for species-specific management (e.g., Driggers et al. 2004). For Cownose Rays, reproductive life history had not been examined year-round until this study because the populations that have been studied previously are migratory or samples were not collected throughout the year (Smith and Merriner 1986; Neer and Thompson 2005; Pérez-Jiménez 2011). Despite these temporal differences and those in methodology, research from the Gulf of Mexico and Chesapeake Bay indicates that there is intraspecific variation in Cownose Ray reproductive life history, including the fact that males can attain maturity below 700 mm DW in some regions (Neer and Thompson 2005; this study) whereas they need to be >800 mm DW in others (Smith and Merriner 1986; Pérez-Jiménez 2011). These differences should be monitored in the context of recently published and ongoing genetic research that suggests the presence of additional *Rhinoptera* species in the western North Atlantic, including the Gulf of Mexico (e.g., Naylor et al. 2012; Hoffmayer, unpublished data). Once these taxonomic issues are resolved, species-specific data can be used to refine geographic differences in Cownose Ray

life history traits by determining the extent of area and species effects (Cope 2006).

As observed for many animals, temperature affects the distribution and survival of Cownose Rays (Smith and Merriner 1987; Neer and Thompson 2005). The Cownose Rays examined in this study were caught in water warmer than 14°C, and studies have indicated that Cownose Rays are rarely collected in water cooler than 15°C (Neer 2005). In Charlotte Harbor, water temperatures rarely fall below 12°C and dead Cownose Rays have been observed after the passage of strong cold fronts (Poulakis et al. 2003; personal observation). These fronts can drop water temperatures below 12°C for several days (Poulakis et al. 2011). This suggests that temperature cues known to prompt emigration of Cownose Rays from temperate latitudes (Smith and Merriner 1987) rarely occur in the subtropical Charlotte Harbor estuarine system. Consistently warm, productive, subtropical estuarine habitats may promote year-round residence of Cownose Rays because the lower lethal temperature is rarely reached, predation is low, and food is plentiful (Castro 2009). For example, Cownose Rays ($n = 12$) tagged with acoustic transmitters were found to occur year-round in the Charlotte Harbor system (Collins et al. 2008). This latitudinal variation in Cownose Ray life history may contribute to the maintenance of subpopulations throughout the species' range if individual- and population-level survival is high. These rangewide intraspecific differences may help maximize the long-term success of this wide-ranging K -selected species, especially as climate change alters environmental conditions (Roessig et al. 2004). Once spatial patterns in life history parameters are better understood, it will be possible to explore some of the potential causes of specific area effects (e.g., current patterns) (Cope 2006).

Management Considerations and Future Research

Many species of rays have received little research attention and their population statuses are unknown. Nonetheless, some appear to have relatively healthy populations, and this presents an opportunity to conduct biological and ecological studies *before* they become overfished or decline for other reasons. For wide-ranging species, obtaining broadly distributed data on life history and population structure, especially before the establishment of fisheries, provides valuable baseline information against which to gauge the effectiveness of management initiatives and recovery efforts (if the latter become necessary) (Cope 2006; Walker 2007). A proactive approach to life history research on these vulnerable elasmobranchs (Stevens et al. 2000), as is being taken for the Cownose Ray, will help prevent situations like the one we are currently in regarding a ray species that was placed on the U.S. Endangered Species List in 2003, namely, the Smalltooth Sawfish *Pristis pectinata*. This species was overfished during the 20th century and became endangered before any detailed life history research was conducted on it, so recovery planning has had to be conducted with minimal information (NMFS 2009). For Cownose Rays, additional life history data are needed on reproduction as well as baseline age

and growth information for populations in the southern portion of the species' range. In addition, a better understanding of the genetic relationship of Cownose Rays in the western Atlantic is needed to clarify interpretation of existing research and to aid future management decisions.

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