## Reproduction of the blacknose shark (*Carcharhinus acronotus*) in coastal waters off northeastern Brazil

Fabio H. V. Hazin Paulo G. Oliveira Matt K. Broadhurst

Departamento de Pesca, Laboratório de Oceanografia Pesqueira Universidade Federal Rural de Pernambuco Av. Dom Manuel de Medeiros, s/n Dois Irmãos, Recife-PE, Brasil, CEP: 52.171-900 E-mail address (for F. Hazin): fhvhazin@elogica.com.br

The blacknose shark, *Carcharhinus acronotus*, is a relatively small carcharinid, typically inhabiting continental shelf areas in the western Atlantic Ocean, from North Carolina throughout the Gulf of Mexico (Bigelow and Schroeder, 1948) and along the South American coast to Rio de Janeiro (Compagno, 1984). The abundance of this shark in nearshore areas throughout its distribution makes it accessible to commercial fishing, mainly from inshore hook-and-line and gill-net fisheries (Trent et al., 1997; Mattos and Hazin<sup>1</sup>).

Aspects of the biology of C. acronotus have been reported by Springer (1938); Bigelow and Schroeder (1948); Clark and von Schmidt (1965); Dodrill (1977); Branstetter (1981); Schwartz (1984); Castro (1993); and Carlson et al. (1999). Schwartz (1984) provided the most comprehensive synopsis, including information on their reproduction and life cycle off North Carolina. Many of the other studies were based on relatively few specimens collected off the southeastern United States and corroborate much of Schwartz's (1984) work, including patterns in spatial and temporal abundances, size at maturity, fecundity, and time of parturition. However, some inconsistencies exist with respect to the duration of the ovarian, gestation, and breeding cycles: i.e. Dodrill (1977) proposed a biennial breeding cycle with gestation taking between 10 and 11 months, Schwartz (1984) suggested a gestation of approximately 9 months, and Branstetter (1981) observed two gravid females

with large ovarian eggs (having concurrent ovarian and gestation cycles, copulation having occurred shortly after parturition).

Because C. acronotus are represented in catches from various inshore fisheries and carcharhinids typically are characterized by low rates of population increase, adequate information about their reproductive cycle is required to facilitate management of stocks. Given the uncertainty of knowledge about reproduction in C. acronotus and the lack of information for the southern part of their range, our aims in the present study were to provide a preliminary overview of the reproductive biology and life cycle of the blacknose shark in coastal waters off northeastern Brazil, using available fishery-dependent data.

### Material and methods

# Fishing gear used and data collected

*Carcharhinus acronotus* (79 females and 45 males) were collected from the catches of commercial gillnetters and vessels using bottom longlines off the coast of northeastern Brazil (approx. 7°30′ to 9°30′S—near Recife) between August 1994 and January 1999. The configuration of fishing gears used remained similar over this period. Gill nets were monofilament, 900 m in length, and had a stretched mesh size of 17 cm and a depth of 70 meshes. Nets were set perpendicular to the beach at depths between 5 and 10 m. Bottom longlines consisted of a multifilament mainline (6 mm in diameter) with up to 100 secondary lines, each approx. 5 m in length and constructed from 3-mm diameter monofilament attached to a wire snood (1 m in length). Types of hooks varied among brands, but relative sizes (i.e. 9/0) remained similar. The main baits were sardine (Sardinella brasiliensis) and mackerel (Scomber spp.), although some other species, including sting ray (Aetobarus narinari) and skipjack tuna (Katsuwonus pelamis) were occasionally used. Longlines were set on the continental shelf at depths between 10 and 60 m, but most sets were shallower than 40 m. All fishing gears were set at dusk and hauled the following morning at dawn.

All specimens were measured (total length [TL] in the natural position) and dissected. Reproductive organs were removed and stored in a solution of 10% formalin in seawater prior to being transported to the laboratory. Data collected from females included weight and width of the oviducal gland and the functional right ovary, maximum ovarian follicle diameter (MOFD), width of the largest uterus, and, if present, the TL, sex, and number of embryos. Using the methods described by Pratt (1993), we examined the oviducal glands of 10 mature females for the presence of spermatozoa. Length and calcification stage of claspers, width of epididymides, and the presence of seminal fluid in the ampullae of the ductus deferens were recorded from males. Reproductive organs were measured to the nearest 0.1 mm with vernier calipers.

Inferences on stages of reproduction were made according to definitions provided in previous studies on carcharinids (e.g. Pratt, 1979; Hazin et al., 2000). Females were categorized into six stages, mainly based on develop-

<sup>&</sup>lt;sup>1</sup> Mattos, S. M. G. and F. H. V. Hazin. 1997. Análise de viabilidade econômica da pesca de tubarões no litoral do estado de Pernambuco. Boletim Técnico-científico do Cepene, 5: 89–114. IBAMA (Instituto Brasiliero de Meio Ambiente e dos Recursos Naturais Renováveis), Rua Samuel Hardman, s/n Tamandaré – PE, Brazil.

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ment of the oviducal gland, ovary, and uterus. Specimens were considered juvenile if they had undeveloped sexual organs, filiform uteri, and no vitellogenic activity in their ovaries. Preovulatory females had relatively larger ovaries with orange vitellogenic follicles, but no uterine eggs. Ovulating females had uterine eggs and ripe ova still in the ovary, whereas in gravid specimens ovulation was complete. Postpartum females showed similar-size ovaries, oviducal glands, and ovarian follicles as those of gravid specimens, but had flaccid uteri that were still slightly enlarged (compared with other nongravid females) indicating recent parturition. Individuals that had similar-size uteri as those of pre-ovulatory and ovulating individuals, but smaller ovarian follicles with little or no vitellogenic activity were termed "resting" females. Male maturation was evaluated according to development of the testes and claspers. Individuals with relatively short, flexible claspers, and filiform ampullae of the ductus deferens were considered juveniles. Adults were characterized by elongate and calcified claspers and relatively large epididymides (compared with juveniles).

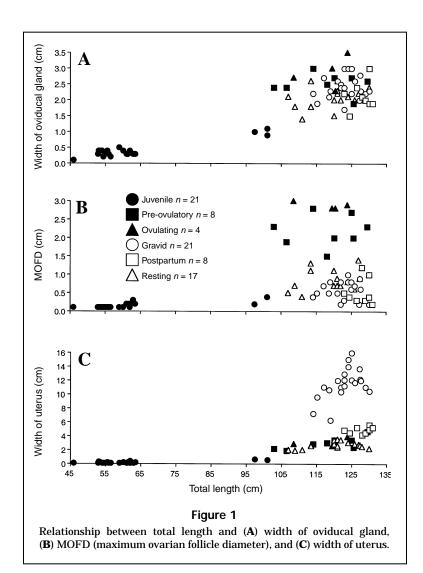
#### Statistical analyses

Size-frequency distributions of males and females were compared by using a two-sample Kolmogorov-Smirnov test (P=0.05). Chi-squared goodness-of-fit tests were used to examine the hypothesis of an equal sex ratio between numbers of juvenile and adult *C. acronotus* sampled and among embryos in gravid females. To help define time of parturition, analysis of variance (ANOVA) was used to investigate the hypothesis of an increase in TL of embryos from near-

term females captured in November and December. To provide a balanced analysis, three gravid females captured on 10 December 1998 were analyzed with three individuals captured almost one month earlier on 11 November 1998. Data were assessed for normality by using the Shapiro and Wilk procedure (Zar, 1996), tested for heteroscedasticity with Cochran's test, and then analyzed in the appropriate one-nested factor ANOVA (Underwood, 1981). Gravid females were considered a random-effects factor nested in months and the four embryos for each gravid female were the replicates.

#### Results

The Kolmogorov-Smirnov test detected significant differences in size-frequency compositions between males and females; proportionally more larger-size females (121–131 cm TL) were captured. Both sexes showed two distinct cohorts. The first consisted of juveniles (46–65 cm) captured by gill nets at depths between 5 and 10 m, and



the second included larger specimens (i.e. 85-131 cm), and mostly adults, captured on bottom longlines in deeper water (10 to 60 m). The ratio of juvenile males to females was not significantly different from 1:1 ( $\chi^2$ =0.027, *P*>0.01), whereas significantly fewer adult males than females were sampled (ratio of 1:2.34) ( $\chi^2$ =14.08, *P*<0.01).

#### Female maturation and reproduction

Juveniles ranged in size from 46 to 101 cm TL and had narrow oviducal glands, light ovaries with undeveloped white follicles, and thin uteri (Fig. 1, A–C, Table 1). Preovulatory specimens ranged in size from 103 to 129.5 cm TL, had well-developed oviducal glands (width of between 1.9 and 3 cm), and mature ovaries with thick orange follicles (1.5 to 2.8 cm in diameter) (Fig. 1, A and B, Table 1). The shortest pre-ovulatory specimens (e.g. 103 and 106 cm TL), had oviducal glands that were substantially wider (i.e. >2×) than those in the longest juveniles (101 cm TL) (Fig. 1A, Table 1); therefore sexual maturity probably was approached within this size range. This should be con-

Characteristic	Juvenile	Pre-ovulatory	Ovulating	Gravid	Postpartum	Resting
Width of oviducal gland	<1.1	1.9–3.0	2.3-3.5	1.7-3.0	1.5-3.0	1.4-2.6
Weight of oviducal gland	2.2-3.0	5.7-8.7	8.6-10.0	2.3-5.0	3.0-5.0	3.0-7.7
Width of uterus	<0.7	1.9-4.7	2.6-3.9	6.3-16	4.2-5.6	1.9 - 3.5
MOFD	0.1-0.4	1.5 - 2.8	2.8-3.0	0.2-1.0	0.2-1.2	0.4 - 1.4
Width of ovary	0.4-1.7	2.3-6.0	4.9-5.7	1.5-3.8	2.2-3.5	1.7 - 4.0
Weight of ovary	2.7 - 4.6	12.2-18.2	20.0-24.6	4.5-16.5	12.0-23.0	4.97-16.5
TL of specimens	46.0-101.0	103.0-129.5	108.5-123.8	114.0-130.0	123.0-131.0	107.0-130.
Number of specimens	21	8	4	21	8	17

Table 1

sidered a preliminary estimate of size at sexual maturity because few individuals were caught between 95 and 105 cm TL and none between 75 and 95 cm TL. Compared with pre-ovulatory females, those in the process of ovulation had slightly wider oviducal glands and heavier ovaries with thicker follicles (2.8-3 cm in diameter) (Fig. 1, A and B, Table 1). Ovulation appeared to occur only when MOFD was at least 2.8 cm (Fig 1B). The majority of gravid, postpartum, and resting females ranged in size from 111 to 131 cm TL and had similar-size oviducal glands (Fig. 1A, Table 1). Gravid females had undeveloped ovaries with no vitellogenic activity (MOFD was less than 1 cm). Uteri in postpartum females were flaccid and slightly distended (4.2-5.6 cm in width), whereas uteri of resting females were comparable to pre-ovulatory and ovulating individuals (Fig. 1C, Table 1). No mating scars were observed on any of the females. There was no evidence of spermatozoa stored in the oviducal glands of 10 females examined.

The temporal abundance of females according to stages of reproduction showed that juveniles were present in catches between January and November, but mainly from February to June (Fig. 2A). Pre-ovulatory and ovulating individuals were sampled between February and April and April and May, respectively (Fig. 2A). Except for one gravid female caught during August, all gravid, postpartum, and resting females were caught between November and January (Fig. 2A). MOFD of mature females was lowest in August and between November and January, after which it steadily increased to May (Fig. 2B).

Examination of the uterine contents of the 22 gravid females (Table 2) revealed that individual litter size was always four with both sexes present in varying proportions, although the total pooled ratio of males to females (1:1.25) was not significantly different from 1:1 ( $\chi^2$ =1.13, *P*>0.05). All embryos from individual gravid females were at similar stages of development and showed small variation in TL. ANOVA detected significant differences in mean TLs of embryos among near-term females (*F*=20.51, *P*<0.01) and for the main effect of months (*F*=10.45, *P*<0.05). ANOVA showed that the mean (±SE) TL of embryos in three nearterm females caught on 10 December 1998 (45.96 ±0.38 cm) was significantly longer than in three near-term females

Table 2
Date of capture and total length (TL) of gravid C. acrono-
tus and the mean TL $(\pm E)$ and sex ratio of embryos.

		Embryos		
Date of capture	TL of gravid specimen	Mean TL (±SE)	Males: females	
17 Aug 94	118.6	29.15 (1.23)	1:3	
11 Nov 98	121	42.50 (0.14)	1:3	
11 Nov 98	122	45.32 (0.60)	2:2	
11 Nov 98	123	40.75 (0.25)	3:1	
11 Nov 98	125	43.25 (0.43)	3:1	
11 Nov 98	129	45.12 (0.43)	3:1	
13 Nov 98	130	38.50 (2.06)	2:2	
17 Nov 98	114	34.27 (0.19)	3:1	
17 Nov 98	127.7	42.50 (0.15)	3:1	
21 Nov 98	115.2	41.37 (0.37)	3:1	
22 Nov 98	127.2	45.47 (0.62)	2:2	
25 Nov 98	126	43.87 (2.01)	2:2	
25 Nov 98	127.5	46.07 (0.22)	1:3	
6 Dec 98	117	44.75 (0.14)	2:2	
6 Dec 98	123	43.37 (0.85)	2:2	
6 Dec 98	123	48.50 (0.28)	2:2	
7 Dec 98	125	46.87 (0.12)	2:2	
10 Dec 98	124	45.62 (0.37)	2:2	
10 Dec 98	124	47.50 (0.28)	3:1	
10 Dec 98	122	44.75 (0.25)	2:2	
12 Dec 98	123	42.50 (0.35)	3:1	
5 Jan 95	114.5	45.37 (0.24)	2:2	

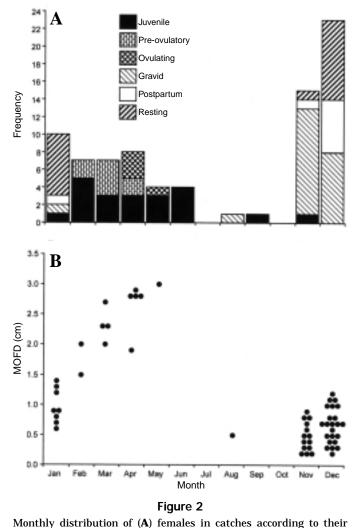
caught on 11 November 1998 (42.31  $\pm$ 0.07 cm), suggesting that embryos continued to develop between these periods.

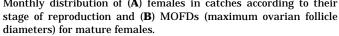
#### Male maturation and reproduction

Of the 45 males examined, 23 were juvenile with thin epididymides (Fig. 3A) and filiform ampullae of the ductus deferens without seminal fluid. Juveniles ranging from 49 to 63 cm TL had relatively short flexible claspers (Fig. 3B) and testes that were not fully differentiated from the epigonal organ. No males between 64 and 87 cm TL were caught. Three juveniles (88, 93, and 94 cm TL) had claspers that were enlarged and beginning to calcify (Fig. 3B) and two larger specimens also had thick epididymides (Fig. 3A). Males appear to approach sexual maturity before 104 cm TL because all specimens longer than this had completely developed sexual organs, including elongate and calcified claspers, thick epididymides, and circumvoluted ampullae of the ductus deferens (Fig. 3).

#### Discussion

Prevalence of adult females in catches was similar to that from observations made by Schwartz (1984) for stocks off the southeastern United States and can be attributed to a reproductive migration involving relatively large num-





bers of gravid individuals (114 to 130 cm TL) into the sampled area. Because there was no evidence of disequilibrium between sexes (i.e. there were equal numbers of male and female embryos and juveniles), large numbers of adult males were probably segregated. It is unlikely males were in the sampled area and not caught because females, although proportionally larger, were captured across the same size range (Fig. 1), implying that the selectivity of the gear encompassed the range of sizes of males.

Evaluation of the maturation stages of males and females showed delineation between juveniles and adults. All females longer than 103 cm TL had enlarged oviducal glands and developed ovaries (Fig. 1, A and B, Table 1), and males longer than 104 cm TL had elongate and calcified claspers and thick epididymides (Fig. 3), indicating that sexual maturity was probably approached at these lengths. Although these estimates were derived from few individuals, they are comparable to those proposed by most researchers for specimens collected off the southeast-

ern United States (e.g. Springer, 1938; Clark and von Schmidt, 1965; Compagno, 1984), with the exceptions of Branstetter (1981) who suggested 110 cm TL for both sexes and Schwartz (1984) who suggested 110 cm TL for males.

Size at birth, number of embryos, sex ratio of embryos, and the time of parturition of *C. acrono*tus in our study are consistent with corresponding data from earlier works (Springer, 1938; Clark and von Schmidt, 1964; Dodrill (1977); Branstetter, 1981; Compagno, 1984; Schwartz, 1984). Size at birth has been suggested to be between 45 and 50 cm TL (e.g. Branstetter, 1981; Castro, 1993) and litter sizes commonly range from 3 to 6 (Springer, 1938; Dodrill, 1977; Compagno, 1984). We observed gravid females caught in November and December (late spring to early summer) with embryos longer than 45 cm TL (Table 2). Given the significant increase in size of embryos between these months (indicating that embryos were still developing) and the capture of several neonates (46-51 cm TL) in February and March (late summer to early autumn), we conclude that parturition off northeastern Brazil probably occurs from December to January (mid to late summer). A similar seasonal timing has been proposed for stocks off the southeastern United States (e.g. during June-Schwartz, 1984) and is generally typical for the majority of carcharhinids (e.g. Castro, 1993).

In contrast to the inference made by Branstetter (1981) but in agreement with observations of Schwartz (1984), we showed that vitellogenisis and gestation occur consecutively in *C. acronotus*. Ovaries of adult females off northeastern Brazil begin to mature (pre-ovulatory stage) in February (late summer) with ovulation occurring two to three months later (Fig. 2, A and B). This sequence of events is illustrated by a rapid increase in MOFD from February (1.5–2 cm in diameter) to May (3 cm in diameter) (Fig. 2B). Given the proposed summer parturition (December to Janu-

#### ary), mating and fertilization during April and May (autumn) would result in a gestation period of approximately 8 months—slightly shorter than that suggested by Schwartz (1984) and Dodrill (1977) (9 and 10 to 11 months, respectively) for stocks off the southeastern United States. Further, the periods required for vitellogenisis and gestation indicate that reproduction of *C. acronotus* off northeastern Brazil could be completed within 10 to 11 months.

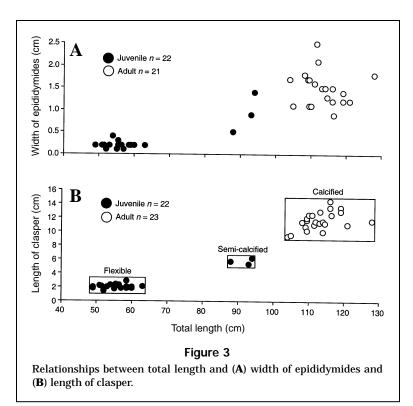
With the simultaneous capture of nongravid and gravid females off Florida, Dodrill (1977) proposed a biennial reproductive cycle for C. acronotus. Although we also collected gravid and nongravid (i.e. postpartum and resting) females together (i.e. mostly during December and January), the latter individuals could have given birth 3 or 4 weeks earlier. If these females subsequently ovulated 2 months later (in April), then reproduction could conceivably be annual. A fast vitellogenic period, combined with clear reproductive progress, is also supportive of a 1-year cycle. An alternative hypothesis that supports biennial reproduction is that the resting females represented some proportion of segregated nongravid females that moved with the relatively large numbers of gravid females into the parturition area. Given the lack of adult fe-

males in catches between June and November (winter to late spring), it is likely, however, that they frequent other areas after copulation. Without additional fishery-independent data, it is impossible to determine these locations and whether some proportion of the female population consists of nongravid or resting individuals throughout the year.

Our evidence suggests a shorter reproductive cycle for C. acronotus than that previously noted in the literature. Given the 6-month difference between times of ovulation and parturition for females off northeastern Brazil and those off the southeastern United States, our results also indicate the existence of at least two separate stocks. Noting temporal differences in the sizes of embryos from females, Schwartz (1984) proposed two partially separated populations off the southeastern United States: one off North Carolina and the other comprising individuals from Florida and the Gulf of Mexico. Additional research is needed to determine if the population in the southwestern Atlantic is separate from those in the north because the existence of a unit stock off northeastern Brazil would require separate management measures according to the status of that stock.

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