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REPRODUCTIVE LIFE HISTORY OF THE ATLANTIC STINGRAY, *DASYATIS SABINA* (PISCES, DASYATIDAE), IN THE FRESHWATER ST. JOHNS RIVER, FLORIDA

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ABSTRACT

A population of the Atlantic stingray, *Dasyatis sabina,* resides in the freshwater St. Johns River system, Florida. The reproductive life history of the species in Lake Monroe near Sanford, Florida, was studied from November 1990 to January 1992. No major differences in reproductive timing or performance were noted between this freshwater population and marine populations studied elsewhere in Florida. Females matured at approximately 22 cm disk width (DW), and mature ovarian eggs were ovulated in early April. Embryos were obtained from pregnant females from 15 May to 17 July, and parturition occurred in late July, when embryos attained approximately 100 mm DW. Males matured at approximately 21 cm DW. Male gonadosomatic index peaked in November and declined continually through the spring, but fluid was retained in the seminal vesicles until May. This population experienced total reproductive failure during the 1991/1992 season. Extremely low conductivity in the lake during the fall and winter of 1991 is suggested as a possible stressor.

There are an estimated 900 to 1,100 species of extant cartilaginous fishes in 170 genera and 51 families. Of these, approximately 42 species in 9 genera and 4 families of sharks and rays enter fresh water. Most freshwater sharks and rays occur in the warm, tropical rivers of South America, Asia, and Australia, but some occur in warm-temperate rivers in North America and Africa (Compagno and Cook, 1995). A marine ancestry for cartilaginous fishes is generally accepted and, on a geological time scale, their invasion of fresh water has been relatively recent (Thorson et al., 1983). "Freshwater" sharks and rays have a wide range of salinity tolerances. Some marginally freshwater-tolerant species are found in marine waters and occasionally enter brackish estuaries and freshwater rivers; others are obligate freshwater species and are not found in marine or brackish-water environments. Several species of euryhaline elasmobranchs tolerate the full range of salinities from sea water to freshwater environments, and some reproduce in freshwater rivers and lakes (Compagno and Cook, 1995). The most completely freshwater-adapted elasmobranchs are rays in the family Potamotrygonidae, which have lost the ability to retain urea in their tissues and can no longer osmoregulate in salinities above 3 parts per thousand (‰) (Thorson et al., 1983).

The Atlantic stingray, *Dasyatis sabina* (Lesueur), is a common coastal ray that ranges in the western North Atlantic from Chesapeake Bay south to Florida and in the Gulf of Mexico southwest to Campeche, Mexico (Bigelow and Schroeder, 1953). This fully euryhaline species occurs in brackish bays and estuaries and has long been known to enter fresh water (Gunter, 1938). The presence of a permanent population in the freshwater portion of the St. Johns River, Florida, was reported by McLane (1955) and Tagatz (1968). Coastal populations of *D. sabina* have been the subject of considerable ecological and life-history research (Breder and Krumholz, 1941; Bigelow and Schroeder, 1953; Murray and Christmas, 1968; Sage et al., 1972; Funicelli, 1975; Schwartz and Dahlberg, 1978; Teaf, 1980; Lewis, 1982; Teaf and Lewis, 1987; Schmid, 1988; Snelson et al., 1988). In contrast, little is known about the biology of freshwater populations of *D. sabina,* and the life history and ecology of the population inhabiting the freshwater St. Johns River has never been studied.

Sharks and rays that occur in fresh water are of interest from an evolutionary perspective in that they show varying degrees of adaptation toward freshwater existence. Assuming a marine ancestry for elasmobranchs, euryhaline species that are able to complete their life cycle in fresh water may be evolutionary "intermediates" between migratory euryhaline species and the truly freshwater forms such as the South American rays of the family Potamotrygonidae. For example, two fully euryhaline elasmobranchs, the bull shark (*Carcharhinus leucas*) and largetooth sawfish (*Pristis perotteti*), differ in the degree to which they have adapted to fresh water. *Pristis perotteti* is known to occur and reproduce in freshwater rivers and lakes in Central and South America (Thorson, 1982). *Carcharhinus leucas,* although found in the same drainages, apparently must enter brackish water to reproduce (Montoya and Thorson, 1982). From an evolutionary perspective, *P. perotteti* is more fully adapted for life in fresh water than is *C. leucas.*

The relative position of *D. sabina* on the evolutionary scale towards adaptation to life in fresh water is not known. There is no physical barrier between the St. Johns River and the Atlantic Ocean, so migration of individuals between fresh and brackish water near the river's mouth is likely. It seems unlikely, however, that rays living in the upper (southern) freshwater parts of the system could traverse the long distances (ca. 300–500 km) required to mingle with the estuarine and marine populations. Schmid (1988) has shown that this species has a high degree of site fidelity and migrates very little in east-central Florida estuaries. These and other observations indicate that rays living in the southernmost reaches of the river represent a permanent population. It is possible that this population was not established by upstream migration but is a remnant of the estuarine population that inhabited the basin when sea levels were higher and when the St. Johns basin was a brackish coastal lagoon, as the Indian River Lagoon is today. Under this scenario, the freshwater population may have been at least partially isolated from coastal populations since the late Pleistocene (Cook, 1939). Given a lengthy freshwater residency and perhaps reduced gene flow with marine populations, morphological and/or physiological adaptations to fresh water may have evolved. For example, Raschi and Mackanos (1989) described differences in the structure of the ampullae of Lorenzini in the freshwater *Dasyatis garouaensis* that may be intermediate steps between sensory adaptations of euryhaline dasyatids and freshwater potamotrygonids.

The purpose of this study was to investigate the reproductive life history of *D. sabina* in the St. Johns River drainage and to identify and compare the differences, if any, between this freshwater population and previously described estuarine/marine populations.

Study Area

The St. Johns River is a shallow, sluggish river flowing northward from its headwaters near Lake Helen Blazes in Brevard County, Florida, to its mouth at Mayport, Florida, a distance of approximately 500 km. The salinity of the river varies, depending on tidal influence, rainfall and run-off, and input from fresh and salt springs located throughout the drainage. Above the tidal influence south of Lake George, the salinity is low, rarely exceeding 1.0‰ except near salt springs.

All collections were made at Lake Monroe near Sanford, Florida, about 258 km from the mouth of the river. Lake Monroe is an "in-channel" basin, essentially a wide spot in the St. Johns River. The lake is shallow, averaging 1 to 2 m in depth, with a soft, mud bottom that becomes firmer and sandy near the littoral zone. The lake supports a large population of freshwater gastropods and mussels and a variety of freshwater fish species typical of the river system (McLane, 1955; Tagatz, 1968). The lake is also inhabited by many species of marine origin. Species that are clearly migratory appear in the lake on a seasonal basis (e.g., striped mullet (*Mugil cephalus*), Atlantic croaker (*Micropogonias undulatus*), and American shad (*Alosa sapidissima*)). Other species of marine ancestry, such as *D. sabina,* maintain year-round populations and apparently complete their entire life cycles in the lake.

These include Gulf pipefish (*Syngnathus scovelli*), clown goby (*Microgobius gulosus*), Atlantic needlefish (*Strongylura marina*), the anthurid isopod *Cyathura polita,* and the xanthid crab *Rhithropanopeus harrisi* (McLane, 1955; Tagatz, 1968).

METHODS

We collected stingrays by pulling a 5-m otter trawl with 1.27-cm mesh behind an outboard skiff. The trawl was towed for 5 min, usually in an area about 100 m from shore where water depth was 2 to 2.5 m. Surface and bottom temperatures were measured to the nearest 0.5°C, salinities were measured to the nearest ‰, and conductivities were recorded to the nearest μMhos/cm. Monthly temperature and conductivity values were averaged for months in which two or more collections were made.

Collections were made semimonthly from February to August, and monthly from September to January. Between November 1990 and January 1992, nearly 500 juvenile and adult stingrays were collected in trawl samples; 212 mature rays were used for this study, and all others were released alive. Sex, disk width and length (to the nearest 0.1 cm), time of day and approximate location of capture were recorded for each ray. Specimens were kept on ice and returned to the laboratory for examination the same day. During the period of gestation, females were euthanized after capture in MS-222 solution to reduce abortion of embryos and uterine eggs. If abortion in the field was unavoidable, embryo and eggs were collected, fixed in 10% formalin, and later transferred to 40% isopropyl alcohol.

In the laboratory, the wet weight of each ray was measured to the nearest 0.5 g. The left ovary and uterus of each female were removed. Testes, epididymi, ducti deferentii, and seminal vesicles of male rays were removed. Tissues from both sexes were fixed in 10% formalin and later transferred to 40% isopropyl alcohol. The presence or absence of fluid in the seminal vesicles or clasper grooves was noted.

Ova were removed from the preserved left ovary of each female, and the wet weight of each ovum was determined to the nearest 1 mg. Most ova examined were elliptical in shape; therefore, the diameter was measured along both the long and short axes to the nearest 0.1 mm and later averaged. The wet weight of each preserved embryo was determined to the nearest 1 mg, and total length from rostrum to end of tail and disk width were measured to the nearest 0.1 mm. Wet weight of the external yolk sac of the embryo was measured to the nearest 1 mg whenever the sac was not ruptured. Egg/embryo measurements were made with dial calipers, and weights were taken on an electronic balance.

Epigonal tissue was removed from the testes of each male, and both gonads were weighed together to the nearest 0.01 gram. The gonadosomatic index (GSI) was calculated by comparing wet weights of both testes with the wet weight of the body as follows:

$$GSI = \text{testis weight/body weight} - \text{testis weight} \cdot 100$$

Data reported in the text are expressed as mean ± one standard deviation unless otherwise noted.

RESULTS

General.—Mean monthly bottom temperatures ranged from a low of 14.0°C in December 1990 to a high of 32.0°C in August 1990. Mean monthly conductivity readings ranged from a low of 300 μMhos/cm in October 1990 to a high of 1550 μMhos/cm in November 1990. Salinity in Lake Monroe never exceeded 1.0‰ during the study.

Rays were collected during all months of the year, and the population was composed of individuals ranging in size from small neonates to large adults. No evidence of migration was noted. These observations suggest that a permanent population resides in this part of the St. Johns River. General characteristics of the Lake Monroe population, including size distribution, sex ratio, seasonal occurrence, abundance, and aspects of ecology, are discussed elsewhere (Johnson, 1992).

Female Reproduction.—Females began to mature at approximately 22–23 cm DW. The smallest mature female, based upon the presence of developing ovarian ova, was 21.9 cm DW. The smallest pregnant female was 23.0 cm DW, and the largest pregnant female was 31.1 cm DW.

In all adult females examined, only the left ovary and uterus were functional. Developing oocytes were absent from the right ovary, and no developing embryos

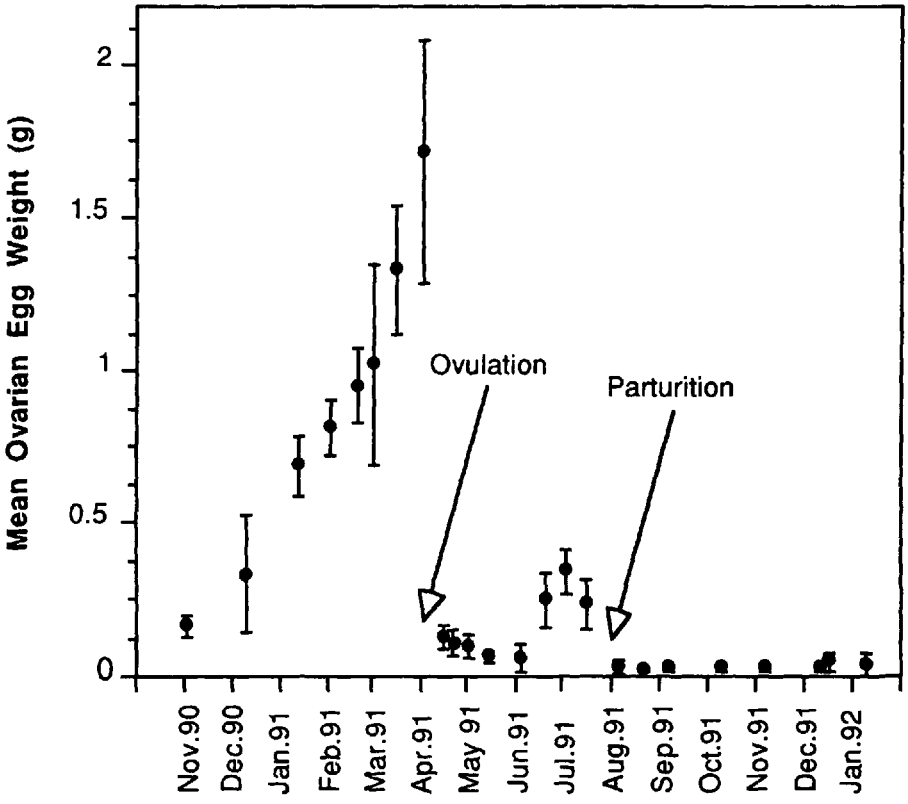

Figure 1. Mean ovarian egg weight for female *Dasyatis sabina* collected November 1990–January 1992. Error bars equal ±1 standard deviation (N = 116).

were found in the right uterus. Mature left ovaries contained an average of 7 oocytes (maximum 16). Beginning in November 1990, between 1 and 4 oocytes (2.36 ± 0.81) were enlarged, representing developing ova for that year. The remaining oocytes were noticeably smaller, rarely more than 0.04 g in weight. These smaller oocytes were white and translucent, whereas developing ova were dark yellow and opaque. Mean weight of developing ova for November was 0.164 ± 0.032 g, and weight increased rapidly until ovulation in mid-April. Mean ovarian egg weight immediately prior to ovulation (April 2) was 1.687 ± 0.396 g, and the largest egg weighed was 2.053 g (Fig. 1). On April 16, after ovulation, the mean ovarian egg weight dropped to 0.129 ± 0.041 g, indicating a relatively synchronous period of ovulation. Based on the presence of egg shell membranes in the uterus of nearly all females collected on 16 April, encapsulation of eggs in the nidamental gland occurred soon after ovulation, between early and mid-April. Spontaneous abortion of uterine eggs by nearly all females captured 2 and 16 April made the examination of newly encapsulated eggs difficult. Many eggs and their delicate, membranous capsules were ruptured by uterine contractions and subsequent abortion.

A second clutch of slightly enlarged ova was observed in the ovaries of most mature females from mid-June to mid-July (Fig. 1). Between 20 June and 17 July, 13 of 16 females sampled contained several enlarged ova. These ova were not as large as ovulated eggs and were absent in all females after July. These ova reached

weights of 0.337 ± 0.070 g in females observed 3 July, about one-fifth the weight of mature eggs. None of the females collected in this study ovulated this second clutch of eggs, which apparently were reabsorbed as corpora lutea atresia. Ovarian egg diameter showed a pattern almost identical to egg weight (Johnson, 1992).

Although uterine trophonemata were not measured, gross inspection of the left uterus revealed that trophonemata were somewhat enlarged in females collected in April and became noticeably thickened and elongated in May. Trophonemata continued to increase in size throughout gestation until parturition. After parturition in late July/early August, uteri and trophonemata were reduced in size in all mature females. Likewise, ovaries became regressed and remained inactive throughout fall and early winter.

Of 30 adult females sampled between 15 May and 17 July, 27 were pregnant with at least one embryo. The three females without embryos were from the 17 July collection and had probably aborted or recently given birth to young, judging from the condition of their uteri. Mean number of embryos per brood was 2.3 (range 1 to 3). Because embryo weight increased with date of collection, it was necessary to include date (as an indicator of relative stage of embryonic development) in the statistical model so that relationships between female weight and mean embryo weight could be determined. Under this ANOVA model, there was no correlation between female weight and embryo weight (partial t-test = −0.249; $P = 0.806$). Likewise, no correlation was found between female DW and embryo DW (partial t-test = −0.638; $P = 0.530$). Regression of the number of embryos per brood on female body weight was statistically significant ($F = 6.323$; df = 26; $P = 0.019$). However, a low r^2 value (0.202) indicated that only a small amount of the variability in brood size was explained by female body weight. The relationship between female DW and brood size was not significant ($F = 2.985$; df = 26; $P = 0.096$).

There were no enlarged, developing ova in females during the winter of 1991/1992. Mean egg weight in four females collected 9 January 1992 was 0.039 ± 0.038 g. The largest mean egg weight was 0.091 g (from a 31.0 cm DW female), nearly eight times smaller than the mean egg weight for females from the January 1991 collection. The left ovaries and uteri of females remained small, inactive, and regressed after parturition in August 1991 until the end of sampling in January 1992 (N = 33). Subsequent field sampling from February to April 1992 revealed no females with mature ova (N = 7) and four females taken in May 1992 had no signs of uterine young.

Embryonic Development.—Only a brief description of embryonic development is presented here, since a thorough examination of embryogenesis in this species was reported by Lewis (1982). Embryos were first recognizable macroscopically on 15 May, approximately 4–6 weeks after ovulation and (presumed) fertilization. The earliest developing embryo examined was 8.6 mm in total length, 1.2 mm in "disk" width, and weighed 0.004 g. At this early stage of development, pectoral and pelvic fins had not yet developed, and the embryo had an elongate, "shark-like" appearance. The eyes had not yet developed, and the olfactory bulb of the head was slightly enlarged. Only minute signs of external gill filaments were evident. Weight of yolk from this embryonic stage averaged 0.733 ± 0.080 g, nearly 10 times the weight of the embryo. At 1.8 mm DW (15 May) the pectoral fins were beginning to expand laterally but were restricted to the posterior half of the body. At this stage the cephalic region was flexed ventrally, and the external gills were further enlarged. Pelvic fins began development at about 3.6 mm DW (15 May), and the pectoral fins had begun to expand anteriorly. By 5.0 mm DW

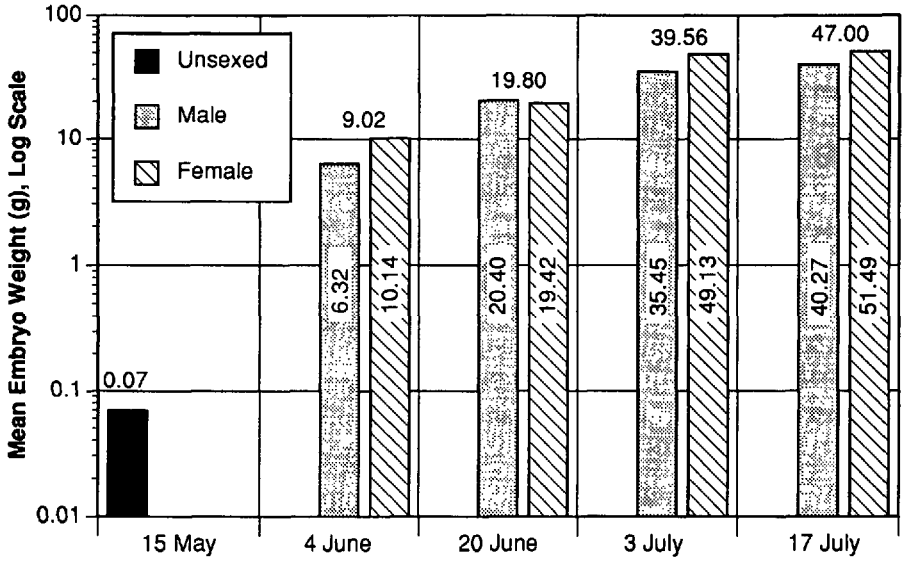

Figure 2. Mean weights of embryos of *Dasyatis sabina* collected 15 May–17 July 1991. The number above each bar is the grand mean weight for the sample with the sexes combined. The number on each bar is the mean weight by sex. Sample sizes in chronological order are 16, 17 (5 M, 12 F), 13 (5 M, 8 F), 10 (7 M, 3 F), and 5 (2 M, 3 F).

(15 May) the eyes had begun to form, and at 7.0 mm DW (15 May) they were pigmented. By 7.0 mm DW the cephalic region became more flattened dorsoventrally. Growth of the embryo during this stage of development was very rapid. The mean embryo weight increased from 0.073 ± 0.080 g on 15 May to 9.018 ± 4.380 g on 4 June, a greater than 100-fold increase in an eighteen-day period (Fig. 2).

By 4 June, embryos were well developed, with fully formed pectoral and pelvic fins, spiracles, eyes, and mouths. Further development was reflected primarily in size increase rather than in conspicuous morphological changes. The external gill filaments were greatly enlarged in embryos up to about 60 mm DW and 11.0 g weight (4 June). In specimens larger than 11.0 g, the external gill filaments were reabsorbed. The tail spine first began to appear in embryos around 60 mm DW but did not become hardened until approximately 70 mm DW (20 June), when embryos were morphologically similar to adult rays. The rate of growth from 4 June until parturition was not as rapid as in the preceding interval. An approximate two-fold increase in weight occurred between both of the semi-monthly collections from 4 June to 3 July. Although this rate of weight increase was smaller than that occurring earlier in development, it represented a large increase in embryo mass. The mean weight gain during the 2-week period between 20 June and 3 July accounted for 42% of the mean weight of the embryo at parturition. During the last 2 weeks of gestation, weight increased only 20%.

In the 4 June and subsequent collections, the gender of embryos was identifiable by the presence or absence of claspers, which were discernable in the embryos at 40 mm DW. Of 45 embryos that could be sexed, the sex ratio was 26F:19M, not significantly different from 1:1 ($\chi^2 = 1.089$, df = 1, $P = 0.297$). In embryos sampled on 20 June, males were slightly heavier and had larger disk widths than females. However, in the other three embryo collections, the average female em-

Table 1. Summary of weights of external yolk and embryo soma for embryos of *Dasyatis sabina* from Lake Monroe (Weights are wet weight in grams given as mean ± SD)

Sample dates	N	External yolk	Embryo soma
15 May	16	0.723 ± 0.167	0.073 ± 0.080
4 June	17	0.109 ± 0.088	9.018 ± 4.379
20 June	13	0.004 ± 0.004	19.799 ± 4.227
3 July	10	0	39.558 ± 15.424
17 July	5	0	47.002 ± 10.606

bryo was significantly larger than the average male embryo (Fig. 2). Analysis of variance of embryo weight (from 4 June to 17 July) indicated that female embryos were significantly larger than the males (2-way ANOVA: $F = 5.906$, $P = 0.020$). The same results were obtained using embryo DW as the dependent variable. Embryo DW measurements by gender were as follows (mean in mm ± SD): 4 June—M(ale) 46.9 ± 5.11 (N = 5), F(emale) 55.4 ± 10.02 (N = 12); 20 June—M 73.8 ± 4.73 (N = 5), F 70.4 ± 7.05 (N = 8); 3 July—M 86.9 ± 10.49 (N = 7), F 100.2 ± 4.26 (N = 3); 17 July—M 91.4 ± 5.09 (N = 2), F 99.9 ± 8.37 (N = 3).

Parturition.—Three of six females collected on 17 July did not carry uterine young, but their uteri were enlarged and trophonemata were well developed, indicating they had recently given birth. No neonates or aborted embryos were collected in the trawl net during that particular tow, suggesting that these females had given birth prior to being captured, rather than having aborted their young in the trawl. Three other females collected on 17 July still retained their young. Mean embryo size was 96.5 ± 8.0 mm DW, and the largest embryo was 110.0 mm DW. None of the five females collected on 5 August 1991 were pregnant, and no aborted young were found in the trawl. Based on these data, parturition occurred between mid-July and early August 1991, after a gestation period of 3.5 to 4 months.

Embryo Nutrition.—The external yolk sac was absorbed within the first 60 days of embryonic development. Ova apparently lost considerable weight between the time of encapsulation and the formation of the yolk sac in early embryos. Mean weight of mature ovarian eggs was 1.687 ± 0.396 g (April 2). Due to uterine contractions and subsequent abortion, newly encapsulated ova were ruptured and could not be weighed. However, assuming little, if any, weight loss between the time of ovulation and encapsulation of the ova by the nidamental gland, a 57% decrease in yolk weight occurred between early April and 15 May, when mean external yolk weight was 0.72 g (Table 1). By 4 June, mean external yolk weight dropped to 0.11 g, representing a 85% decrease in weight in the two-week period. The weight was further reduced to <0.01 g in embryos taken on 20 June, and by 3 July the external yolk supply was exhausted in all embryos examined (Table 1).

Male Reproduction.—Males were mature at approximately 21 cm DW, as indicated by enlargement and calcification of claspers and presence of milky fluid in the seminal vesicles (the expanded distal end of the ductus deferens; Lewis, 1982) of males collected during the winter and spring. Other researchers have documented the presence of active sperm in the seminal fluid during this time period (Lewis, 1982; Snelson et al., 1988). The smallest male with seminal fluid present was 21.7 cm DW and was collected 13 January 1991.

Testicular weight of males peaked in November 1990; mean gonadosomatic

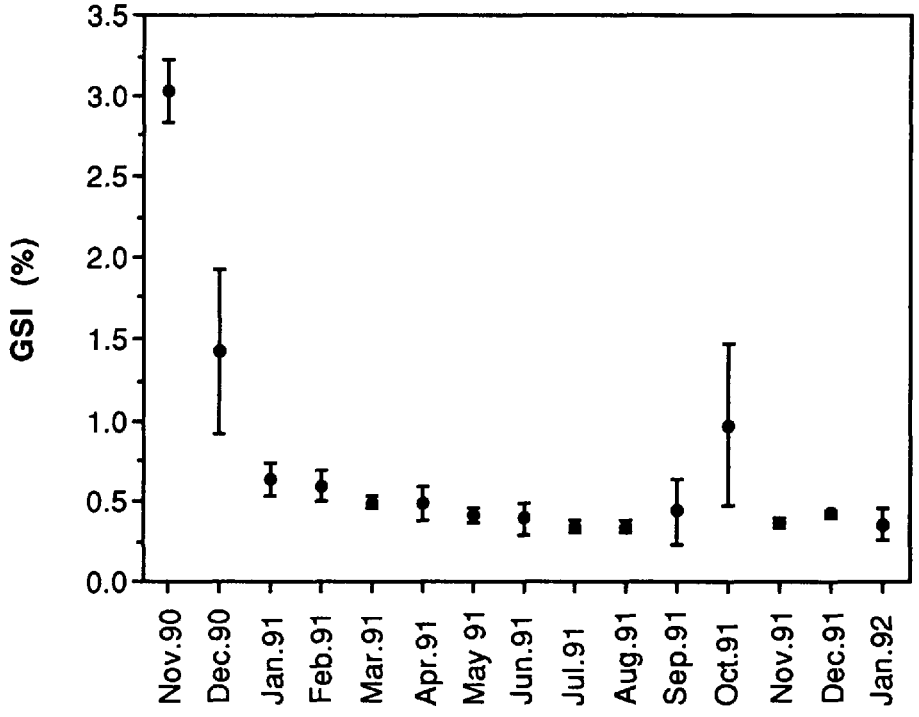

Figure 3. Mean gonadosomatic index (GSI) for male *Dasyatis sabina* collected Nov. 1990–Jan. 1992. Error bars equal ±1 standard deviation (N = 71).

index (GSI) at that time was 3.00 ± 0.20 (Fig. 3). During this month, both left and right testes were active, as indicated by well-developed testicular lobes and presence of fluid in both seminal vesicles. On 11 December 1990, GSI decreased to 1.40 ± 0.50, but a copious amount of fluid was present in both seminal vesicles. Testes continued to regress during the succeeding months despite the presence of at least some fluid in the seminal vesicles as late as 2 May 1991.

Male GSI remained low during the spring and summer months, began to increase in September, and reached a small peak in October 1991 (1.00 ± 0.50). However, this peak was only about one-third the mean GSI measured in the preceding November. The GSI dropped again to 0.40 ± 0.03 in November 1991, and testicular weight remained low throughout the remainder of the study. Four of the five mature males collected in the October sample had a small amount of fluid in the seminal vesicles and moderately enlarged testes. The one male (26.0 cm DW) without seminal fluid had the lowest GSI for that month (0.50). One other male (22.2 cm DW), collected on 17 December 1991, had fluid in the seminal vesicles. Overall, only five of twenty-six males sampled between September 1991 and January 1992 had fluid in the seminal vesicles or epididymi. Subsequent periodic sampling in the spring of 1992 revealed only two males with seminal fluid (N = 16).

Discussion

Reproductive Comparisons.—Several life history traits of six populations of *D. sabina* from the southeastern US are compared in Table 2. The size of females at sexual maturity in the St. Johns River population, about 22 cm DW, was similar

Table 2. Comparison of life history traits of female *Dasyatis sabina* from the St. Johns River with those of female *D. sabina* from several marine populations

Location	Mating	Ovulation	Gestation period	Egg size at ovulation	Mean brood size	Embryo DW at parturition	Female DW at maturity
St. Johns River, FL (present study)	Nov.–Mar.	Late Mar.–early Apr.	Apr.–early Aug.	15–16 mm	2.3	95–105 mm	22 cm
Indian River Lagoon, FL (Snelson et al., 1988)	Oct.–Mar.	Late Mar.–early Apr.	Apr.–early Aug.	15 mm	2.6	100–130 mm	23 cm
Northeastern Gulf of Mexico (Lewis, 1982)	Oct.–Mar.	Apr.	Apr.–early Aug.	13–14 mm	2.0	100–135 mm	22 cm
Georgia and North Carolina (Schwartz and Dahlberg, 1978)	Spring–Winter?	Late Apr.?	Apr.–Sept.	n/a	n/a	101–130 mm	n/a
West Coast of Florida (Breder and Krumholz, 1941)	n/a	n/a	n/a	n/a	n/a	99–100 mm	22 cm
Northern Gulf of Mexico (Murray and Christmas, 1968)	n/a	n/a	n/a	n/a	2.09	100–120 mm	n/a

to that reported for marine populations. Breder and Krumholz (1941) indicated that females matured between 22.0 and 23.0 cm DW. Snelson et al. (1988) reported that females from the Indian River Lagoon begin to mature at about 23 cm DW, and Lewis (1982) gave a range of 22.3 to 24.0 cm for females in the northeastern Gulf of Mexico.

Lewis reported the size of males at sexual maturity in the northeastern Gulf of Mexico population to be between 20.5 and 21.5 cm DW, based upon well-developed reproductive tracts and active spermatogenesis (Lewis, 1982). Snelson et al. (1988) reported the size of males at maturity in the Indian River Lagoon population to be between 20 and 25 cm DW, based on clasper structure and gonadal condition. Ripe males, 21.1 to 26.5 cm DW, were reported from Georgia estuaries from April to August, and two were reported during mid-November (Schwartz and Dahlberg, 1978). However, ripe males were considered those that contained mucus in the clasper grooves; this may not necessarily imply spermatogenic activity. Size of males at sexual maturity in Lake Monroe was estimated to be approximately 21 cm DW, based on presence of fluid in the seminal vesicles and enlargement of testes of males collected during the winter and spring.

The results of this study indicate that the St. Johns River population has a distinct annual reproductive cycle. Early works by Gunter (1945) and Bigelow and Schroeder (1953) reported *D. sabina* to have a year-round breeding season, but more recent studies of marine populations show a strictly annual cycle (Breder and Krumholz, 1941; Murray and Christmas, 1968; Sage et al., 1972; Schwartz and Dahlberg, 1978; Lewis, 1982; Snelson et al., 1988).

Vitellogenesis began in November with the development of one to four oocytes that were noticeably enlarged and filled with yolk. The fate of the remaining oocytes within the ovary was not determined in this study. Lewis (1982) examined the ovarian tissue of *D. sabina* histologically and determined that most of these smaller oocytes underwent atresia and were reabsorbed as corpora lutea. Ovarian eggs reached a maximum size during early April, and eggs were ovulated by all females examined prior to 16 April, indicating relatively synchronous ovulation. This is similar to what was found in the populations studied from both the northeastern Gulf of Mexico (Lewis, 1982) and the Indian River Lagoon (Snelson et al., 1988). Schwartz and Dahlberg (1978) mentioned collecting females with yellowish eggs in late April, but they failed to specify whether these were ovarian or uterine eggs.

What appeared to be a second clutch of enlarged ova was observed in the left ovaries of all mature females collected from 20 June 1991 to 17 July 1991. These ova were a fraction of the size of mature ova and apparently none were ovulated. These findings are much like those of Snelson et al. (1988), who also found several females with enlarged ova in June and July but found no evidence of a second period of ovulation. Lewis (1982) observed a similar occurrence of enlarged ovarian oocytes in late July. However, some females in Lewis' study carried uterine egg capsules containing degenerate ova. Lewis suggested that these ova represented anomalous oocytes that were ovulated but not fertilized. A significant difference between Lewis' observations and the observations in this study and those of Snelson et al. (1988) is that we and Snelson et al. found no females with ovulated eggs or shell membranes in the uterus after this second period of oocyte enlargement. In addition, in the present study all females that carried enlarged ovarian eggs in midsummer were also pregnant with uterine young at the time. After parturition in late July, no evidence of enlarged ovarian eggs was found. Because this midsummer group of enlarged ovarian ova coincides with the latter stages of gestation, they may play a hormonal role in late gestation and/or parturition.

We did not examine eggs histologically to determine the exact time of fertilization. However, it can be assumed that fertilization could not have occurred before ovulation, nor after encapsulation of the egg in the nidamental gland (Snelson et al., 1989). Based on this assumption, fertilization occurred between early and mid-April 1991. This is close to the estimated time of fertilization for females in the northeastern Gulf of Mexico and the Indian River populations (Lewis, 1982; Snelson et al., 1988).

The brood sizes in this study matched those reported for marine populations. Murray and Christmas (1968) reported mean brood size of 2.09 young per female in Mississippi Sound, and Lewis (1982) reported brood sizes ranging from 1 to 4 and a mean of 2.0 young per female in the northeastern Gulf of Mexico population. Snelson et al. (1988) found that brood size ranged from 1 to 4, with an average of 2.6 young per female in the Indian River. In the St. Johns population the mean brood size was 2.3 young per female, and no female was found with more than 3 young.

Babel (1967) indicated that larger females (DW) of *Urolophus halleri* produced larger brood sizes than did smaller females. Snelson et al. (1988) found no correlation between brood size and maternal size (DW), but they did not analyze female body weight as a factor. There was a modest relationship between female body weight and brood size in this study, and yet the same analysis using female DW as the independent variable did not show a significant relationship. Lewis (1982) also mentioned a significant relationship between maternal size and brood

size, but he did not clarify how maternal size was measured. We found no correlation between embryo size (either DW or mass) and maternal size. It appears that heavier females may have some capacity to produce more young per clutch but not larger young.

Development of embryos followed that reported by Lewis (1982) in most respects. We examined an embryo in an earlier stage of development (8.6 mm total length, 1.2 mm disk width) than had previously been reported. By early June, embryos were well developed morphologically and resembled free-living juveniles. Lewis (1982) reported that claspers were identifiable in male embryos at 17 mm DW. We assume that clasper formation occurred at approximately the same size in embryos from Lake Monroe, but growth was so rapid during this two-week period that embryos between 7.5 mm and 43.1 mm DW were missed.

The sex ratio of embryos was not significantly different from 1:1 in this study, which is in agreement with data reported by Lewis (1982) for the northeastern Gulf of Mexico population. Snelson et al. (1988) did not state the sex ratio of embryos collected from the Indian River Lagoon. Other researchers have reported slightly more male than female embryos, but none of them indicated whether or not this was statistically significant (Breder and Krumholz, 1941; Murray and Christmas, 1968).

With all field collections of embryos examined together, female embryos tended to be larger than male embryos. The tendency for free-living female rays to have a faster rate of growth than males has been documented in rays from the Texas Gulf Coast (Sage et al., 1972). Presumably, females incur greater physiological and energetic demands in reproduction than do males, rendering large size an evolutionary advantage. Apparently this size difference between males and females begins during embryonic development in *D. sabina.*

Breder and Krumholz (1941) reported full-term embryos of 99.6 mm DW being taken on July 11 off the west coast of Florida. Murray and Christmas (1968) reported that in Mississippii Sound, size shortly after parturition (16 July) was between 100 and 120 mm DW. Sage et al. (1972) reported females with mature embryos in July but gave no indication when parturition occurred off the Texas Gulf Coast. Schwartz and Dahlberg (1978) reported the capture of a pregnant female with fetuses of 111 to 120 mm DW in a Georgia estuary on 16 August. Lewis (1982) suggested that births of neonates between 100 and 135 mm DW occurred from mid-July to mid-August in the northeastern Gulf of Mexico. Snelson et al. (1988) estimated parturition to occur from late July to early August in the Indian River population, and that neonates were between 100–130 mm DW. In Lake Monroe, gestation lasted 3.5 to 4 months, and neonates measured approximately 100 mm or slightly larger between mid-July and early August. Time of gestation and parturition appears remarkably similar in all populations of *D. sabina* studied.

Lewis (1982) reported that testicular weight began to increase and small amounts of sperm were present in males from August to November. From September through February, testes reached maximum size and the epididymus and ductus deferens were enlarged and contained sperm. Between February and April, the testicular weight and size of the epididymus began to decline, but the ductus deferens and seminal vesicle remained enlarged and engorged with fluid. The testes became inactive and the epididymus and ductus deferens were regressed from April to May. However, the seminal vesicle contained small amounts of sperm during this period. From May through August the gonads were in a latent stage. Snelson et al. (1988) found that the gonadosomatic index of males from the Indian River Lagoon began to increase in September and peaked in October.

Although the GSI began to decline in November, the ductus deferens remained enlarged and sperm continued to be produced throughout the winter and spring. They observed copulation in March, and active sperm were present in the cloaca and vagina of several females in December and January. Snelson et al. (1988) reported that sperm was absent from male reproductive tracts after April and that the GSI was minimal until August.

The male reproductive cycle of the St. Johns River population followed closely that reported for other populations. GSI was highest during November 1990 and decreased abruptly in December 1990. Ducti deferentii and seminal vesicles remained enlarged and contained fluid until early May 1991. The obvious difference between the male reproductive cycle of the St. Johns River population and other populations studied was the failure of the GSI to increase during the late summer and fall of 1991. A slight increase in GSI occurred in September and October. Based on the presence of small quantities of fluid in the seminal vesicles and the small size of the testes, ducti deferentii and seminal vesicles, this was a period of limited sexual development in males. This period of moderate increase in GSI probably represented a period when testicular development normally begins in most years. Except for one adult male collected in December 1991, seminal fluid was not found in any males from November to January 1992. Explanations as to the possible causes of this reproductive failure are discussed below.

Embryonic Nutrition.—According to Wourms (1977), the characteristic mode of development in myliobatoid rays is aplacental viviparity. Although details are obscure, the mode of nutrition for the embryo is reported to be similar in all species. Embryos in the early stages of development are dependent upon yolk supplied from the extra-embryonic yolk sac via the yolk stalk. At some point early in gestation, trophonemata lining the inner wall of the maternal uterus begin to secrete histotroph, or uterine milk, which probably is at first absorbed through the yolk sac (Ranzi, 1934 in Lewis, 1982). As the embryonic digestive tract forms, this fluid enters the mouth and perhaps the spiracles, at first supplementing yolk nutrition, but eventually replacing it during the latter stages of development (La Marca, 1961; Babel, 1967; Lewis, 1982). Lewis (1982) detailed the histology of the glandular epithelium of trophonemata in *D. sabina* and, like previous authors, suggested that the secretion of histotroph by these cells was important in embryo nutrition. Lewis (1982) also found that trophonemata increased in size as gestation proceeded.

In *D. sabina,* the yolk within the yolk sac was absorbed completely by the embryo by about 60 days into gestation. The greatest amount of absorption of yolk occurred between mid-May and early June. During this period the embryos also showed the greatest growth. By 3 July, no external yolk sac remained in any embryo examined. An internal yolk sac forms from an enlargement of the terminal end of the vitelline duct in some species of sharks (Wourms, 1977). In the oviparous spotted dogfish, the internal yolk sac functions as a storage site for yolk mobilized from the external yolk sac (Lechenault et al., 1993). There is no internal yolk sac in *D. sabina.* Instead, the vitelline duct empties directly into the gut at a point between the pyloric end of the stomach and the valvular intestine (FFS and E. Amesbury, unpubl.). Thus, when the external yolk sac has been absorbed, about three-fourths of the way into gestation, all yolk has been passed into the embryo's digestive system and metabolized. At this point, the embryo must become entirely dependent on histotroph and/or materials stored in the liver for nutritional support.

In *U. halleri,* the absorption of the external yolk sac occurs relatively later in

development (Babel, 1967). Babel noted that about half of the yolk within the yolk sac still remained at two-thirds of the way into gestation, and he suggested that histotroph probably supplements yolk in supplying nutrients early in development. However, Lewis (1982) suggested the role of histotroph in *D. sabina* was more important in late embryonic development. Other than Wourms and Bodine's (1983) brief abstract on *Gymnura micrura,* we know of no published papers dealing with the nature of histotroph or its exact role in embryonic nutrition (although work is underway in several laboratories at this time). Clearly, additional studies are needed to clarify the relationship between yolk and histotroph nutrition of embryos during gestation in dasyatid rays.

Reproductive Failure.—Data from both males and females indicate that there was a complete reproductive failure in the St. Johns population of *D. sabina* in the spring of 1992. Egg weight remained low throughout the fall and winter, and the largest ovarian eggs measured never exceeded a fraction of the average size of ova from the previous year. According to Lewis (1982) and Snelson et al. (1988), oocytes for the next breeding season begin to enlarge during the summer and fall and are greatly enlarged and yolky during the late fall and early winter. This was the pattern we found in Lake Monroe in the fall and winter of 1990. However, there was no significant enlargement of ovarian eggs in the fall and winter of 1991. Subsequent periodic sampling in the spring and early summer of 1992 indicated that females did not produce mature ova or uterine young in that year. All studies thus far published indicate that most, if not all, mature females of *D. sabina* reproduce annually (Sage et al., 1972; Schwartz and Dahlberg, 1978; Lewis, 1982; Snelson et al., 1988). The reproductive failure in the females coincided with the observation that only two males collected in the spring of 1992 contained fluid in the seminal vesicles. Thus, the absence of reproduction in 1992 is assumed to be an abnormal event.

Decomposing stingray carcasses were collected in trawl tows during the fall and winter of 1991. Between September and December, approximately 30 carcasses of both sexes and of various sizes were collected. In addition, many of the live rays sampled during this period were emaciated (MRJ, unpubl. data). In contrast, prior to September 1991, no dead rays were ever collected in trawl samples, and animals all appeared to be in good condition. Furthermore, unpublished data on hepatosomatic index and liver lipids indicated a severe depletion of hepatic lipid reserves that coincided with the reproductive failure during the late fall and winter of 1991 (Johnson, 1992).

The reasons for the reproductive failure and mortalities are not clear, but probably resulted from some type of physiological stress. The only factor that we identified that may have been a stressor is water conductivity. Low ($\leq$700 μMhos/cm) conductivity readings in Lake Monroe persisted from July 1991 to February 1992, and data obtained from the Florida Game and Freshwater Fish Commission indicated that August through November 1991 had the lowest conductivity values in 8 years. At only five other times during this 8-year period did conductivity values drop below 600 μMhos/cm (Johnson, 1992).

Odum (1953) gave evidence that invasion of marine species into low-salinity waters in Florida was possible due to the presence of Pleistocene salt deposits that contribute to increased chlorinity levels in otherwise freshwater systems. Blue crab (*Callinectes sapidus*), tarpon (*Megalops atlanticus*), snook (*Centropomus undecimalis*), and the Atlantic stingray are a few species listed by Odum that commonly enter "freshwater" systems such as the St. Johns River. However, chloride levels and other ions such as calcium and magnesium fluctuate with

changes in season and rainfall. Sudden drops in chlorinity may stress or kill marine invaders that are already on the fringes of their ecological and physiological tolerance ranges. Extremely low conductivities in Lake Monroe from August 1991 to January 1992 may have played a part in the reproductive failure and mortalities of the rays in the 1991/1992 season.

There are other possible explanations for the physiological stress the rays experienced. Although the low ionic concentrations in the lake may have directly compromised the ray's osmoregulatory ability, it may have also affected the survivability of its prey. The rays may have been forced to switch to a less desirable prey or to one that may have had a lower nutritional value. Analysis of stomach contents taken from the rays may help to determine whether or not food consumption and/or prey type changed during the study. Water temperatures were about the same during the falls and winters of 1990 and 1991, suggesting that temperature does not explain the phenomena. The St. Johns River population of *D. sabina* may represent a population that is living on the fringes of its ecological tolerances and is periodically confronted with stresses that limit reproduction and survival.

Conclusions

We evaluated several aspects of reproductive life history in a population of *Dasyatis sabina* that inhabits the freshwater portion of the St. Johns River, Florida. Our data, associated anecdotal evidence, and other published reports all indicate that this is a permanent population that completes its entire life cycle in fresh water. Despite being at least partially isolated in a markedly different habitat, perhaps since the late Pleistocene, this population shows no significant divergence in reproductive characteristics when compared to populations from nearby marine habitats. The species is living near the edge of its physiological/ecological tolerance in Lake Monroe, and there may be occasional years of high mortality and reproductive failure.

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