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Mating behavior of southern stingrays, *Dasyatis americana* (Dasyatidae)

Demian D. Chapman[a], Mark J. Corcoran[a], Guy M. Harvey[a], Sonita Malan[b] & Mahmood S. Shivji[a]
[a]*Guy Harvey Research Institute and Oceanographic Center, Nova Southeastern University, 8000 N. Ocean Drive, Dania Beach, FL 33004, U.S.A. (e-mail: mahmood@nova.edu)*
[b]*P.O. Box 903, Grand Cayman, Cayman Islands, British West Indies*

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Synopsis

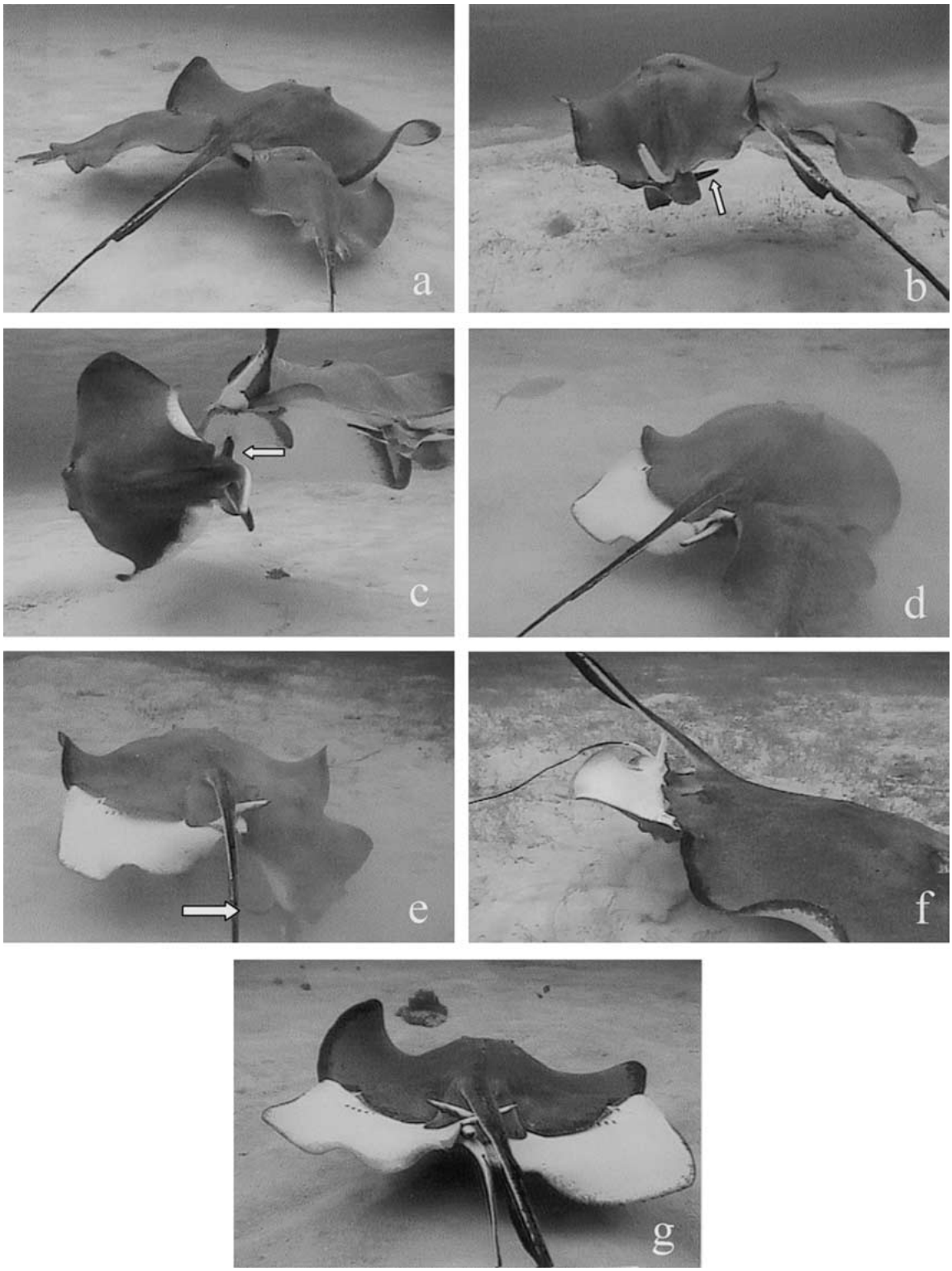
We document in detail the first complete sequence of mating events in the southern stingray, *Dasyatis americana*, based on observations of four matings (five separate copulations) at Grand Cayman, British West Indies and Bimini, Bahamas. These observations are significant because almost nothing is known about this important aspect of the life cycle of batoids, due to the rarity of encounters with mating animals in natural settings. Similar to mating behavior described in the manta ray, *Manta birostris*, the mating sequence of *D. americana* can be characterized as (1) 'close-following', (2) 'pre-copulatory biting', (3) 'insertion/copulation', (4) 'resting' and (5) 'separation'. Additional information gained from these observations includes the fact that (1) two of the females were mated very shortly (i.e. within minutes–hours) after parturition and (2) one of the mating events involved a single female that copulated (unforced) with two males in rapid succession. The latter observation suggests that polyandry and multiple paternity may be elements of the mating system of *D. americana*.

Introduction

Only five of the more than 450 species of batoids (skates and rays) have been observed copulating in the wild, leaving most aspects of their natural sexual behavior and mating systems unknown (Brockman 1975, McCourt & Kerstitch 1980, Tricas 1980, Young 1993, Nordell 1994, Yano et al. 1999, Carrier & Pratt 2001). The southern stingray, *Dasyatis americana*, though common in shallow waters of the western Atlantic, is no exception to this generalization. Other than producing litters of 2–10 pups twice a year in captivity (Henningsen 2000), little else is known of the reproductive natural history of this species, including details of its mating behavior. Brockman (1975) observed a possible copulation between two individuals of this species near the surface, but clasper insertion was not confirmed. DeLoach (1999) provided a brief account and two photographs of a mating event in southern stingrays, whereby one female was chased by seven or eight smaller males, forcibly held to the substrate and then mated by two of them in succession. Here we report the first detailed, fully photographically documented, complete sequence of mating events in free-living southern stingrays. The behaviors we describe were observed during two separate mating events that were captured on underwater video at Grand Cayman Island in the British West Indies. The first event involved a single female and two males, with successful mating achieved by both males without forcibly restraining the female (i.e. two complete copulations were observed). The second videotaped observation of mating involved one male and one female, resulting in a single copulation. We also report on two mating events observed in a semi-captive situation in Bimini, Bahamas, each involving a single male and female.

Observations

The first videotaped observation was made on 6 September, 2001 (Sony TRV 9 camera with a mini DV cassette) in approximately 2 m of water over a sand bottom on the seaward edge of the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North Sound of Grand Cayman Island. Three rays, one large female and two males each approximately one-third the female's size, were initially seen swimming together at 08:24 h. Both males swam slightly posterior to the female (<10 cm), one on each side of her (Figure 1a). Despite the close proximity of the two males, the female continued to swim slowly, approximately 1 m off the substrate. At 08:25 h, male 1 (on the female's left side and identifiable throughout the event by his shortened tail) grasped the rear margin of the female's left pectoral disc in his mouth and attempted to copulate by arching his body so that his pelvic region curled towards the urogential opening (cloaca) of the female, with both of his claspers rotated forward relative to their resting position (Figure 1b). Soon after, male 2 grasped the right side of the female's pectoral disc in his mouth, slightly anterior to the right pelvic fin, also arching his body and rotating both of his claspers forward. Male 1 soon managed to successfully insert his right clasper into the female's cloaca (Figure 1c). He immediately flipped upside down so that he was in a 'ventral to ventral' position (Yano et al. 1999) with the female, while maintaining his oral grasp of her disc (Figure 1d) and then vigorously thrust his pelvic region in rapid succession. The female swam slowly in a small circle during the first few seconds of copulation and then hung almost vertically with her rostrum on the sand, male 1 still beneath her. This rapid thrusting lasted 24 s. Immediately before the male ceased this thrusting movement, a plume of white, viscous material that was likely semen was observed to drift out from the female's cloaca. Male 2, who had maintained his oral grasp of the right rear margin of the female's disc throughout her copulation with male 1, was then observed to insert his right clasper, move into the 'ventral to ventral' position and copulate with the female (Figure 1e and f). Male 1 maintained his oral grip of the female's disc throughout this second copulation, while rapidly fluttering his gills. It also appeared, but because the film provides only a dorsal view difficult to confirm, that the clasper of male 1 also remained inserted over this period. The female settled onto the substrate, both males on their backs beneath her. The rapid thrusting motion of male 2 lasted for 33 s. Like the first male, he then maintained his oral grip of the female's disc while rapidly fluttering his gills. Another probable semen plume was seen trailing from the female's cloaca. The female swam slowly over the substrate, still towing both males in the 'ventral to ventral' position (Figure 1g). At 08:26 h, male 2 withdrew his clasper, released his oral grip and continued to closely follow the female, just above her dorsal surface. Male 1 released his oral grip a few seconds later and swam away rapidly, while male 2 continued to follow about 1 m behind the female. The duration of this following behavior or subsequent behavioral events were not captured on video. The entire sequence of mating events is also available for viewing in streaming video format (http://www.nova.edu/ocean/ghri/stingray/).

The second mating sequence captured on underwater video occurred at the 'Sandbar', a popular Cayman Islands stingray feeding tourism site. A lone female stingray was observed in approximately 1.3 m of water over a sand substrate, amongst a large group of human waders. A smaller male approached the female from the right and grasped the lower margin of her right pectoral fin in his mouth, while arching his body and rotating his left clasper forward. Copulation was once again in a 'ventral to ventral' position with the male beneath the female. Photographs of this particular mating are not shown because the sequence of events and behaviors observed are essentially identical to each of the copulations described in the previous account (refer to Figure 1a–d).

Two additional mating events were observed at Bimini, Bahamas, the first on the 20 August 2000,

Figure 1. Sequence of polyandrous mating events in southern stingrays. Arrows indicate clasper orientation. (a) 'Close following': The posterior orientation of two males to the mature female. Male 1 (left) can be identified throughout the sequence by his shortened tail. (b) 'Pre-copulatory-biting': Male 1 initiates copulation by grasping the margin of the female's pectoral disc in his mouth and arching his body so that his rotated claspers point towards the females urogential opening (cloaca). Male 2 remains on the female's right side. (c) 'Insertion/copulation': Male 1 inserts his right clasper into the females cloaca. Note that Male 2 is also attempting insertion with his left clasper. (d) 'Insertion/copulation': Male 1 copulates in a 'ventral to ventral' (Yano et al. 1999) posture with the female. (e) 'Resting': Male 1 finishes copulating, but his clasper remains inserted as he rapidly flutters his gills. Male 2 grasps the females disc, arches his body and rotates his clasper into position (denoted by white arrow). (f) Male 2 achieves insertion and copulates with the female. Male 1 maintains his oral grip and, possibly, the insertion of his clasper throughout (Male 1 is not visible because he is behind the female in this frame). (g) Both males maintain an oral grip while rapidly fluttering their gills. Female swims slowly, towing both males. Soon after (not shown), both males withdraw their claspers from the cloaca and move away from the female (male 1 departs and male 2 continues to swim with the female slightly above the middle of her disc).

the second on 2 September 2001. In both cases, gravid female stingrays captured on seagrass flats were placed in a seawater container on a boat for transport to a 10 m diameter, circular pen constructed from dark green, plastic mesh fencing (1.5-in. mesh size) situated on a nearby flat. Presumably due to handling stress, the females gave birth immediately before being placed in this shallow pen (depth ca. 1.3 m). In the 2000 event, a viscous yellow fluid was observed flowing from the post-partum female's cloaca. Although no males were observed in the immediate vicinity before the female gave birth, at least six male stingrays (no females) approached from the surrounding flats and circled the pen from 35 min to 2 h after parturition. Similarly, in the 2001 observation one male appeared 25 min after the female gave birth. In both cases, a single male ray circling the enclosure was collected by handheld dipnet and placed in the pen with the post-partum female (approximately 2 h after parturition in 2000 and approximately 30 min after parturition in 2001). In each case the males promptly initiated copulation by grasping the posterior margin of the female's disc, while simultaneously arching their bodies and rotating their claspers forward. In the 2000 observation, subsequent details of the copulation were obscured by a sediment cloud generated by the thrusting movements of the male. In the 2001 observation, however, the male clearly oriented in a 'ventral to ventral' position with the female, at which time she stopped swimming and rested on the bottom during the 10 s copulation. Once again, the sequence of events and behaviors observed are very similar to those shown in Figure 1a–d.

Discussion

The sequence of events of southern stingray mating observed at both Grand Cayman and Bimini can generally be characterized as follows: (1) 'close-following': the posterior orientation of the male(s) to the female; (2) 'pre-copulatory biting': oral grasping of the female's pectoral disc by the male(s), with anterior rotation of one or both claspers and forward arching of the pelvic region such that the claspers are oriented towards the cloaca; (3) 'insertion/copulation': insertion of a clasper followed by vigorous thrusting of the male's pelvic region, lasting (in our observations) from 10 to 33 s; (4) most commonly followed by a 'resting phase', characterized by a rapid fluttering of the male's gills while maintaining clasper insertion and oral grip of the females disc; and (5) 'separation': the male's release of his oral grip, clasper withdrawal, and movement away from the female. Between steps (3) and (5) we typically observed what we believe to be semen trailing from and eventually drifting out of the females urogential opening. Given the consistency of this general sequence of events across all five observed copulations, we suggest that this mating behavior is likely typical of the southern stingray.

This mating sequence matches closely the template presented by Yano et al. (1999) used to characterize mating behavior of manta rays, *Manta birostris*. Despite extreme differences in the habitat and life style of the pelagic manta and demersal southern stingray, the mating behavior of these two species appears to be very similar. In at least four of the five observed copulations, the southern stingrays adopted a 'ventral to ventral' position, supporting the suggestion by Yano et al. (1999) that this mating posture may be conserved amongst the batoids.

The two matings observed at Bimini demonstrate that female batoids are able to copulate within minutes–hours after parturition and are evidently attractive to males at this time. It is unknown whether immediate post-partum copulations in *D. americana* are reproductively successful (i.e. fertilization can occur). In the related Atlantic stingray *Dasyatis sabina*, mating begins in October and continues until late March to early April when the annual ovulation and fertilization take place. Since female *D. sabina* are not known to store sperm over this protracted period, copulations months in advance of ovulation are hypothesized to induce the reproductive cycle of the female (Maruska et al. 1996). A similar ovulation inducement mechanism may explain the post-partum copulation observed in southern stingrays. Alternatively, if *D. americana* females in the wild have two reproductive cycles per year as they do in captivity (Henningsen 2000), the interval between parturition and the next mating season in this species may be very short.

The relatively rapid appearance of male stingrays around the Bimini holding pen containing an immediate post-partum female suggests that the process of parturition in *D. americana* might produce some form of olfactory attractant for male rays. Kajiura et al. (2000) suggested that organic molecules originating from the cloaca of female *D. sabina* may act as sexual pheromones. Olfactory cues were also suggested as responsible for the establishment of mating pair

formations in the blacktip reef shark, *Carcharhinus melanopterus* (Johnson & Nelson 1978).

One of the female southern stingrays we observed in the wild at Grand Cayman was mated in quick succession by two different males, without the apparent chase and forcible multiple-male restraint prior to polyandrous mating described by DeLoach (1999). This behavior suggests that polyandrous mating may also be female-driven (i.e. females can choose to, as opposed to being forced to, mate with more than one male) in this species. Polyandry occurs commonly across the animal kingdom and is beginning to be documented in some species of elasmobranchs (Birkhead 2000, Carrier & Pratt 2001). Regardless of whether polyandrous mating by female southern stingrays occurs through forcible restraint by multiple males or by choice, it appears that ejaculates from more than one male can occur simultaneously in their reproductive tract. Given this situation, it is possible that post-copulatory paternity selection operates in this species (i.e. sperm competition and/or sperm selection). Although multiple paternity of litters has been genetically documented in three shark species (lemon, *Negaprion brevirostris* (Feldheim et al. 2001), nurse, *Ginglymostoma cirratum* (Ohta et al. 2000, Saville et al. 2002) and blue, *Prionace glauca* (P. Prodohl, D. Chapman & M. Shivji, unpubl. data)), there are no such studies of parentage in the closely related batoids. In light of the first of our mating observations and that reported by DeLoach (1999), it is possible that polyandrous mating could facilitate multiple paternity in litters of the southern stingray.

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