

Reproductive behavior of three mobulid species (Mobula mobular, Mobula thurstoni and Mobula munkiana) in the Southern Gulf of California, Mexico

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Abstract

We examined the reproductive behavior (courtship and mating) and its seasonality in three mobula species, spinetail, bentfin, and monk's devil rays (*Mobula mobular*, *M. thurstoni*, and *M. munkiana*) in the southwestern Gulf of California, Mexico, using boat surveys (with drones and in-water observation) (n = 69 survey days), spotter planes (n = 428 flights) and citizen science observations (n = 31). We examined whether (1) reproductive grounds existed within the area for any of these species, (2) reproductive behavior followed seasonal patterns and (3) if this behavior was similar among species. We observed reproductive behavior in 221 sessions in 2017 and 2021–2022, for *M. mobular* (n = 10), *M. thurstoni* (n = 3), and *M. munkiana* (n = 208) dispersed along 312 km of the eastern Baja California Peninsula between 4 m to 6.3 km away from the coast. Most events (n = 209) occurred in the La Ventana and Ensenada de Muertos area. Courtship was observed for *M. mobular* and *M. thurstoni* and mating for *M. munkiana*, with reproductive behavior following a seasonal pattern occurring from March to August, with a peak during May. *M. munkiana* displayed a previously undescribed behavior, the “courtship vortex”, where 122 individuals were observed circling in a clockwise direction for five hours with mating groups joining and leaving the main vortex formation. This study highlights the area of La Ventana and Ensenada de Muertos as a critical habitat for reproductive behavior of three endangered and vulnerable mobulid species.

1. Introduction

The reproductive behavior of many species of sharks and rays (elasmobranchs) is poorly understood due to the difficulties associated with observing courtship and mating events in the wild (Pratt and Carrier 2001). Much of the research on elasmobranch reproductive behavior has been conducted using fresh fisheries carcasses (Serrano-Lopez et al. 2021) where reproductive behavior is inferred from mating scars on females or the state of male claspers (Marshall and Bennett 2010; Rangel et al. 2022; Whitehead et al. 2022), or from sporadic observations in the wild (Whitney et al. 2010; Arnés-Urgellés et al. 2018) or captivity (Uchida et al. 1990). Few studies report specific reproductive grounds based on direct and repeated observations of courtship and mating behavior in the field with exceptions including whitetip reef shark, reef manta rays and basking sharks (Whitney et al. 2004; Marshall and Bennett 2010; Deakos 2011; Stevens et al. 2018a; Sims et al. 2022). It is particularly important to identify the timing and location of elasmobranch reproductive behavior as such areas may be critical in providing specific conditions that support reproductive success. These areas may be important for elasmobranch conservation and management measures to ensure the viability of future elasmobranch populations (Hyde et al. 2022; Palacios et al. 2023).

Mobulid rays (manta and devil rays) are filter feeding batoids distributed in all oceans from temperate to tropical waters (Couturier et al. 2012; Stevens et al. 2018b). The Mobulidae family has the lowest fecundity of all elasmobranchs (Stevens et al. 2000; Dulvy et al. 2014), giving birth to just one pup per gestation period that lasts up to 12 months (Deakos 2011; Marshall and Bennett 2010; Stevens 2016). Their reproductive cycles normally involve resting periods, with a two to seven year interval between

pregnancies (Deakos 2011; Marshall and Bennett 2010). Mobulids mate using internal fertilization (Conrath and Musick, 2012) occurring after courtship behaviors that can last from several minutes to days (Marshall and Bennett 2010; Stevens et al., 2018a). Studies of the reproductive behavior (courtship and mating) of mobulids have focused on a limited number of species (*Mobula birostris*, *M. alfredi*, *M. tarapacana*) (Marshall and Bennett 2010; Deakos 2011; Stevens et al. 2018a, b; Mendonça et al. 2020) with anecdotal observations of other mobulid species (*M. mobular*, *M. thurstoni* and *M. kuhlii*) (Duffy and Tindale 2018; McCallister et al. 2020; Carpenter and Griffiths 2023). These studies examined all or some of the seven stages described for mobulid courtship and mating (Stevens 2016; Stevens et al. 2018a). Courtship behavior includes the first four stages: initiation, endurance, evasion, pre-copulatory positioning, while mating behavior refers to the three stages when copulation, post-copulation holding, and separation occurs (Stevens et al. 2018a). During courtship events, several individuals are involved, with one or two females chased by males, in a formation described as a mating train, numbering from just a few to up to 26 males (Marshall and Bennett 2010; Stevens et al. 2018a). Mobulid courtship aggregations have been described for oceanic islands, seamounts, ridge systems, coral reefs, feeding aggregation sites and cleaning stations (Yano et al. 1999; Marshall and Bennett 2010; Sobral 2013; Stevens et al. 2018a; Germanov et al. 2019; Mendonça et al. 2020; Palacios et al. 2023). While reproductive behavior has been described for the larger mobulids (e.g., *M. birostris*, *M. alfredi*), there is only one observational description of courtship behaviors for the pygmy devil ray (*M. kuhlii*) (Carpenter and Griffiths 2023). However, no mating behavior has been described for the smaller species (*M. hypostoma*, *M. kuhlii*, *M. eregodoo* and *M. munkiana*). Lack of information currently exists on how or where these mobulid species mate or whether they follow the same courtship behaviors described for the better-known species (Childs 2001, Notarbartolo-di-Sciara et al. 2019). In this study we use direct field observations to examine the courtship and mating behavior of three lesser known mobulid species: *M. mobular*, *M. thurstoni*, and *M. munkiana*.

Due to their conservative life history traits (Dulvy et al. 2014) and anthropogenic threats including target fisheries and bycatch (Croll et al. 2016; Lezama-Ochoa et al. 2019), all mobulids species are listed as Endangered or Vulnerable on the IUCN's Red List of Threatened Species (IUCN, 2023). In Mexico, the most common mobulid species include *M. mobular*, *M. thurstoni*, and *M. munkiana* (Notarbartolo-di-Sciara 1988; Serrano-Lopez et al. 2021). Mobulids have been protected in Mexico since 2006, with their primary threats including incidental capture in artisanal fisheries using gillnets (Del-Valle-González-González 2018) as well as bycatch in industrial fisheries, especially in the tuna purse seine fishery (Croll et al. 2012; Croll et al. 2016; Lezama-Ochoa et al. 2019). In recent years, emerging nonregulated ecotourism activities based on observation and the snorkeling with mobulid aggregations have brought new economic opportunities to the local communities in the southern Gulf of California, Mexico. However, increasing ecotourism, particularly during vulnerable life history periods (e.g., mating, nursery), could potentially become a problematic source of disturbance (Murray et al. 2020).

M. mobular and *M. thurstoni* are found circumtropically and can reach up to 3.20-m and 1.83-m disc width (DW), respectively (Stevens et al. 2018b, Stewart et al., 2018). Courtship for these species has been reported from direct observations at offshore areas of New Zealand (*M. mobular*) (Duffy and Tindale

2018) and in Brazil (*M. thurstoni*) (McCallister et al. 2020). In the southern Gulf of California, Mexico, the reproductive season for these species occur during June and July, inferred from morphometry and histology studies of fisheries caught individuals (Notarbartolo-di-Sciara 1988; Serrano-Lopez et al. 2021). *M. munkiana* is distributed in the eastern tropical Pacific and reaches a maximum DW of 1.3-m (Stewart et al. 2018). No direct observations of *M. munkiana* reproductive behavior have been reported for this species; however, Palacios et al. (2021) speculated that they mate in the southern Gulf of California from April to June, based on courtship observations and the presence of sperm in the developed claspers of males captured at a nursery area.

Diver avoidance and a lack of survey effort focused on these three mobulid species has resulted in significant knowledge gaps in important life history parameters and behaviors, particularly reproduction. However, recent citizen science efforts, collaborations with tourism companies using spotter seaplanes, and the use of scientific drones has facilitated field observations in remote or inaccessible areas (Stevens et al. 2018a; Ehemann et al. 2022; Rambahiniarison et al. 2022) and the collection of important behavioral and demographic data on mobulid species (Setyawan et al. 2020, 2022).

We examined the distribution and seasonality of reproductive events for three mobulid species (*M. mobular*, *M. thurstoni*, and *M. munkiana*) in the southern region of the Gulf of California, Mexico, to determinate (1) if reproductive grounds exist within the Gulf of California area for any of these species, (2) if reproductive behavior of the three species follows a seasonal pattern and (3) if courtship and mating behavior is similar among species. We present the first observations of courtship behavior for *M. mobular* and *M. thurstoni* and courtship and mating observations for *M. munkiana* in the Gulf of California from direct observations from citizen scientists, spotter seaplanes, and from research surveys by boat using drones and in-water observations. We clarify and extend the duration of the reproductive season previously proposed for *M. mobular*, *M. thurstoni*, and *M. munkiana* in the Gulf of California, describe a novel courtship behavior for *M. munkiana*, and an important reproductive area for *M. munkiana* in La Ventana and Ensenada de Muertos area, Gulf of California.

2. Methods

2.1 Study area

All reproductive behavior observations described in this study occurred in the southern region of the Gulf of California, along the east coast of the Baja California Peninsula, Mexico (Fig. 1, a, b). The area is characterized by sandy and rocky coastlines with deep depths (> 3700-m), small tidal ranges, and sea surface temperatures ranging between 20–30° C (Brusca et al. 2005). The ocean productivity in the area is influenced by a monsoonal wind pattern, with northwesterly winds causing upwelling events during the cold season (December to May), with an average primary production of 10-mg m⁻³ of sea surface chlorophyll a (Santamaría-del-Ángel et al. 1999, Lavín and Marinone 2003). During the warm season (June to November), strong thermal stratification occurs, with warm water from the Pacific entering the southern Gulf of California, and southeast winds create weak upwelling on the peninsula east coast with

an average primary production of 0.1-mg m^{-3} (Santamaría-del-Ángel et al. 1999, Lavín and Marinone 2003).

2.2 Data Collection

2.2.1 Boat surveys

Between May 2021 and June 2022, a total of 69 survey-days (between 2 to 24 surveyed days per month) were conducted in La Ventana and Ensenada de Muertos area (Fig. 1, b). Each survey consisted of a non-systematic transect of at least five hours (7am- 12pm) of observations from a small boat (panga) covering a fixed study area (Fig. 1, b) during conditions of Beaufort sea state ≤ 2 . Once an individual or mobulid group were located by sight (by their breaching behavior and/or swimming activity at or near the surface), the research boat remained $\sim 20\text{-m}$ distant from the animals with the motor switched off or in neutral to record group information. For each sighting, we recorded date, time, location, species observed and estimated number of individuals, behavior, and when possible, pregnancy status and maturity stage based on body size and presence of mating scars (females) or size of claspers (males). Following the collection of this information, a drone (DJI Mavic Pro 2) was launched from the boat and aerial observations were conducted flying at an altitude between 20 to 50 meters above ocean surface for 10–20 minutes. Finally, when possible, in-water observations were conducted by free divers taking video and/or still images using a GoPro7.

2.2.2 Citizen science data

Photographs and video files of mobulid reproductive events were collected from ecotourism guides and ecotourism boat captains in Baja California Sur (from La Paz to Cabo San Lucas, along the western Gulf of California coast) during 2017, 2021, and 2022. Images were elicited during public educational talks with local communities and submitted via email with information on the sighting date and location. All reported sightings for *M. mobular* and *M. thurstoni* were accompanied by photographic material to verify the accuracy of the data. *M. munkiana* sightings from citizen scientists that did not have associated photographic evidence were only considered if provided by guides trained ($n = 5$) in the collection of this data. These guides were trained on species identification and behavior recognition with the lead author of this study. All video and photographic materials are used with permission and include owner credit.

2.2.3 Seaplane data

During 2021–2022, spotter flights operated by private tourism companies were conducted on the southeastern portion of the Gulf of California, originating from La Paz, Baja California Sur. Although these flights did not follow a standardized transect, they were designed to spot megafauna in the area and all sightings of marine animals were recorded during the flight by trained observers with location, date, species, number of individuals or size of the group and, behavior. A total of 428 flights were conducted, each flight lasted between two to four hours for a total of 858.3 flight hours between January 2021 and December 2022. The total study area covered by these flights was $8,317\text{-km}^2$, and flights were

conducted during all months of the year (Fig. 1, b **Seaplane I**). During May-August 2022 seven flights covered an additional area of 8547-km² along the coast from Cabo San Lucas to Loreto (Fig. 1, b **Seaplane II**). The weather conditions during surveys were Beaufort sea state ≤ 3 , no clouds, with light and gentle breeze. The aircraft used for these flights were an SLA Seaplane or a Citabria flying at an altitude of $\sim 500\text{--}1000$ feet (152–305-m) at a ground speed between $\sim 45\text{--}69$ knots (83–128-km⁻¹).

2.3 Behavioral data and analysis

Reproductive behavior refers to courtship and mating events and was distinguished from other behaviors such as feeding and cruising following the criteria proposed by Stevens et al. (2018a) for mobulids. Near-term pregnancy was identified by the distended abdominal area on the dorsal and ventral surfaces in females (Marshall and Bennett 2010, Stevens et al. 2018a). Individuals observed in videos and photographs were counted using the software ImageJ (ImageJ) using the multipoint tool. To estimate effort-corrected reproductive observations we divided the number of observations per week (from boat surveys) and per month (from seaplane observations) between the days at sea for each method. Maps were created using Surface Mapping System (Golden Software, Inc., <https://www.goldensoftware.com/products/surfer>) and the coastline data was extracted from GEODASNG (National Geophysical Data Center, 2000).

3. Results

A total of 221 direct observations of courtship and mating behavior were recorded between March and August in 2017 ($n = 1$), 2021 ($n = 13$), and 2022 ($n = 207$). We observed 207 courtship events and one mating event for *M. munkiana*, ten courtship events for *M. mobular*, and three courtship events for *M. thurstoni*. We recorded 126 courtship events by seaplane, 64 by boat surveys, and 31 by citizen science images (Table 1). The single mating event we observed for *M. munkiana* was recorded during boat surveys using the drone. All courtship and mating events were observed between the surface and $\sim 5\text{-m}$ depth.

Table 1
Number of reproductive events (courtship and mating behavior) for each species and type of data collection method.

Species	Data collection method	Number of reproductive behavior events
<i>Mobula mobular</i>	Citizen science	3
	Seaplane	7
<i>Mobula thurstoni</i>	Citizen science	3
<i>Mobula munkiana</i>	Boat surveys	64
	Citizen science	25
	Seaplane	119
Total Events		221

3.1. Distribution and seasonality of courtship and mating behavior

All courtship and mating observations occurred during spring and summer months (Fig. 2), coinciding with the transition between the cold season (December to May) and warm season (June-November) in the southern Gulf of California. The peak of reproductive observations occurred during the month of May.

Sightings were dispersed along 312-km of the east coast of the Baja California Peninsula, from Cabo San Lucas to Ensenada de Cortes at the entrance of San Jose Island channel (SJI); however, the majority (n = 209) occurred within La Ventana (LV) and Ensenada de Muertos (EM) area (**Fig. 3**). Observations occurred between 4-m and 6.3-km of the coast, while the plane flew from 0 to 60 km offshore.

M. mobular

Courtship behavior was observed for *M. mobular* on ten occasions (Table 1) during the months of May (n = 5) in 2017, 2021–2022, July (n = 4) in 2022, and August (n = 1) in 2022. Sightings were dispersed more than 312-km along the east coast of Baja California Peninsula, from Cabo San Lucas to Ensenada de Cortes at the northern entrance of San Jose Island channel (**Fig. 3, a**). During a courtship event observed in May 2021, one of the females was pregnant in the last stages of gestation (evidenced by a highly distended abdomen).

M. thurstoni

Courtship behavior was observed for *M. thurstoni* on three occasions (Table 1) during the months of June (n = 1) in 2021, July (n = 1) in 2022, and August (n = 1) in 2022. Sightings were dispersed more than 43-km along the east coast of Baja California Peninsula, from El Saltito to Punta Arenas (**Fig. 3, b**).

M. munkiana

Courtship behaviors were observed for *M. munkiana* on 208 occasions (Table 1) during the months of March (n = 4) in 2022, April (n = 5) in 2022, May (n = 177), June (n = 16) and July (n = 3) during 2021–2022, and finally in August (n = 3) in 2022. The peak of sightings was during the month of May in both years. During this peak, increased courtship activity was observed for the last 10 days of the month. Sightings were dispersed more than 184-km along the east coast of Baja California Peninsula, from El Saladito in the Bay of La Paz to Cabo Pulmo (**Fig. 3**). Mating behavior was observed (n = 1) during a boat survey (28 May 2022) and filmed with the drone. In addition, the following day during a boat survey (29 May 2022) a large number of mating trains were observed in La Ventana over an extended area. A seaplane flight flying in a straight transect of 8.06-km in the area counted 102 mating trains on 29 May 2022.

3.2 Reproductive Behavior Description

Detailed descriptions of the courtship behavior of *M. mobular* and *M. thurstoni* are limited, while the courtship and mating behavior of pygmy devil ray species (particularly *M. munkiana*) have not been previously reported. Here we provide detailed description of reproductive behavior for these species.

M. mobular

The number of individuals involved in *M. mobular* courtship groups (n = 10 events) ranged from two to nine individuals, with the sex of individuals determined for three of these events (recorded by citizen science observations) (Fig. 4). Three of the seven stages of courtship and mating behavior (Stevens et al. 2018a) were recorded. Detailed descriptions of events are provided below.

On 5 May 2017 at Cabo San Lucas (Fig. 4, a-d) a group of nine individuals (two females and seven males) were observed engaging in courtship behavior. One of the females was chased by seven males at high-speed close to the surface (endurance), with one of the males approaching the female by the ventral side trying to push her towards the surface. The female started to perform backward somersaults, mirrored by two males (evasion) and later reduced speed, and stopped at the surface (pre-copulation positioning), while one of the males approached her from underneath.

Figure 3 (a) Southern portion of Baja California Peninsula, with reproductive events (colored dots) observed between 2017–2022 for *M. mobular* (green), *M. thurstoni* (yellow), *M. munkiana* (red). Black polygons indicate MPAs in the region: CSL, CP, ESA **(b)** Expanded detail in the area of La Ventana (LV), Ensenada de Muertos (EM), and Cerralvo Island (CI) as indicated by the yellow polygon in (a)

On 20 May 2021 at the Espiritu Santo Archipelago (Fig. 4, e-h), one near-term pregnant individual, was chased by three adult males (endurance), one of the males subsequently positioned himself on top of the female's dorsal surface attempting to reach and bite the left pectoral fin of the female (pre-copulation positioning). After two failed attempts the female performed backwards somersaults mirrored by the same male (evasion). Another event was observed in proximity and on the same day as Event 2 (Fig. 4, i-j). It involved one female and one male performing erratic movements and backward somersaults

(evasion) and the positioning of the male on top of the female, trying to reach the female's left pectoral fin (pre-copulation positioning).

M. thurstoni

The number of individuals in *M. thurstoni* courtship groups ranged between three to five, although courtship occurred within bigger groups (> 10) that included individuals not engaging in reproductive behavior. Three of the seven stages of courtship and mating behavior (Stevens et al. 2018a) were recorded for *M. thurstoni*. Detailed descriptions of events are provided below.

On 28 June 2021 at El Saltito beach (Fig. 5, a-d) a group of five individuals (one female and four males) were engaged in courtship behavior at the surface. A female with mating scars on her left pectoral fin was chased at high speed by three males (endurance). The female performed erratic movements, changes of direction, and somersaults (evasion). During these movements, the female was mirrored by a male directly behind her during the entire observation period. This male pushed the female towards the surface by approaching her from underneath, and then positioned himself on top of the female (pre-copulation positioning).

On the 1 July 2022 at Punta Arenas (Fig. 5, e-h) a female with fresh mating scars on her left pectoral fin was chased by three males in a mating train (endurance), while performing turns along its axis (evasion). The third male in the formation advanced position and speed to a position on top of the female's dorsal surface (pre-copulation positioning).

On the 5 August 2022 in La Ventana (Fig. 5, i-j) within a group of *M. thurstoni* (> 10), a mating train of two males was recorded chasing a female and performing backward somersaults (evasion).

M. munkiana

We observed a maximum of 29 *M. munkiana* individuals in reproductive groups. We recorded six of the seven stages of courtship and mating behavior (Stevens et al. 2018a) *M. munkiana*. Detailed descriptions of events are provided below.

On 19 May 2021 at Ensenada de Muertos (Fig. 6, a-c) a vortex formation at the surface was observed during a five-hour period (**Supplementary material, Video 1**). During this event, females and males (n = 122) were swimming at a consistent speed, circling in a clockwise direction, swimming up and down to a maximum of 5-m depth in the column of water. No feeding was observed, yet numerous small mating trains (> 20) where a female was chased by one to three males regularly occurred (Fig. 6, a). The individuals in mating trains did not separate more than 5–10-m from the main formation, returning to the courtship vortex after a few seconds of chase (endurance). No visible pregnant females were observed within the group; however, maturity in males was estimated by the presence of elongated claspers beyond their pelvic fins. Females presented mating scars and recent abrasions on the lower area of their dorsal side probably produced by the pre-copulatory positioning of the male on top of them. Breaching behavior was not observed in the vortex during the five-hour observation period.

On 28 May 2022 at La Ventana (Fig. 6, f-i) a group of 29 individuals (one female and 28 males) were engaging in courtship behavior (**Supplementary material, Video 2**). The group was observed for 45 minutes and included one female swimming very slowly within a small area at the surface while the rest of individuals (28) were circling, chasing her, and swimming from behind positioning themselves on top of her, at least 135 times during the observation period. Male individuals chased each other away when in proximity to the female. On some occasions one male lifted the female to the surface from underneath while others rushed from behind and swam on top of her (pre-copulation positioning). Up to three males swam on top of the female at the same time. However, the most common behavior was when males, one at a time, lifted the female from the ventral part and then slid along one of the female's pectoral fins and swam on top of her. When on top of the female, males erected their tail dorsally at 90°, bending their pelvic area and making rapid pelvic thrusts. On one occasion, we observed a successful copulation attempt where the male swam on top of the female and started to make rapid pelvic thrusts, they both subsequently sank to the bottom while spinning around (copulation) for 14 seconds. The pair then separated, and the female swam off to the surface (separation). Immediately after, the same courtship behavior continued with this female and the rest of the males.

On 29 May 2022 at La Ventana (Fig. 6, d-e), 102 mating groups were observed at the surface. Courtship and mating behavior consisted of mating trains (initiation, endurance, and evasion) dispersed within La Ventana area. Mating groups occurred at the surface, while larger groups of *M. munkiana* remained a few meters below. On many occasions several courtship events occurred in close proximity. Mating trains were dynamic with males switching chasing one female to another nearby female.

3.3 Associated observations

Pregnancy

M. mobular (n = 1) and *M. munkiana* (n = 10) females showing evidence of later-term pregnancy with a distended abdominal area on both the dorsal and ventral surfaces (Marshall and Bennett 2010) were observed engaging in courtship behaviors during the months of May and June 2021–2022 (Fig. 7, a). We confirmed our external evidence of late-term pregnancy using ultrasound on *M. munkiana* individuals (Palacios unpubl data).

Mating Scars

Parallel scratches and abrasion on females' left pectoral fins (dorsal or ventral) resulting from the teeth of conspecific males were observed in *M. thurstoni* (Fig. 7, b, c). We recorded old mating scars on a female on the ventral part of the left pectoral fin at El Saltito beach during a courtship event (June 2021) (Fig. 5, a-d), and fresh mating scars on a female engaging in mating train at Punta Arenas (July 2022) (Fig. 5, e-h).

We recorded mating scars in *M. munkiana* on the left pectoral fins (Fig. 7, d) as well as the posterior half of the disc on their dorsal side (Fig. 7, d-f) in near-term pregnant females or in adult females. Pregnant

females showing abrasions on their lower dorsal area didn't always have pectoral fin mating scars.

Breaching behavior

We define breaching behavior as an arial behavior where individual mobulids accelerate rapidly towards the surface, propelling themselves clear of the water. We only recorded breaching groups in *M. munkiana*, although single individuals of *M. thurstoni* were also frequently sighted breaching out of the water. During boat surveys (n = 64), *M. munkiana* breaching groups were recorded (n = 118) between 2021–2022. We observed breaching behavior in individuals of all maturity stages (juveniles, adults, pregnant) and sexes (male and female) (Fig. 8, a-d). During some of the breaches, the individuals shook their pectoral fins when they had a remora attached to their bodies. During courtship and mating behavior, breaching was not observed among individuals that were engaging in mating. Near-term pregnant females breaching were a common sight during the months of May and June (Fig. 8, d-f). Different kinds of jumps were observed for this species, breaching forward to land on the ventral surface, breaching and landing on one side, as well as breaching to land on the dorsal surface.

4. Discussion

Distribution and seasonality

This study extends the reproductive season described for three (*M. mobular*, *M. thurstoni*, *M. munkiana*) of the five mobulid species present in the Gulf of California. Previously, *M. mobular* and *M. thurstoni* were reported to reproduce during June and July, inferred from dead specimens from artisanal fisheries (Notarbartolo-di-Sciara 1988; Guerrero-Maldonado 2002; Serrano-Lopez et al. 2021). However, based on direct observations of living animals in this study, reproductive behavior occurs from May to August for *M. mobular*; two months longer than previously reported. We found that the reproductive season for *M. thurstoni* occurs from June to August; one month longer than previously reported (Serrano-Lopez et al. 2021). *M. munkiana* was observed displaying reproductive behavior from March until August; an extension of three months (Palacios et al. 2021). In the south-west Gulf of California, these three species are found in greatest abundance during the spring and summer (March-July) (Notarbartolo-di-Sciara 1988; Palacios et al. 2021; Serrano-Lopez et al. 2021), likely correlated to the abundance peak of their main prey, the euphausiid *Nyctiphanes simplex* (Gendron 1992; Sampson et al. 2010). This time frame also coincides with the transition from the cold season (December-May), when northwest winds lead to lower sea surface temperature (SST) between 21–24 °C, to the warm season (June-November), when weaker winds from the southeast bring warm tropical water from the Pacific and SST are between 27–31 °C (Herguera et al. 2001; Lluch-Cota et al. 2007).

The extension of these reproductive seasons could be the result of the combination of our larger study area throughout the year than previous studies (Notarbartolo-di-Sciara 1988; Guerrero-Maldonado 2002; Serrano-Lopez et al. 2021; Palacios et al. 2021) and the different observational methodologies used (drone, in-water observation, citizen science, and seaplanes) wich have proven to be useful in the collection of behavioral data (Fiori et al. 2017; Stewart et al. 2018; Oleksyn et al. 2021; Palacios et al.

2023). Direct observations of courtship events for *M. mobular* and *M. thurstoni* have been previously reported at offshore areas and remote archipelagos (Duffy and Tindale 2018; McCallister et al. 2020). In our study, reproductive events occurred between 4-m to 6.3-km from the Baja California Peninsula coastline or adjacent islands. The southern Gulf of California is characterized by steep slopes, with a narrow shelf and deep basins where enriched waters contribute to a high primary production close to the coast (Lavín and Marinone 2003; Lluch-Cota et al. 2007). The greatest number of reproductive events occurred at La Ventana, a channel with a maximum width of 17-km and 293-m depth between the peninsula coast and the island of Cerralvo (Nava-Sanchez et al., 1995), and at Ensenada de Muertos, where the ocean floor falls to 300-m within the first 2-km from the coast. These bathymetric features, in conjunction with high seasonal productivity during spring (Lluch-Cota et al. 2007), may favor high abundance of food and optimal conditions for the aggregation of large numbers of mobulids, facilitating the encounter of potential mating partners; similar to other reproductive aggregations in other elasmobranchs (Sims et al. 2022; Palacios et al. 2023).

Reproductive behavior

Reproductive behavior for manta ray species is well established in seven stages (Stevens et al. 2018a) of which we observed six during this study; endurance, evasion, and pre-copulation positioning were the most common. However, during observed pre-copulation positioning, males first lifted the female towards the surface by positioning themselves underneath, instead of positioning themselves on top of the female's back, as previously described (Stevens et al. 2018a). This could be a strategy to trap the female at the surface and avoid movement into deeper waters that may more easily facilitate evasion.

Male biting of the pectoral fins of the female during reproductive events is a common behavior in elasmobranchs (Klimley 1980; Uchida et al. 1990; Pratt and Carrier 2001; Marshall and Bennett 2010), and in mobulids is included in the pre-copulation positioning phase (Stevens et al. 2018a). This behavior enables the proper positioning of the male for the insertion of the claspers in the female cloaca during copulation (Kajiura et al. 2000) and results in female pectoral fin abrasions, wounds, and permanent scars (Yano et al. 1999; Marshall and Bennett 2010; Stevens et al. 2018a). In contrast to manta rays (*M. birostris* and *M. alfredi*), devil ray species have teeth on both jaws (Notarbartolo-di-Sciara 1987; Stevens et al. 2018b), leaving mating scars on both sides of the females' pectoral fins when biting occurs. Mating scars (Marshall and Bennett 2010; Stevens et al. 2018a) (Fig. 7, b-d) were present in *M. thurstoni* individuals on the dorsal and ventral sides of their pectoral fins, while *M. munkiana* individuals presented scars on the posterior half of the disc. Since we did not observe males rubbing the backs of the females with their cephalic fins at any time (Stevens et al. 2018a), it is possible that these dorsal scars could be the result of the swimming on top of the female from behind as the males attempt to copulate, as recorded on 28 May 2022 at La Ventana (Fig. 6, f-i). During mating, *M. munkiana* males did not always wait until pre-copulation positioning (e.g., biting the female's pectoral fin) before erecting their tail, bending their pelvic area and making rapid pelvic thrusts for copulation. Whether *M. munkiana* always needs to engulf the female's pectoral fin for a successful insertion of the clasper into the cloaca remains unclear based on our observations of mating behavior. This is also supported by our observation of

pregnant females with dorsal scars but without biting scars on their pectoral fins (Fig. 7, f), as well as the high flexion capability of elasmobranch claspers (Tricas 1980; Pratt and Carrier 2001), which is up to 180° for cownose ray (*Rhinoptera javanica*), a similarly sized species (Uchida et al. 1990).

For *M. munkiana*, we describe a new courtship behavior: the courtship vortex. Vortex formations are regularly performed by *M. munkiana* in the La Ventana area, probably for predator avoidance and as a coordinated feeding strategy (Palacios et al. in prep), similar to the cyclone feeding of *M. alfredi* in Maldives (Stevens 2016; Armstrong et al. 2021). However, we hypothesize that the courtship vortex observed in this study has a reproductive function based on the absence of feeding behavior or visible predators in the area (Higuera-Rivas et al. 2023) throughout the five hours of the encounter. Further, the presence of only sexually mature individuals of both sexes (males with elongated claspers and females with mating scars) and the observation of mating trains entering in and out of the main formation indicates that this may represent a social and reproductive aggregation where adults assess and chose potential mates before engaging in individual mating trains and copulation. The structure and speed of the vortex allowed physical contact among most of the individuals within the vortex, especially those situated in the center. Similar behaviors have been studied for basking sharks (*Cetorhinus maximus*) (Sims et al. 2022) but have never been reported for mobulid species (Yano et al. 1999; Pratt and Carrier 2001; Marshall and Bennett 2010; Deakos 2011; Stevens 2016; Stevens et al. 2018a; Mendonça et al. 2020).

Breaching behavior

Breaching behavior, or jumping, is a commonly observed behavior in elasmobranchs, with several species (thresher shark, basking shark, white shark, eagle rays, and blacktip sharks) breaching for various reasons including feeding, mating, parasite removal, and predator avoidance (Curtis and Macesic, 2011, Berthe et al. 2018; Gore et al. 2019). Breaching behavior for the mobulidae family has been hypothesized as a form of signaling mechanism to aggregate for reproduction (Marshall and Bennett 2010; Medeiros et al. 2021; Stevens et al. 2018a), where the sound produced in the water after the jump helps the animals communicate their position and size during courtship events (Stevens 2016). While breaching behavior may be related to reproductive behavior, it is important to note that we observed all maturity stages (juveniles, adults, and pregnant females) and sexes breaching. Additionally, we found that breaching occurred in all months we observed mobulids in the Gulf of California, including outside of the reproductive season. Based on this evidence we hypothesize that most breaching events for *M. munkiana* may be a form of communication meant to attract other groups or individuals to a certain area to feed, perhaps cooperatively, or as a predator avoidance mechanism. These functions have been suggested for *M. alfredi* during coordinated feeding events (cyclone feeding) in Maldives (Stevens 2016; Armstrong et al. 2021) and for *M. birostris* in estuarine environments in Brazil (Medeiros et al. 2021). Breaching behavior may also be related to parasite removal: during some of the breaching events, we observed *M. munkiana* actively shake pectoral fins where remoras were attached, and similar behavior has been observed for other elasmobranchs (Ritter and Brunnschweiler 2003; Brunnschweiler 2006).

5. Conservation implications

The southwestern Gulf of California is a reproductive area for *M. mobular*, *M. thurstoni*, and *M. munkiana*, based on our observations and on histological and morphological studies previously conducted in the area (Notarbartolo-di-Sciara 1988; Serrano-Lopez et al. 2021). Reproductive behavior for these three species has been observed in this area from March to August. This period extends three months outside of the currently established elasmobranch fishing ban in the Mexican Pacific, which extends from the first of May to the first of August. Although mobulids have been protected from target fisheries in Mexico since 2006 (NOM-029-PESC-2006), gillnets, longlines and simpleras (bottom-fixed lines with baited hooks) are used in the Gulf of California by artisanal and industrial fisheries to legally fish for stingrays and sharks during the other nine months of the year (Bizzarro et al. 2009). These fisheries, combined with industrial purse seiners targeting tuna (Croll et al. 2016; Del-Valle-González-González 2018; Lezama-Ochoa et al. 2019), shrimp boats, illegal fisheries targeting mobulids in the area (Palacios unpubl data), and mobulid habitat overlaps with that of commercial species, are the main threats to mobulids in the study area (Croll et al. 2016; Lezama-Ochoa et al. 2019). *M. mobular* and *M. thurstoni* are the dominant species captured as bycatch in industrial fisheries in the region (Lezama-Ochoa et al. 2019). While the extent of the impact of bycatch and target fisheries on *M. munkiana* is unknown across its range (Alfaro-Cordova et al. 2017, Lezama-Ochoa et al. 2019), high rates of mobulid bycatch in artisanal gillnets have been observed within the study area (Del-Valle-González-González 2018; Palacios unpubl data). The lack of spatial management for shark and ray fisheries (Bizzarro et al. 2007) and the use of non-selective gear (gillnets) in Mexico, coupled with the limited enforcement of existing regulations potentially puts critical habitats at risk, even for protected species like mobulids (Salomón-Aguilar 2015; Jabado et al. 2023). Establishing greater spatial and temporal restrictions on the use of gillnets along the coast and islands at the southwestern Gulf of California, especially around critical habitats such as reproductive grounds (La Ventana and Ensenada de Muertos area) and nursery areas (Espíritu Santo Archipelago) (Palacios et al. 2021) may help reduce the impact of bycatch of these species during key life periods.

The results of this study indicate reproductive areas are within 6.3-km of the coast, where we observed surface aggregations of the three mobulid species. This distribution makes them vulnerable not only to bycatch, but to other anthropogenic threats such as an increasing coastal development in the region and the associated sound and chemical pollution, habitat loss, and boat traffic. During our surveys we observed reproductive behavior at the surface, creating the potential for individuals engaging in reproduction to be exposed to boat strikes potentially resulting in lethal or sublethal injuries (Womersley et al. 2022; Strike et al. 2022). Furthermore, currently unregulated tourist activities offering free diving and snorkeling with mobulids, especially (*M. munkiana*), is growing in the area, providing new economic opportunities to local communities. Currently, little is known about the extent to which these activities affect the reproductive behavior and movement patterns of these species. However, unregulated tourism has negatively impacted manta rays in the Maldives, Australia (Venables 2013; Venables et al. 2016) and Mexico (Gómez-García et al. 2021), showing to disrupt or stop natural behaviors during 37% of the observations (Murray et al. 2020). The establishment of management measures, including studies of the optimal carrying capacity for mobulid tourism (Zekan et al. 2022), and codes of conduct to observe and

interact these species may help mitigate the negative impacts of tourism activities (Murray et al. 2020) at reproductive grounds while offering economic benefits to local communities (O' Malley et al. 2013).

Only four of the 222 observations of reproductive behavior occurred inside a marine protected area (MPA) (Espiritu Santo Archipelago and Cabo Pulmo), with only Cabo Pulmo being a strict no-take MPA. This is of potential concern because mobulids are believed to give birth immediately after mating (Stevens et al. 2018a). This is supported by our observations of heavily pregnant *M. mobular* and *M. munkiana* females engaging in courtship behavior indicating it is likely that reproductive areas are located adjacent to these nursery areas, as previously described for *M. munkiana* (Palacios et al. 2021). This suggests that further spatial protection of reproductive areas could be useful to simultaneously protect both nursery and mating areas. Recently, several Important Shark and Ray Areas (ISRA) (Hyde et al. 2022) have been established in the Mexican Pacific and Gulf of California (Jabado et al. 2023), however, some of the critical habitats for reproductive behavior reported in this study such as the Ensenada de Muertos area are not covered inside the designated polygons of ISRA.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethical approval

Research was conducted under the research permit PPF/DGOPA-024/20 issued by Comisión Nacional de Acuacultura y Pesca with authorization of Comisión Nacional de Áreas Naturales Protegidas. No specimens were collected during this research. The drone used in this study is registered at the AFAC under the folder MX-F-2-2303-036.

Data availability

The dataset of reproductive events observations used during the current study is available from the corresponding author on reasonable request.

Contributions

M.D.P., J.D.S., M.R.C., N.L.O., K.M.Z., R.G.A., F.G.M., and D.A.C. conceived of the paper. M.D.P., S.V.H., S.A.K.H.M. conducted the fieldwork. M.D.P. wrote the main manuscript text. M.D.P., and A.T.R. designed and elaborated the figures. All authors contributed to writing and editing the manuscript. All authors contributed to the article and approved the submitted version.

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Figures

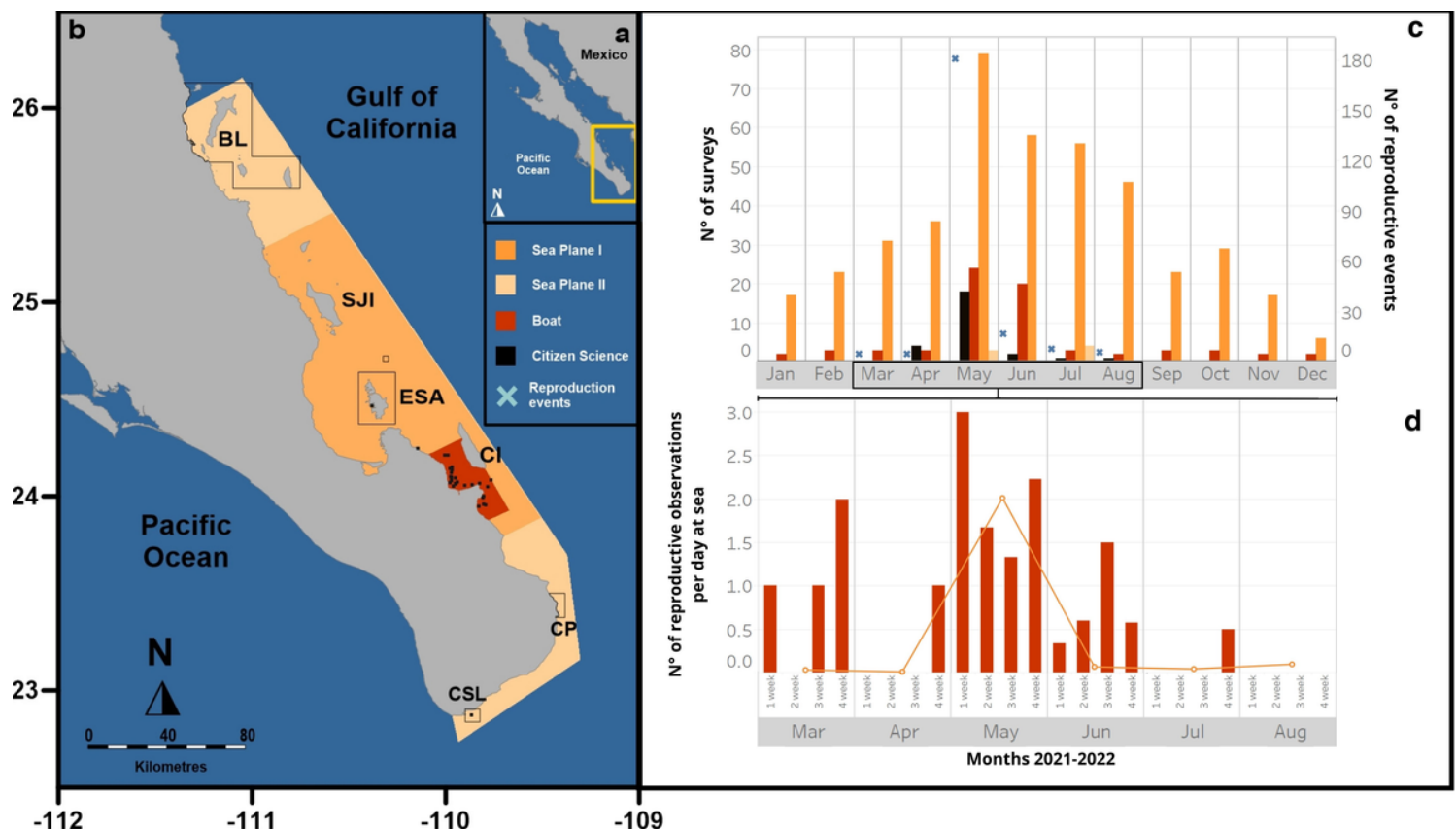


Figure 1

(a) Location of Baja California Peninsula in Mexico **(b)** Study area in the southwestern region of the Gulf of California. Black polygons indicate MPAs in the region: Cabo San Lucas (CSL), Cabo Pulmo (CP), Espiritu Santo Archipelago (ESA) and Bahía de Loreto (BL). Orange and yellow polygons are the areas

surveyed by seaplanes, the red polygon is the area covered by boat surveys near Cerralvo Island (CI) and the black points are sightings reported by citizen scientists. **(c)** Survey effort (number of surveys, left axis) during 2021-2022 for two seaplanes, boat surveys, and sightings days from citizen scientists (colors correspond to the legend in panel b), and the number of reproductive events (right axis) observed (blue cross x). **(d)** Effort-corrected reproductive observations by two seaplanes (orange line) and boat surveys (red bars) from March to August

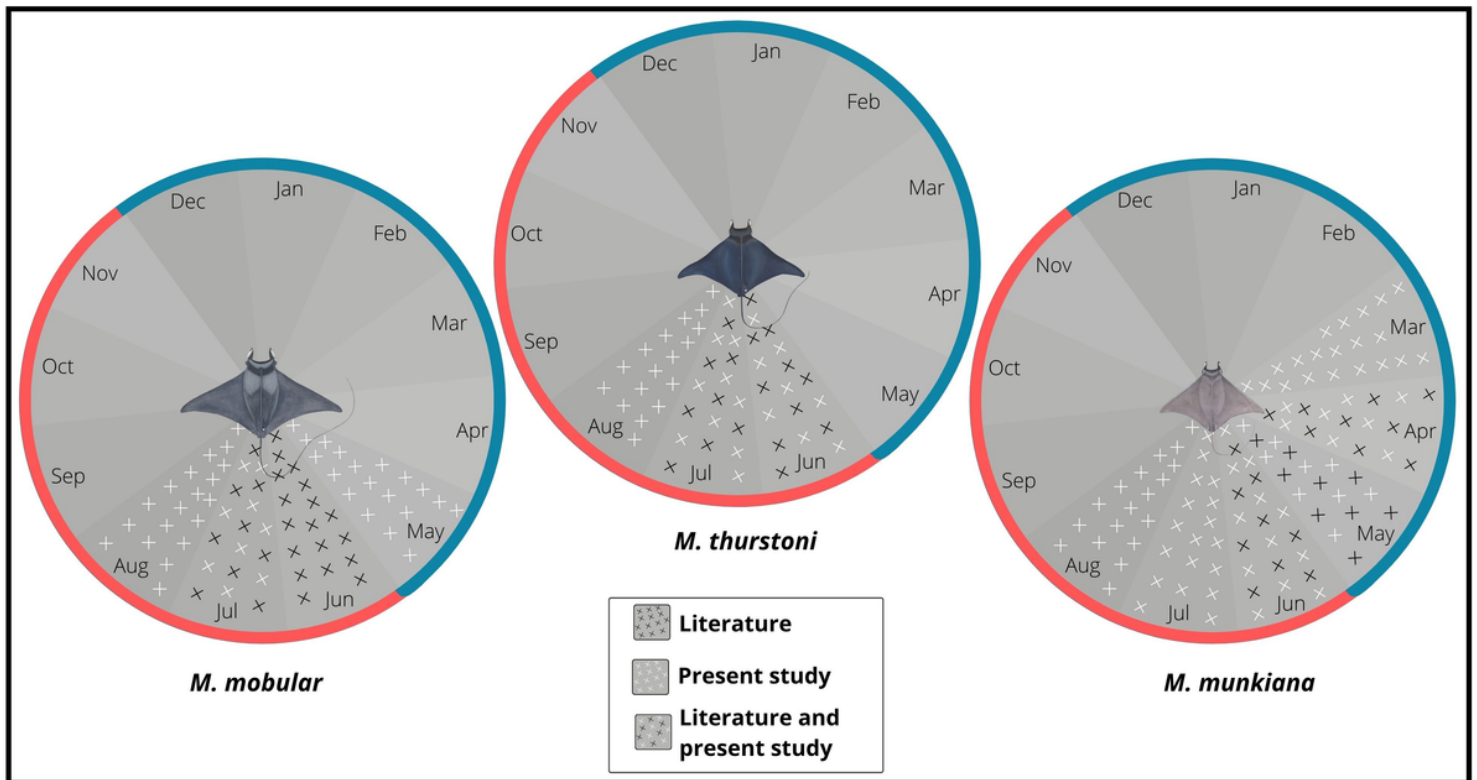


Figure 2

Courtship and mating seasonality of *M. mobular*, *M. thurstoni* and *M. munkiana* in the southern Gulf of California based on fisheries information from previous research (Notarbartolo-di-Sciara 1988; Guerrero-Maldonado 2002; Serrano-Lopez et al. 2021) and from direct observations reported on this study. Red indicates warm water months, while blue indicates cold water months. Black crosses (x) indicate courtship and mating from the literature, and white crosses (x) indicate courtship and mating observations in the present study. Mobula species illustrations by Julie Johnson, Life Science Studio

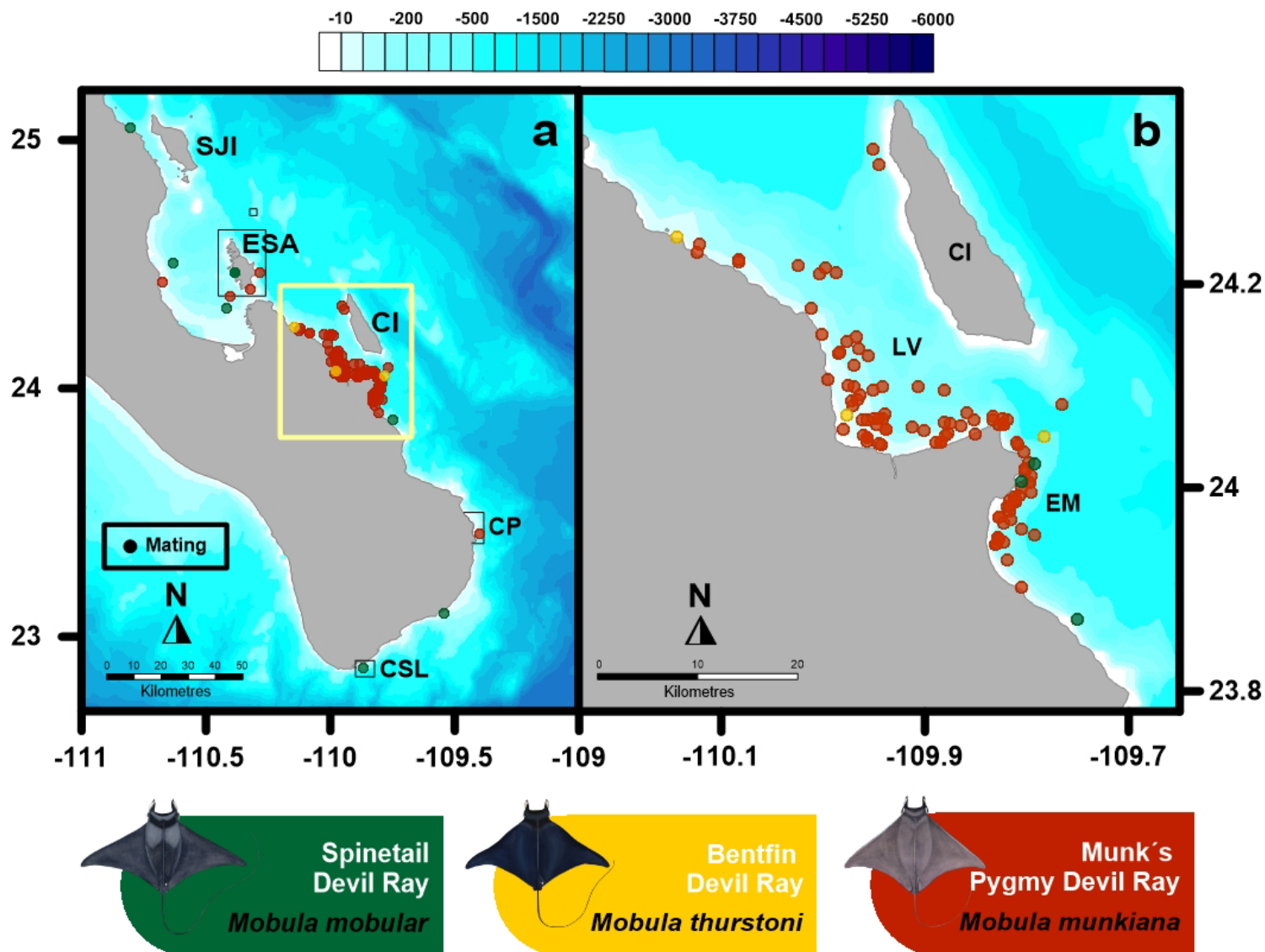


Figure 3

(a) Southern portion of Baja California Peninsula, with reproductive events (colored dots) observed between 2017-2022 for *M. mobular* (green), *M. thurstoni* (yellow), *M. munkiana* (red). Black polygons indicate MPAs in the region: CSL, CP, ESA **(b)** Expanded detail in the area of La Ventana (LV), Ensenada de Muertos (EM), and Cerralvo Island (CI) as indicated by the yellow polygon in (a)



Figure 4

M. mobular courtship events (a, b) endurance, (c) evasion, (d) pre-copulation positioning. Photo © Marta D. Palacios (e) endurance, (f, g) pre-copulation positioning, and (h, i) evasion and (j) pre-copulation positioning. Photo © Paulo Gómez Aldana



Figure 5

M. thurstoni courtship events. **(a, b)** endurance, **(c)** evasion, **(d)** pre-copulation positioning. Photo © Adriá Bosch-Soler **(e)** endurance, **(f, g, h)** evasion and **(g)** pre-copulation positioning. Photo © Maru Brito **(i)** evasion and **(j)** pre-copulation positioning. Photo © Afelandra González-Cibrián

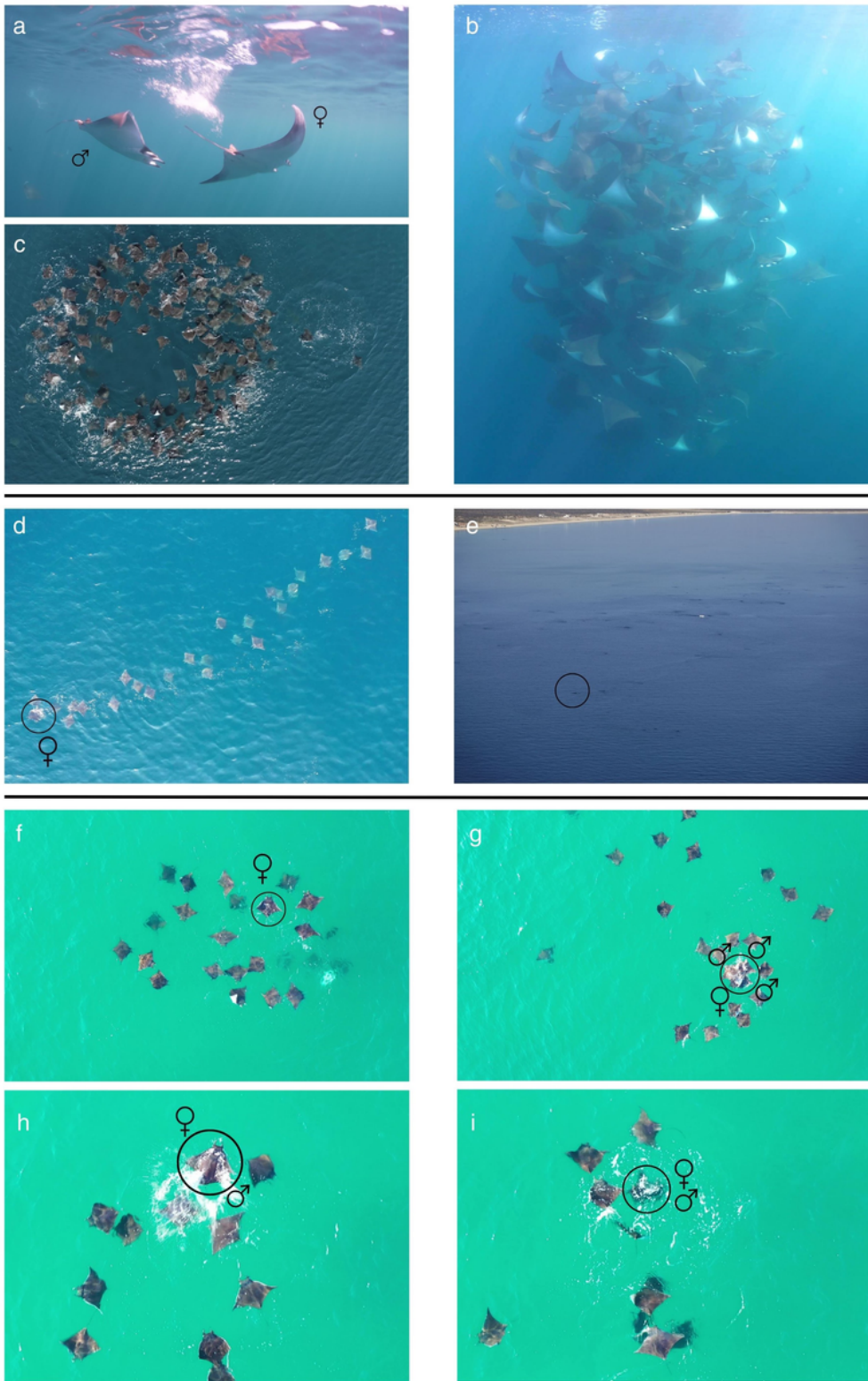


Figure 6

M. munkiana courtship and mating event **(a)** endurance and **(b, c)** courtship vortex. Photo © Shawn Hendrich and Jay Clue **(d)** endurance and **(e)** view of mating trains from the seaplane in a circle. Photo © Sidharta Velázquez-Hernández. **(f)** Evasion, **(g, h)** pre-copulation positioning and **(i)** copulation. Photo © Marta D. Palacios

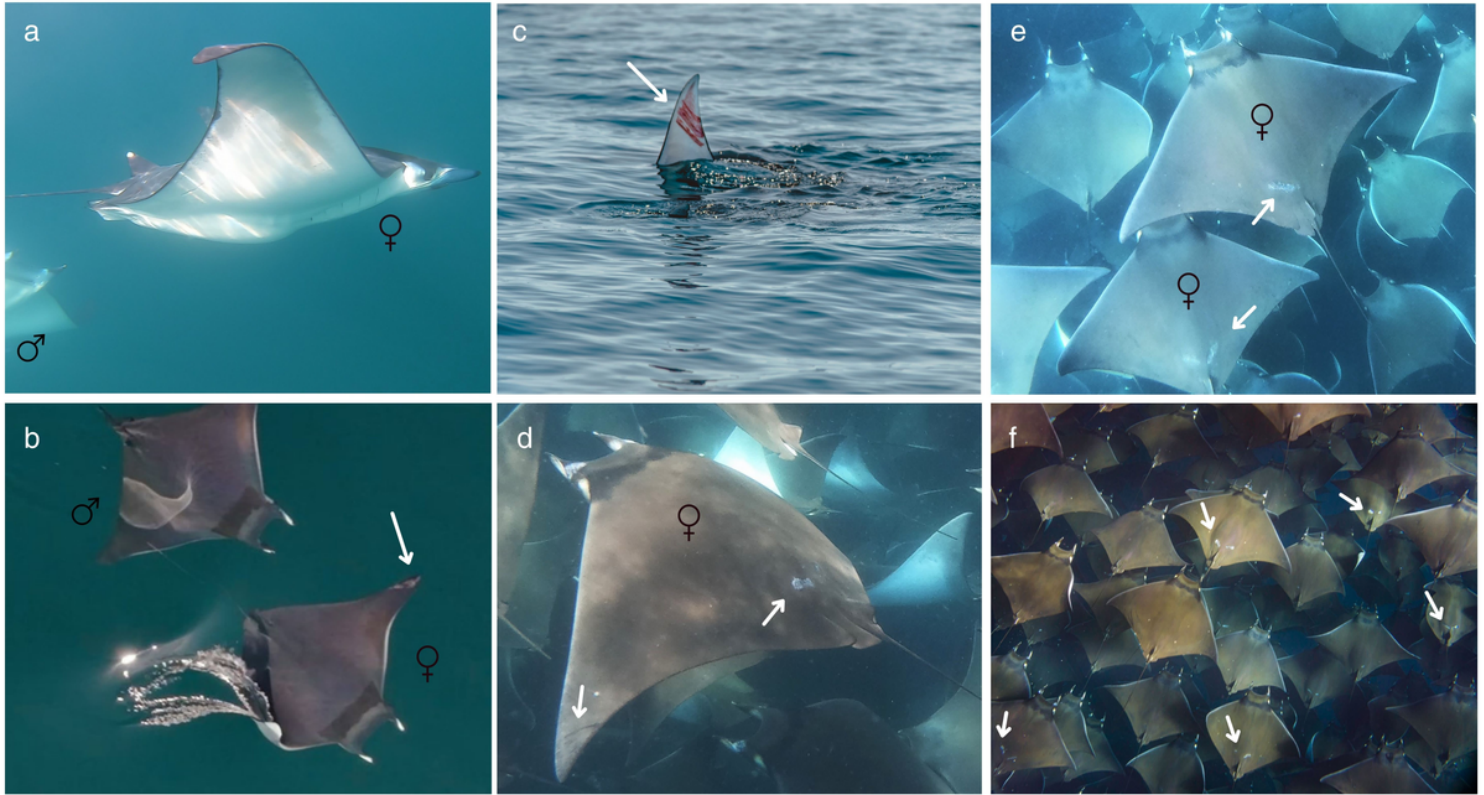


Figure 7

(a) Near term *M. mobular* engaging in a courtship event in May 2021. Photo © Paulo Gómez Aldana **(b, c)** Female *M. thurstoni* with fresh mating scars indicated by white arrows. Photos © Maru Brito and Cecilia Mar-Ruiz **(d)** *M. munkiana* females with posterior dorsal scars and bite mating scars on pectoral fin indicated by white arrows. Photo © Marta D. Palacios **(e, f)** *M. munkiana* females with posterior dorsal scars indicated by white arrows. Photos © Marta D. Palacios and Karissa Nanetta

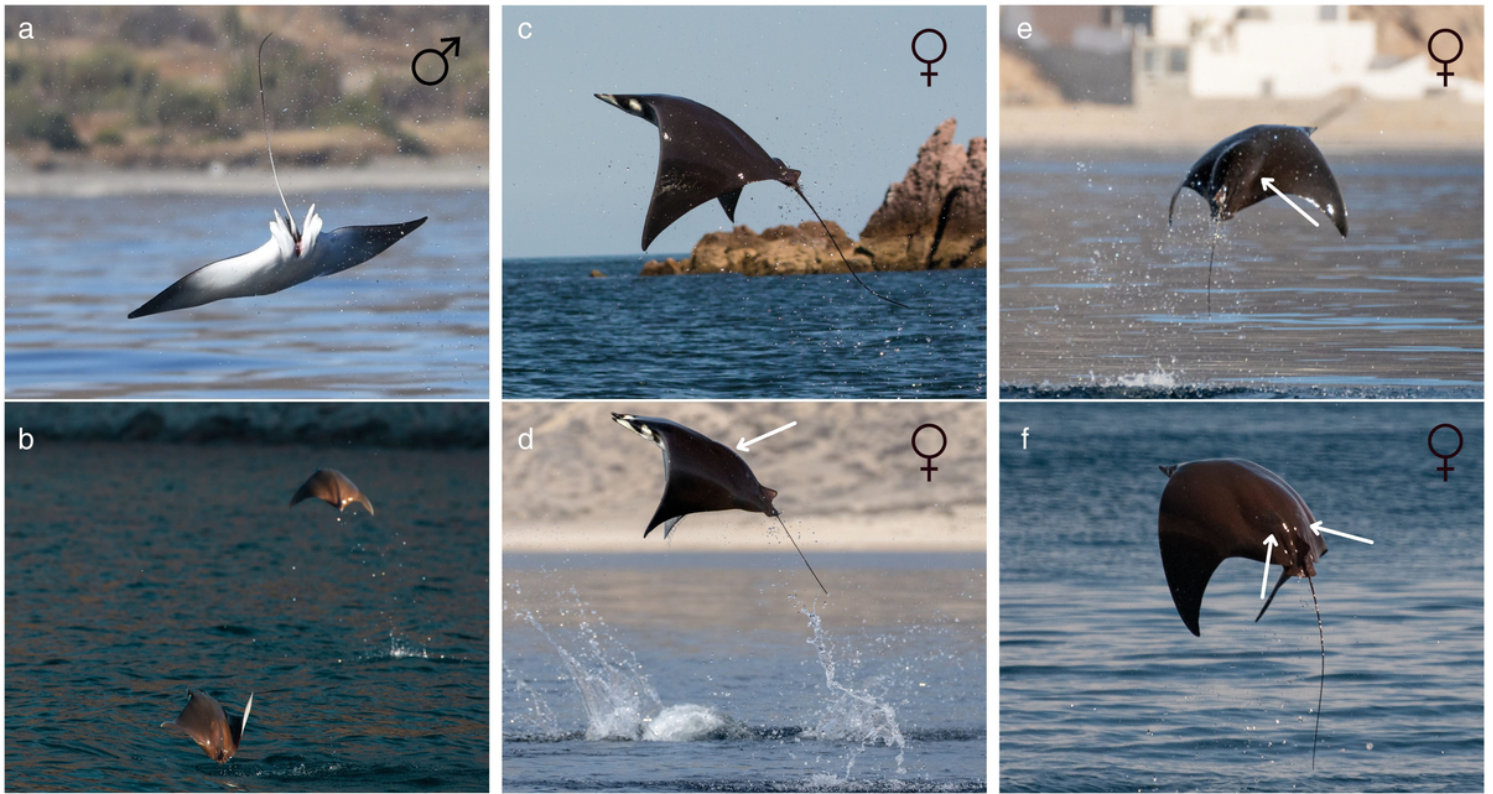


Figure 8

M. munkiana breaching behavior **(a)** Adult male. Photo © Blanca Idalia González-Garza **(b)** Juveniles. Photo © Juan Camilo Mora-Parra **(c)** non-pregnant female. Photo © Antoni Murcia **(d, e)** Pregnant females with fetal bulge on their dorsal side indicated by white arrows. Photos © Jay Clue **(f)** Pregnant female with fetal bulge and mating scars on its dorsal side indicated by white arrows. Photo © Antoni Murcia

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