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# Observations of reproductive behaviors in the Critically Endangered bowmouth guitarfish *Rhina ancylostoma*

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ABSTRACT: Wedgefishes, family Rhinidae, are collectively identified as one of the most threatened groups of chondrichthyan fishes globally by the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species. Within the family Rhinidae, bowmouth guitarfish *Rhina ancylostoma* have been designated by the IUCN as Critically Endangered since 2018. Bowmouth guitarfish were first introduced to the Association of Zoos and Aquariums (AZA) in 2005 when the Newport Aquarium, Newport, KY, USA, acquired a single female. In 2007, a male bowmouth guitarfish was added into the collection with the goal of establishing an institutional breeding program and to generate foundational knowledge for future breeding programs. This study presents an ethogram of mating behaviors exhibited by a mixed sex group of bowmouth guitarfish using behavioral observations from December 2017 through August 2022. The observed interactions between male and female bowmouth guitarfish led to the identification of 5 male reproductive behaviors: hovering, chasing, wrapping up, flipping/rolling, and copulation. These 5 behaviors were directly linked with successful reproduction, e.g. active breeding and live parturition, in the imperiled species *R. ancylostoma*. The breeding behavioral knowledge presented within this study provides the first step to a viable breeding program, a critical tool for conserving wild populations.

 $KEY WORDS: \ Shark \ ray \cdot Wedge fish \cdot Breeding \ program \cdot Reintroduction \cdot Elasmobranch \ breeding \cdot Behavioral \ ethogram$ 

## 1. INTRODUCTION

Whether living *in situ* or *ex situ*, reproduction is undeniably the key to the survival of all species (Comizzoli et al. 2019). Because of the increasingly altered status of wildlife habitats, to mitigate extinction, conservation often includes *ex situ* strategies, such as breeding programs in aquariums and zoos (Chargé et al. 2014). The majority of elasmobranch reproductive behaviors reported in literature have been observed in animals under human care, with reference to 100 chondrichthyans known to have exhibited reproductive behaviors or reproduced in aquariums and zoos

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(Henningsen et al. 2004). Two significant aspects of elasmobranch life history benefiting from aquarium collections are reproductive biology and the characterization of mating behaviors (Feldheim et al. 2022).

*Rhina ancylostoma*, commonly known as bowmouth guitarfish or shark rays, were first described in 1801 (Bloch & Schneider 1801). However, the species is rarely observed *in situ*, and little data exists detailing its life history. With most referenced sightings emanating from either targeted fisheries or as by-catch within the fisheries industry (Devadoss & Batcha 1995, Hartoko et al. 2020, Kyne et al. 2020), bowmouth guitarfish are not often observed in their natural habitat

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(S. Venables pers. comm.). According to the International Union for Conservation of Nature's (IUCN) most recent assessment (Kyne et al. 2019), it is estimated that the bowmouth guitarfish has undergone an 80% population decline over the last 3 generations (45 yr). This information has been calculated using data collected for the giant guitarfish *Glaucostegus typus*, in which an estimated 15 yr generational length was applied to all large rhinid species, including bowmouth guitarfish (White et al. 2014).

Following their listing as Critically Endangered, the Association of Zoos and Aquariums (AZA) identified bowmouth guitarfish as a species of interest in 2019 and established a studbook program documenting the pedigree and demographic history of the managed population. As of its 2022 publication, the Bowmouth Guitarfish AZA Regional Studbook indicated a total of 17 (7.10.0; male.female.unknown) bowmouth guitarfish managed across 9 AZA institutions (Hazeres 2022). The European AZA (EAZA) also published a studbook listing the total number of bowmouth guitarfish as 4.6.0 individuals distributed between 7 EAZA institutions (Walenciak 2022). Collectively, these 16 institutions represent facilities in North America, Europe, and Asia. Additionally, while these studbooks identify 27 individual specimens, only 7 of those institutions have managed mixed sex populations.

Understanding the reproductive strategies of any species is an integral aspect of both breeding programs and conservation. Reproductive behaviors of the bowmouth guitarfish have been observed at Newport Aquarium since the pairing of a single male and female in 2007. The initial acquisition of the first female occurred in 2005, followed by a male in 2007. The second female and male were separately introduced into the exhibit in 2013, with the female entering in February and the male in March. This timeframe represents the longest documented period of mixed sex management of bowmouth guitarfish in both AZA and EAZA history. By maintaining the bowmouth guitarfish in an environment that allows them to successfully breed, Newport Aquarium has had the unique opportunity to observe and detail reproductive behaviors for more than one estimated generational length that are otherwise rarely witnessed in threatened elasmobranchs. The ethogram presented in this paper is the first detailed description of successful breeding behavior of bowmouth guitarfish. By documenting these behaviors, this study aims to contribute to future breeding programs and the potential augmentation of critically endangered wild populations.

## 2. MATERIALS AND METHODS

#### 2.1. Habitat

The bowmouth guitarfish observed in this study were collectively managed within a 1457384l closed salt water system ranging in depths from 1.2 to 6.1 m (Fig. 1). The exhibit, identified as 'Shark Tank', features flat open areas of crushed aragonite substrate and vertical rock structures. A series of 3 acrylic tunnels run through the tank dividing the space, with the shallowest depth measuring 1.2 m. The largest area of open tank floor within the exhibit is located north of the tunnels. This space has a depth of 3.1 m and represents an area of 195 m<sup>2</sup>. The system also includes a deep well located underneath the easternmost of the 3 tunnels. This area is 6.1 m at its deepest and is openly accessible to collection animals by swimming underneath the third acrylic tunnel. During the study timeframe, water temperature ranged from 21.8 to 25.4°C, with an average temperature of 23.5°C. Seasonal changes in temperature were minimal with temperatures in the summer averaging only 0.5°C higher than those in the winter months. The exhibit light cycle was generally maintained on a 12 h photoperiod.

### 2.2. Study organisms

Throughout the study, bowmouth guitarfish individuals were not continuously housed in the Shark Tank, resulting in 2 phases with differing sex ratios. In Phase 1, the sex ratio of wild-caught mature bowmouth guitarfish within the exhibit was 2.2.0. This phase occurred from December 13, 2017, to January 10, 2018, and June 15 to September 18, 2018. Dates from January 10 to June 15, 2018, were excluded because individuals were intermittently removed to alternative sites. The second phase of the study began when the second male, on an AZA breeding loan, was returned to the loaning institution on September 18, 2018. For the remainder of the study (herein referred to as Phase 2), animals were observed in a 1.2.0 ratio.

From December 2017 to August 2022, the animals were individually handled for morphometric data collection on a minimum of 1 to a maximum of 6 occasions. Newport Aquarium biologists have developed capture techniques with bowmouth guitarfish that have proven successful in both handling and transport. For example, bowmouth guitarfish were maintained throughout each exam using tonic immobility (TI), a technique used in elasmobranch handling where the animal is rolled to an inverted

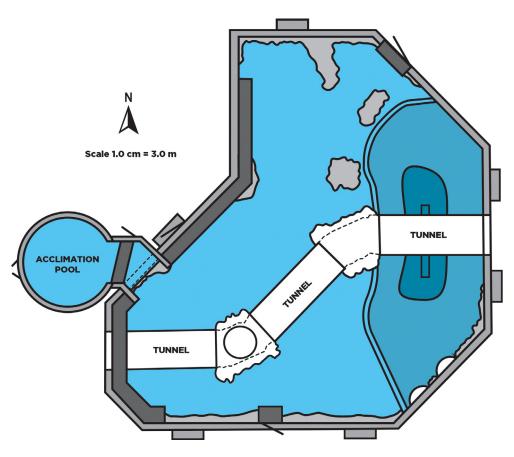


Fig. 1. Newport Aquarium (Newport, Kentucky, USA) Shark Tank schematic

position (Henningsen 1994, Páez et al. 2023). While in TI, animals were restrained by 3 staff members at the following locations: rostral bow, mid-body coelomic region, and the tail. Measurements were gathered using a flexible or soft measuring tape and, for consistency, were obtained from the individual's ventral aspect. Fork length morphometrics were taken as a linear measurement from the center of the rostrum to the fork in the tail where the upper and lower lobes of the caudal fin meet. Pectoral width measurements spanned from the tip of the left pectoral fin to the tip of the right. Girth was taken as a circumference at the caudal end of the central thorn ridge. Additionally, male clasper lengths were measured from the interior aspect.

Bowmouth guitarfish total length measurements at Newport Aquarium ranged from 217 to 226 cm for mature females and 195 to 210 cm for mature males (Table 1). Weight measurements varied from 67 to 97 kg for the males and 101 to 124 kg for the females. The females of the species present as larger bodied in comparison to the males represented in this study, with their average girth, pectoral width, total length, and body weight measurements, respectively, 13, 6, 25, and 9% greater than the conspecific males.

For the study animals acquired as juveniles, ages were estimated using growth data from bowmouth guitarfish pups born at Newport Aquarium — specifically, total length and weight measurements at ages 0 to 505 d. Using this information, the 1.2.0 bowmouth guitarfish (herein referred to as Male 1, Female 1, and Female 2) ages at the initiation of the study were calculated to be 12, 13, and 9 yr, respectively. The second male (referred to as Male 2) was acquired as a mature individual, eliminating the ability to estimate age.

### 2.3. Animal care

With consideration to the demersal nature of these animals (Hartoko et al. 2020), bowmouth guitarfish at the Newport Aquarium were fed a diet comprised of both crustaceans and teleosts. The crustacean portion was primarily Caribbean spiny lobster *Panulirus argus*, while the teleost portion rotated between salmon *Oncorhynchus* sp., bonito *Sarda* sp., and Spanish

Table 1. Morphometrics from mature bowmouth guitarfish
housed at Newport Aquarium (Newport, Kentucky, USA)
from December 2017 to August 2022

Measurement	Sample size	Mean ± SD	Range	
Female 1				
Fork Length (cm)	3	$213 \pm 2.65$	210-215	
Girth (cm)	4	$102 \pm 1.71$	100 - 104	
Pectoral width (cm)	1	129		
Total length (cm)	1	226		
Weight (kg)	3	$115.57 \pm 7.31$	111 - 124	
Female 2				
Fork length (cm)	3	$201 \pm 1$	200 - 202	
Girth (cm)	6	$99.67 \pm 3.44$	95-103	
Pectoral width (cm)	1	128		
Total length (cm)	1	217		
Weight (kg)	4	$105.9 \pm 6.63$	101-115	
Male 1				
Clasper left (cm)	3	$37 \pm 1$	36-38	
Clasper right (cm)	3	$38 \pm 0$	38-38	
Fork length (cm)	6	$189.17 \pm 2.99$	185-193	
Girth (cm)	5	$83.6 \pm 2.41$	81-87	
Pectoral width (cm)	3	$114.33 \pm 7.23$	106-119	
Total length (cm)	2	$196 \pm 1.41$	195-197	
Weight (kg)	6	$70.95 \pm 3.24$	67-74	
Male 2				
Clasper left (cm)	1	38		
Clasper right (cm)	1	35		
Fork length (cm)	1	205		
Girth (cm)	1	92		
Pectoral width (cm)	1	127		
Total length (cm)	1	210		
Weight (kg)	1	97		

mackerel *Scomberomorus regalis*. Dietary consumption for each individual was tracked using computer aided software (Tracks Software) allowing for routine monitoring of trends from the group as well as each animal. This data included type of diet offered, quantity offered, and quantity of diet consumed. Each individual was offered approximately 4% of their body weight weekly, divided among 5 successive, daily feeding sessions. Mazuri Shark and Ray Supplement tablets (PMI Nutrition International) were included with the diet at a recommended dosage of one 1.5 g tablet per 226 g food. Additional oral supplements included 1000 mg vitamin C tablets, krill oil, and fish oil capsules as needed.

Diet portion size and composition were estimated per feed and were not altered seasonally. Portion size per feed, calculated as the total weight offered to all individuals during 1 feeding session, remained 2.77  $\pm$ 0.28 kg (average  $\pm$  SD) throughout the study. The composition of the diet was calculated as the weight of teleosts divided by the weight of crustaceans offered to the group per feed, and reported as the ratio of teleosts to crustaceans. This ratio changed from mostly teleosts (1.13  $\pm$  0.26) before June 2020 to mostly crustaceans (0.68  $\pm$  0.13) after June 2020 (*t*-test, p < 0.005).

## 2.4. Ethogram development and behavioral observations

The Newport Aquarium bowmouth guitarfish breeding behavior ethogram was developed using 10 yr of observations of behaviors in a mixed sex group. During this time, iterative male behaviors directed towards females were noted and described. Using these informal observations and confirmations of viable pregnancies, an ethogram was established that captured the sequential behavioral stages in bowmouth guitarfish breeding behaviors.

Starting in December 2017, the established ethogram was used to document breeding behaviors observed during the daily management and care of the bowmouth guitarfish. Each period of data collection occurred during the scheduled feed and ranged in time from 2 to 10 min. For each observation, the presence/absence of each behavior was recorded as well as the individuals involved in the interaction. Through this process, almost 5 yr of bowmouth guitarfish breeding behaviors were cataloged.

#### 2.5. Data analysis

The breeding behavior presence/absence data were divided into 2 phases based on the occupancy in the tank (see Section 2.2). During each of these phases, the percent occurrence of each breeding behavior (i.e. the number of observations with breeding activity divided by the total number of observations multiplied by 100%), as well as the proportion of the observations that were targeted (i.e. only 1 female was involved in the breeding activity of a male), were recorded for each combination of male and female pairing.

To examine the potential impact of diet composition and time of year on breeding behavior, the monthly frequency of breeding behavior and corresponding monthly ratio of teleost to crustacean weight were calculated. The proportion of breeding activity per stage was also calculated per month over Phase 2 of the study. A regression analysis was performed to examine a potential relationship between diet (specifically average weight of food offered per feeding and the monthly ratio of teleost to crustacean weight) and the monthly frequency of breeding behavior. To further examine whether diet composition influenced behavior, a Wilcoxon rank sum test was used to compare the monthly frequency of breeding behavior of Male 1 before and after the shift in diet composition from a teleost- to crustacean-focused diet (see Section 2.3).

Lastly, the potential impact of visitor volume on breeding behaviors was examined by calculating Pearson's correlation coefficient between the relative monthly volume of visitors and the monthly frequency of breeding behavior. Newport Aquarium provided monthly visitor data from January 2019 to August 2022. The relative monthly volume of visitors was calculated as the number of people visiting the aquarium that month divided by the maximum monthly number of visitors observed during that time period. The percentile ranks of the monthly breeding frequencies while the aquarium was closed due to COVID (April and May 2020) were also calculated to investigate potential impact of the shutdown on breeding behavior frequency.

#### 3. RESULTS

#### 3.1. Breeding behavior ethogram

Bowmouth guitarfish breeding behavior was observed as a sequence of male-driven interactions that included the following stages: hovering, chasing, wrapping up, flipping/rolling, and copulation (see Video S1 in the Supplement at www.int-res.com/ articles/suppl/n053p429\_supp/). 'Hovering', the initiation of breeding, is defined as the male swimming over top of the female (Stage 1, Fig. 2A). Following hovering behavior, the male began 'chasing', i.e. quickly and relentlessly pursuing the female with apparent intention of catching up to her (Stage 2, Fig. 2B). After chasing and catching up to the female, the male then engaged in 'wrapping up' behavior, where he aligned himself directly above yet slightly caudal to the female and attempted to corral her by wrapping his pectoral fins around the circumference of the female's coelomic cavity (Stage 3, Fig. 2C). If the male successfully wrapped his pectoral fins around the female, he then exhibited 'flipping/rolling' behavior, i.e. he attempted to flip her, exposing her ventral side and thus orienting his claspers to the female's cloaca (Stage 4, Fig. 2D.). In the final stage, 'copulation', the male aligned his claspers to the female's cloaca for insertion (Stage 5, Fig. 2E.). The final stage was only observed during the informal observation period as the ethogram was developed.

Successful bowmouth guitarfish breeding at Newport Aquarium has been confirmed through diagnostic ultrasound recordings of viable pregnancies on 5 separate occasions with 2 mature females. Three of the 5 pregnancies led to live parturition in 2014 (Female 1), 2016 (Female 1), and 2017 (Female 2). Pup litter size and sex ratio for each of these birthing events were 3.3.0 (2014), 3.6.0 (2016), and 1.0.0 (2017). Percent survivability (>30 d) within each litter equated to 33, 89, and 0%, respectively. Subsequent confirmation of aquarium breeding events through paternity analysis between potential sires and pups using microsatellite markers indicated that all instances of live parturition were sired by Male 1 (K. Feldheim pers. comm.).

#### 3.2. Breeding stage prevalence and trends

During Phase 1 of the study (2.2.0 occupancy), the males differed in the frequency and intensity of their breeding behaviors (Table 2). Male 1 exhibited breeding activity at 3 times the frequency of Male 2. Once engaged in these behaviors, Male 1's breeding activity reached higher stages than Male 2's. Male 1 more often targeted 1 female rather than splitting his attention between both females during the same feeding session, while Male 2 never focused on 1 female. There were no observations where both males simultaneously exhibited breeding behaviors.

During Phase 2 of the study (1.2.0 occupancy), Male 1 showed patterns of breeding behaviors similar to his behaviors during Phase 1 (Table 3). Male 1 exhibited breeding activity during approximately 12% (Phase 1) and 9% (Phase 2) of observations. Male 1 slightly favored Female 1 (Phase 1: 57%, Phase 2: 65% of targeted breeding interactions), and was more likely to target 1 female than both females during feeding observations (Phase 1: 88%; Phase 2: 60% of feeding sessions with breeding activity recorded).

Breeding behaviors occurred most frequently in September and October across Phase 2 of the study; however, the highest proportion of elevated breeding stages, i.e. wrapping and flipping/rolling, were observed in January (Fig. 3). Copulation was not observed during Phase 1 nor Phase 2 of the study.

## 3.3. Influence of husbandry and environmental parameters

There was no significant linear relationship between the monthly breeding frequency and the amount of

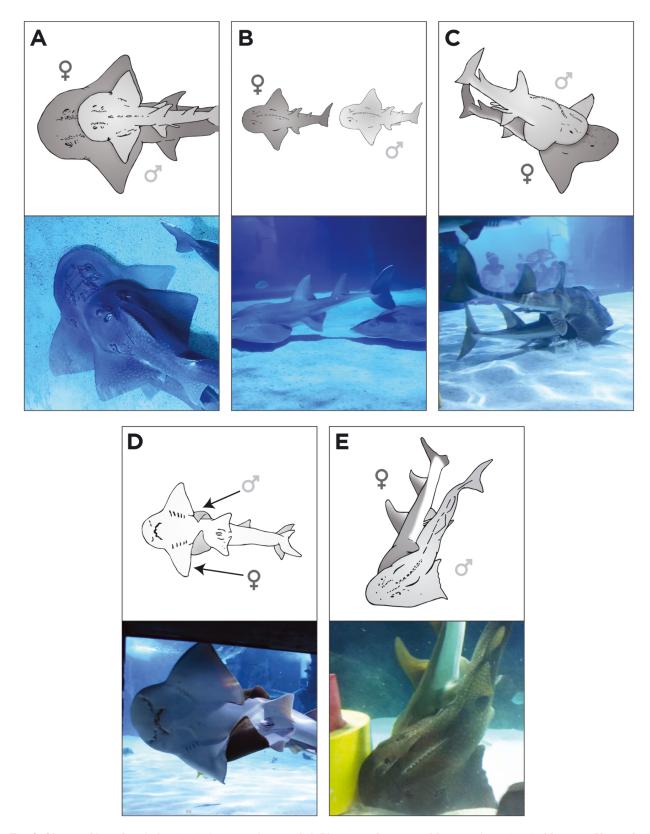


Fig. 2. Observed breeding behaviors in bowmouth guitarfish *Rhina ancylostoma* at Newport Aquarium in Newport, Kentucky, USA. Breeding behavior followed a pattern of stages: (A) Stage 1: hovering; (B) Stage 2: chasing; (C) Stage 3: wrapping; (D) Stage 4: rolling; and (E) Stage 5: copulation

Male	Female	Frequency of breeding activity (percent of total observations)	Intensity of breeding activity during observation (number of observations)			
			Hover	Chase	Wrap	Roll
Male 1	Female 1	4 (5.5%)	0	1	3	0
	Female 2	3 (4.1%)	0	2	0	1
	Both females	2 (2.7%)	0	2	0	0
	Total	9 (12.3%)	0	5	3	1
Male 2	Female 1	0	0	0	0	0
	Female 2	0	0	0	0	0
	Both females	3 (4.1%)	2	1	0	0
	Total	3 (4.1%)	2	1	0	0

Table 2. Breeding interactions per observation period under a 2.2.0 (male.female.unknown) ratio

Table 3. Breeding interactions per observation period under a 1.2.0 (male.female.unknown) ratio

Male	Female	Frequency of breeding activity (percent of total observations)	Intensity of breeding activity during observation (number of observations)			
			Hover	Chase	Wrap	Roll
Male 1	Female 1	32 (3.7%)	14	8	7	3
	Female 2	17 (1.9%)	7	6	4	0
	Both females	33 (3.8%)	16	10	5	2
	Total	82 (9.4%)	37	24	16	5

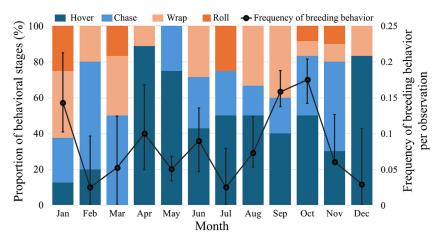


Fig. 3. Breeding behavior of Male 1 by month. Black line: monthly frequency of breeding behavior displays; error bars: the 25th and 75th percentiles of monthly frequency of breeding behavior displays. Stacked bars represent the proportion of the highest behavioral stage per observation of all breeding behaviors observed during that month throughout the study period

food offered (regression analysis, p = 0.12) nor the teleost:crustacean composition of the diet (regression analysis, p = 0.27, Fig. 4). There was also no significant difference in the monthly frequency of breeding behavior observed before versus after the change in diet composition in June 2020 (Wilcoxon rank sum, p = 0.83).

Attendance at the aquarium did not appear to influence the frequency of breeding behavior. There was a very weak negative correlation between breeding behavior frequency and visitor volume (Pearson's correlation coefficient = -0.08). The monthly frequencies of breeding behavior during the time period of the COVID shutdown were in the 30th (April 2020) and 40th (May 2020) percentiles of observed monthly breeding behaviors. This indicates that breeding behavior frequencies with a lack of visitorsalthough less than the median - were within the interguartile range of the months with visitors present.

## 4. DISCUSSION

This study presents the observed breeding behaviors of the Critically Endangered *Rhina ancylostoma* while under human care at Newport Aquarium. The observations and resulting ethogram have led to the identification of 5 stages of breeding behavior for the

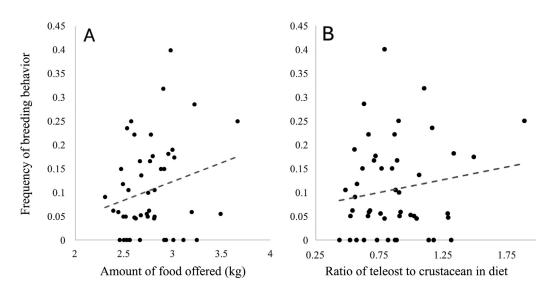


Fig. 4. Relationship between (A) average daily amount of food offered and (B) ratio of fish to crustacean in the diet and the monthly breeding behavior frequency. Dashed line: insignificant linear relationships (p > 0.05) between diet and breeding behavior frequency

species: hovering, chasing, wrapping up, flipping/ rolling, and copulation. Of the 5 identified stages of breeding, wrapping up presents as the most unique to this species. The term 'wrap' has previously been defined as a species-typical behavior where the male of a small shark species may wrap the total length of his body around the female to facilitate copulation (Pratt & Carrier 2001). This definition has focused on 3 shark species, the small-spotted catshark Scyliorhinus canicula (Bolau 1881, Hardy 1959), the cloudy catshark Scyliorhinus torazame (Uchida et al. 1990), and the grey bamboo shark Chiloscyllium griseum (Dral 1980), where the male's entire length is engaged in a snake-like coiling around the female's body allowing for controlled copulation. In contrast, male bowmouth guitarfish wrap their pectoral fins in a fullcircumference grip of the female's coelom while not engaging the tail for physical restraint. This difference allows the male to control the swimming direction of the female while continuing to use his tail for propulsion.

Commonly, elasmobranch breeding sequences involve biting by the male to gain physical control of the female (Kajiura et al. 2000, Pratt & Carrier 2022). Within the aquarium environment, bite wounds and marks on both the male and female bowmouth guitarfish were noted, especially associated with the dorsal aspect of the caudal fin and periodic bite abrasions on the pectoral fins. The presentation of bitemarks, however, in bowmouth guitarfish, have not been directly attributed to indications of breeding. Throughout the timeframe of this study, the use of the mouth for holding or biting by the male to gain leverage or control of the female was not observed as part of the breeding sequence. The male bowmouth guitarfish instead used his large pectoral fins to wrap and physically control the female.

Functional constraints of diverse aquarium environments can alter adaptations of social dynamics and behavioral interplay within species. A single occurrence of successful copulation during the development of the ethogram was witnessed at Newport Aquarium. In that instance, after flipping and rolling the female into ventral-to-ventral positioning, the male pinned her against hard-surfaced tank décor, leading to speculation as to whether the species is restricted to the benthos for breeding. Similar positioning for copulation in this species was also observed at Georgia Aquarium (Atlanta, Georgia, USA); however, this occurred mid-water column without the aid of a solid structure (L. Neal pers. comm., Video S2). In contrast, after the conclusion of this study, staff at Osaka Aquarium Kaiyukan in Osaka, Japan, observed successful clasper insertion where the male and female aligned in a parallel, side-byside, orientation. During this copulation event, the male used his right pectoral fin to lift the female off the tank floor and appeared to maintain control of the female by biting her left pectoral fin (Video S3). While the ventral-to-ventral positioning coincided with confirmed pregnancies, no pregnancies were confirmed between the mating pair of bowmouth guitarfish exhibiting parallel positioning (Y. Miyagawa pers. comm.). The complexities of the aquarium environment may amplify or suppress sexual courtship; however, this effect on breeding behaviors is beyond the scope of the observations identified within this paper.

This study found corroborating evidence that breeding behavior frequency was directly related to breeding success. Male 1 exhibited breeding behaviors 3 times more often than Male 2 in this study and sired all live pups. Furthermore, Male 1 favored Female 1 in his breeding interactions, and Female 1 carried the majority of viable pregnancies. This supports the use of the ethogram from this study to identify breeding behavior. Noting increases in frequency of mating behaviors can allow for the preparation of potential pregnancies, identification of conditions leading to enhanced breeding interactions, and recognition of sexually active individuals.

It is unclear why the frequency of breeding behavior peaked in September and October. This seasonality could not be attributed to changes in light regime, temperature, diet, or aquarium visitor volume. Continued research including studies of reproductive hormonal patterns in conjunction with ultrasonography focusing on gonadal development would aid in determining reproductive maturation, gestational period, and seasonality of breeding for bowmouth guitarfish.

Elasmobranch breeding programs similar to the institutional bowmouth guitarfish breeding program established by Newport Aquarium in 2007 have been garnering increased recognition and support. Currently, the AZA manages 11 elasmobranch species with dedicated studbook programs focused on their demographic and genetic survivability. Breeding initiatives in general are a well-recognized conservation strategy where threatened or endangered species are selectively managed to achieve sustainable genetic diversity. Upon successful breeding and reproduction, the reintroduction of species to their native environments following established IUCN guidelines has also been positively undertaken (IUCN Species Survival Commission (IUCN/SSC 2013). With confirmed viable pregnancies as well as documented breeding behaviors, this study provides guidance for the development of a successful breeding program.

The work presented in this study has served as the foundation for ongoing global collaboration focused on bowmouth guitarfish breeding programs with the goal of reintroduction. If successful, this initiative represents the second example of elasmobranch conservation through reintroduction. The precedent to this program is the *Stegostoma tigrinum* Augmentation Recovery (StAR) project, founded in 2019, which

focusses on the reintroduction of the Endangered zebra shark (www.reshark.org). The StAR project is a global cooperative effort that serves as a model for the conservation of other elasmobranch species.

The conservation of a species can only be undertaken as a successful endeavor when the biology of that species is understood. Many aspects of biology and behavior can be studied within aquarium settings and collections that would otherwise be difficult if not impossible in the wild. The work done at Newport Aquarium presented here provides the only published information on the reproductive behavior of this species to date. Lack of such scientific knowledge often hinders management of endangered populations both ex situ and in situ (Swaisgood 2004). Our goal in presenting this study is the promotion of shared knowledge associated with the imperiled bowmouth guitarfish. Identifying and sharing the breeding behaviors of any animal is crucial to improving the performance and management of conservationbased breeding programs (Bussolini et al. 2023).

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#### LITERATURE CITED

- Bloch M, Schneider J (1801) Systema ichthyologiae iconibus cx illustratum. Berolini, Sumtibus Austoris Impressum et Bibliopolio Sanderiano Commissum, Berlin
- Bolau H (1881) Über die Paarung und Fortpflanzung der Scyllium-Arten. Z Wiss Zool 35:321–325
- <sup>\*</sup>Bussolini LT, Crates R, Magrath MJL, Stojanovic D (2023) Identifying factors affecting captive breeding success in a critically endangered species. EMU Austral Ornithol 123:161–169

- Chargé R, Teplitsky C, Sorci G, Low M (2014) Can sexual selection theory inform genetic management of captive populations? A review. Evol Appl 7:1120–1133
- Comizzoli P, Brown JL, Holt WV (2019) Reproductive science as an essential component of conservation biology: new edition. Adv Exp Med Biol 1200:1–10
  - Devadoss P, Batcha H (1995) Some observations on the rare bow-mouth guitar fish *Rhina ancylostoma*. Mar Fish Inf Serv T & E Ser 138:10–11
  - Dral A (1980) Reproduction en aquarium du requin de fond tropical *Chiloscyllium griseum* Müll. et Henle (Orectolobidés). Rev Fr Aquariol 7:99–104
- Feldheim KA, Wyffels JT, Lyons K (2022) The role of aquaria in the advancement of elasmobranch reproductive biology. Front Mar Sci 9:963542
  - Hardy A (1959) The open sea: its natural history. Houghton Mifflin Harcourt, Boston
- Hartoko A, Pringgenies D, Anggelina AC, Matsuishi T (2020) Morphology and molecular biology of benthic Java sea shark ray *Rhina ancylostoma* Bloch and Schneider 1801 (Elasmobranchia: Rhinidae). Annu Res Rev Biol 35:19–31
- Hazeres J (2022) Bowmouth guitarfish (*Rhina ancylostoma*) AZA Regional Studbook. Newport Aquarium, Newport, KY
- Henningsen A (1994) Tonic immobility in 12 elasmobranchs: use as an aid in captive husbandry. Zoo Biol 13:325–332
  - Henningsen AD, Smale MJ, Gordon I, Garner R, Marin-Osorno R, Kinnunen N (2004) Captive breeding and sexual conflict in elasmobranchs. In: Smith M, Warmolts D, Thoney D, Hueter R (eds) The elasmobranch husbandry manual: captive care of sharks, rays and their relatives. Ohio Biological Survey, Columbus, OH, p 237–248
  - IUCN/SCC (IUCN Species Survival Commission) (2013) Guidelines for reintroductions and other conservation translocations. V 1.0. IUCN Species Survival Commission, Gland

Editorial responsibility: David Ebert (Guest Editor), Moss Landing, California, USA Reviewed by: 3 anonymous referees

- Kajiura S, Sebastian A, Tricas T (2000) Dermal bite wounds as indicators of reproductive seasonality and behavior in the Atlantic stingray, *Dasyatis sabina*. Environ Biol Fishes 58: 23–31
- <sup>K</sup>Kyne PM, Rigby CL, Dharmadi, Jabado RW (2019) *Rhina* ancylostoma. The IUCN Red List of Threatened Species 2019:e.T41848A124421912. doi:10.2305/IUCN.UK.2019-2. RLTS.T41848A124421912.en (accessed 12 Sep 2022)
- Kyne PM, Jabado RW, Rigby CL, Dharmadi and others (2020) The thin edge of the wedge: extremely high extinction risk in wedgefishes and giant guitarfishes. Aquat Conserv 30:1337–1361
- Páez AM, Padilla EMH, Klimley AP (2023) A review of tonic immobility as an adaptive behavior in sharks. Environ Biol Fishes 106:1455–1462
- Pratt H, Carrier J (2001) A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. Environ Biol Fishes 60: 157–188
  - Pratt HL Jr, Carrier JC (2022) Elasmobranch courtship and mating behavior. In: Hamlett WC (ed) Reproductive biology and phylogeny of Chondrichthyes. CRC Press, Boca Raton, FL, p 129–169
  - Swaisgood R (2004) What can captive breeding do for conservation and what can behavior research do for captive breeding? Conserv Behav 2:3–6
  - Uchida S, Toda M, Kamei Y (1990) Reproduction of elasmobranchs in captivity. In: Pratt HL Jr, Gruber SH, Taniuchi T (eds) Elasmobranchs as living resources: advances in biology, ecology, systematics, and status of the fisheries. NOAA Tech Rep NMFS 90:211–237
  - Walenciak O (2022) Bowmouth guitarfish (*Rhina ancylos-toma*). EAZA Regional Studbook, EA LIFE, Oberhausen
- White J, Simpfendorfer CA, Tobin AJ, Heupel MR (2014) Age and growth parameters of shark-like batoids. J Fish Biol 84:1340–1353

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