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New records on intestinal eversion in a free-ranging nurse shark (*Ginglymostoma cirratum*) and a free-ranging, breaching-spotted eagle ray (*Aetobatus narinari*)

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Abstract Although intestinal eversion has been documented as an important behavior in elasmobranchs, it is rarely observed in wild animals. Here we describe the first published record of an intestinal eversion in a shark with a ring type of valvular intestine, the nurse shark (*Ginglymostoma cirratum*), and the first record of an intestinal eversion during breaching events in the spotted eagle ray (*Aetobatus narinari*), a ray with a conicospiral type of valvular intestine. These observations contribute to the scientific knowledge on this interesting and important behavior in elasmobranchs and also expand the proposed hypotheses for breaching behavior in rays.

Keywords Behavior · *Ginglymostoma cirratum* · *Aetobatus narinari* · Field observation · Intestinal rinsing

Intestinal eversion has been documented as an important behavior in elasmobranchs (sharks and batoids) (e.g., Christie 2012), for instance, to facilitate the expulsion of indigestible prey parts (e.g., shell remains and bones), mucous, and parasites from the spiral valve (Crow et al. 1990; Wiersma et al. 2015). Elasmobranchs have a remarkable intestinal adaptation, the spiral valve, which considerably increases the internal surface area (Ebert et al. 2021). The spinal valve takes three main forms: a conicospiral valve, angled anteriorly in the intestine and resembling an auger; a ring valve, with numerous short

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turns resembling a stack of washers; or a scroll valve, unlike the other types, the valves are longitudinally rolled and attached to the intestinal wall (found in requiem and hammerhead sharks) (Ebert et al. 2021).

Although intestinal eversion has been observed in several captive elasmobranchs, this behavior is rarely observed in the wild, mainly due to its short duration in non-stressful situations and the logistical challenges of recording a rare event (e.g., Weideli and Papastamatiou 2021). Before the field records were documented, this mechanism was believed to be related only to stressful situations in captivity (e.g., Crow et al. 1990, 1991; Henningsen et al. 2005). However, as described to voluntary stomach eversions (Sims et al. 2000; Brunnschweiler et al. 2005), recent observations in the field suggests that intestinal eversions occur naturally and fulfills a cleansing function (Wiersma et al. 2015; Weideli and Papastamatiou 2021).

While there are several published observations of this phenomenon in 12 shark species and 4 batoid species in captivity, there are only four observations of intestinal eversion in free-ranging elasmobranchs, including in two shark species (Wiersma et al. 2015; Weideli and Papastamatiou 2021), and in two manta ray species (Clark et al. 2008; Stevens et al. 2018) (Table 1). Therefore, considering the rarity of such published reports, we report two new records of intestinal eversions in free-ranging elasmobranchs. The first observation was of a nurse shark *Ginglymostoma cirratum* (Bonnaterre 1788), a species with a ring type of valvular intestine; the second observation was of a spotted eagle ray *Aetobatus narinari* (Euphrasen 1790) during a breaching event, a species with a conicospiral type of valvular intestine.

Table 1 Published records of intestinal eversion in elasmobranchs to date. Intestine morphology description was based on Compagno (1988): conicospiral (a series of cones), ring (flattened rings), and scroll (a series of parchment-like rolls)

	Order	Species	Intestine morphology	Captive/wild	Location	Reference
Sharks	Carcharhiniformes	*Carcharhinus galapagensis*	Scroll	Captive	UZG	Crow et al. (1990)
		Carcharhinus leucas	Scroll	Captive	OEA	Crow et al. (1990)
		Carcharhinus longimanus	Scroll	Wild	RSE	Weideli and Papastamatiou (2021)
		Carcharhinus melanopterus	Scroll	Captive	WAQ	Crow et al. (1990)
		Carcharhinus obscurus	Scroll	Captive	OEA	Crow et al. (1990)
		Carcharhinus plumbeus	Scroll	Captive	OEA	Crow et al. (1990)
		Negaprion acutidens	Scroll	Captive	WAQ	Crow et al. (1990)
		Negaprion brevirostris	Scroll	Captive	SWC	Crow et al. (1990)
		Rhizoprionodon terraenovae	Scroll	Captive	MGA	Christie (2012)
		Triaenodon obesus	Scroll	Captive	WAQ	Crow et al. (1990)
	Hexanchiformes	*Notorynchus cepedianus*	Conicospiral	Wild	FBSA	Wiersma et al. (2015)
	Orectolobiformes	*Ginglymostoma cirratum*	Ring	Wild	FNBR	This study
Batoids	Rhinopristiformes	*Pristis pectinata*	Conicospiral	Captive	NAB	Henningsen et al. (2005)
	Myliobatiformes	*Aetobatus narinari*	Conicospiral	Wild	RJBR	This study
		Mobula alfredi	Conicospiral	Wild	MA	Stevens et al. (2018)
		Manta birostris	Conicospiral	Wild	HI	Clark et al. (2008)
		Potamotrygon motoro	Conicospiral	Captive	DAQ	Christie (2012)
		Rhinoptera bonasus	Conicospiral	Captive	DAQ	Christie (2012)

UZG (Ueno Zoological Gardens, Tokyo, Japan); OEA (Okinawa Expo Aquarium, Okinawa, Japan); RSE (Red Sea, Egypt); WAQ (Waikiki Aquarium, Honolulu, HI); SWC (Sea World, San Diego, CA); MGA (The Rainforest and Aquarium at Moody Gardens, Galveston, TX); FBSA (False Bay, South Africa); FNBR (Fernando de Noronha Archipelago, Brazil); NAB (National Aquarium in Baltimore, Baltimore, MD); RJBR (Rio de Janeiro, Brazil); MA (Maldives Archipelago); MHI (Maui, Hawaiian Islands); DAQ (Dallas Zoo and Aquarium at Fair Park, Dallas, TX).

The intestinal eversion in the juvenile male nurse shark was photographed during a scuba dive at the Buraco das Cabras, Fernando de Noronha Archipelago (3º52' S, 32º25' W), 345 km off northeastern Brazil, on November 23, 2020. The record was obtained after we started a Citizen Science Program of Fernando de Noronha Archipelago. The program was launched in May 2021 and aimed to collect elasmobranch records provided by scuba divers, underwater photographers, and snorkelers. The care of animals within this program complies with Brazilian animal welfare laws, guidelines, and policies as approved by SISBIO (ICMBIO/SISBIO #12064–28). The nurse shark interacted with a small group of scuba divers, at a depth of 14 m, and its behavior was photographed on camera (Nikon D90). In the photograph it is possible to observe the everted intestine with the excretion of a milky fluid into the water (Fig. 1). However, it was not possible to observe the exact time that the intestine everted or quantify the duration of the evertion, as the nurse shark interacted with the divers for a few seconds before swimming away.

The evertion event in the female spotted eagle ray was filmed on camera (Sony A7siii + len Sony 70–200 f4), on Jacone Beach, *Laje da Manitiba* (22.93º28.82'S, 27 42º 58.45'W), Saquarema City, Rio de Janeiro, southeastern Brazil, on May 29, 2021. The spotted eagle ray was observed from the beach breaching, at least, three times. The last two jumps were recorded in 20 s of the video. On the video, the intestine is already everted at the first recorded jump and clearly visible on the second jump before the ray re-entered the water (Fig. 2; see video supplementary).

Here we describe the first published record of an intestinal eversion in a free-ranging nurse shark and a free-ranging, breaching spotted eagle ray (Table 1). Despite the difficulties of researchers to be in the field at the right time to record such rare events, citizen science comes as a way to increase the probability of these records. Our record of intestinal eversion in the nurse shark contrasts Crow et al. (1990) theory that sharks with ring valve intestines would have difficulty to evert it because of their shorter and more robust pyloric stomach compared to long and slender pyloric stomachs of sharks with a scroll valve intestine. While the present study reports the first occurrence of intestinal eversion for the spotted eagle ray, previous studies have described eversions in other ray species from the same order (Myliobatiformes, Table 1) and for rays with the conicospiral type of valvular intestine. The first recorded intestinal eversion on a conicospiral valvular intestine of an elasmobranch was in the smalltooth sawfish *Pristis pectinata*, highlighting the similarity to carcharhinid sharks of long pyloric stomachs (Henningsen et al. 2005).

Although intestinal eversion during a breaching event has not been documented to our knowledge, breaching behavior is commonly observed in myliobatid rays. Such behavior in rays has been proposed as mating displays, avoiding underwater predators (e.g., sharks), parturition and removing attached remoras (e.g., Marshall and Bennett 2010). However, it is also possible that breaching events at the time of intestinal eversion could be explained by the following hypotheses: (1) to facilitate the expulsion of indigestible materials from the intestine and (2) to escape from smaller predators (e.g., small teleost fish) while exploiting ejected digested material

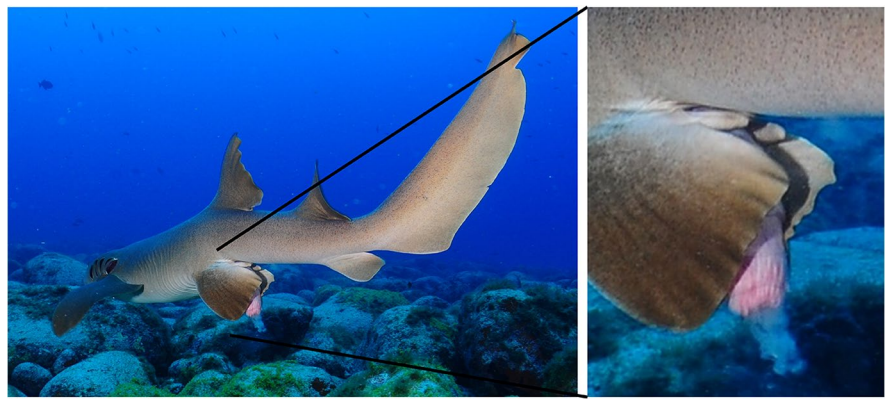

Fig. 1 Intestinal eversion with a visible mucous by a male nurse shark (*Ginglymostoma cirratum*). Photo by Ciliares

Fig. 2 Intestinal rinsing in a free-ranging female spotted eagle ray (*Aetobatus narinari*) observed during a breaching event. See video supplementary

(e.g., Weideli and Papastamatiou 2021). Attacks by smaller predators during the intestinal eversions have been documented to cause severe injuries and even mortalities (Crow et al. 1991; Henningsen et al. 2005; Christie 2012).

Our study provides the first record of intestinal eversions of a nurse shark and a breaching spotted eagle ray, providing evidence that this behavior may be widespread and an important mechanism for elasmobranchs. We also present the first case of a ring valve intestine eversion contributing to the scientific knowledge necessary to better understand this event. Additionally, these observations expand the proposed hypotheses for breaching behavior in rays.

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Authors' contributions BSR envisioned the study. RV recorded the video and NX is responsible for the photo. BSR and VB wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability No further data than that presented in this manuscript are available.

Declarations

Ethical approval No animal testing was performed during this study.

Conflict of interest The authors declare no competing interests.

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