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Clasper flaring in a guitarfish (*Zapteryx brevirostris* Elasmobranchii, Rhinobatidae) under anesthesia

This report describes the details of the *flaring* condition and aggressive behavior observed in a wild male guitarfish suspected of being initiated by exposure to an anesthetic agent. This animal was part of a study investigating the utility of eugenol (clove oil) to induce in this species a stage of anesthesia characterized by reduced opercula movements, decreased swimming ability and loss of equilibrium (Neiffer & Stamper 2009). The project was approved by the Ethics Committee on Animal Use at the Federal University of Paraná (no. 776/2014 UFPR).

Ten adult male guitarfish (*Zapteryx brevirostris*) were accidentally caught by artisanal fishermen. The fish were individually held in 130 L tanks, with salinity maintained at 35 practical salinity units and temperature at 20 °C, and pH and nitrate were monitored. The animals were not fed. After 24 hours of acclimation, the fish were individually anesthetized by immersion in eugenol (Biodinâmica Ltda, RJ, Brazil) diluted in 95% ethanol (1:10) to 50 mg L^{-1} in a bath (56.4 cm × 38.5 cm × 37.1 cm), under constant aeration (dissolved oxygen 8.5 mg L^{-1}).

Reduction of spiracle movement and a lack of response to tail grabbing or touching were observed after the fish were immersed in the anesthetic solution for approximately 8 minutes. At this point, one male (length 57 cm, disk width 23.3 cm, mass 740 g) exhibited *flaring* at the distal end of both claspers (Fig. 1a). The male was not anesthetized and exhibited aggressive behavior, characterized by continuous contraction of the pectoral fins, body and tail whipping, urinary incontinence and persistent mouth opening. The fish was immediately returned to the permanent tank and left without disturbances for 7 hours. After this time, the *flaring* condition and aggressive behavior, characterized by evasive swimming, persisted. After careful examination, the animal was considered responsive and in good physical condition, and it was released into its natural habitat in accordance with the approved protocol. Since it is an endangered species, maintenance in a laboratory for a long period is not approved, so there was no follow-up.

Male elasmobranchs anchor the clasper within the oviduct of the female during copulation to increase the efficiency of sperm transfer. When guitarfishes mate, the male flexes one clasper by contraction of the extensor muscle during insertion into the cloaca of the female. The anchoring of the clasper occurs by contraction of the dilatator muscle that flexes the terminal cartilages. The return of these terminal elements to the resting position (Fig. 1b) occurs by relaxation of the dilatator muscle and concomitant contraction of the outer lip muscle. When this condition occurs without sexual stimulation it is referred to as *flaring*. It has been described in clearnose skates (*Raja eglanteria*) and in a whitetip reef shark (*Triaenodon obesus*) facing electrical stimulation and human presence, respectively (Luer & Gilbert 1985; Ritter & Compagno 2013). There is no information in these publications indicating return to a relaxed/flaccid state.

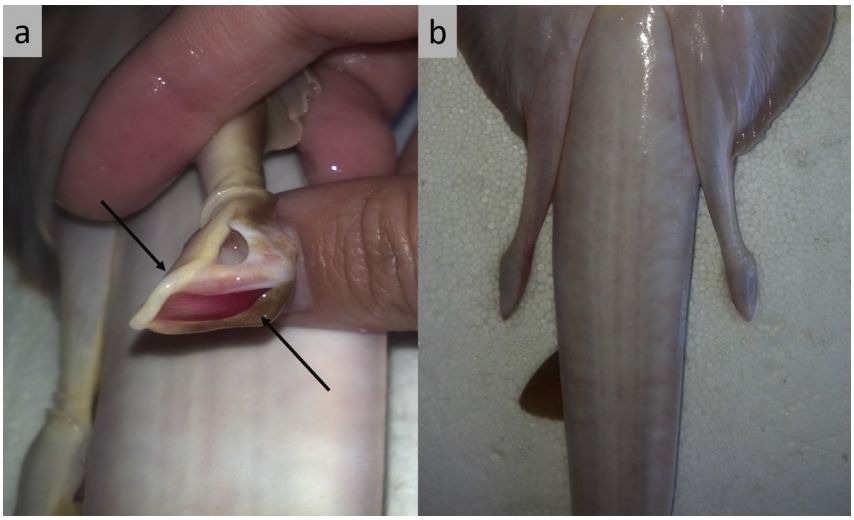

Figure 1 (a) Flaring condition of a male guitarfish (Z. *brevirostris*) after immersion in an anesthetic solution of eugenol and ethanol. The dilated glans at the distal end of a clasper reveals two side openings and the arrows point to the dorsal and ventral cartilages. (b) Appearance of claspers in a resting state in another fish.

Persistent penile erection (priapism) in horses has been reported, mainly associated with the administration of phenothiazine derivatives (Pearson & Weaver 1978). Many causes, including drugs, have been associated with priapism in humans. It is believed that long-acting drugs may trigger the condition, and the prolonged effects in the parasympathetic nervous system lead to an inability to retract the member even after complete drug metabolization (Krause 2007). In mammals, the condition is often related to abnormal blood flow to the member. However, since the mechanisms involved in guitarfish's copulation are more related to muscular and cartilaginous dynamics of the clasper, the *flaring* may be a result of nervous system stimulation rather than ischemia.

In the last decade, the use of eugenol in fish was popularized due to its low cost and rapid metabolization, reducing the respiratory and cardiac risks imposed by anesthesia (Neiffer & Stamper 2009). More than 100 Z. *brevirostris* were anesthetized and carefully examined in the past 5 years in our laboratory. Evacuation and urinary incontinence have been observed during exposure to eugenol; however, this fish is the only one identified with *flaring*. The information available cannot confirm the association between eugenol and the condition observed. As little is known about the effects of eugenol in elasmobranchs, documentation of the physical responses is important to evaluate the significance of events such as clasper modification, evacuation and urinary incontinence.

Persistent *flaring* has the potential to result in tissue damage, possibly affecting mating capacity or even progressing to death. Various treatment options are available for treating priapism in mammals, but these would be difficult to implement in aquatic animals. A detailed examination and diagnosis of any alterations observed during drug administration in fish should be recorded. This will increase knowledge in this relatively new area, thereby helping veterinarians, researchers and aquarium keepers to improve anesthesia protocols and monitoring of anesthetized wildlife.

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Authors' contributions

NW: study design, conducting experiments, data acquisition, photography, preparation of manuscript. BSR: study design, conducting experiments, data acquisition, preparation of manuscript. RAM: clasper

morphological analysis, preparation of the manuscript. CAF: funding, study design, preparation of manuscript.

Conflict of interest statement

Authors declare no conflict of interest.

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Haemodynamic changes occurring in a loggerhead sea turtle (*Caretta caretta*) during mechanical ventilation under general anaesthesia

We wish to report the occurrence of significant haemodynamic changes during mechanical ventilation in a sea turtle under general anaesthesia.

A 67-year-old loggerhead sea turtle (*Caretta caretta*), weighing 94 kg, was referred for a 16-week period of inappetence. Physical appearance was unremarkable. A computed tomography scan revealed the presence of an enterolith causing intestinal obstruction, requiring surgical intervention. The turtle was anaesthetized with 6 mg kg^{-1} of ketamine (Anaestamine; Animalcare, UK) and 0.025 mg kg^{-1} of dexmedetomidine (Dexdomitor; Vetoquinol, UK), injected into the left cervical sinus. An additional 2 mg kg^{-1} of propofol (PropoFlo Plus; Abbott, UK) was administered via this route to allow for orotracheal intubation with a size 9 endotracheal tube. The latter was connected to a circle breathing system (Model 2800C; Mallard Medical, CA, USA) and mechanical ventilation was initiated with a tidal volume of 5 mL kg^{-1} and a respiratory rate ranging between 8 and 12 breaths $minute^{-1}$. Anaesthesia was maintained with desflurane (Suprane; Baxter, UK) vaporized in oxygen (end-tidal percentage: 7.4–8.4%). Morphine (0.3 mg kg^{-1}, morphine sulphate; Martindale Pharmaceuticals, UK) was administered intramuscularly before performing the celiotomy. Intravenous access was obtained in the left cervical sinus, and 2 mL kg^{-1} $hour^{-1}$ of Hartmann's solution was administered throughout the anaesthesia. An Datex-Ohmeda S/5 multiparametric monitor (Datex-Ohmeda; WI, USA) was used to monitor physiological parameters. Throughout the anaesthetic, end-tidal carbon dioxide partial pressure ranged from 15 to 27 mmHg (2.00–3.60 kPa) and the heart rate (HR)