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Short Note

Capture-Release Protocol for Araguaian River Dolphins (*Inia araguaiaensis*) and Preliminary Physiological Parameters: An *In Situ* Approach for Research and Conservation

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The Araguaian river dolphin (*Inia araguaiaensis*), or Araguaian boto, is the only cetacean species endemic to Brazil, and is restricted to the Araguaia-Tocantins River system (Hrbek et al., 2014). The species was described in 2014 by Hrbek and colleagues, who analyzed samples from two extremes of the *Inia* distribution. Despite the molecular and osteological evidence presented in the study, the Committee on Taxonomy of the Society for Marine Mammalogy (2017) questioned the species-level designation of *Inia araguaiaensis*. However, most South American scientists support its classification as a distinct species (Melo-Santos et al., 2019, 2020; Rodrigues et al., 2019; Brum et al., 2021; da Silva et al., 2023). Reflecting this consensus, the Brazilian government, where the species exclusively occurs, recognizes its taxonomic status and classifies it as "Vulnerable to Extinction" (Brasil, 2022). The International Union for Conservation of Nature's *Red List* (da Silva et al., 2018) acknowledges the existence of *Inia araguaiaensis* but currently provides a conservation status ("Endangered") only for *Inia geoffrensis*.

Inia araguaiaensis forms groups of up to 20 individuals, with an average of five, and primarily preys on fish (Hrbek et al., 2014; Melo-Santos et al., 2020; Echeverria et al., 2022). Major threats to the species include overfishing, bycatch, illegal hunting, habitat degradation and loss, obstruction of river corridors by hydroelectric dams, and contamination from organochlorines and heavy metals (Paschoalini et al., 2020; Brum et al., 2021).

Araguaian river dolphins are apex predators that contribute to the balance of aquatic ecosystems and trophic chains (Brum et al., 2021; Echeverria et al., 2022). As such, they can also be considered sentinel species, acting as indicators of environmental health in alignment with the One Health concept (Bossart, 2011; Brum et al., 2021; Silva et al., 2023). These dolphins exhibit tolerant behavior towards anthropogenic activities, making them approachable and interactive with humans, which attracts media and tourist attention (Alves et al., 2011, 2012; Rodrigues et al., 2019). However, such interactions can lead to undesirable behavioral changes and pose health risks due to the potential transmission of zoonoses (Buck et al., 1993; Samuels & Spradlin, 1995; Moreno et al., 2008; Waltzek et al., 2012).

Capturing and collecting biological samples from live river dolphins *in situ* presents significant challenges as some methods can stress the animals, potentially resulting in mortality risks. Nevertheless, the information obtained is crucial for understanding population health, monitoring diseases, obtaining germplasm for biobank formation, and tracking hormone levels (Gulland & Hall, 2007; Barratclough et al., 2019; Pizzutto et al., 2021a, 2021b). Despite their interactions with humans, reports on capture-release efforts for river dolphins, particularly those of the genus *Inia*, remain scarce (da Silva & Martin, 2000; de Mello & da Silva, 2019; de Mello et al., 2021). Such protocols are indispensable for advancing research on biological and health parameters (de Mello & da Silva, 2019; de Mello et al., 2021), life history traits (Martin & da Silva, 2018), telemetry and movement patterns (Campbell et al., 2023), and habitat use through mark-recapture studies (Mintzer et al., 2016, 2020). These studies provide critical data that directly inform conservation efforts for river dolphins.

In this short note, we describe a specific protocol for the capture and release of Araguaian river dolphins, enabling the collection of biological material for studies in accordance with the One Health and One Conservation concepts (Rocha et al., 2021). Any such protocol requires careful planning and adherence to ethical and legal guidelines to ensure the well-being and conservation of these animals. Thus, our general protocol was performed in the following three steps: (1) *Permits and Permissions:* we obtained all necessary permits and permissions from relevant authorities, ensuring compliance with local, national, and international regulations regarding the capture and handling of protected species; (2) *Capture Team:* we assembled a team of experienced personnel, including veterinarians, biologists, and trained fieldworkers, who have expertise in dolphin capturing and handling; and (3) *Equipment:* we ensured all capture and handling equipment was in good working condition, including dolphin-safe nets or stretchers.

The capture was conducted at the market in the town of Mocajuba in Pará, Brazil (2° 35' 0.19" S, 49° 30' 41.32" W), in the lower Tocantins River, eastern Amazon. The procedures were approved by the Animal Research Ethics Committee of the Federal Rural University of the Amazon (CEUA-UFRA No. 5342120922), the Chico Mendes Institute for Biodiversity Conservation (SISBIO Licenses No. 44915-16 and 94110-1), and the Pará State Environmental Secretariat (No. 5331/2023).

From September to October 2023, a 10-d expedition was conducted to evaluate the population of Araguaian river dolphins that regularly visit the town's market (Melo-Santos et al., 2019). These dolphins have a long history of interacting with the local population and tourists, primarily attracted by the provision of fish—a behavior that has persisted for over 40 y (Rodrigues et al., 2019). To avoid recapture during the study, the research team visually identified individual animals using unique natural marks. These animals are known to our team as we have been monitoring them for over 10 y.

Only one animal was captured per day as it was necessary to wait for the tide to recede to form a beach section where the animals would be restrained and examined, and the animals became skittish after one of the group members was captured. With all animals, biological material was collected for parallel studies conducted by associated researchers, including blowhole sprays, nasal secretions, blood (collected via pectoral and caudal fins), feces, and various swabs, including oral, genital, anal, skin lesions, blowhole, mouth, and mammary slits. Acoustic recordings of sounds produced by dolphins were made by positioning a hydrophone directly on the melon. Ultrasound examinations of the thoracic, abdominal, ocular, and melon regions were also performed. A fecal sample was obtained from only one animal (an adult female) that spontaneously defecated during restraint.

The capture method was adapted from the technique described by da Silva & Martin (2000). A seine net made of cotton threads, measuring 36 × 30 × 50 × 100 m (mesh 25; thread 48), was used. The animals are accustomed to our research team as we have been conducting studies in the area for the past 10 y (Melo-Santos et al., 2014, 2019; Rodrigues et al., 2019; Echeverria et al., 2022). However, since we had never used seine nets before, we habituated the animals to the net over a 2-d period (Figure 1A & B). Because tourists and locals feed the animals, they typically approach humans, which allowed us to collect biological material (e.g., swabs from skin lesions, oral swabs, sprays on Petri dishes) and acoustic recordings, and to conduct ultrasound examinations with and without capturing the animals.

A team of 15 to 17 people, including veterinarians, biologists, and trained fieldworkers (local fishermen), formed a human circle

Figure 1. Sequence of steps for dolphin capture in Mocajuba, Pará, Brazil: (A) habituation of the animals to the team, with encirclement without the containment net; (B) habituation of the animal to the stretcher in the water (arrow); (C) encirclement of the animal with the net raised (arrow); (D) animal attempting to escape the encirclement with the net lowered; (E) preparation of the beach area to receive the captured dolphin (moistened mattress on a wooden pallet covered with blue plastic sheeting); (F) captured animal being lifted by the team using the stretcher; (G) transport from the river to the examination and sample collection area; and (H) animal restrained in right lateral recumbency for access to the left pectoral fin vein. (Photos provided by Alessandro Falco)

around the target dolphin holding a net above the water (Figure 1C). Each animal was individually attracted to the human circle by slapping the water surface with our hands, in the same manner that locals and tourists attract them (Figure 1C). When the desired animal entered the circle, and upon command from a team member, the net was dropped and the bottom line held down with the feet of each team member to prevent the boto's escape (Figure 1D). One researcher attracted the dolphin to the circle center, while another one distracted any other animal outside the circle that could potentially disrupt the capture.

With the animal trapped in the circle, a group of six to seven people detached from the larger circle and surrounded the dolphin, forming a smaller circle around it and preparing for its capture (Figure 1F). Then, using a stretcher made of cotton straps (2 × 2.20 m; capacity up to 1 ton), this team lifted the dolphin out of the water, physically restraining its jaws and fluke to avoid accidents (Figure 1H). The team then carefully transported the dolphin to the beach, where a platform of wooden pallets covered with plastic sheeting and a moistened mattress had been set up (Figure 1E & G).

With the animal contained on the mattress, a rigorous protocol was conducted to continuously evaluate its condition. River water was sprayed using regular gardening sprayers to keep the animal moist throughout the procedure, and it was shielded from direct sunlight with a makeshift tent, created by people holding wet towels to block the sunlight. The animal was continuously monitored, with respiratory and heart rates recorded every 5 min. Heart rate was assessed using a veterinary vascular Doppler (DV 610 V; Medmega, Franca, São Paulo, Brazil). To protect the eyes and minimize stress, a moist gauze was placed over the animal's eyes. After all procedures were complete, we released the dolphin gently into the water, ensuring minimal disruption and allowing sufficient time for the individual to acclimate and re-establish normal behaviors. All capture-release procedures were demonstrated beforehand in a video (the supplemental video for this short note is available in the "Supplemental Material" section of the *Aquatic Mammals* website).

Total body length and thoracic circumference measurements were obtained using a measuring tape, while sex determination was based on the presence of mammalian slits around the genital region. Ultrasound examinations (C10RL Dual-Probes Multipurpose Ultrasound; Konted Medical Ultrasound, Beijing, China) were used to assess the reproductive condition of adult females (non-pregnant/non-lactating, pregnant, or lactating) and to evaluate organ morphology. To minimize disturbance and accommodate time constraints, ultrasound data were collected while the dolphins were unrestrained in the water. The prioritization of biological sample collection limited the scope of additional ultrasound assessments.

Swabs (oral, anal, genital, respiratory opening, mammary slits, and skin lesions), blood, nasal secretions, skin tissue, and water samples were collected and transported at 4°C in isothermal boxes. Samples intended for fungal and bacterial analysis were transported in a pre-enrichment medium. Water sample pre-enrichment was performed in the laboratory, where all microbiological samples were plated onto selective media and incubated. Blood samples were processed immediately for hematological tests in a field laboratory. Other field samples, such as water and respiratory sprays (collected in Petri dishes), were stored in isothermal boxes, then transferred to a refrigerator at -20°C, and later moved to a -80°C freezer. Hydrophones (sampling rate: 576 kHz; Soundtrap High Frequency; Ocean Instruments, Auckland, New Zealand) were used to record sounds from the dolphins' melons as well as sounds of dolphins in the water during and after capture.

Animals were classified by age based on total length and sexual maturity (de Mello & da Silva, 2019). During the 10-d expedition, 12 individuals were observed frequenting the capture site, including one adult male, six adult females, three juveniles (two males and one female), and two calves (one male and one female). These individuals have been monitored yearly by the same team since 2013 (Melo-Santos et al., 2019; Rodrigues et al., 2019). The established protocol resulted in the capture of 42% (5 of the 12 individuals) of the dolphins that frequented the site during the expedition. Of the five captured individuals, three were males (the largest adult in the group and two juveniles) and two were females (one adult and one juvenile).

It is important that initial capture attempts began only on the third day of the expedition, allowing the animals to become accustomed to the presence of the net and capture stretchers. Dolphins were restrained for an average of 21.6 ± 5.4 min (range: 15 to 27 min) to collect biological materials (Table 1). This capture time was shorter than the 30 min (range: 18 to 45 min) reported by de Mello & da Silva (2019) using seine net techniques. The reduced handling time in our study is likely due to improvements in capture protocols, smaller group sizes, and more efficient handling methods, which streamlined the process and minimized stress. Reducing handling time is essential for safeguarding animal welfare during research activities.

Biometric data and physiological parameters, including respiratory and heart rates, are summarized in Table 1 and Figure 2. Juvenile dolphins exhibited a lower average respiratory

Table 1. Capture duration and biometric data according to age class and sex of Araguaian river dolphins (*Inia araguaiaensis*; *n* = 5) in Mocajuba, Pará, Brazil

Individual	Age class	Sex	Capture time (min)	Total length (cm)	Thoracic circumference (cm)
Coti	Juvenile	Male	27	175	87
Manga	Adult	Female	15	179	95
Cuíras	Juvenile	Male	26	164	97
Tiff	Juvenile	Female	17	159	91
Double tooth	Adult	Male	23	236	129
Mean ± SD (Minimum – Maximum)			21.60 ± 5.37 (15 – 27)	182.60 ± 9.32 (159 – 236)	99.8 ± 16.77 (87 – 129)

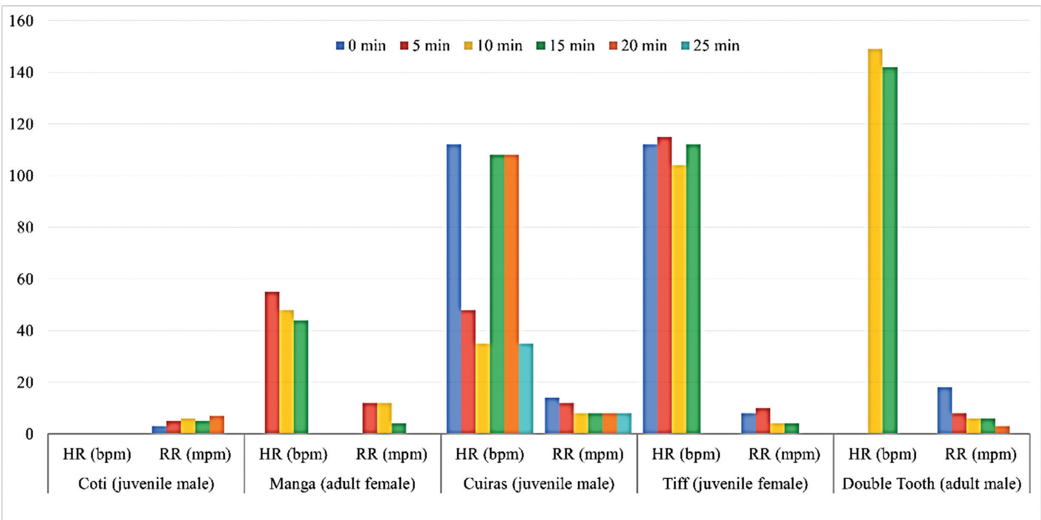

Figure 2. Heart rate (HR; beats per minute [bpm]) and respiratory rate (RR; movements per minute [mpm]) during each capture and restraint of the Araguaian river dolphin (*Inia araguaiaensis*) in Mocajuba, Pará, Brazil

rate of 7.3 ± 3.0 movements per minute (range: 3 to 14 mpm) compared to adult dolphins, whose average respiratory rate was 8.6 ± 5.0 mpm (range: 3 to 18 mpm). This finding aligns with observations in harbor porpoises (*Phocoena phocoena*) by Eskesen et al. (2009) in which elevated respiratory rates were linked to thoracic pressure caused by surface contact during handling. The slightly higher respiratory rates in adults in our study likely reflect tachypnea as a compensatory response to hindered lung filling when removed from the water. In contrast, de Mello & da Silva (2019) reported a similar respiratory rate range (3 to 18 mpm) for both juvenile and adult *I. geoffrensis*.

Heart rate analysis revealed that juveniles had a higher average heart rate (88.9 ± 34.5 bpm; range: 35 to 115 bpm) compared to adults (79.6 ± 43.6 bpm; range: 44 to 142 bpm). These results differ from Eskesen et al. (2009) who suggested elevated heart rates might be caused by fluke overheating, while bradycardia could result from body flexion during stress. The higher heart rates observed in juveniles in our study may reflect age-related physiological differences or heightened sensitivity to stress. Similarly, de Mello & da Silva (2019) reported juvenile heart rates ranging from 110 to 118 bpm and adult rates from 60 to 113 bpm under moderate stress conditions. These variations may be attributed to differences in procedural techniques, environmental conditions, or individual physiological variability within the species.

The dolphins in our study exhibited moderate stress levels, as defined by de Mello & da Silva (2019), characterized by mild resistance to handling, occasional vocalizations, and moderate responsiveness to external stimuli. These stress indicators are crucial for interpreting the observed physiological responses. The differences in respiratory and heart rates between age groups highlight the complexity of physiological responses to stress. Factors such as age, handling protocols, stress levels, and environmental conditions are likely to interact to influence these parameters. For instance, juveniles' higher heart rates may reflect greater sensitivity to stress or age-related physiological differences, whereas adults' slightly elevated respiratory rates may serve as a compensatory mechanism for thoracic pressure when out of the water.

We acknowledge that limitations, including a small sample size and disparities in data collection methods, reduce the robustness of the comparisons presented. Nevertheless, these findings offer valuable preliminary insights into the physiological responses of river dolphins to capture and handling, and contribute to a growing understanding of the physiological effects of research activities on freshwater cetaceans.

Future studies with larger sample sizes, standardized protocols, and refined handling techniques will be essential to confirm these findings. Such efforts will not only enhance scientific understanding but also ensure that research activities prioritize the welfare of the animals involved.

In marine environments, the capture protocol for *in situ* common bottlenose dolphins (*Tursiops truncatus*) involved chasing the animals with boats, then encircling them with a seine net, with people around the net's circumference performing the restraint. The dolphins are then placed inside a boat for biological material collection (Fair et al., 2006, 2014). Although the capture and release of marine and river dolphins involves distinct protocols due to their different environments and behaviors, both require careful planning and execution, tailored to their specific conditions/realities. Thus, minimizing stress and ensuring quick recovery are paramount in all capture-release operations.

Unfortunately, capturing the population of Araguaian river dolphins is essential due to the multiple threats they face. The town of Mocajuba in Pará, Brazil, is a popular tourist destination, which increases the risk of zoonotic diseases through close human–dolphin interactions such as swimming with the animals. Additionally, these dolphins inhabit the Tocantins River, the most human-impacted river system in the Amazon, which is subjected to illegal fishing, mining, agriculture, deforestation, and large vessel traffic (Goulding et al., 2003). With a population of only around 1,000 individuals (Paschoalini et al., 2020), the species is particularly vulnerable.

Given these challenges, gathering scientific knowledge through biological data obtained during capture-release operations is crucial. These data will not only enhance our understanding of the species but also inform conservation strategies, especially in response to extreme events such as the 2023 drought, which led to the death of hundreds of dolphins. By following a structured protocol, researchers and conservationists can minimize stress, maximize health and safety, and ensure the successful collection of valuable data. Furthermore, the analysis of the samples collected through this protocol can provide crucial insights to guide the development of public policies by government agencies, supporting the long-term conservation and management of Araguaian river dolphins.

Note: The supplemental video for this short note is available in the "Supplemental Material" section of the *Aquatic Mammals* website: https://www.aquaticmammalsjournal.org/supplemental-material.

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