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CAPTURE AND HANDLING STRESS IN INCIDENTALLY CAPTURED RAYS FROM SMALL-SCALE FISHING: A PHYSIOLOGICAL APPROACH

Bianca de Sousa Rangel[1]*, *Alexandre Rodrigues*[2], *Renata Guimarães Moreira*[1]

[1] Universidade de São Paulo, Instituto de Biociências, Departamento de Fisiologia, Laboratório de Metabolismo e Reprodução de Organismos Aquáticos. Rua do Matão, travessa 14, 321, CEP 05508-090, Cidade Universitária, São Paulo, SP, Brazil.

[2] Universidade Estadual Paulista, Instituto de Biociências de Botucatu, Laboratório de Biologia e Genética de Peixes. Rua Professor Doutor Antonio Celso Wagner Zanin, s/n, CEP 18618-689, Botucatu, SP, Brazil.

E-mails: biarangel.sharks@gmail.com (*corresponding author); tubabiomar@hotmail.com; renata.fish@gmail.com

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Abstract: Incidental capture is the most common threat to rays worldwide, by both artisanal and industrial fishing. To better understand this threat, we evaluated the capture and handling stress in three incidentally captured benthopelagic ray species: American cownose ray (*Rhinoptera bonasus*), Brazilian cownose ray (*Rhinoptera brasiliensis*), spotted eagle ray (*Aetobatus narinari*), and one benthic species, the longnose stingray (*Hypanus guttatus*). Through analyzing secondary stress physiological variables (plasma lactate and glucose), our results revealed a similar physiological stress response in benthopelagic rays, suggesting they are resilient to capture using beach seine fishing. We also demonstrated that handling for research can increase the stress in both American cownose and spotted eagle rays, suggesting that more stringent handling protocols for research should be required. Findings from this study expands on the number of ray species for which stress to capture and handling has been evaluated, providing recommendations for appropriate research and management.

Keywords: Incidental capture; elasmobranchs; non-lethal research; beach seine fishing; batoid; stingrays.

Batoids (rays, skates and their relatives) are the most diverse and endangered group of elasmobranchs (Last *et al.* 2016). Their life-history traits (*i.e.* slow growth, late sexual maturity, low fecundity and long gestation period) coupled with increasing anthropogenic pressures (*e.g.* habitat degradation and fisheries interactions) makes them particularly vulnerable to overexploitation and incidental capture (Stevens *et al.* 2000). Incidental capture is the most common threat to batoids worldwide, by both artisanal and industrial fishing (*e.g.* Dulvy *et al.* 2017). For the species that are captured alive, hypoxia, hypercapnia, and exhaustive exercise during capture and handling can compromise their fitness and survival, reducing the efficiency of compensatory release (Cicia *et al.* 2012, Wosnick *et al.* 2018). Physiological response to capture and handling has received more attention in sharks (Skomal & Mandelman 2012, Marshall *et al.* 2012). However, few researches have been conducted on rays, which are routinely captured and released or discarded as incidental capture in ground fishing operations (e.g. Cicia *et al.* 2012, Lambert *et al.* 2018).

Understanding how rays respond to fisheries interactions and handling may elucidate patterns of vulnerability and resilience to fishing and non-lethal research. For example, it may help in identifying species that are more sensitive and need to be released quickly (*e.g.* hammerhead sharks, Gallagher *et al.* 2014) or even those with high resistance and survival when exposed to capture and handling stress (*e.g.* the guitarfish *Zapteryx brevirostris*, Wosnick *et al.* 2018). Stress-induced blood parameters such as lactate and glucose have been commonly used to assess the secondary response in elasmobranchs (Marshall *et al.* 2012, Jerome *et al.* 2017). Glucose is an important metabolic fuel, which is mobilized rapidly from liver and muscle (through glycolysis and gluconeogenesis) during stress in response to increased circulating glucocorticoid hormones (*e.g.* Ruiz-Jarabo *et al.* 2019). However, several studies have been shown that plasma lactate concentration is the most informative and predictive physiological marker to evaluate stress response in elasmobranchs (Cicia *et al.* 2012, Lambert *et al.* 2018). Increases in plasma lactate concentrations, a metabolite resulting from the anaerobic metabolism, usually occur after the stress of capture and air exposure (*e.g.* Hoffmayer *et al.* 2012, Lambert *et al.* 2018).

In this study, we evaluated two physiological markers of secondary stress response (plasma lactate and glucose) of four ray species frequently captured as incidental capture by beach seine fishing: American cownose ray *Rhinoptera bonasus* (Mitchill, 1815), Brazilian cownose ray *Rhinoptera brasiliensis* (Müller, 1836), spotted eagle ray *Aetobatus narinari* (Euphrasen, 1790), and longnose stingray *Hypanus guttatus* (Bloch & Schneider, 1801). Given that the beach seine fishing has been previously reported to allow high survival rates post-capture (Rangel *et al.* 2018), our objectives were (i) to evaluate the differences in the stress response during capture among ray species; and (ii) to assess the physiological profile during handling for research in both American cownose and spotted eagle rays, the two most captured species in the study area compared to the others.

The rays were opportunistically collected from January 2016 to February 2017 in Bertioga, Guaibe Sector, a marine protected area located in the state of São Paulo, Southeastern Brazil (23°49’35.02”S; 46°5’41.69”W). Specimens were sampled following incidental capture by fisher with beach seine, using a 400 x 11 m fishing net, mesh-size of the 80 mm between knots in the wings and 70 mm in the bag, thrown at 400-600 m from the beach and the gathered by manual traction, with approximately six fishers in each of the trawl ropes. Fishing duration was approximately 40 minutes, with the main target fishes being *Centropomus* spp., *Mugil* spp., and *Scomberomorus* spp. (see Rangel *et al.* 2018 for details). The research was conducted under permits provided by the SISBIO (ICMBIO/SISBIO # 48572–1) and the Animal Ethics Committee (CEUA; # 258/2016) of the Institute of Biosciences, University of São Paulo.

Following capture, rays were immediately removed from the net and individually placed in plastic containers (50 L) filled with seawater (2 or 3 individuals per box). Following the recording of biometric data *i.e.* disc width (DW) and weight (data not shown in the present study), blood samples (~1 mL) were taken by caudal venipuncture. After all procedures, rays were individually released (from 5 to 30 minutes). Then, after approximately four hours, blood samples were centrifuged for 5 minutes (655.2 g) to separate the plasma. Plasma samples were stored at -80°C until analysis. Lactate and glucose levels were measured in plasma using commercial kits (Labtest®, Brazil) with colorimetric enzymatic reaction using a spectrophotometer ELISA (Spectra Max® 250, Molecular Devices).

To evaluate the differences in the stress response to capture among ray species, the difference of plasma lactate and glucose levels were assessed using one-way ANOVA followed by the Dunn’s *post hoc* test. To assess the physiological profile handling for research in both American cownose and spotted eagle rays, they were separated into two groups: handling time 1: the first rays sampled after capture (approximately 5 minutes), handling time 2: rays sampled later (confinement of 10-20 minutes followed by handling for sample collection). The Student t test (unpaired two-sample comparison) was used to test whether mean plasma lactate and glucose differed between handling time 1 and time 2. Individual lactate concentration of American cownose rays over the course of handling exposure in each day (on March 23th 2016; December 22th 2016; February 7th 2017, and February 22th 2017), *i.e.* handling sequence 1 (~5 min), 2 (~10 min), 3 (~15 min) and 4 (~20 min), in which no statistical test was performed.

Statistical significance was declared at $p < 0.05$, and analyses were conducted in PAST 3.12 (EFB; www.essential-freebies.de).

A total of 45 individual rays of 4 total species were sampled, number and sizes of rays sampled were as follows: American cownose ray (N = 28; 49.1 ± 8.83 cm DW, mean ± SD), Brazilian cownose ray (N = 4; 50.4 ± 3.35 cm DW), spotted eagle ray (N = 7; 59.4 ± 18.25 cm DW) and longnose stingray (N = 6; 47.1 ± 14.10 cm DW). The low number of samples for Brazilian cownose ray, spotted eagle ray, and longnose stingray was due to the low capture rate on the days of sampling.

No significant differences were verified among the plasma lactate levels of rays sampled (Table 1, Figure 1a). Longnose stingrays showed significantly lower glucose values than American cownose rays and spotted eagle rays (Table 1, Figure 1b). Plasma lactate increased in American cownose rays with handling sequence (Figure 2a). Later sampled rays (handling time 2) showed significantly higher lactate values than rays sampled before (handling time 1), for American cownose rays (Student t test, $p = 0.004$; Figure 2b) and spotted eagle rays (Student t test, $p = 0.037$; Figure 2c). There were no significant differences in glucose values between the handling time in American cownose rays (time 1: 49.0 ± 14.38 mg dL^{-1}; time 2: 60.7 ± 15.98 mg dL^{-1}; Figure 2d) and spotted eagle rays (time 1: 58.4 ± 16.90 mg dL^{-1}; time 2: 59.9 ± 6.49 mg dL^{-1}; Figure 2e).

Our findings revealed a similar physiological stress response in incidentally captured rays from small-scale fishing. We also found changes in physiological disturbance during handling for research in both American cownose and spotted eagle rays. This study represents the first investigation to consider the impacts of capture and handling stress on the physiological alterations of these species. Our results suggest that beach seine fishing does not seem to have an alarming effect on the physiological stress response, corroborating previous studies reporting a high survival rate (Rangel *et al.* 2018). However, we suggest caution because our sample size was low for 3 of the 4 species, and therefore, the power of the analysis in finding significant results was low. Additionally, our results also revealed that lactate is the most informative and predictive physiological marker, again corroborating previous studies (*e.g.* Cicia *et al.* 2012, Lambert *et al.* 2018).

The similar physiological stress response found in these ray species may be due to their phylogenetic proximity (Adnet *et al.* 2012) and similar lifestyles to benthopelagic rays with relatively high-movement behavior (*e.g.* Ajemian & Powers 2014). An exception was found in the longnose stingray, which showed lower glucose levels (average 40 mg dL^{-1}), indicating a different aerobic response compared to other species which may be because of their more sedentary lifestyle staying motionless during stressful activities (*i.e.* capture; Lambert *et al.* 2018). Previous studies have shown a similar glucose concentration in other demersal ray species, for example the Southern stingray *Hypanus americanus* (Cain *et al.* 2004); smalltooth sawfish *Pristis pectinata*

Table 1. Lactate and glucose concentration (mean ± standard deviation) and results of ANOVA test performed to evaluate the differences among species: American cownose ray (*Rhinoptera bonasus*), Brazilian cownose ray (*Rhinoptera brasiliensis*), spotted eagle ray (*Aetobatus narinari*), and longnose stingray (*Hypanus guttatus*). t values for Dunn's post hoc. Significant ($p < 0.05$) results shown in bold.

	Lactate (mg dL^{-1})	**Brazilian cownose ray**	**Spotted eagle ray**	**Longnose stingray**
American cownose ray	34.8 ± 20.43	0.879	0.748	0.702
Brazilian cownose ray	36.3 ± 30.38	--	0.931	0.694
Spotted eagle ray	33.2 ± 24.64	--	--	0.580
Longnose stingray	42.0 ± 30.53	--	--	--
	Glucose (mg dL^{-1})			
American cownose ray	57.6 ± 18.16	0.930	0.680	**0.009**
Brazilian cownose ray	57.8 ± 14.10	--	0.838	0.057
Spotted eagle ray	59.3 ± 10.82	--	--	**0.015**
Longnose stingray	40.2 ± 3.26	--	--	--

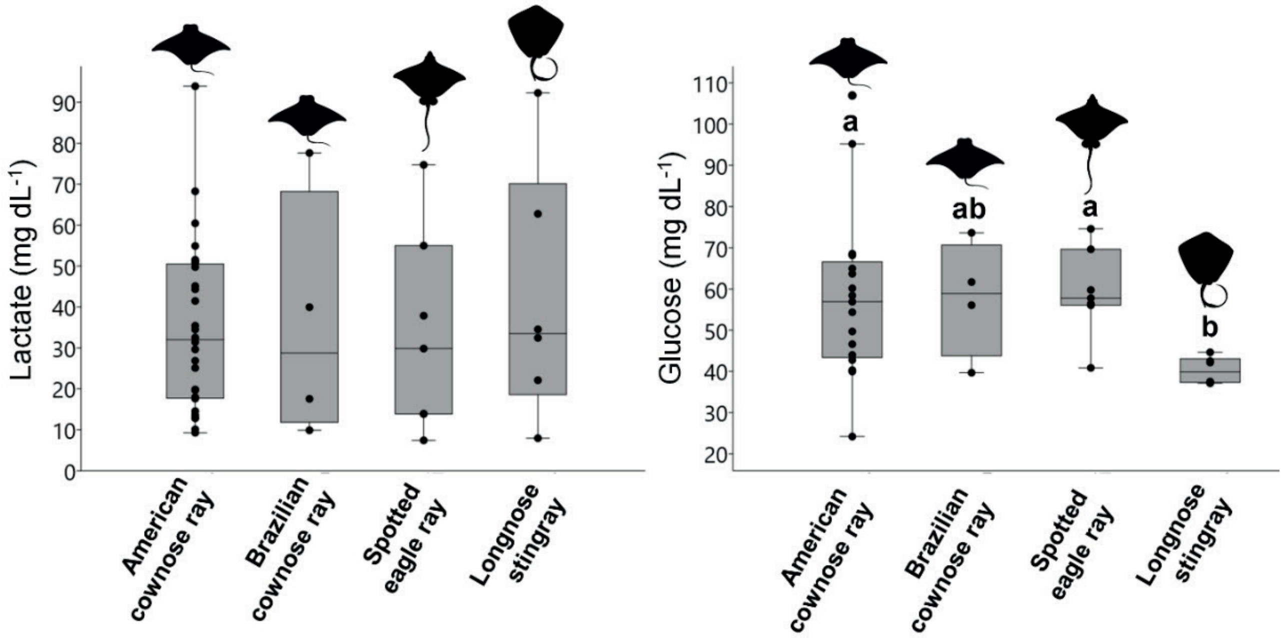

Figure 1. Boxplots of **(a)** lactate concentration **(b)** and glucose concentration of American cownose ray (*Rhinoptera bonasus*), Brazilian cownose ray (*Rhinoptera brasiliensis*), spotted eagle ray (*Aetobatus narinari*), and longnose stingray (*Hypanus guttatus*). ^{abc} Significant difference among species (ANOVA, $p < 0.05$).

(Prohaska *et al.* 2018); Atlantic stingray *Hypanus sabinus* (Lambert *et al.* 2018).

Our results showed that the large intraspecific variation found in lactate concentrations (7-93 mg dL^{-1}) is a result of handling time. Significant increases in lactate levels were recorded in later sampled rays (10-20 minutes post-capture / handling time 2). In addition, the results showed a gradual increase in lactate over time. Elevated lactate levels suggest continued physiological disruptions for as long as the rays are kept in confinement. Similar findings have demonstrated a consistent increase in lactate concentration over the course of the stressor (Cicia *et al.* 2012, Lambert *et al.* 2018). Since American cownose and spotted eagle rays are highly mobile, this effect may be potentiated by exhaustive exercise associated with capture and handling, and therefore, increased contribution of anaerobic functioning to satisfy energy demands (Bouyoucos *et al.* 2019). Despite this considerable increase, the values are below those described in moribund and dead elasmobranchs (> 180 mg dL^{-1}; Moyes *et al.* 2006, Wosnick *et al.* 2018), indicating that rays are able to recover after release. Given that lactate is one of the best predictors of post-release mortality (Moyes *et al.* 2006, Gallagher *et al.* 2014, Jerome *et al.* 2017), constant monitoring of this physiological marker may indicate at which life-stages and seasons the rays are most vulnerable to capture.

When not associated with research, immediate release is recommended. Considering the lactate values of the first sampled rays (9-35 mg dL^{-1}), and therefore, results closely related to capture response, our findings suggest that these ray species have a low stress response in this small-scale fishing. Indeed, the high survival rates reported in our previous study (*i.e.* 98.8 %; Rangel *et al.* 2018) confirm that this fishing gear does not have an immediate impact on the physiological condition of rays. Nevertheless, further studies should consider the post-release mortality and sub-lethal effects on growth and reproduction (*e.g.* Wilson *et al.* 2014, Wheeler *et al.* 2020).

Our findings expand on the number of ray species for which stress to capture and handling has been evaluated and provide recommendations for appropriate research, management and conservation efforts. Although limited, our results showed that these species are resilient to capture using beach seine fishing, however, they demonstrate that associated research can increase the stress caused by capture. Consequently, more stringent handling protocols for research should be required to reduce the physiological stress. Since the highly mobile rays appear to have a more

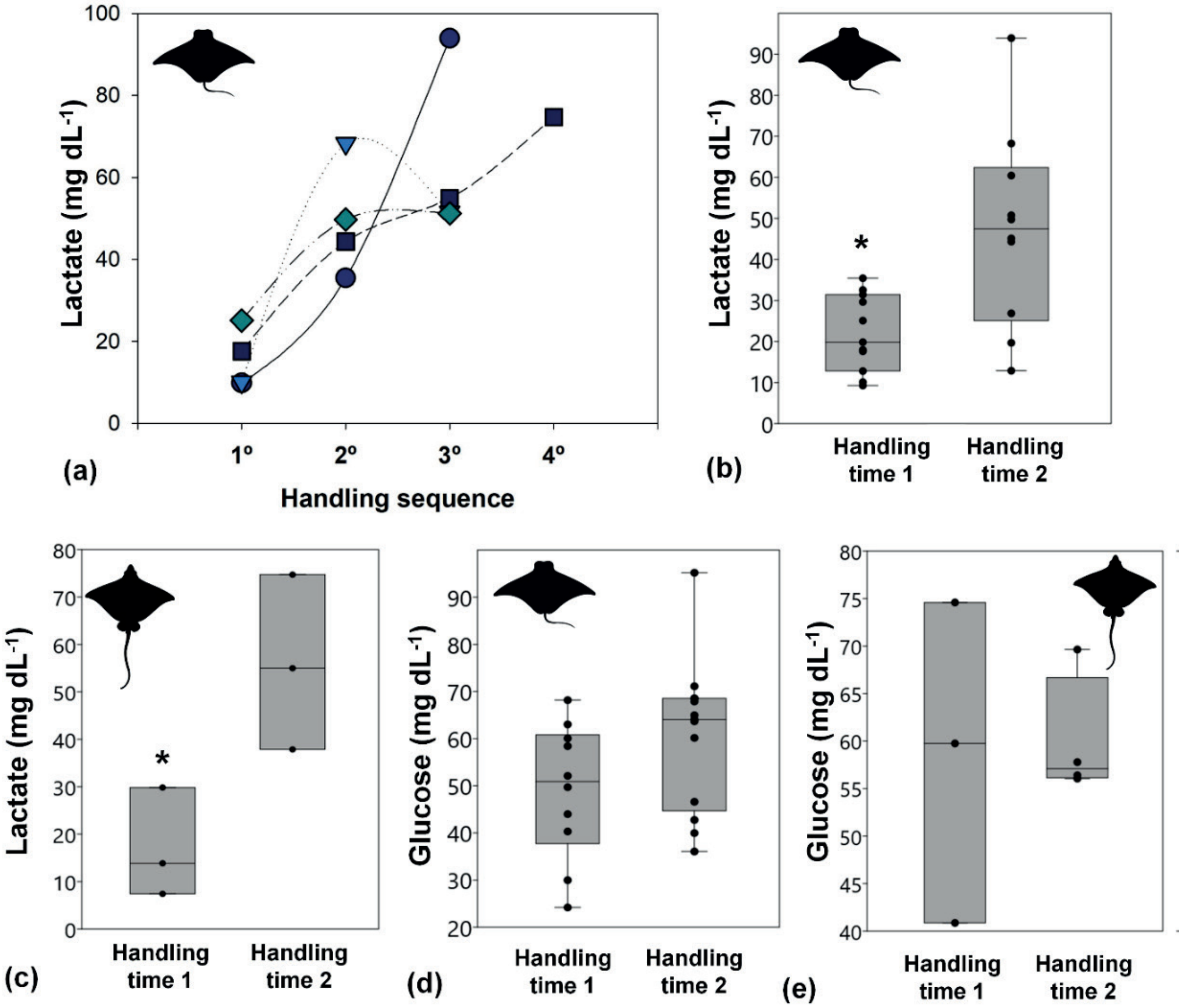

Figure 2. **(a)** Individual lactate concentrations of american cownose ray (*Rhinoptera bonasus*) over the course of handling exposure in each day (●March 23th 2016; ▼ December 22th 2016; ■ February 7th 2017 and ◊ February 22th 2017). Handling sequence: 1 (~5 minutes), 2 (~10 minutes), 3 (~15 minutes) and 4 (~20 minutes). Boxplots of **(b)** lactate concentration of American cownose ray **(c)** lactate concentration of spotted eagle ray (*Aetobatus narinari*), **(d)** glucose concentration of american cownose ray and **(e)** glucose concentration of spotted eagle ray. * Significant difference between handling time 1 and 2 (Student t test, $p < 0.05$).

pronounced response (aerobic metabolism) when compared to more sedentary species, future field and laboratory experiments should investigate the vulnerability of these species to specific release recommendations, *e.g.* releasing highly mobile rays first. For example, active or R.A.M ventilating sharks experience higher mortality than benthic species (Skomal & Mandelman 2012). Batoids exhibit plastic responses to capture, *e.g.* surviving several hours out of the water (Wosnick *et al.* 2018), and generally have remarkably high post-capture survival. Such evidence reinforces the need for further studies with other batoid species and dissemination of programs such as participatory monitoring to encourage the immediate release, which will be essential for the improved management and conservation of this threatened group.

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