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## COMMUNICATION

### FIRST RECORD OF A MORPHOLOGICALLY ABNORMAL AND HIGHLY METAL-CONTAMINATED SPOTBACK SKATE *ATLANTORAJA CASTELNAUI* (RAJIFORMES: ARHYNCHOBATIDAE) FROM SOUTHEASTERN RIO DE JANEIRO, BRAZIL

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## First record of a morphologically abnormal and highly metal-contaminated Spotback Skate *Atlantoraja castelnaui* (Rajiformes: Arhynchobatidae) from southeastern Rio de Janeiro, Brazil

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**Abstract:** This paper reports the first record of a morphologically abnormal and highly metal-contaminated Spotback Skate *Atlantoraja castelnaui* (Ribeiro, 1907) (Elasmobranchii, Rajidae) in Rio de Janeiro, Brazil. Incomplete fusion of the right pectoral fin with the head was observed, while a radiography indicated muscle sheaf discontinuity near the rostrum. Extremely high contamination by several elements, including teratogenic As, Hg and Cd in the individual was detected. The observed morphological deformity may be due to high concentrations of teratogenic elements in the environment, possibly playing a role in abnormal embryonic development in egg cases exposed to high environmental concentrations of these contaminants. *Atlantoraja castelnaui* is the least biologically understood member of the genus *Atlantoraja*, and this paper furthers both morphological observations and ecotoxicological assessments on this species.

**Keywords:** Altered embryonic development, Arhynchobatidae, food safety, metal contamination, morphological abnormality,

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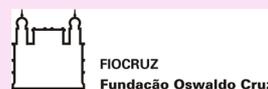
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**Ethics statement:** Elasmobranch sampling is permitted under SISBIO license 70078-1. All samples were bought from local artisanal fishers and no animals required sacrifice.

**Competing interests:** The authors declare no competing interests.

For **Author details**, **Author contribution** and **Acknowledgements** see end of this article.



## INTRODUCTION

The Spotback Skate *Atlantoraja castelnaui* (Ribeiro, 1907), Arhynchobatidae, is endemic to the southwestern Atlantic Ocean, between Rio de Janeiro, Brazil, and northern Argentina (Hozbor et al. 2004; Figueiredo & Menezes 2015). *A. castelnaui* can reach 1.5m and occurs between 10 and 500 meters in depth, with benthic habits, oviparous reproduction mode and feeds on teleost fish, cephalopods, decapods and other elasmobranchs (Moreira et al. 2011; Barbini & Lucifora 2012; Figueiredo & Menezes 2015). It is especially vulnerable to trawl fisheries due to its benthonic habits (Ebert & Sulikowski 2009). In addition, owing to its large size, this taxon achieves high commercial values and trawl experiences along the coast of Uruguay and Argentina have indicated a 75% drop in biomass between 1994 and 1999 (Hozbor et al. 2004). As such, many populations are overexploited throughout their distribution, *A. castelnaui* is listed as “Endangered” by the IUCN and currently undergoing decreasing population trend (Hozbor et al. 2004). In fact, the vulnerability of large skates and rays to overexploitation and, consequently, stock depletion, is well documented (Dulvy & Reynolds 2002). Given this scenario, alongside the fact that this species is the least biologically understood member of the *Atlantoraja* genus (Moreira et al. 2011), information on the basic biology of *A. castelnaui* is required to support fisheries management and conservation actions (Ribeiro-Prado et al. 2008).

The most common morphological abnormality in skates (order Rajiformes) is the non-fusion of the pectoral fins to the head or rostrum (Mejía-Falla et al. 2011) (Figure 1), and some studies have reported such abnormalities for the Arhynchobatidae family (Casarini et al. 1996; Ribeiro-Prado et al. 2008).

In order to contribute towards biological knowledge on *A. castelnaui*, the aim of this study was to describe a morphological abnormality in a very young specimen captured in southeastern Brazil, where no conservation measures are in place for this species (Hozbor et al. 2004), through morphometric measurements, radiography and chemical analyses.

## MATERIAL AND METHODS

An abnormal and very young male *A. castelnaui* specimen was collected during regular field studies of elasmobranchs caught by artisanal fishing gillnets at Tamoios, Cabo Frio, southeastern Brazil (Image 1) on 12

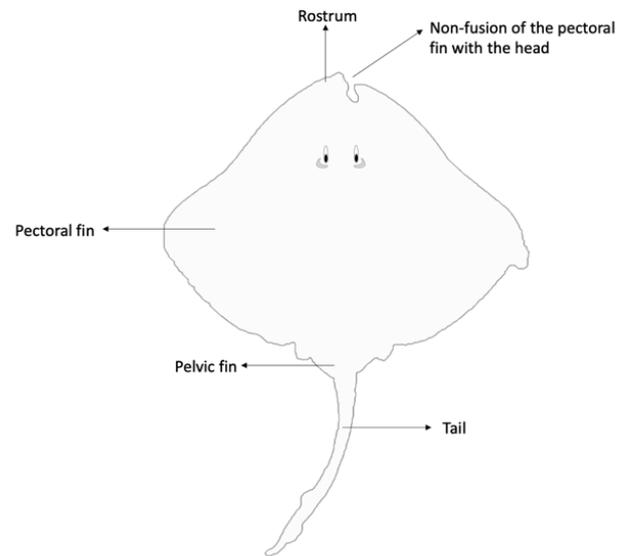


Figure 1. Schematic example of the non-fusion of the pectoral fins to the head in skates and rays.

October 2019.

The ray specimen is deposited at the Fish, Chelonian, Seabird, and Marine Mammal Tissue Collection, at the Instituto Oswaldo Cruz, Fiocruz, under identification code CTPQAMM #01-2019. At the laboratory, the following morphometric measurements were taken: total length (TotL); disk length (DL); disk width (DW); total weight (TW); tail length (TailL). Bilaterally symmetric structures were also measured on the right and left sides, as follows: gill length (GL); eye height (EH); eye diameter (ED), spiracle height (SH); spiracle diameter (SD); pelvic fin length (PFL); pectoral fin length (PectFL). All measurements were taken to the nearest mm using a caliper.

The abnormal specimen was then submitted to a radiography for further abnormality assessments.

A ventral muscle sample was removed with the aid of a stainless-steel scissors and metals, metalloids and rare earth elements were determined by inductively coupled plasma mass spectrometry (ICP-MS). Briefly, approximately 150mg of the sample were placed in a 15mL screw-capped polypropylene tube and mixed with concentrated sub-boiled bidistilled nitric acid (Merck, Rio de Janeiro). This mixture was then left to stand overnight at room temperature in the closed tube. After 12 hours, the acid decomposition was completed by heating the sample at 100°C, for 4h in the closed vessel, avoiding volatilization of volatile elements, such as Hg and Se. The sample was then diluted with ultra-pure water (resistivity > 18.0 MΩ cm) obtained from a Merck Millipore purifying system (Darmstadt, Germany) to

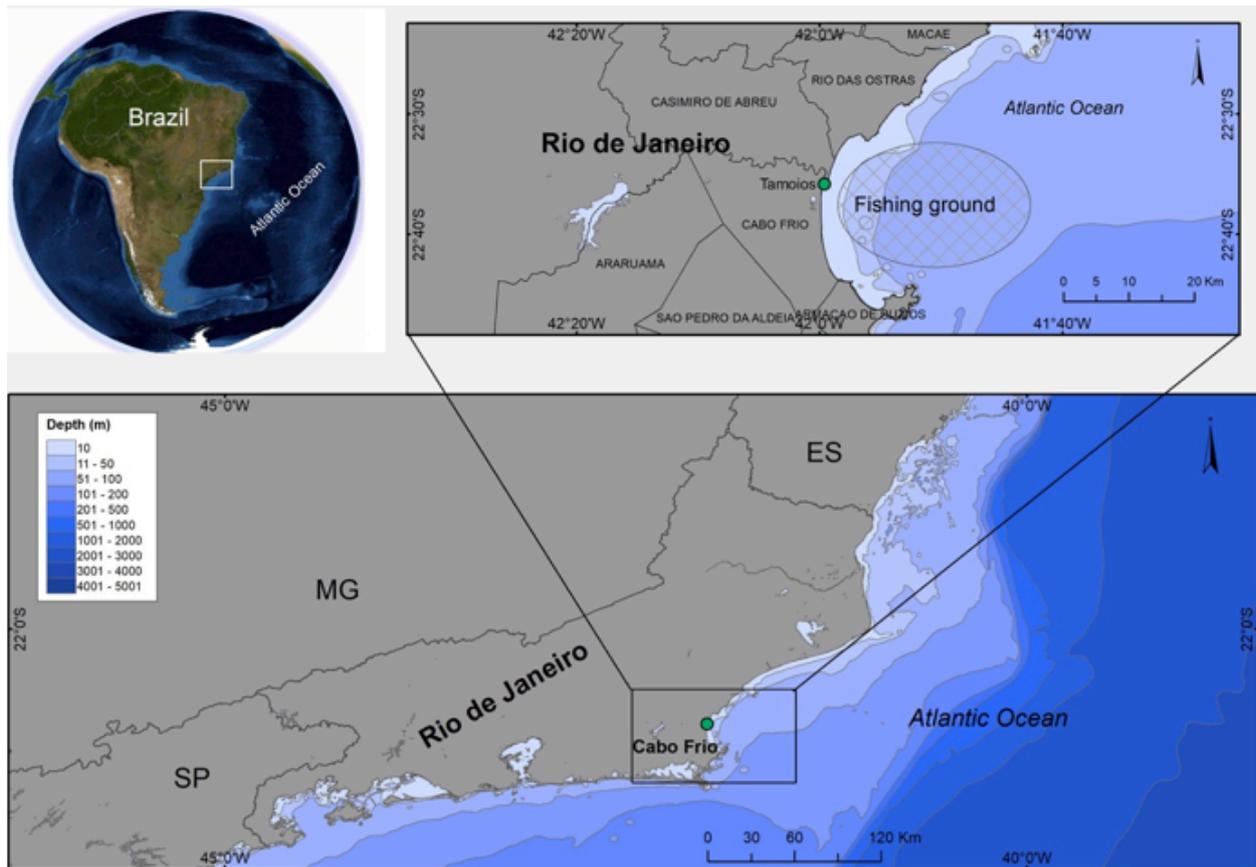


Image 1. The sampling location of the *A. castelnaui* specimen, at Tamoios, Cabo Frio, southeastern Brazil.

10mL. Metals, metalloids and rare earth elements were determined, in quintuplicate, using multi-elemental external calibration, by appropriate dilutions of a mixed standard solution (Merck IV) and using  $^{102}\text{Rh}$  as the internal standard at  $20 \text{ mg L}^{-1}$ . The determinations were conducted on a Nexlon 300 Perkin Elmer ICP-MS (Norwalk, CT, USA). Method accuracy was verified with procedural blanks and by the parallel analysis of the certified reference material (CRM) ERM<sup>®</sup>- BB422 (fish muscle) in triplicate. All CRM recovery values were within acceptable Eurachem standards (Eurachem 1998).

## RESULTS

The *A. castelnaui* abnormality consisted of the incomplete fusion of the right pectoral fin with the head, resulting in cleft between the pectoral fin and the rostrum (Image 2). No anophthalmia was observed.

The morphometric measurements of the *A. castelnaui* specimen are displayed in Table 1.

Bilaterally symmetric structures were also measured,

in order to assess possible variations, displayed in Table 2.

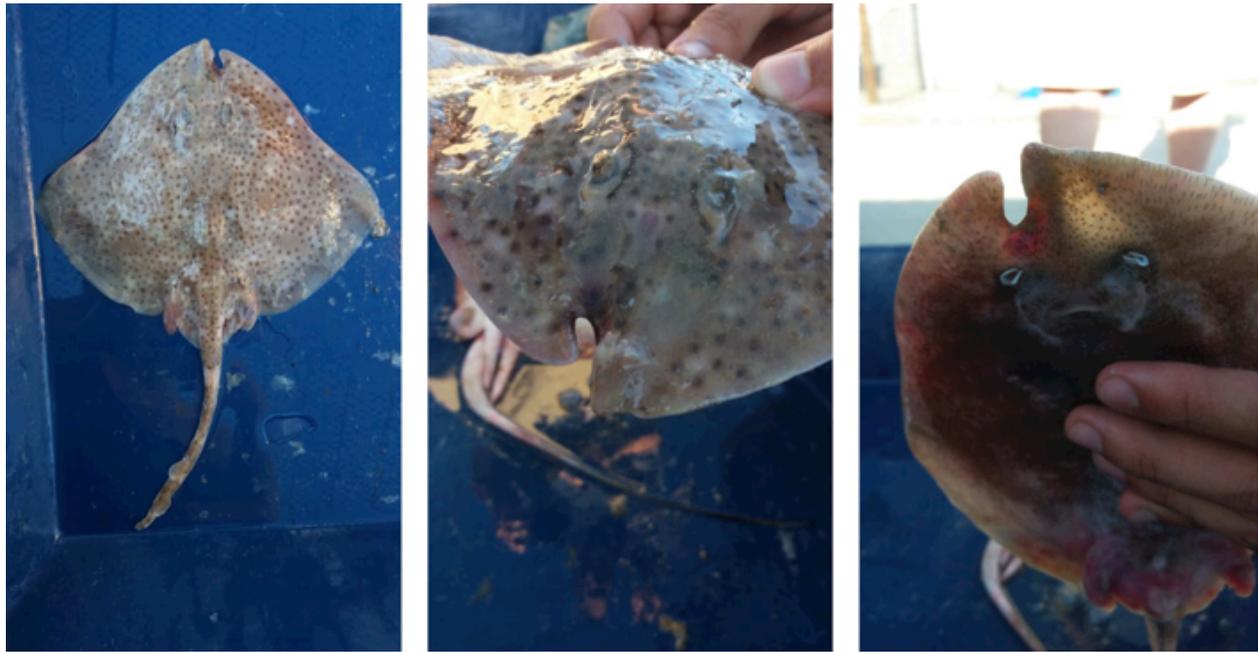
The radiography image of the specimen is displayed in Image 3. Muscle sheaf discontinuity is noted near the rostrum, while a very discrete radio-opacity, possibly indicative of arthrosis, is also observed.

The metal, metalloid and rare earth element concentrations detected in the muscle tissue sample are displayed in Table 3.

The metals Bi, Cd, In, Nb and Re were all below their respective LQ of 0.024, 0.035, 0.008, 0.029 and 0.0005  $\text{mg kg}^{-1}$  wet weight, while the rare Earth elements Nd, Pr and Th were below their LQ of 0.0001, 0.0003 and 0.014  $\text{mg kg}^{-1}$  wet weight, respectively.

## DISCUSSION

It appears that pectoral fins non-adherent to the head are the most frequently recorded abnormality in Rajidae species worldwide (Ribeiro-Prado et al. 2008), where the pectoral fin fails to fuse together at the front of the head during early development stages (Ahlstrom



**Image 2.** Incomplete fusion of the right pectoral fin with the head in a very young *A. castelnaui* specimen from Cabo Frio, Rio de Janeiro, southeastern Brazil. © Salvatore Siciliano and Catarina Amorim Lopes.

**Table 1.** Morphometric body measurements of a very young *A. castelnaui* specimen from Cabo Frio, Rio de Janeiro, southeastern Brazil.

Morphometric body measurements	
Total length (cm)	34.50
Total weight (g)	115.00
Disk length (cm)	15.00
Disk width (cm)	20.90
Tail length (cm)	16.50

**Table 2.** Bilaterally symmetric structure measurements of the assessed *A. castelnaui* specimen from Cabo Frio, Rio de Janeiro, southeastern Brazil.

Measurement	Right	Left
Length of the 1 <sup>st</sup> gill arch	3.80	3.80
Length of the 2 <sup>nd</sup> gill arch	2.90	3.30
Length of the 3 <sup>rd</sup> gill arch	3.20	3.36
Length of the 4 <sup>th</sup> gill arch	3.00	3.30
Gill arch length means	2.20	2.40
Eye diameter	6.37	6.00
Eye height	10.03	10.53
Spiraculum diameter	4.66	6.13
Spiraculum height	4.43	4.06
Pectoral fin width	105.6	106.83
Pelvic fin width	40.80	38.66

& Bigelow 1963). Records of such abnormalities are available for *Atlantoraja cyclophora*, *A. platina*, *Raja asterias*, *R. brachyura*, *R. clavate*, *R. miraletus*, *R. radiata*, *R. radula*, *R. richardsoni*, *Rioraja agassizi* and *Rostroraja alba* (see Ribeiro-Prado et al. 2008 for more details). For *A. castelnaui*, a previous record of incomplete pectoral fin fusion is noted for the state of São Paulo, also located in southeastern Brazil, in one sub-adult specimen (total length and disk width of 87.5cm and 61cm, respectively), albeit for the left pectoral fin (Ribeiro-Prado et al. 2008).

Fluctuating asymmetry, defined as random deviations from perfect bilateral symmetry due to developmental disturbances during early life, is a valuable tool to quantify stress during early developmental stage (Jagoe & Haines 1985). In the present study, most right-side structures were slightly smaller compared to the left-side structures, with the exception of the 1<sup>st</sup> gill arch (same size), eye diameter (larger), spiraculum height (higher) and pelvic fin width (larger). Although the sample size is of only one individual, the observed differences in bilaterally symmetric structure may be indicative of developmental disturbances, and future studies in the study area should also carry out this analysis in order to build a fluctuating asymmetry database for this and other species.

It has been postulated that unfavorable environmental conditions, such as high pollutant loads, probably play a role in occurrence of abnormalities (Casarini et al.

**Table 3. Metal, metalloid and rare earth element concentrations (mg kg<sup>-1</sup> wet weight) in the muscle of the assessed *A. castelnaui* specimen from Cabo Frio, Rio de Janeiro, Southeastern Brazil. LQ – Limit of Quantification (mg kg<sup>-1</sup> wet weight), defined as the lower limit that elements can be accurately quantified.**

Metals and metalloids					
Element	LQ	Sample	Element	LQ	Sample
Ag	0.003	0.178	Pb	0.010	2.288
Al	0.101	82.74	Pd	0.003	0.113
As	0.015	61.64	Rb	0.002	4.626
Au	0.001	0.006	Sb	0.002	0.052
Ba	0.014	2.442	Sc	0.087	0.82
Br	1.022	265.55	Se	0.428	7.951
Co	0.002	0.17	Sn	0.007	0.149
Cr	0.034	13.59	Sr	0.018	635.162
Cs	0.001	0.098	Ta	0.003	0.007
Cu	0.018	5.45	Ti	0.163	39.40
Fe	2.642	378.24	Tl	0.001	0.002
Ga	0.002	0.12	U	0.006	0.022
Ge	0.020	0.12	V	0.006	3.39
Hg	0.009	0.487	W	0.019	0.046
Mn	0.022	8.17	Y	0.001	0.352
Mo	0.009	0.197	Zn	0.206	256.37
Ni	0.010	4.19	Zr	0.014	0.076
Rare earth elements					
Element	LQ	Sample	Element	LQ	Sample
Ce	0.004	0.176	La	0.001	0.085
Dy	0.001	0.012	Lu	0.001	0.001
Er	0.000	0.007	Sm	0.001	0.015
Eu	0.001	0.032	Tb	0.000	0.001
Gd	0.001	0.028	Tm	0.000	0.001
Ho	0.000	0.001	Yb	0.001	0.005

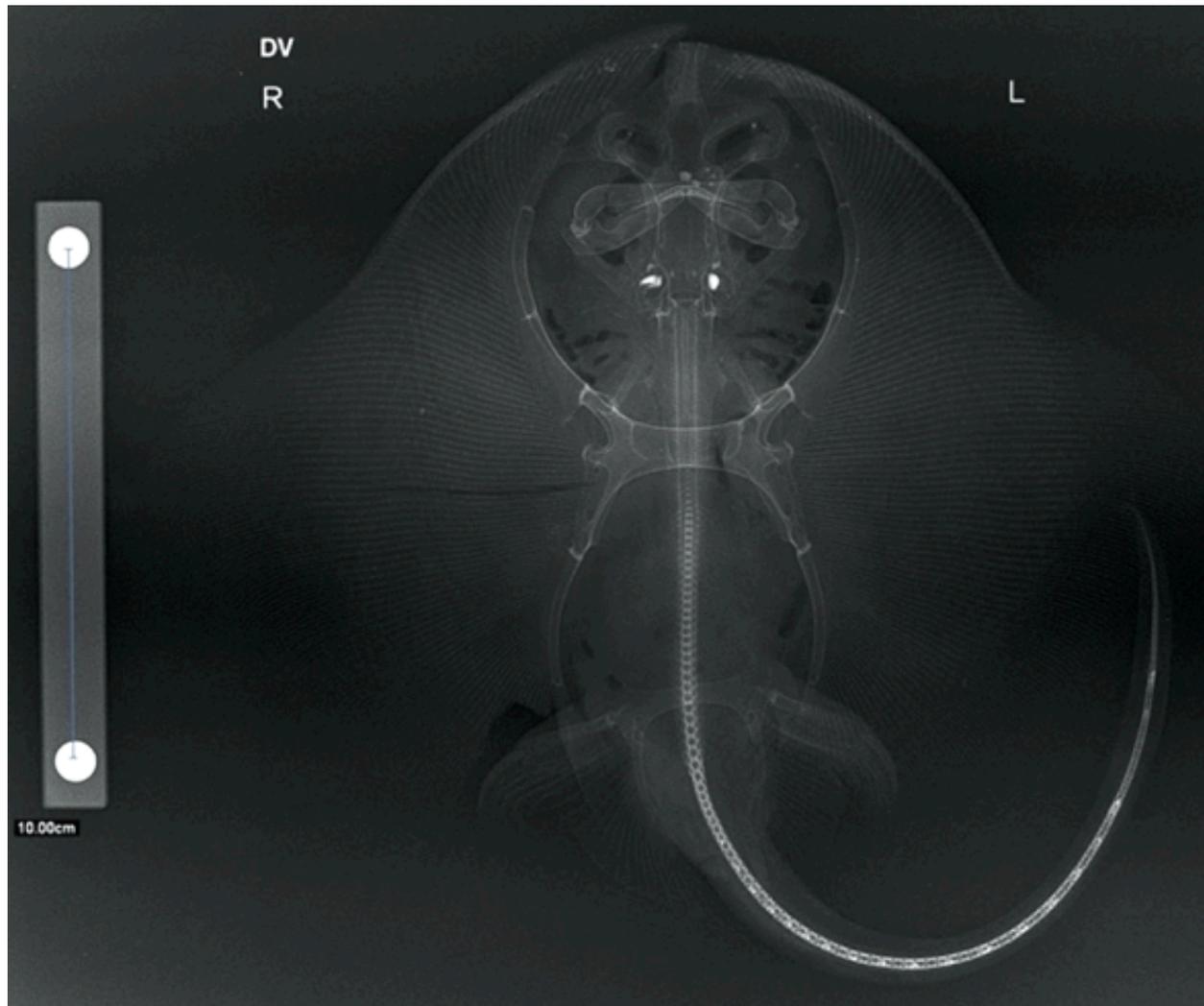
1996; Ribeiro-Prado et al. 2008), especially during early developmental fish stages, which are considered particularly sensitive to water pollution toxicity (Osman et al. 2007; Jezierska et al. 2009; Zhang et al. 2012). *In vitro* exposure to metals, in particular, has been proven as responsible for increasing the frequency of several types of body malformations of fish embryos (Cheng et al. 2000; Flik et al. 2002; González-Doncel et al. 2003; Hallare et al. 2005; Jezierska et al. 2009), confirming the teratogenic and genotoxic properties of metals in fish. In addition, several field studies have also been carried out and have associated the genotoxic potential of these compounds to morphological abnormalities in fish (Ferrante et al. 2017; Braga et al. 2019). This shall be further discussed ahead.

This hypothesis was assessed by a screening of metals, metalloids and rare earth elements in the muscle

tissue of this individual prior to fixation in alcohol.

The specimen assessed herein was a very young individual. *A. castelnaui* juveniles and females have been reported as inhabiting more coastal areas in Brazil (Oddone et al. 2008). This leads to high exposure to environmental contamination from anthropogenic activities in these individuals. In addition, *A. castelnaui* feeds mainly on bony fish, followed by decapods, elasmobranchs, mollusks, and cephalochordates, with crustaceans present in this species diet in greater amounts in smaller individuals, while cephalopods, elasmobranchs, and echinoderms predominate in higher class sizes (Barbini & Lucifora 2012). Therefore, this skate is at high risk for the bioaccumulation of several contaminants, including metals, through the dietary route.

Morphological deformities in several fish species



**Image 3.** Dorsal view radiography image of the assessed *A. castelnaui* specimen from Cabo Frio, Rio de Janeiro, southeastern Brazil.

have been related to water quality and contamination, including metal concentrations (Hiraoka & Okuda 1983; Sun et al. 2009; Alavi-Yeganeh et al. 2019). For example, altered spinal curvatures in Rainbow Trout *Oncorhynchus mykiss* larvae hatched from Cd-incubated eggs has been reported (Woodworth & Pascoe 1982), as well as spinal and cranial malformations and jaw underdevelopment in common carp larvae exposed to Cu during embryonic development (Stouthart et al. 1995). Other assessments have verified various types of vertebral deformities and two-headed morphological abnormalities in Cu- and Zn-exposed White Sucker *Catostomus commersoni* larvae (Munkittrick & Dixon 1989), skeletal kinking, improperly formed mouth, head and eyes and reduced brain size, among others, in Zn-exposed Fathead Minnow *Pimephales promelas* embryos (Dawson et al. 1988), eye and optic capsules malformations and jaw and branchial arch deformities in Zn-exposed Atlantic Herring *Clupea*

*harengus* eggs who hatched into larvae (Somasundaram et al. 1984), and several spinal cord deformations in Cu-exposed common carp embryos (Flik et al. 2002). In addition, Zebrafish *Danio rerio*, widely applied as a model bioindicator species concerning metal effects, assessments concerning Cd exposure in embryos have reported several morphological alterations, such as head and eye hypoplasia, altered axial curvature and tail malformations (Cheng et al. 2000), helical bodies, hooked tails, tail degeneration and abnormal body posture (Hallare et al. 2005), and severe stunting, ocular deformities (microphthalmia, anisophthalmia and anophthalmia) and dystrophic jaws (synarthrosis) (González-Doncel et al. 2003).

Besides *in vitro* assessments, real environmental scenarios have also indicated that metals are most likely causative of morphological abnormalities in fish. For example, spinal deformities in natural Grass Goby

*Zosterisessor ophiocephalus* populations from the Gulf of Gabès in Tunisia have been associated to metal (Cd, Cu and Zn) accumulation, as higher frequencies of deformities were observed in metal-contaminated areas compared to non-contaminated areas (Messaoudi et al. 2009); a high frequency of vertebral deformities in Fourhorn Sculpin *Myoxocephalus quadricornis* exposed to heavy metal pollution in the Gulf of Bothnia (Baltic Sea) has been verified (Bengtsson & Lithner 1988), and higher frequencies of skeletal anomalies (deformed fins, the lack of one or more fins and pelvic girdle, pugheadedness, asymmetric cranium, shortened operculae, fused and deformed vertebrae and spinal curvatures) were observed in Bream *Abramis brama* sampled from a polluted area (River Rhine) compared to a control area (Lake Braassem) (Slooff 1982). In addition, one assessment carried out on Mediterranean Killifish, *Alphanius fasciatus*, from different unpolluted and polluted areas off the coast of Tunisia reported deformed specimens only from the polluted sampling areas, presenting higher Cd concentrations in their livers and spinal columns when compared to normal specimens, also indicating significantly higher Cd bioaccumulation factors in the former (Kessabi et al. 2009). In another study carried out by the same group also associated skeletal deformities in the vertebral column of Mediterranean Killifish from the Tunisian coast to high concentration of heavy metals (Cd, Cu and Zn) (Kessabi et al. 2013). In another study, many different skeletal deformities in the vertebral column, cranium, operculum, fins and jaws of tilapia (*Oreochromis* spp.) sampled from different rivers in Taiwan were correlated to Hg, Zn, Pb, Cu and Cr concentrations (Sun et al. 2009).

In addition, some assessments have evaluated genotoxicity effects of several metals comparing polluted and non-polluted sites and associated this with morphological abnormalities in fish. For example, an assessment carried out concerning the ichthyofauna from polluted and non-polluted/protected estuaries located on the São Paulo coast, Brazil, reported several genotoxic alterations (nuclear abnormalities in erythrocytes) in two teleosts, *Centropomus paralelus* and *Diapterus rhomneus* due to high Zn, Co, Cr, and As concentrations (Braga et al. 2019), while another assessment observed a clear and significant correlation between two genotoxic biomarkers of effect (micronuclei and nuclear abnormalities) and Cd, Cr, Hg and Pb, as well as to an overall degree of metal pollution index, in a benthic teleost species, the Rusty Blenny *Parablennius sanguinolentus* (Ferrante et al. 2017).

These assessments, however, have all been

carried out in teleosts, and studies in this regard for elasmobranchs are severely lacking. To the best of our knowledge, no assessments in this regard are available in the literature concerning this group, indicating a significant knowledge gap that must be bridged.

Furthermore, morphological abnormalities are more frequently observed in oviparous species compared to viviparous species (Ribeiro-Prado et al. 2008), as embryos developed in egg cases maintain direct contact with environmental conditions, including contaminants, while embryos that develop inside the womb are protected from external influence up to a certain extent. Feeding solely only on yolk, as *A. castelnaui* embryos do (Dulvy & Reynolds 1997), produced through lipid mobilization from the mother's liver during vitellogenesis (Rossouw 1987), also allows for high maternal transfer of several contaminants, including metals.

Regarding the contaminant concentrations observed herein, almost no studies regarding rare Earth elements (REE) in elasmobranchs are available. This group of elements, comprising scandium, yttrium, lanthanum and the 14 chemical elements following lanthanum, termed lanthanoids (Redling 2006), consists of non-essential elements for living systems and have been reported as presenting low to moderate toxicity, including substitution of bone calcium by certain REE, due to their same oxidation state, carcinogenic properties (Rim et al. 2013) and the ability to result in cytotoxicity and genetic damage through oxidative stress (Huang et al. 2011; Jha & Singh 1995). In addition, long-term REE intake has been postulated as resulting in chronic poisoning (Hirano & Suzuki 1996). The sum of the Rare Earth Elements ( $\Sigma$ REE) detected herein did not reach the only maximum permissible concentration available worldwide, of  $0.7 \text{ mg kg}^{-1}$  (China 2005), although this has been established only for animal feeds and no other limits are available for other matrices. REE are found in the geological composition of sediments (Hu et al. 2006; Laveuf & Cornu 2009) and, as *A. castelnaui* is a benthic species, it may ingest sediment during feeding, accounting for the levels detected in muscle tissue. Higher REE concentrations have, in fact, been previously reported as being higher in benthic species (Guo et al. 2003; Mayfield & Fairbrother 2015), suggesting that they experience higher REE exposure due to their feeding habits, as REEs in aquatic environments are preferentially adsorbed to sediments and to fine suspended sediment particulates compared to the dissolved water column phase (Yang et al. 1999; Moermond et al. 2001; Taylor et al. 2012).

Although certain essential elements, such as Cu, Fe, Mn, Se and Zn, when present in high amounts can also

lead to negative biota and consumer effects, three of the most noteworthy environmental contaminants, As, Hg and Pb were observed at extremely high concentrations in the evaluated specimen. Thus, we shall focus on these elements, as they are known carcinogenic and teratogenic compounds.

Arsenic, a dangerous teratogen (Eisler 1988a) at almost  $62\text{mg kg}^{-1}$  w.w., was astonishingly high. This element, however, is usually present in its non-toxic form arsenobetaine, which comprises over 90% of total As, in fish (Gao et al. 2018; Ruelas-Inzunza et al. 2018). This demonstrates the need to carry out arsenic speciation analyses, in order to quantify both the toxic inorganic fractions and nontoxic organic fractions in fish. Nevertheless, even when taking this percentage into account, about  $6\text{mg kg}^{-1}$  w.w. would still be present in the toxic inorganic form, over the threshold for adverse aquatic organism effects reported as ranging from 1.3 to  $5\text{mg kg}^{-1}$  w.w. (Eisler 1988a). Arsenic exposure has been directly associated to skeletal abnormalities in fish. In one study, adult Mummichog *Fundulus heteroclitus* were exposed to  $230\text{mg kg}^{-1}$  of arsenic, an environmentally relevant in drinking water and aquatic environments in several areas worldwide, resulting in an average arsenic body burden of  $74.6\mu\text{g kg}^{-1}$  (one order of magnitude lower than the observed value of  $6\text{mg kg}^{-1}$  in toxic form calculated herein, albeit for muscle only) for 10 days immediately prior to spawning, and the hatchlings of exposed fish presented significantly increased incidence of curved or stunted tails (Gonzalez et al. 2006). In addition, this is also six-fold higher the maximum amount stipulated by the Brazilian ANVISA and the Codex Alimentarius ( $1.0$  and  $0.5\text{mg kg}^{-1}$  w.w., respectively), indicating significant consumer health risks for humans who consume this species (Codex Alimentarius Commission 2009; ANVISA 2013).

Concerning Hg, a potent neurotoxin, concentrations as low as  $0.008\text{mg kg}^{-1}$  w.w. in muscle have been reported as enough to alter biochemistry and gene expression, while the threshold for negative reproductive, histological and growth effects is of about  $0.135\text{mg kg}^{-1}$  w.w. in muscle (Sandheinrich & Wiener 2011). Morphological abnormalities have been previously reported in Hg-exposed fish. For example, one study assessed Hg-exposed Mummichog *Fundulus heteroclitus* and reported various eye vesicle malformations, ranging from partially fused eyes with two separate lenses to cyclopia and severe gross malformation of the craniofacial, cardiovascular and skeletal systems (Weis & Weis 1977), indicating the direct effect of this element on embryo development. Therefore, the concentration

observed herein indicates significant biota health effects, as well as potential consumer risks, since the maximum amount stipulated by the Brazilian ANVISA and the Codex Alimentarius for total mercury amounts in fish is of  $0.5\text{mg kg}^{-1}$  (Codex Alimentarius Commission 2009; ANVISA 2013), almost the same as the  $0.487\text{mg kg}^{-1}$  detected in the present study.

Regarding Pb, there is no safe threshold for exposure to this carcinogen and neurotoxin for any organism (ATSDR 2017). Dietary levels as low as  $0.1$  to  $0.5\text{mg kg}^{-1}$  have been linked to learning deficits in vertebrates (Eisler 1988b), and Pb effects range from neurotoxic and immunological to physiological and behavioral (ATSDR 2017). Pb exposure in fish has also been directly linked to diverse embryonic organogenesis malformations. For example, one study carried out in Pb-treated Common Carp *Cyprinus carpio* reported craniofacial anomalies, yolk sac malformation, vertebral shortening and curvatures and cardiac malformations (Jeziarska et al. 2009), while another verified scoliosis in Pb-exposed brook trout (*Salvelinus fontinalis*) eggs who hatched into larvae (Holcombe et al. 1976). Regarding human consumption, the FAO/WHO permissible level for Pb of  $0.3\text{mg kg}^{-1}$  (Codex Alimentarius Commission 2009) was exceeded almost 100 times in the present study, indicate severe human consumption risks for this toxic element.

On a side note, Ti, although not considered a classic environmental contaminant, has emerged in recent decades as a contaminant of increasing concern in the form of titanium dioxide nanoparticles applied to many personal care products. These compounds have been reported as eliciting deleterious effects in marine trophic webs, although scarce data is available for either Ti or its nanoparticle forms in the marine environment (Frenzilli et al. 2014). In the present study, it is noteworthy that Ti concentrations were an order of magnitude higher than observed in marine mammal muscle, liver, and kidneys (Holsbeek et al. 1998, 1999), which are long-lived animals highly exposed to metals through the dietary route and expected to bioaccumulate more contaminants than a very young skate. Thus, Ti contamination is probable, and should be further assessed in future studies.

Other assessments concerning pollutant concentrations for elasmobranchs carried out in only one specimen are available in the literature. For example, one study assessed metals, persistent organic pollutants and polonium in the muscle and liver of a rare filter-feeding shark specimen, the Megamouth *Megachasma pelagios*, found stranded on the central-north coast of the Rio de Janeiro, Southeastern Brazil (de Moura et al. 2015), while another assessment was carried out in one

shortfin Mako Shark *Isurus oxyrinchus* specimen and one Big-eye Thresher *Alopias superciliosus* specimen, also from Brazilian waters, concerning persistent organic pollutant concentrations in muscle (Azevedo-Silva et al. 2009), although the studies did not aim to verify the causes of morphological abnormalities. Another report verified metal concentrations in the liver of one specimen from three marine mammal species (one Orca, one Pygmy Killer Whale and one Franciscana Dolphin) (Lemos et al. 2013). Thus, even though discussion with the literature is hampered, reports concerning only one specimen of threatened species are also important to create baseline data for threatened species.

## CONCLUSIONS

*Atlantoraja castelnaui* is an endangered species displaying a current decreasing population trend and especially vulnerable to trawl fisheries due to its benthonic habits. In addition, no conservation measures are in place for this species in Brazil. This study is the first record of a specimen displaying incomplete pectoral fin fusion with the head in Rio de Janeiro, southeastern Brazil. A radiography indicated disordered muscle sheafs near the rostrum, while a metal, metalloid and rare-earth screening indicated extremely high contamination by teratogenic elements such as As, Hg, and Cd. The observed morphological deformity may in fact be due to the high concentrations of these elements in the Cabo Frio environment, also indicating high environmental contamination and significant human health risk concerns for populations who consume this species regularly in southeastern Brazil. It should be noted that this coastal environment undergoes under a strong influence of the so-called Cabo Frio upwelling system, an oceanographic anomaly that significantly enriches these waters, yielding locally higher fish catches. This paper furthers both morphological observations and ecotoxicological assessments on this relatively biologically unknown species in Brazil, paramount for future conservation measures. Although only one specimen was assessed herein, environmental contamination cannot be discarded as a possible cause for the observed deformity, and the extremely high contaminant levels observed indicate the need for further assessments for the species, both with regard to deleterious effects on the species itself and in a public health context.

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