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Short Communication

Clinical and hematological assessment during rehabilitation of a wild green turtle (*Chelonia mydas*) with fibropapillomatosis in Argentina

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ABSTRACT. Fibropapillomatosis (FP) is a debilitating, transmissible neoplastic disease that affects all sea turtles, particularly green sea turtles (*Chelonia mydas*). Although FP is endemic to tropical and subtropical regions, it was recently reported in Argentina. This study presents the management, rehabilitation, and clinical and hematological findings of a wild juvenile green sea turtle affected by FP that was found stranded in the Río de la Plata estuary. We established baseline data for managing FP in stranded turtles in Argentina and detailed the hematological and electrolyte effects associated with the turtles' presence in the estuary. These imbalances were successfully resolved during rehabilitation. Our findings suggest that environmental degradation of the estuary likely led to a diluted diet and subsequent ingestion of plastic, as evidenced by the recovery of plastic fragments from fecal material and the significant improvement in hematological parameters post-rehabilitation. This data is critical for developing strategies to monitor green turtles' health and evaluate environmental conditions in Argentina. The coastline of Buenos Aires, Argentina, and the Rio de la Plata estuary, that also is boundary with Uruguay, are vital feeding grounds for green turtles. However, they are also major recipients of transboundary industrial and domestic pollution. These results emphasize the urgent need for collaborative conservation efforts among countries in the Southwest Atlantic region.

Keywords: fibropapilloma treatment; hematology; biochemistry; rehabilitation; homeostasis; pollution

Green turtles (*Chelonia mydas* Linnaeus, 1758) are found in tropical and subtropical oceans and migrate extensively during their life stages. Most South Atlantic green turtles forage in the pelagic and neritic areas from northern Brazil to the Patagonia in Argentina (Vélez-Rubio et al. 2018). Despite their non-malignant nature, fibropapillomatosis (FP) outgrowths frequently result in a debilitating clinical condition that impairs the physiological health and long-term viability of affected sea turtle populations (Page-Karjian et al. 2014, Buenrostro-Silva et al. 2022). FP is defined by the growth of cutaneous tumor lesions, typically on areas of soft skin, but also appears on the carapace, plastron, or internal organs (de Deus Santos et al. 2015). FP has been strongly associated with chelonid alphaherpesvirus 5 (ChHV5) (Jones et al. 2016, Zamana et al. 2021). Several cofactors, including environmental pollutants, immunosuppression, and genetic predisposition, have been linked to tumor growth and disease development (Jones et al. 2016, Zamana et al. 2021). Low-quality environments with low diversity and opportunistic algae have been linked to FP (dos Santos et al. 2010, Bastos et al. 2022).

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In contrast, turtles inhabiting high-quality environments with thick-stemmed algae are free of FP (Bastos et al. 2022, Buenrostro-Silva et al. 2022). The stranded green turtle was admitted into the rehabilitation center after being found stranded on February 8, 2019 (summer season) at the Río de la Plata estuary (34º70.53'S, 58º23.22'W), an important feeding area for juvenile green turtles. However, this area is under considerable stress due to urban and industrial pollution (primarily domestic and industrial plastic and waste), harmful algal blooms, and hypoxic zones (Vélez-Rubio et al. 2018). Turtles in this area are mainly threatened by litter ingestion, bycatch, and industrial bottom trawling (González-Carman et al. 2011, Vélez-Rubio et al. 2018). Green turtles are currently classified as Endangered on the IUCN Red List (Broderick & Patricio 2019). The individual was evaluated at Fundación Mundo Marino Rehabilitation Center (FMMRC), where a physical examination, including in- and out-of-water assessments, was performed. The out-of-water evaluation included an assessment of the responsiveness, activity level, respiratory rate and sounds (that could indicate airway obstruction, or pulmonary trauma or diseases), the species identification, life stage (neonate, juvenile, or adult; and in adults, gender identification), body condition, the presence of fisheries debris (entanglement or ingestion) or contaminants (such as plastic or oil), signs of interaction with animals or environmental elements (such as bites or carapace lesions), and the presence of epibiota or ectoparasites (species ID, quantity and location in the body) (Tristan & Norton 2017, Pereira-Figueroa et al. 2022). The in-water assessment was performed in the rehabilitation tank (described below) with water at 22°C. It included the assessment of individual swimming ability, diving capabilities, buoyancy pattern, respiratory capacity, feeding behavior, visual acuity, flap movements, defecation patterns, and water-based behavior cues (Pereira-Figueroa et al. 2022).

The individual weighed 9.4 kg and based on the curved carapace length of 44 cm, was a juvenile specimen (Tristan & Norton 2017). Turtle sex was not determined due to the absence of sexual dimorphism at this life stage. The individual had a thin body condition, as determined by a slightly sunken plastron, few fat deposits in the shoulders, mildly sunken eyes, a sunken neck and prefemoral space, and a visible supraoccipital crest (Pereira-Figueroa et al. 2022). Three wart lesions were observed in the dermis, one pigmented lesion (4 cm) on the left lower eyelid and two ventrocaudal on the left pectoral flipper, where one lesion was pigmented (3 cm). The other was non-pigmented (2.5 cm, Fig. 1a). According to the protocol described by Work & Balazs (1999), it received a mild tumor involvement score of 1. Following the out-of-water assessment, the turtle was placed in a circular tank (1.20 m diameter × 0.60 m height) containing natural freshwater at 22ºC for 24 h. Freshwater immersion can be used as a form of fluid therapy through ingestion and possible cloacal absorption (Pereira-Figueroa et al. 2022). Then, it was transferred to a natural saltwater (22.4 g L^{-1}) pool (3.2×2.0×0.6 m height) where it remained until its release. The water was replaced every other day and kept between 22 and 25ºC with a pH of approximately 7.7. The in-water examination was performed daily, and a general physical examination (out of water) was conducted once a week. To prevent contamination spread, all materials that contacted the turtle or the pool water, along with the discarded pool water itself, were disinfected for 10 min using a 3% hypochlorite solution (Pereira-Figueroa et al. 2022).

Since admission, a good swimming, immersion, and buoyancy pattern was observed. The first intake was recorded 15 days after admission. Thereafter, a regular feeding pattern was observed, with daily intakes ranging from 50 to 130 g of frozen-thawed skinless fillet (*Macrodon ancylodon*) and spirulina (*Arthropira platensis*). Analysis of the total fecal samples collected during the rehabilitation identified eleven plastic fragments. Since the color of ingested plastic material is a suggested factor in determining the likelihood of ingestion (Petry et al. 2021), we noted the colors of the recovered fragments. Ten of the plastic fragments were sheet-type; of these, six were black, three were transparent, and one was red. The 11th piece was a unique find, a distinctively bullet-shaped, hard, white object (Fig. 2). The accidental ingestion of plastic by marine turtles, whether due to debris mixed with their food or mistaken as prey, can have lethal effects, such as damage to their digestive system or obstruction. More commonly, it can cause a range of sublethal effects due to the prolonged time plastic spends in the gut and stomach (Nelms et al. 2016).

Blood samples were collected from the external jugular vein with a 21G×1.5" needle coupled to a 20 cm^3 syringe at 11 and 54 days after admission (Fig. 1b). Lithium-heparin anticoagulant tubes were used for hematological evaluation, and tubes without anticoagulant were used for serum biochemistry. A hematocrit and hemogram analysis (total erythrocyte and leukocytes count, as well as an absolute differential formula) were performed at the Rehabilitation Center laboratory using conventional reptile cell counting tech-

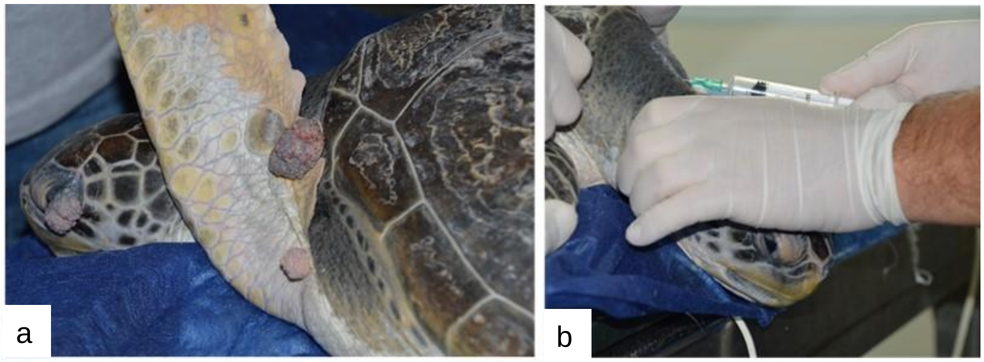

Figure 1. Out-of-water evaluation of the juvenile green turtle *Chelonia mydas* stranded in the Río de La Plata estuary, Argentina. a) Macroscopic appearance of the fibropapilloma lesions, b) blood sample collection.

Figure 2. Plastic debris collected from the feces of a juvenile green turtle (*Chelonia mydas*) stranded from the Río de La Plata estuary, Argentina. Several pieces of sheet-type plastic fragments and one hard plastic (black arrow) recovered during its rehabilitation period.

niques. The hematocrit percentage was determined by filling a microcapillary tube with heparinized blood, centrifuging the sample for 5 min, and reading on a microhematocrit card (Giumelli Z-12, Argentina). Natt and Herrick's solution was used as the dilutant for counting erythrocytes and leukocytes in the Neubauer counting chamber. For differential leukocyte counts, smears were stained with Tinction 15 (Biopur, Argentina) and examined using standard procedures (de Mello & Álvarez 2020). Immediately after blood collection, tubes were transported at 10ºC to Dr. Buszczak's private laboratory for biochemical and electrolyte determinations. Biochemical analyses were performed using an automated biochemistry analyzer (Wiener Lab Analyzer CM250, Argentina), and electrolyte concentrations were determined using an electrolyte analyzer (Diestro 103AP V4, Buenos Aires, Argentina), following standard procedures. The results of the hematological and biochemical analyses, along with the species- and developmental-stage-specific reference values are shown (Table 1).

The initial blood sodium concentration measured (139 mmol L^{-1}) was below the reported range for estuarine regions: 154-158 mmol L^{-1} (de Mello & Alvarez 2020) and 145.7-165 mmol L^{-1} (de Deus Santos et al. 2015). Additionally, blood chloride values (98.5 mmol L^{-1}) were also below the reference range of 105.5 to 127.3 mmol L^{-1} (de Deus Santos et al., 2015). Significant changes in sodium efflux, as well as subsequent ionic and osmotic imbalances, had been documented in green turtles within just 25 h of exposure to freshwater (Ortiz et al. 2000). The results of this individual showed that the turtle had already been in the estuary for several days before the stranding. By the time of the second blood sample, 53 days after exposure to saltwater, blood sodium and chloride concentrations (150.80 and 104.30 mmol L^{-1}, respectively) were within reference values (Table 1).

The eyelid tumor was surgically removed eight weeks after admission (Fig. 3). The patient fasted for 24 h before surgery, and prophylactic antibiotics (amikacin sulfate 3 mg kg^{-1} IM, Richet Laboratories, Buenos Aires, Argentina) were administered twice, two days apart, beginning one day before the surgery. The excised tumor mass was subjected to histopathological and molecular analyses. The results of these analyses are detailed in Origlia et al. (2023). Briefly, after the tumor was surgically removed, an identification plate (UUW905) was placed in the right fore flipper. Four weeks later, once the surgical wound had healed, the turtle was released at Faro Punta Médanos in Buenos Aires (36°53.00'S, 54°40.40'W). This location is considered a less contaminated foraging area for juvenile

Table 1. Hematology and serum biochemistry of the juvenile green turtle (*Chelonia mydas*) affected with fibropapillomatosis FP (tumoral score 1), compared with reference values from the literature. Values indicated by an asterisk (*) were restored during the rehabilitation process.

Determination	Sampling day		Reference intervals	
	11	54	de Deus Santos et al. (2015) (n)	Mello et al. (2020)
Creatine phosphokinase (U L^{-1})	1932	335	234.9-3429.0 (83)	539-1166
Aspartate aminotransferase (U L^{-1})	211	164	91.8-442.3 (94)	119-168
Alanine aminotransferase (U L^{-1})	2	17	0.0-31.1 (92)	2.2-5.2
Alkaline phosphatase (U L^{-1})	40	44	14.5-91.2 (76)	28-42
Gamma-glutamyl transpeptidase (U L^{-1})	0	0	0.0-6.1 (91)	2.22-3.1
Triglycerides (mg dL^{-1})	46	82	11.3-209.48 (89)	158-271
Creatinine (mg dL^{-1})	0.23	0.34	0.0-0.5 (88)	0.41-0.61
Urea (mg dL^{-1})	18	77	5.0-156.2 (90)	54-74
Glucose (mg dL^{-1})	87	128	59.6-120.2 (91)	102-125
Cholesterol (mg dL^{-1})	223	189	15.0-212.7 (93)	181-223
Uric acid (mg dL^{-1})	0.63	1.00	0.0-2.5 (94)	1.68-2.32
Total protein (g dL^{-1})	3.40	4.38*	1.3-5.5 (92)	2.91-3.48
Albumin (g dL^{-1})	1.24	1.38	0.1-1.7 (88)	0.87-1.01
Globulin (g dL^{-1})	2.16	3.00	0.4-4.6 (84)	1.99-2.53
Albumin globulin ratio	0.57	0.46	0.1-1.1 (84)	
Total bilirubin (mg dL^{-1})	0.21	0.32	0.0-0.3 (95)	0.36-0.85
Direct bilirubin (mg dL^{-1})	0.01	0.01	0.0±0.1 (94)	
Indirect bilirubin (mg dL^{-1})	0.20	0.31	N/D	
Amylase (U L^{-1})	1496	1490	92.3-1020.1 (86)	
Lactate dehydrogenase (U L^{-1})	222	353	3.7-351.2 (93)	81-438
Calcium (mg dL^{-1})	7.22	6.56	5.4-12.0 (85)	5.1-5.8
Phosphorus (mg dL^{-1})	FP 5.60	7.06	2.7-8.7 (87)	6.7-8.1
Chlorine (mEq L^{-1})	98.50	104.30*	105.5-127.3 (89)	
Sodium (mEq L^{-1})	139.10	150.80*	145.7-165.0 (93)	151-158
Potassium (mEq L^{-1})	4.19	4.73	3.3-5.8 (93)	
Ferritin (µg dL^{-1})	33	58	N/D	
Hematocrit (%)	42	38	19.2-45.1 (91)	33-35
Erythrocytes (10^6 µL^{-1})	0.35	0.30		0.45-0.5
Total white cell count (cells µL^{-1})	8200	8780		3200-4130
Absolute Leucocyte formula (cells µL^{-1}):				
Lymphocytes	5950	6160		635-876
Heterophils	2030	2370		2410-3120
Basophils	150	170		2.51-11.11
Monocytes	70	80		18-41
Eosinophils	0	0		27-65

green turtles in the southwestern Atlantic (Gonzalez-Carman et al. 2014).

The coastal waters of Argentina are an important feeding and developmental area for juvenile green turtles (González-Carman et al. 2011). Since 2001, the FMMRC has assisted with 735 sea turtle strandings and entanglements involving the three species that forage along this coast: 257 green turtles (*C. mydas*), 304 loggerheads (*Caretta caretta*), and 174 leatherbacks (*Dermochelys coriacea*). Of these, only 230 were rescued alive for rehabilitation. Interestingly, the current case was the only one with signs of FP disease. A study of 27 green turtles and two loggerheads incidentally caught in artisanal gillnets in Argentina found no evidence of FP lesions, either macroscopically or microscopically (Albareda et al. 2007). Green turtles undertake extensive migrations between oceanic and neritic zones, showing their ability to adapt to changing environments. The ability to osmoregulate across a range of salinities depends on the regulation of blood sodium concentration (Whiting et al. 2007). Thus, reference intervals for blood sodium concentrations in juvenile green turtles demonstrate notable variability across different habitats. Studies in coastal environments reported ranges from 151 to 158 mmol L^{-1} for the Lagaman estuary lagoon, Brazil (de Mello & Alvarez 2020), 145.7 to 165 mmol L^{-1} along the southeastern coast of Espírito Santo, Brazil (de Deus Santos et al. 2015), while turtles sampled at Puerto Manglar and Tortuga bay, Puerto Rico exhibited

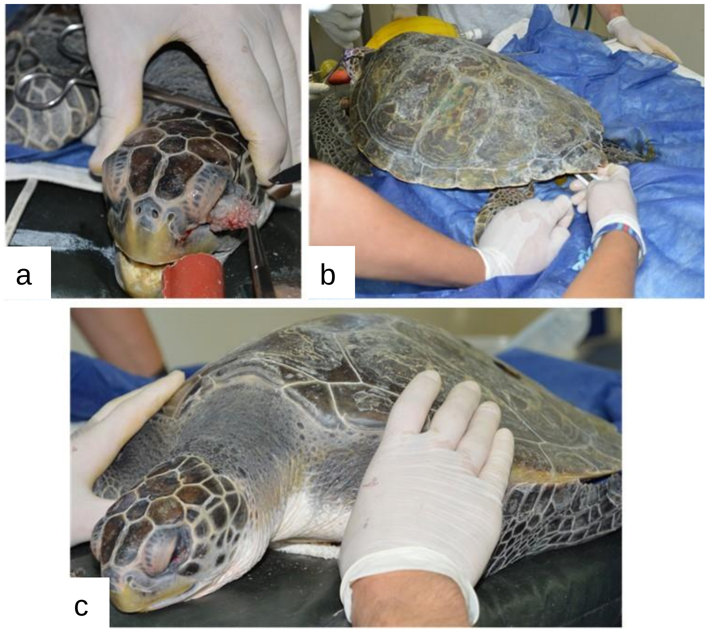

Figure 3. Juvenile green turtle *Chelonia mydas* stranded in the Río de La Plata estuary, Argentina. a) Surgical removal of the eyelid fibropapilloma lesion, b) monitoring during surgery, c) control of the surgical lesion.

a range of 143.08 to 167.01 mmol L^{-1} (Page-Karjian et al. 2015). Those from Fog Bay, Australia, ranged from 105 to 172 mmol L^{-1} (Whiting et al. 2007). The initial blood sodium concentration obtained for this turtle (11 days after admission) was 139 mmol L^{-1}, which is at the lower end of the minimal values reported for estuarine regions (132 mmol L^{-1}; Brazil (de Mello & Alvarez 2020)) and in neritic habitats (134 mmol L^{-1}; Fog Bay, Australia (Whiting et al. 2007)). This hyponatremia was observed together with low chloride concentrations (98.5 mmol L^{-1}, reference range 105.5 to 127.3 mmol L^{-1} (de Deus Santos et al. 2015). The water-electrolyte imbalance was successfully reversed during rehabilitation by maintaining the turtle in an appropriate saline environment. As described for Kemp's ridley sea turtles, exposure to freshwater induces acute hyponatremia, an adaptive mechanism to the hypoosmotic environment, with electrolyte levels returning to normal levels upon returning to salt water (Ortiz et al. 2000).

For this individual, both total white blood cells and lymphocytes were above reference values (Table 1), a finding potentially associated with FP. Moreover, the initial total protein concentration of 3.40 mg dL^{-1} is consistent with those reported in green turtles that showed greater FP involvement. Specifically, this value falls within the range associated with moderate and severe tumor involvement in studies from southern Brazil (score 2: 3.70 g dL^{-1}; score 3: 3.30 g dL^{-1} (de Deus Santos et al. 2015) and closely aligns with the value reported for severe tumor involvement in Hawaii (score 3: 3.5 g dL^{-1}) (Aguirre & Balazs 2000). However, the turtle in this study exhibited a mild tumor. The observed improvement in total protein concentration during rehabilitation (rising to 4.38 g dL^{-1}) suggests that the low initial value may have been due to a systemic decline, likely stemming from reduced dietary intake, rather than solely to FP severity.

Therefore, all hematological values must be carefully interpreted in conjunction with a comprehensive clinical evaluation. The biochemistry values were consistent with the ranges reported for juvenile green turtles from southern Brazil (dos Santos et al. 2010, de Deus Santos et al. 2015, de Mello & Alvarez 2020). The slight variation observed was likely due to dietary differences, as the turtle showed no other signs of disease. Specifically, cholesterol, triglyceride, urea, creatinine, and liver enzyme levels are likely diet-related. This finding is consistent with reports on the stomach contents of juvenile green turtles along the South Atlantic coast of Argentina, which exhibit an omnivorous diet based mainly on gelatinous zooplankton and macrophytes (Vélez-Rubio et al. 2016). Consequently, further research in Argentina is essential to improve our understanding and fully determine the significance of these biochemical evaluations within the region.

During the rehabilitation process, sheet and hard plastic fragments were found in the feces. The limited visibility in estuarine regions, such as the Río de la Plata, makes the incidental ingestion of plastic fragments common for green turtles (González-Carman et al. 2014). Their preferred diet of seagrass or algae may further increase the observed ingestion of plastics. Studies in southeastern Brazil have already shown that the most ingested items were plastic sheets and hard plastic fragments (Petry et al. 2021). While knowledge gaps exist for Argentina, the observation of debris accumulations at the bottom of the Río de la Plata estuarine system, where their primary prey items are found, strongly suggests an increased risk of plastic ingestion in this area as well (González-Carman et al. 2014, Vélez-Rubio et al. 2018). Plastic ingestion can lead to malnutrition because the debris reduces stomach capacity and the feeding stimulus, thereby diluting the diet (Nelms et al. 2016). The resulting weakness might impair the turtle's ability to avoid predators and escape incidental capture by fisheries (Nelms et al. 2016).

Other studies of green sea turtles have linked tumor development to coastal habitat, affecting recruitment, and underlying immune suppression (Page-Karjian et al. 2014, dos Santos et al. 2015, Bastos et al. 2022). Consequently, turtles affected by FP are often clinically debilitated and cachectic, exhibiting anemia of chronic disease (Page-Karjian et al. 2014). Additionally, the prevalence of FP was linked to warm water temperatures, with significant increase in cases during warmer seasons (Page-Karjian et al. 2014, Manes et al. 2022). Although UV radiation has been considered a potential risk factor, recent meta-analyses suggest that it does not significantly impact the prevalence of the disease (Dujon et al. 2021). Instead, evidence indicates that environmental processes, such as eutrophication and toxic phytoplankton blooms, have a far greater impact on the development of fibropapillomatosis than UV radiation does (Duffy & Martindale 2019). The main factors previously linked to FP development or occurrence are present in the Río de la Plata estuary and may have contributed to the disease in this case. Given that turtles rely heavily on vision for feeding, the eyelid tumor lesion was surgically removed to improve the animal's prognosis; evidence indicates that turtles without ocular FP have an eight-fold increase in survival compared to those with the condition (Page-Karjian et al. 2014). Management of FP remains a debated topic (Page-Karjian et al. 2014, Duffy & Martindale 2019, Pereira-Figueroa et al. 2022). While natural tumor regression, potentially driven by cell or humoral immunity, has been documented, the current clinical consensus is to surgically remove tumors that severely impair vital functions, particularly vision. The main limitation of this treatment is its poor efficacy in overall case outcomes (Page-Karjian et al. 2014).

Furthermore, a long-term prognosis is critically limited by factors beyond surgical control, including a high rate of tumor recurrence, due to a persistent viral infection, and the presence of internal tumors (Duffy & Martindale 2019, Zamana et al. 2021). Consequently, treatment is expanding, with new medical interventions, including anticancer drugs such as 5-fluorouracil (5-FU), and advanced approaches emerging from precision oncology (Brunner et al. 2014, Donnelly et al. 2019, Duffy & Martindale 2019). Further studies are needed to address the prevalence of FP in the southwestern Atlantic and the relationship between environmental conditions and turtle health in this region. This information will be crucial for developing more effective strategies to monitor sea turtle health and assess environmental conditions.

CONCLUSION

This study provides baseline information for the management and rehabilitation of a stranded green turtle with FP in Argentina. Our findings demonstrate that the rehabilitation process successfully corrected the hematological and electrolyte abnormalities acquired by the turtle in the low-salinity environment of the Río de la Plata estuary. This data is crucial for developing strategies to monitor the health of green turtles in relation to environmental conditions in the region. In this case, the turtle health deterioration was likely linked to environmental degradation in the Río de la Plata estuary system. This degradation may have led to a diluted diet and enabled the ingestion of plastic debris. The Río de la Plata is a critical foraging area for sea turtles and a productive fishing area (González-Carman et al 2011). It is also a major recipient of industrial and domestic pollution from Argentina and Uruguay. This overlap underscores the urgent need for transboundary conservation efforts, including habitat restoration.

All procedures were approved by the Institutional Animal Care and Welfare Committee from Mundo Marino Oceanarium and complied with Regulations of the Government of the Province of Buenos Aires (Ley Provincial de Pesca 11.477 and Decreto Reglamentario 3237/95).

Credit author contribution

J.D. Loureiro: conceptualization, methodology, investigation, writing and original draft; J.A. Origlia: methodology; J.P. Loureiro: investigation and method-

ology; K.C. Alvarez and S. Rodríguez Heredia: methodology; R. Nuñez Favre: writing, review and editing.

Conflict of interest

The authors declare no conflict of interest.

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