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Pseudomonas aeruginosa sepsis in a neonate Franciscana dolphin (*Pontoporia blainvillei*): clinical approach and laboratory findings

Marzia Antonelli[1,*], Mateus Rocha Ribas[2], Rafael Meurer[1], Sandro Sandri[1], Jussara Kasuko Palmeiro[2], Thaís Cristine Marques Sincero[2], and Cristiane Kiyomi Miyaji Kolesnikovas[1]

[1]Associação R3 Animal, Florianópolis, Brazil
[2]Laboratório de Microbiologia Molecular Aplicada (MiMA), Universidade Federal de Santa Catarina, Florianópolis, Brazil

*Corresponding author: marziaantonelli@gmail.com

Abstract

A neonate (4,2 kg and 71 cm total body length) female Franciscana dolphin, *Pontoporia blainvillei,* was found stranded alive on a beach in Santa Catarina, Southern Brazil, with respiratory signs (dyspnea, crackles during thoracic auscultation and changes in buoyancy) suggestive of pneumonia. Hand-rearing and rehabilitation efforts were unsuccessful, and the neonate died three days after it was rescued. Postmortem investigation was conducted using diagnostic imaging techniques, such as endoscopy and computed tomography. A complete necropsy and histopathology were performed, revealing bronchopneumonia as well as neutrophilic and discrete otitis media. A pure *Pseudomonas aeruginosa* culture was obtained from the blowhole, bronchoalveolar lavage, and blood using the API NE System. Our results indicate the cause of death to be related to bacterial sepsis. Further understanding of the main causes of death of Franciscana dolphin calves is warranted for conserving this threatened and understudied species.

Keywords:
bacterial septicemia, cetacean, otitis, pneumonia, *Pseudomonas aeruginosa*, rehabilitation, stranding event

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Introduction

The Franciscana dolphin (*Pontoporia blainvillei*) is a small cetacean endemic to the South Atlantic Ocean and restricted to coastal waters, spanning from Brazil to Argentina. The species is the only member of the family Pontoporiidae and one of the most threatened cetaceans in South America, listed as Vulnerable according to the International Union for the Conservation of Nature – IUCN (Zerbini et al., 2018) due to its well-acknowledged susceptibility to bycatch and as threatened in the Brazilian Red List (Sucunza et al., 2023).

Rehabilitation of cetaceans poses significant challenges due to the aquatic environment, stress related to stranding, and physiologic adaptations. Furthermore, deficiencies in the immune system make neonatal cetaceans susceptible to disease, and mortality is a recognized concern in calves (Sweeney et al., 2010; Flower et al., 2018).

Pseudomonas aeruginosa is an ubiquitous Gram-negative bacterium commonly distributed across terrestrial and aquatic environments globally, and it is prevalent in the natural microbiota of numerous marine animals, including cetaceans. Under conditions of immunosuppression, these bacteria may transition to a pathogenic state, leading to the development of consequential diseases, such as pneumonia and sepsis (Buck et al., 2006; Pérez et al., 2015; Faure et al., 2018; Li et al., 2019; Pelegrin et al., 2021; Reynolds & Kollef, 2021). Otitis is often reported in domestic animals, caused by a wide variety of pathogens, but is rarely documented in marine mammals (Siebert et al., 2001; Wohlsein et al., 2019; Ready et al., 2021).

Clinicopathological evaluation in Franciscana dolphins is complicated due to the non-existing normal range for blood

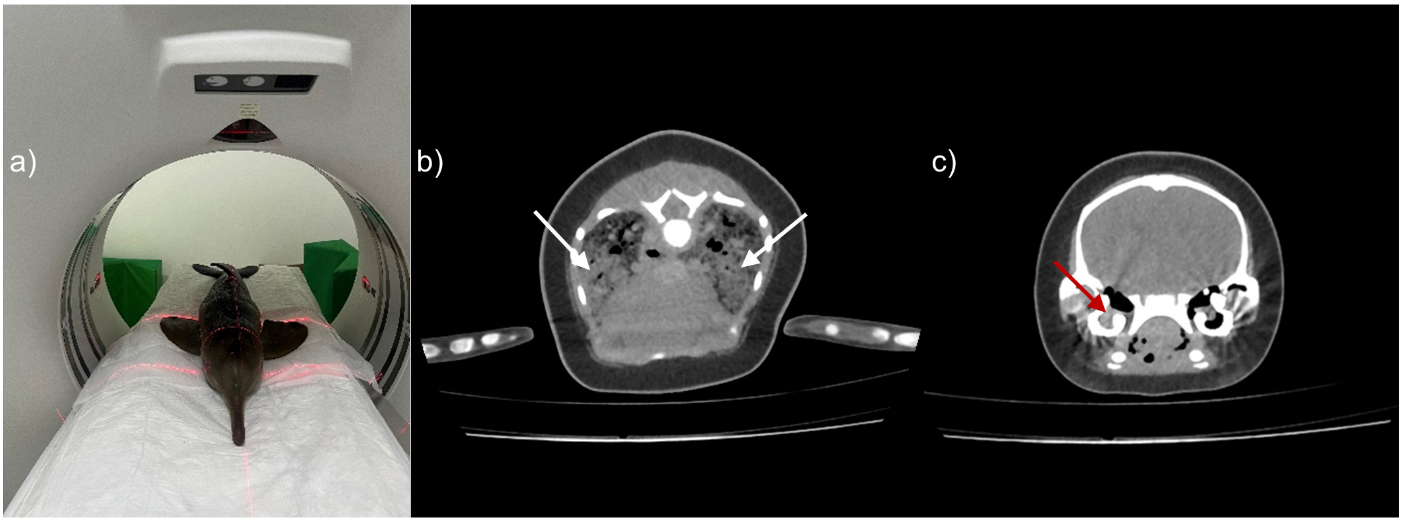

Figure 1. Computed tomography (CT) findings in a neonate female Franciscana dolphin *Pontoporia blainvillei* found stranded alive on a beach in Santa Catarina, Southern Brazil. (a) The carcass was placed in a prone position for CT examination. (b)Transverse view from CT scan of the lungs showing consolidation in the lower lungs (c) Transverse view from CT scan of the skull in *P. blainvillei* depicting occlusion of the left tympanic bulla (red arrow).

values and imaging findings. Additionally, there are only a few reported cases of Franciscana's clinical management (Baldassin et al., 2007; Kolesnikovas, 2022; Meegan et al., 2022). Here, we describe the rehabilitation efforts and clinical, pathologic, microbiological, and diagnostic imaging findings in a Franciscana dolphin which stranded in Santa Catarina State, Southern Brazil.

Materials and Methods

Case history and clinical findings

A female Franciscana dolphin estimated to be a newborn, around one week old (total body length: 71 cm, body weight: 4,2 kg, with umbilical cord, vibrissae on the rostrum and fetal lines), stranded alive on the coast of Florianopolis, Santa Catarina State, Brazil (27°39'05"S, 48°28'02"W) in November 2023. No other animals were observed in the area, and the neonate was transported to the R3 Animal Rehabilitation Facility for medical care. Upon admission, the dolphin was extremely weak, and significant signs included dehydration, hypoglycemia, crackles during thoracic auscultation, and dyspnea. The animal showed changes in buoyancy and was unable to stay afloat unassisted. The dolphin was placed in a seawater pool at 26 - 28°C, with a flotation device to aid in buoyancy, and it was monitored 24 hours a day. Several diagnostic tests (hematological and blood gas analysis, bacterial isolation, antimicrobial susceptibility test from the blowhole swab) and diagnostic imaging (thoracic ultrasound and radiography) were performed to establish the most appropriate treatment. The initial respiratory rate was five breaths per minute, and the heart rate was 150 beats per minute. Respiratory rate was measured every five minutes, and heart rate was measured when the animal was fed (every hour). Preliminary blood analysis showed mild respiratory alkalosis (pH: 7.635, and pCO_2: 31.9 mmHg) and hemogram values (Table 1) similar to a previous study (Baldassin et al. 2007). Blood glucose level was 50 mg/dl. Thorax radiographs revealed a mild interstitial pattern and thoracic ultrasound showed focal areas of a moderate alveolar interstitial syndrome. During rehabilitation, antibiotics, fluid therapy, and glucose were administered, including intramuscular injection of ceftriaxone (15 mg/kg s.i.d) and long-acting penicillin (50.000 IU every 72 hours). The neonate was tube-fed with 2 ml/kg of fluids and milk formula (Zoologic® Milk Matrix 30/52 PetAg Inc., Hampshire, IL, USA, filtered water, dried egg whites and cetacean multivitamin) hourly. On the third day in rehabilitation, the dolphin's condition had further deteriorated. It showed signs of abdominal and respiratory discomfort, dyspnea, and began to vomit. Feeding was temporarily halted and only fluids were administered orally. Intramuscular antiemetic, metoclopramide (0,1 mg/kg), oral antispasmodic, and simethicone (2,0 mg/kg) were given twice. Nebulization with aminophylline (2 mg/kg) and 0.9% NaCl (3 mL) driven by oxygen was conducted q.i.d. However, the animal went into respiratory arrest and died on the same day.

Post-mortem diagnostic procedures

The dolphin carcass was refrigerated, and seven hours later an endoscopy of the oral and nasal cavity and upper gastrointestinal tract was performed using a rigid optic Hopkins® telescope with a 30° vision angle, diameter of 2.7 mm, length of 18 cm (Karl Storz SE & Co.), following standard protocols. After the endoscopy, the dolphin was transported to a veterinary clinic with a diagnostic imaging service, where a whole-body scan (Figure 1a) was conducted using computed tomography (CT) (Revolution CT from GE Healthcare).

Necropsy and histopathology

A complete necropsy was performed after the post-mortem imaging procedures, the carcass was examined for external lesions, and all organ systems were analyzed macroscopically. Samples from most major organs and lesions were collected and fixed in 10% neutral buffered formalin for histological examination. Tissue samples were embedded in paraffin-wax, sectioned at 5 μm, and stained with hematoxylin and eosin for

Table 1. Hematological and blood gas values of neonatal female Franciscana dolphin (*Pontoporiablainvillei*), found stranded alive on a beach in Santa Catarina, Southern Brazil.

Layer (Type)		**This study**	**Baldassin, P. *et al.*, 2007**		
Hematology and blood gas	**SI Units**	2023-11-05	2006-01-09	2006-01-11	2006-01-13
RBC	**10^{12} L-1**	4.03	4.23	4.56	4.77
Hemoglobin	**g L-1**	158.8	155	163	177
GV	**(%)**	46	47	51	55
TTP	**g L-1**	72	NA	NA	NA
MCV	**f L-1**	114.14	111.1	111.84	115.30
MCH	**pg**	39.40	36.64	35.74	37.10
MCHC	**g L-1**	345.2	329.7	319.6	321.8
WBC	**10^9 L-1**	4.50	4.20	3.50	0.90
Neutrophils	**%**	61	60	60	68
Neutrophils	**10^9 L-1**	2.74	2.52	2.10	0.612
Lymphocytes	**%**	36	35	30	28
Lymphocytes	**10^9 L-1**	1.62	1.47	1.05	0.252
Monocytes	**%**	0	2	5	3
Monocytes	**10^9 L-1**	0	0.084	0.175	0.027
Eosinophils	**%**	3	2	3	0
Eosinophils	**10^9 L-1**	135	0.084	0.105	0
Basophils	**%**	0	0	0	0
Basophils	**10^9 L-1**	0	0	0	0
Platelets	**10^9 L-1**	60.60	189	155	120
pH		7.635	NA	NA	NA
PCO2	**mmHg**	31.9	NA	NA	NA
PO2	**mmHg**	82	NA	NA	NA
TCO2	**mmol L-1**	35	NA	NA	NA
HCO3	**mmol L-1**	34	NA	NA	NA
BEecf	**mmol L-1**	1	NA	NA	NA
sO2	**%**	98	NA	NA	NA
Sodium	**mmol L-1**	156	NA	NA	NA
Potassium	**mmol L-1**	4.3	NA	NA	NA
iCa	**mmol L-1**	1.12	NA	NA	NA
Glucose	**mmol L-1**	3.33	NA	NA	NA

RBC - red blood cell count; GV - globular volume; TTP - total plasma proteins; MCV - mean cell volume; MCH - mean corpuscular hemoglobin; MCHC - cellular hemoglobin concentration mean; WBC - white blood cell count; PCO2 - carbon dioxide partial pressure; PO2 - oxygen partial pressure; TCO2 – total carbon dioxide; HCO3 – bicarbonate; BBecf - buffer base extracellular fluid; sO2 - oxygen saturation; iCa - ionized calcium; NA – not available.

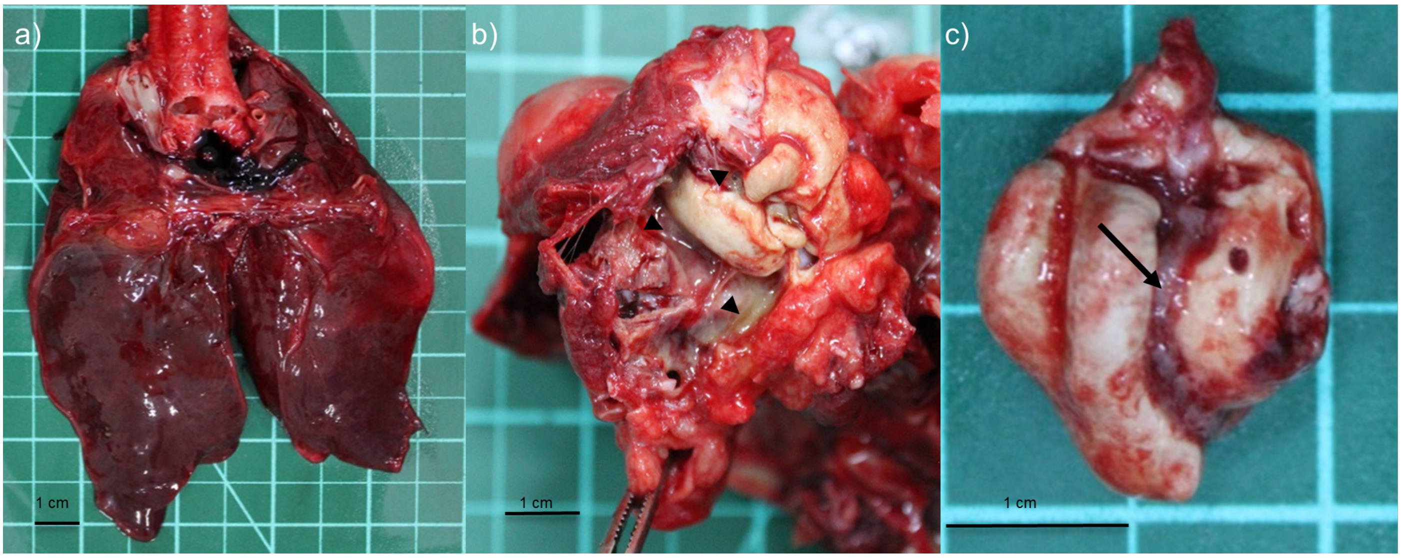

Figure 2. Gross lesions of a neonate female Franciscana dolphin, *Pontoporia blainvillei,* found stranded alive on a beach in Santa Catarina, Southern Brazil diagnosed with pneumonia and otitis (a) lungs, ventral view, showing congestion (b). Left tympanic bulla with suppurative material (black head arrows), view upon the ventral aspect of the skull. (c) Left tympanic bulla removed from the skull, showing suppurative material content (black arrow).

microscopic analysis.

Microbiology and molecular diagnosis

During the necropsy, the blowhole, blood, and bronchoalveolar lavage samples were aseptically collected and submitted for bacteriological culture. Blowhole and bronchoalveolar lavage samples were inoculated onto Columbia agar (Kasvi) with 5% sheep blood and MacConkey agar (Kasvi) and incubated overnight at 35±2°C. Blood samples were aseptically collected from the heart and stored in blood culture bottles (NewProv) broth at 35±2°C for 24 h before subculturing onto the culture as mentioned above. After isolation, the bacterial strain underwent identification using the API NE System (bioMérieux). After identifying the bacterial isolate, antimicrobial susceptibility testing was conducted using the disk diffusion method (Bauer et al., 1966). Discs (Oxoid and Liofilchen) containing Cephalosporins (Cefepime 30 µg, Ceftazidime 30 µg), Carbapenems (Imipenem 10 µg, Meropenem 10 µg), Monobactam (Aztreonam 30 µg), Quinolone (Ciprofloxacin 5 µg), and Aminoglycoside (Amikacin 30 µg) were utilized for the tests. The zone diameter breakpoints were evaluated in accordance with the CLSI (2023) guidelines. Tympanic bulla swabs were not collected due to contamination of the site after opening the skull during the necropsy examination. Reverse Transcription Polymerase chain reaction (RT-PCR) testing for *Morbillivirus* sp. and PCR testing for *Brucella* spp. were performed on frozen samples (adrenal gland, brain, heart, liver, lungs, lymph nodes, and spleen) as previously described (Tong et al., 2008; Batinga et al., 2018).

Results

The endoscopic evaluation of the cranial esophagus revealed the presence of milk formula and mild esophageal hyperemia, suggesting gastro-esophageal reflux, and the presence of anterolateral mechanical papillae were observed on the tongue apex. Slight streaks of blood were found through blowhole endoscopy. The CT scan revealed pneumonia in the cranioventral lung regions (Figure 1b), fluid material in the esophagus, and soft tissue opacity in the left tympanic bulla (Figure 1c).

At the necropsy, the subcutaneous tissue was pale yellow and congested. The intrathoracic pressure was decreased, and the main lesions were observed in the lungs. The tissue of the caudal dorsal quadrant collapsed, and multifocal, white, small punctate nodules were observed in the parenchyma, along with congestion of both lungs (Figure 2a). The esophageal mucosa was hyperemic, and pasty content was found within the lumen. The liver was enlarged. The intestines, kidneys, and stomach were congested. The left tympanic-periotic complex was extracted from the paraotic sinus, revealing congestion and yellow purulent material in tympanic bullae (Figure 2b, c). No internal or external parasites were recorded.

Microscopically, there was moderate congestion of the mucosa and serosa of the large intestine, and in the gastric mucosa and submucosa. Additionally, splenic, renal, thymic, and brain parenchyma were congested. A discrete infiltration of neutrophils permeated by discrete eosinophilic fibrillar material (fibrin) was observed in the left tympanic bulla. The lungs showed moderately expanded alveoli, with discrete to moderate neutrophil infiltration and macrophages permeated with fragments of laminar keratin.

All bacterial cultures revealed pure growth of *Pseudomonas aeruginosa*, and the result of the antimicrobial susceptibility testing showed no resistance to any of the tested antimicrobials. PCR tests were all negative for *Morbillivirus* sp. and *Brucella* spp.

Discussion

The Franciscana dolphin is a threatened cetacean whose main cause of strandings and death is bycatch. Most cases of live-stranded Franciscana dolphins are neonates, which survive only a few hours or days. Providing food for neonatal cetaceans that meets nutritional requirements and has good digestibility is a challenge. We tested a formula based on published work (Baldassin et al., 2007; Flower et al., 2018), but the results found suggest the need for new adaptations of the current formula recipe and future studies on nutritional protocols for *P. blainvillei* calves. To date, only two juvenile Franciscana dolphins have been successfully released in Brazil, and every effort to rehabilitate a live animal provides valuable information for future strandings (Kolesnikovas, 2022; Meegan et al., 2022).

In marine mammals, bacterial respiratory system infections significantly contribute to morbidity and mortality. Pneumonia is one of the most common pathologies reported in dolphins, and several pathogens have been associated with respiratory diseases in cetaceans (Bogomolni et al., 2010; Venn-Watson et al., 2012; Venn-Watson, 2016; Elfadl et al. 2017). Aspiration pneumonia, gastroesophageal reflux, infections, and sepsis are among the principal problems in neonates (Sweeney et al., 2010; Pérez et al., 2015; Díaz-Delgado et al., 2018; Flower et al., 2018). Herein, an endoscopic examination of the oral cavity demonstrated the presence of anterolateral mechanical papillae arranged on the tongue apex, described in neonatal and juvenile individuals of *P. blainvillei* (Tostado-Marcos et al., 2024). Following what we observed in the reported case, thoracic ultrasound is a valuable diagnostic method for detecting pulmonary diseases (Smith et al., 2012), and endoscopy and CT can be used for post-mortem investigation (Venn-Watson et al., 2012; Tsui et al., 2020; García de los Ríos y Loshuertos et al., 2021; Ready et al., 2021).

Diagnosing diseases in marine mammals is often challenging, even when clinical signs are present (Souter et al., 2024). Timely and appropriate therapy frequently fails to yield successful outcomes. The coexistence of bacteria with viruses and parasitic infections complicates determining their role as primary or secondary pathogens (Simeone & Stoskopf, 2018; Haebler & Moeller, 2021). In cases of bacterial respiratory disease, antibiotic selection should follow routine culture and sensitivity characterization, often starting before test results are available. Broad-spectrum antibiotics effective against Gram-negative organisms are commonly used initially (Simeone & Stoskopf, 2018).

However, the rapid progression of infections, coupled with clinical deterioration exacerbated by factors like stress, adds complexity to clinical management. Unfortunately, bacterial culture and susceptibility testing results are often known only after an animal's death.

The initial treatment selected for the reported animal included the administration of ceftriaxone, followed by penicillin. These antimicrobials can effectively target beta-lactam-susceptible *Enterobacterales* and penicillin-susceptible Gram-positive bacteria (Cheng et al., 2016). This enables the treatment of primary and secondary infections associated with a diverse range of pneumonia-associated agents (da Trindade & Salgado, 2018). Additionally, ceftriaxone can be utilized in combating erysipelas, caused by *Erysipelothrix rhusiopathiae*, which is considered one of the most severe bacterial infections in cetaceans worldwide (Fidalgo et al., 2002; Tryland et al., 2018; Lee et al., 2022).

Despite efforts to use antimicrobials, post-mortem results indicated that the cetacean exhibited a widespread infection caused by *Pseudomonas aeruginosa.* This agent is a known opportunistic bacterium found in marine mammal infections (Venn-Watson et al., 2012) and was one of the most common species isolated in humans and domestic animals diagnosed with otitis (Shyamala R & Reddy, 2012; de Melo, 2019). Furthermore, this bacterium presents a broad range of intrinsic antimicrobial resistance in its chromosome, and the use of Penicillin and Ceftriaxone was not effective in combating this infection (Karruli et al., 2010).

Unfortunately, it was not possible to identify the bacterial species before the rapid clinical progression of the infection. In this scenario, using ceftazidime or cefepime as substitutes for ceftriaxone could have been more effective in treating the infection and, at the same time, impacting beta-lactam-susceptible *Enterobacterales*. Both medications are applicable in the treatment of infections in cetaceans, indicating that an adaptation to the internal protocol could represent a viable alternative to encompass a wider variety of pneumonia-causing agents in these animals (Simeone & Stoskopf, 2018). Furthermore, maintaining the use of Penicillin can aid in combating potential infections of *E. rhusiopathiae* (Reboli & Farrar, 2010).

Otitis media is an accumulation of exudate and debris in tympanic bulla and represents a disease often reported in domestic animals caused by a wide variety of etiologies. In contrast, it is rarely documented in marine mammals, with most cases being post-mortem findings on stranded cetaceans and incidentally findings in live stranded pinnipeds (Siebert et al., 2001; Ready et al., 2021). In *Phocoena phocoena*, otitis was reported to be associated with parasite infestation, chronic inflammation, hyperplastic, and metaplastic epithelial changes (Wohlsein et al., 2019). *Penicillium* spp. was identified by molecular analysis in a *Tursiops truncatus* with pyogranulomatous and necrotizing otitis media, and mycotic otitis has also been seen in *P. phocoena* (Seibel et al., 2010; Attig et al., 2018; Van Elk et al., 2019). In our reported case, no parasites or fungal hyphae were found in the tympanic cavity and ear structures. Otitis was initially identified by CT, a useful technique to diagnose middle ear diseases in domestic and marine mammals (Parlak et al., 2021; Ready et al., 2021).

Diagnostic imaging findings, clinicopathological evaluations, and microbiological assessments allowed the diagnosis of *P. aeruginosa* pneumonia and sepsis in the Franciscana dolphin. Additionally, the neonate exhibited mild to moderate neutrophilic and histiocytic bronchopneumonia associated with fragments of laminar keratin, though this can be an incidental finding in calves (Van Elk et al., 2007). Neonate's immature immune system may have contributed to otitis media and septicemia. There is a scarcity of information about *Pontoporia blainvillei's* natural parameters and pathogens, and further research is needed to advance our understanding of their natural causes of disease and mortality. Furthermore, trying to rehabilitate individuals of such endangered populations would contribute to future conservation efforts.

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