

## MORPHOLOGICAL AND PATHOLOGICAL ASPECTS OF CETACEANS STRANDED ON THE BLACK SEA SHORE

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### Abstract

The aim of this paper is to analyse the pathology which can be observed in four cases of stranded cetaceans in the costal area of the Black Sea, in order to highlight the main causes of mortality and the associated lesions patterns. Those cases included severe respiratory diseases (parasitism, bacterial pneumonia), endocrine disorders associated with chronic stress (adrenal hyperplasia), acute head trauma and, also, specimens in an advanced state of decomposition. The necropsy examination was completed with histopathological and microbiological investigations and the results obtained was providing a perspective on the interactions of biological, anthropogenic and environmental factors that are involved in cetaceans' mortality. The study points out the necessity for multidisciplinary monitoring and early intervention in the conservation of marine mammals.

**Key words:** cetaceans, stranded, Romania, Black Sea.

### INTRODUCTION

Along the Romanian Black Sea coast, dozens of cetacean stranding events are reported annually. A primary contributing factor is accidental bycatch in fishing nets (Benke et al., 2001) targeting turbot and other resident fish species, in addition to bacterial and viral infections. Anthropogenic pollution further exacerbates dolphin mortality (Bilgin et al., 2018).

All three cetacean species occurring in the Black Sea - the harbour porpoise (*Phocoena phocoena*), the bottlenose dolphin (*Tursiops truncatus*), and the common dolphin (*Delphinus delphis*) - are currently endangered (Dede et al., 2023).

Investigating the etiological factors underlying strandings is of particular significance given the ecological role of cetaceans and the increasing likelihood of human-cetacean interactions, which also entails heightened zoonotic risks. Consequently, systematic necropsies are routinely performed on stranded individuals to establish definitive causes of death. Necropsy findings allow for differential diagnoses, while complementary investigations such as histopathology or bacteriology facilitate the determination of precise etiological diagnoses. Collectively, these methods also contribute to the monitoring of population health status within Black Sea dolphin communities.

Pathogenic agents affecting marine mammals include bacteria, viruses, and parasites. Among bacterial infections, actinomycoses - particularly nocardiosis - are frequently reported, manifesting as cutaneous or subcutaneous abscesses and localized or systemic pyogranulomatous lesions (Arbelo et al., 2022). This pathology is increasingly recognized as a health concern in free-ranging marine mammals. Diagnosis is challenging and typically established post-mortem through histopathological or cytological analyses. Infections with *Actinomyces* spp. and *Arcanobacterium* spp. have been widely reported, with *Arcanobacterium animalium* isolated predominantly from porpoises (Field, 2022a). Another bacterium frequently identified in both captive and free-ranging cetaceans is *Brucella ceti*. Additional isolates include *Clostridium* spp., *Erysipelothrix rhusiopathiae*, *Mycobacterium* spp., and *Pasteurella multocida*.

Viral pathogens of concern comprise *Morbillivirus* and *Poxvirus*. Furthermore, other potentially pathogenic viruses include *Papillomavirus*, *Hepadnavirus* (hepatitis B-like), and endogenous gamma retroviruses, identified in the genomes of bottlenose dolphins, porpoises, and other delphinids (Field, 2022c). Marine mammals are also susceptible to parasitic infestations from all major taxa -

nematodes, trematodes, cestodes, mites, and acanthocephalans - which may also contribute to stranding events. Among acanthocephalans, *Bolbosoma* spp. are notable, with cetaceans serving as definitive hosts; diagnosis relies on fecal examination for parasite ova (Field, 2022b). Other examples include *Halocercus lagenorhynchi*, affecting the lungs with evidence of prenatal transmission, and *Contracaecum* spp., identified in the stomachs of wild Baltic Sea dolphins (*Lagenorhynchus albirostris*) (Campbell et al., 1991). Additionally, *Crassicaudata* spp. have been reported in cetacean kidneys, mammary ducts, large blood vessels, and cranial sinuses, inducing trabecular osteolysis (Duignan et al., 2020). Another parasitosis frequently associated with stranded dolphins is toxoplasmosis, transmitted via oocysts contaminating marine waters (Di Guardo et al., 2013).

The aim of this study is to elucidate the causes of dolphin mortality, to monitor the health status of resident cetacean populations, and to determine the proportion of cases in which entanglement in fishing nets constitutes the primary cause of death.

## MATERIALS AND METHODS

For the purpose of this study, four stranded cetacean specimens from the Black Sea coast were examined. All safety regulations concerning the handling of stranded specimens were strictly observed. Field records were compiled, documenting the stranding location, hydrometeorological conditions, and specimen-specific data such as species, condition and body weight, sex, and morphometric measurements. The initial evaluation of dolphins plays a crucial role in individual identification and in assessing their nutritional and physiological status, information that can support the determination of an accurate cause of stranding. Environmental conditions were also documented, as these significantly influence the assignment of the body condition code. Necropsy procedures and sample collection were carried out using protocols specific to marine mammals, integrated with methodologies taught in the Pathology curriculum. Tissue samples were collected for histopathological, bacteriological, and

parasitological examinations, as well as for the quantification of microplastics.

Histopathological analyses were performed using an Olympus BH2 microscope. Microscopic imaging was conducted with the CellSens Entry software, a specialized research and diagnostic tool widely employed in laboratory settings.

## RESULTS AND DISCUSSIONS

### Case No. 1

#### Macroscopic description

Upon opening the thoracic cavity, approximately 50 mL of sanguinolent serous fluid was observed (interpreted as a thawing artifact). The lungs were incompletely collapsed, exhibiting a reddish-violet coloration and containing a significant amount of foamy fluid, present both on cut surface and within the bronchial tree, consistent with drowning. On section, diffuse whitish nodules resembling calcifications were identified, along with numerous intrapulmonary parasites, which were collected for parasitological examination.

#### Microscopic description

Pulmonary architecture was severely altered by a diffuse inflammatory infiltrate rich in macrophages and neutrophils, admixed with fibrillar proteinaceous material, within which numerous coccoid bacterial colonies were identified. Additionally, acellular fluid was present in the broncho-alveolar spaces, alternating with compensatory emphysematous areas. Marbled regions were also noted, characterized by dark red to blackish discolorations and consolidation, consistent with bronchopneumonia. Microscopically, areas of atelectasis and necrosis associated with inflammatory infiltrates were also observed, findings consistent with parasitic migration pneumonia (Figure 1). Microbiological analysis of pulmonary samples confirmed the presence of *Klebsiella* spp. and  $\beta$ -haemolytic *Streptococcus*. Pulmonary nematodes are frequently reported in cetaceans, with *Halocercus lagenorhynchi* identified in this specimen. Pulmonary nematode infestation may also be diagnosed via coprological examination or by analysis of bronchiolar mucus in live animals. Such parasitism is generally subclinical for extended

periods, with clinical signs emerging only when the host becomes debilitated, favouring the development of secondary pulmonary infections, which may ultimately result in death.

### Hypothesized cause of death

Although case-specific data are limited, correlation of macroscopic and microscopic findings supports pulmonary parasitism, compounded by secondary bacterial pulmonary infections, as the most likely cause of death.

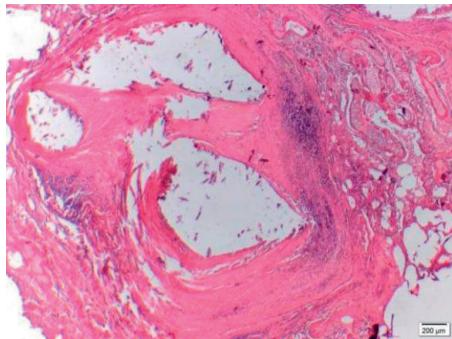


Figure 1. Lung, **objective 2x**, H&E stain, parasitic migration area (Original)

### Case No. 2

#### Macroscopic description

On external inspection, signs of human interaction were noted. Multiple linear lesions were observed on the ventral skin, suspected to have been caused by contact with shoreline shells, along with injuries to the caudal fin, possibly inflicted by fishing nets (Figure 2).



Figure 2. Skin with linear lesions (Original)

The absence of the right pectoral fin was also documented. On sectioning of the lungs, small whitish, firm nodules were identified. In

addition, the specimen presented gastric parasites and parasite-derived cysts, which were collected for further examination.

#### Microscopic description

Microscopic examination of the prescapular lymph node revealed hyperplasia with an increased lymphocyte population, without evidence of other pathogens. The testis exhibited atrophy, with seminiferous tubules of variable size and moderate cellularity; binucleated and multinucleated cells were occasionally observed, suggesting mild testicular atrophy. No additional lesions were identified, and neoplastic changes at this site are rarely reported in cetaceans (Baumgartner et al., 2005). Examination of the kidney revealed preserved renal architecture and eosinophilia, indicative of autolysis, together with capsular fibrosis. The adrenal gland showed cortical hyperplasia, a change typically associated with stress. The normal cortico-medullary ratio ranges from 1:1 to 2:1. In this specimen, the ratio was 3.84:1, indicating marked adrenal cortical hyperplasia, consistent with chronic stress (Figure 3). Such stress predisposes marine mammals to harmful factors potentially leading to death, including secondary infections, panic responses, disorientation, and ultimately drowning and stranding (Clark et al., 2006).

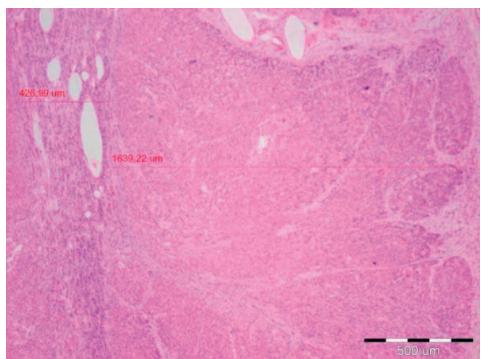


Figure 3. Adrenal gland, **objective 2x**, H & E stain, cortical hyperplasia (Original)

#### Hypothesized cause of death

Based on the correlation of macroscopic and microscopic findings, death is suspected to have resulted from chronic stress, predisposing the organism to various diseases. Additionally, panic responses and potential drowning

following entanglement in fishing nets may also be implicated as contributing factors leading to stranding.

### Case No. 3

#### Macroscopic description

Symmetrical, rectilinear lesions were observed on the pectoral fins, while the mandibular region displayed a large erosive wound containing shell fragments. In addition, periocular skin loss was noted around the left globe, likely attributable to opportunistic scavenger activity. Cranial to the penile orifice, a circular lesion with marked tissue loss was documented. A contusion was also observed in the temporal region, associated with the formation of an epidural hematoma, a lesion potentially resulting from intra- or interspecific interactions (Figures 4 and 5) (Andrade et al., 2020).

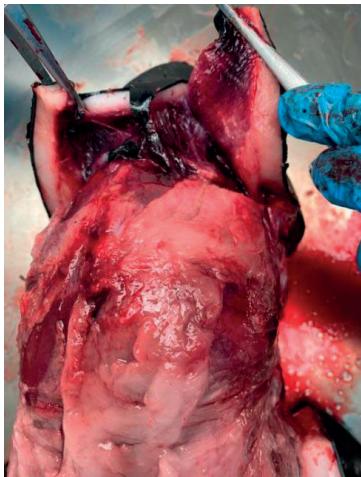


Figure 4. Temporal bone contusion and epidural hematoma (Original)

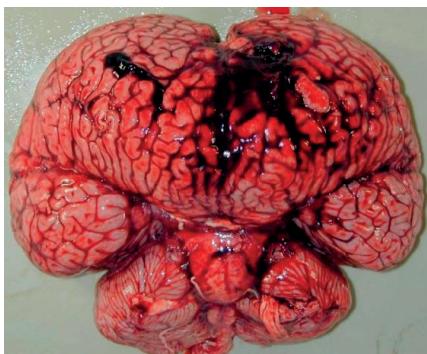


Figure 5. Post-traumatic cerebral haemorrhage (Original)

Opening of the abdominal cavity revealed a moderate quantity of sanguinous fluid, suggestive of internal haemorrhage caused by trauma. Parasites were also identified within the tympanic bulla and were collected for parasitological analysis.

#### Microscopic description

The prescapular lymph node exhibited hyperplasia with germinal centres outlining lymphoid follicles. In the lungs, parenchymal architecture was preserved; however, alveolar spaces were filled with homogeneous, eosinophilic fluid, accompanied by marked oedema. Additionally, hyperaemia of interstitial and alveolar capillaries was observed, suggestive of agonal death. The liver displayed both micro- and macrovesicular steatosis, with preserved lobular architecture. Diffuse hepatocellular micro- and macrovacuolar degeneration, together with intrahepatic bile plugs and bile canalicular stasis, indicated hepatic lipidosis with impaired biliary drainage.

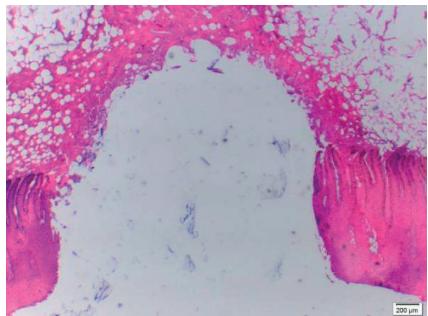


Figure 6. Cutaneous epithelium, **objective 2x, H & E staining**, ulcerative dermatitis with haemorrhagic infiltrate in adipose tissue (Original)

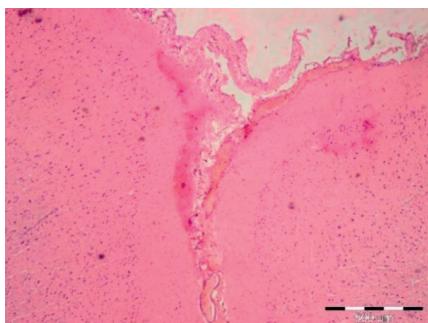


Figure 7. Meningeal and brain haemorrhage, post-traumatic injury, **objective 2x, H & E stain** (Original)

The epithelial tissue collected from the mandibular region showed a full-thickness skin defect involving all epidermal layers and extending into the superficial dermis. Adjacent to the lesion, localized hemorrhagic infiltrate was observed in the dermal adipose tissue (ulcerative dermatitis without evidence of a pathogen-which are frequently observed to be ciliated protozoa (Lipscomb & Schulman, 1999), possibly of mechanical origin (Figure 6).

### **Hypothesized cause of death**

In this specimen, traumatic injury is suspected as the cause of death, most likely associated with human interaction or accidental events. Dolphins commonly become entangled in fishing nets while foraging, and fishermen frequently attempt to drive them away, thereby inflicting traumatic lesions.

### **Case No. 4**

#### **Macroscopic description**

Advanced decomposition: the carcass was excessively bloated, with disintegrated skin, non-differentiable internal organs, and visible skeleton. Macroscopic examination also revealed various hematomas on the body. The first teeth of the upper jaw were absent, and subsequent teeth exhibited increased mobility. Multiple linear skin lesions were identified, consistent with accidental entanglement in fishing nets.

This case was not subjected to necropsy due to the advanced stage of autolysis. The specimen was classified as Condition Code 4 (advanced decomposition: excessively bloated carcass, disintegrated skin, non-differentiable internal organs, and visible skeleton).

It is well-known that aquatic animals, particularly marine mammals, possess a significant subcutaneous fat layer, which serves as a protective mechanism against caloric loss and body cooling. This layer can accelerate autolysis, often rendering examination or sample collection for cause-of-death determination impossible.

#### **Hypothesized cause of death**

Due to the advanced state of decomposition, the cause of death could not be determined for this specimen. This case highlights the critical

importance of carcass freshness and performing necropsies as soon as possible after death.

## **CONCLUSIONS**

Pulmonary parasitism and secondary infections represent one of the most frequent causes of mortality in stranded dolphins from the Black Sea, reflecting chronic exposure to aquatic pathogens.

Chronic stress can lead to significant endocrine alterations with systemic effects, even in the absence of overt lesions. Adrenal cortical hyperplasia is a reliable morphological indicator of prolonged stress.

Severe cranial trauma constitutes a direct cause of acute death, frequently associated with anthropogenic activities (marine traffic, conflictual interactions with fishermen).

Advanced decomposition severely limits postmortem diagnostic value, emphasizing the need for an efficient system for carcass recovery, preservation, and triage.

Stranded cetacean mortality is often multifactorial, requiring an integrated approach: necropsy, histopathology, microbiology, toxicology, and ecological analysis - in line with the One Health concept.

The establishment of a national rapid-response protocol for cetacean strandings is recommended, involving veterinary faculties, environmental organizations, and coastal authorities, to ensure population conservation and a better understanding of emerging risks.

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