

# Lesions associated with drowning in bycaught penguins

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**ABSTRACT:** Fisheries bycatch, the incidental mortality that occurs as a result of entanglement in fishing gear, is an important conservation threat to penguins and other seabirds. Identification of entanglement and drowning in beach-cast carcasses of seabirds remains a challenge, as it is still unclear what lesions are to be expected in a bycaught seabird. We necropsied 2 Magellanic penguins *Spheniscus magellanicus* that were entangled and drowned in gillnets. Marked distension of the lungs with foamy red fluid and marked oedema of the dorsal visceral pleura were prominent lesions consistent with those described in cases of ‘wet drowning’ in humans. On the other hand, the air sacs contained very small quantities of liquid, suggesting that absence of water in the air sacs might not be a reliable sign to exclude drowning. Other relevant findings included cutaneous lacerations and bruising in one bird and cervical and pectoral rhabdomyolysis in both birds. While cutaneous or subcutaneous hematomas may be an indication of bycatch, especially if linear or cross-linear patterns consistent with fishing nets are present, these lesions might not always be discernible and their absence does not suffice to exclude the possibility of entanglement in fishing nets. Additionally, our findings suggest that the histological examination of skeletal muscles, particularly of the neck, may provide additional clues to corroborate the diagnosis of drowning in penguins.

**KEY WORDS:** Fisheries · Gillnet · Mortality · Pathology · Forensic science · Asphyxia · Spheniscidae

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## INTRODUCTION

Fisheries bycatch is an important threat to the conservation of marine megafauna (Brothers et al. 1999, Lewison et al. 2004, Soykan et al. 2008). Because of their dependence on breathing air, sea turtles, seabirds, and marine mammals will drown if they become hooked or entangled in fishery hooks or nets underwater. As a result, whenever carcasses of air-breathing marine animals are beach-cast, it is important to consider drowning due to fisheries bycatch among the potential causes of death (Read & Murray 2000, Schlatter et al. 2009, Mannocci et al. 2012).

A number of studies have investigated fisheries bycatch of seabirds (Brothers et al. 1999, Moore & Zydalis 2008, Zydalis et al. 2009), including penguins (Gandini et al. 1999, Yorio & Caille 1999, Norman 2000, Majluf et al. 2002, Tamini et al. 2002, Cardoso et al. 2011, Pütz et al. 2011). In these studies, information was primarily obtained directly or indirectly from the fishery industry, whereas the fewer studies that have attempted to diagnose entanglement and drowning post-mortem in beach-cast seabirds have relied primarily on external lesions compatible with entanglement in nets (Darby & Dawson 2000, Hocken 2000, 2005) or on the presence of water in

the lungs or air sacs (Daoust et al. 1998, Simeone et al. 1999, Hocken 2000, 2005, Schlatter et al. 2009, Cannell et al. 2016).

Identification of drowning in human forensic medicine is often challenging due to the difficulty in differentiating drowning and post-mortem submersion, and there are many aspects of the pathophysiology of drowning that remain unclear (Salomez & Vincent 2004, Piette & Letter 2006). This may be further complicated in marine animals due to their unique anatomical and physiological adaptations for diving. For instance, drowned dolphins rarely have water in their lungs, and it is unclear whether this occurs because these species' physiological adaptations for diving apnoea prevent them from inhaling significant quantities of water (Dierauf & Gulland 2001) or because, unlike in humans, dolphin entanglement drowning occurs without repeated breathing at the surface (Knieriem & Hartmann 2001). Because the respiratory tract lesions are inconsistent and the presence of water in the lungs cannot be relied upon, the diagnosis of entanglement/drowning in beach-cast cetaceans has relied primarily on the observation of external entanglement lesions (Read & Murray 2000, Dierauf & Gulland 2001, Boer et al. 2012, Mannocci et al. 2012).

The avian respiratory system differs profoundly from that of mammals, as birds rely on relatively small, rigid and flow-through lungs and a system of air sacs that allows for continuous air flow through the lungs (Fedde 1998, Powell 2000). It is not clear how these anatomical characteristics can affect the pathogenesis of drowning in birds or if there are substantial differences in the pathology of drowning between diving and non-diving birds. As a result, determining whether or not beach-cast seabird carcasses are the result of fisheries bycatch remains a challenge and it is still unclear what lesions are to be expected in a bycaught seabird.

In this study, we describe the gross and microscopic findings in 2 Magellanic penguins *Spheniscus magellanicus* that were necropsied after having been entangled and drowned in fishing nets, and discuss the implications for the diagnosis of entanglement and drowning in diving seabirds.

## MATERIALS AND METHODS

On 17 September 2014, a Magellanic penguin was entangled and drowned in a bottom gillnet (depth = 12 m) near the coast of Peruíbe, SP, Brazil (24° 20' S, 46° 58' W) (Case A). Another 4 Magellanic penguins

were entangled in a floating gillnet (depth = 3 m) on 2 June 2015 near the coast of Mongaguá, SP, Brazil (24° 07' S, 46° 40' W); 1 bird survived and was immediately released by the fishermen whereas 3 birds drowned, one of which was brought ashore for necropsy (Case B).

Necropsy was conducted within 6 to 10 h after carcass retrieval following standard protocols (Hocken 2002, Rae 2003). Age was determined on the basis of feathering (Williams 1995). Sex was determined through the identification of gonads. Samples of organs and tissues were fixed in 10% neutral buffered formalin for 36 h, then transferred to 70% ethanol. Tissues were then embedded in paraffin and 3 to 5 µm sections were obtained, stained with hematoxylin-eosin (H&E) and phosphotungstic acid hematoxylin (PTAH) (to assist the diagnosis of necrosis in skeletal muscles) and examined by light microscopy (Prophet et al. 1992). Skeletal muscles sampled for histopathology were

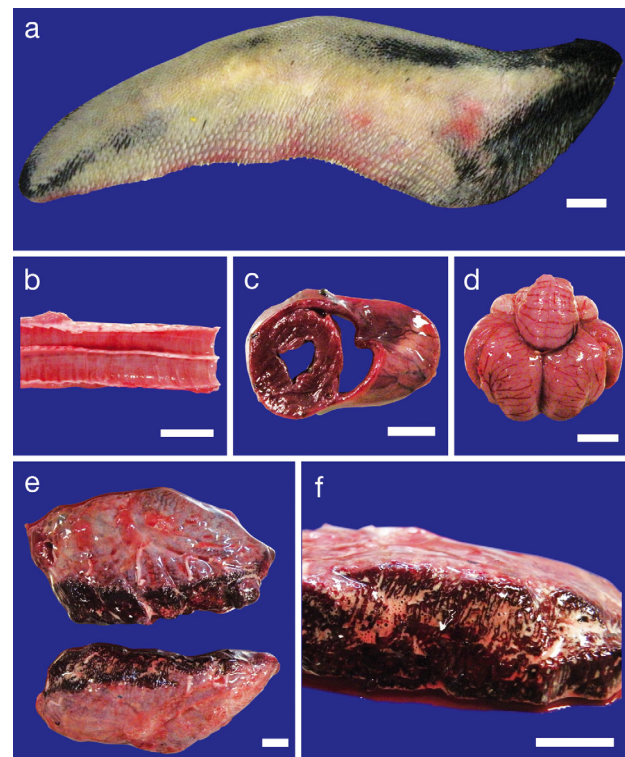


Fig. 1. Gross lesions observed in bycaught Magellanic penguins *Spheniscus magellanicus*. (a) Ventral surface of the left flipper: bruising and hematomas; (b) longitudinal section of the trachea: mucosa congestion; (c) transversal section of the heart: dilation of the right ventricle; (d) dorsal surface of the encephalon: cerebral and cerebellar congestion; (e) dorsal surface of the lungs: oedema and congestion; (f) cut surface of the left lung: oedema and congestion with extravasation of a foamy red fluid. Scale bars = 1 cm

m. pectoralis (chest) and m. rectus capitis lateralis (neck) (Watson 1883).

## RESULTS

Both examined penguins were juvenile females. Body masses were 2.40 kg (Case A) and 2.34 kg (Case B), and in both cases pectoral musculature was moderately atrophied (poor body condition). Bill lengths were 52.7 mm (Case A) and 52.3 mm (Case B). The only external lesions in Case A were superficial lacerations and bruising on the skin of the ventral surface of both flippers (approximately 10 to 20% of the surface area) (Fig. 1a); Case B did not exhibit external lesions. Mild infestation ( $\leq 50$  ind.) of chewing lice *Austrogoniodes* sp. was noted in Case A.

Unless stated otherwise, the following description applies to both cases. Internally, the most prominent changes were within the lungs. Both lungs were markedly distended, with approximately 150% of the volume that would be expected in a similarly-sized healthy penguin (R. E. T. Vanstreels pers. obs.). A 1 to 3 mm-thick layer of gelatinous tissue covered the surface of the lungs (especially dorsal), and as a result the lungs could be easily dissected from the ribcage, and the low relief impression that the ribcage usually produces on the dorsal surface of the lungs was barely visible (marked oedema of the visceral pleura) (Fig. 1e). Lung parenchyma was diffusely dark red with clearly visible white/reddish foam in the parabronchi (oedema) (Fig. 1f). Thin red fluid flowed abundantly when the lungs were cut (congestion). Both lungs floated in formalin, however less than 5% of the lung volume remained above the surface.

All air sacs were transparent. A small amount of red to yellow foamy fluid was present in the thoracic and abdominal air sacs (<1 ml in each air sac in Case A, <5 ml in each air sac in Case B); this characteristic foamy appearance was transient and vanished as the necropsy progressed. The tracheal mucosa was diffusely red (consistent with congestion) (Fig. 1b); in Case B, petechial haemorrhage and a small quantity (<1 ml) of red to yellow foamy fluid was within the distal third of the trachea. The mucosa of the nasal cavity and paranasal sinuses

was diffusely red (consistent with congestion of the upper respiratory tract mucosa).

There were no signs of subcutaneous bruising, fractures or internal haemorrhage, but skeletal muscles of the dorsal neck near the shoulders were mildly dark red (consistent with congestion). There was almost no subcutaneous fat, however small quantities of visceral and pericardial fat were noted. Other necropsy findings included diffusely red subcutaneous tissue and skeletal muscle, with most large veins visibly distended (external jugular veins, vena cava, and coronary veins); dilation of the right ventricle (Fig. 1c); diffusely red coloration both externally and on the cut surface of the brain (consistent with congestion) (Fig. 1d); and multifocal petechiae and ecchymoses in the pancreas. Case A had 3 approximately  $1.5 \times 0.5 \times 0.1$  cm caseous plaques adhered to the caudal palate whereas Case B had a 3 mm diameter caseous nodule on the caudal palate. The stomachs contained a small quantity of white-yellow doughy material (partially digested fish); in Case B, a small quantity of squid beaks and crab legs were also present in the stomach. Mild infestation ( $\leq 20$  ind.) by gastric nematodes (consistent with *Contracaecum* sp. and/or *Cosmocephalus* sp.) was noted in both cases, but no lesions were visible on the gastric mucosa.

Histological findings are listed in Table 1 (and Table A1 in the Appendix, and the most relevant

Table 1. Histopathological lesions related to drowning in bycaught Magellanic penguins *Spheniscus magellanicus*. Superscript letters indicate the case(s) (A or B) in which lesions were recorded

Organ/tissue	Histopathological findings
Trachea	Congestion, diffuse, mild to moderate <sup>A,B</sup> Haemorrhage, segmental, mild <sup>A,B</sup> Tracheitis, granulocytic, diffuse, mild <sup>A,B</sup>
Lungs	Congestion, diffuse, moderate to severe <sup>A,B</sup> Oedema, diffuse, moderate to severe <sup>A,B</sup> Micro-haemorrhage, diffuse, moderate <sup>A,B</sup> Deposition of proteinaceous material, alveolar and parabronchial, diffuse, moderate, with occasional erythrocytes, granulocytes and bacterial colonies <sup>A,B</sup>
Heart	Cardiomyolysis, multifocal, mild to moderate <sup>B</sup> Congestion, diffuse, mild to moderate <sup>A</sup>
Cerebrum	Congestion, diffuse, mild to moderate <sup>A,B</sup> Oedema, diffuse, mild to moderate <sup>A,B</sup>
Cerebellum	Congestion, diffuse, mild to moderate <sup>A,B</sup>
Skeletal muscle	Rhabdomyolysis, multifocal, mild to moderate <sup>A,B</sup> Congestion, diffuse, mild to moderate <sup>A,B</sup> Oedema, diffuse, mild <sup>A</sup>
Liver	Congestion, diffuse, mild <sup>A,B</sup>
Adrenal glands	Congestion, diffuse, mild <sup>A</sup>
Kidney	Congestion, diffuse, mild to moderate <sup>A,B</sup>

lesions are presented in Fig. 2. All tissues presented an increased quantity of granulocytes within blood vessels. The following tissues were microscopically unremarkable: abdominal aorta, gall bladder, thyroid, parathyroid, supraorbital gland, uropygial gland and ovary. Tissue autolysis was considered mild, and is not thought to have compromised histological analysis.

## DISCUSSION

### Respiratory tract

The presence and type of pathological changes associated with drowning typically depends on whether

or not water enters the lungs (wet vs. dry drowning) (Salomez & Vincent 2004, Piette & Letter 2006). Wet drowning corresponds to the majority of human drowning deaths, whereas dry drowning is common in cetaceans and occurs when laryngospasm prevents inhalation of water (Orlowski et al. 1989, Salomez & Vincent 2004, Lunetta & Modell 2005). The hypoxia that occurs in both dry and wet drowning leads to ischemic damage to the myocardium and central nervous system, culminating in death (Salomez & Vincent 2004, Lunetta & Modell 2005). In seawater wet drownings, the hypertonic liquid can draw protein-rich fluid from the vascular space into the pulmonary alveoli, damaging the basement membrane and leading to rapid pulmonary oedema, dilution and washout of surfactant (Lunetta & Modell 2005).

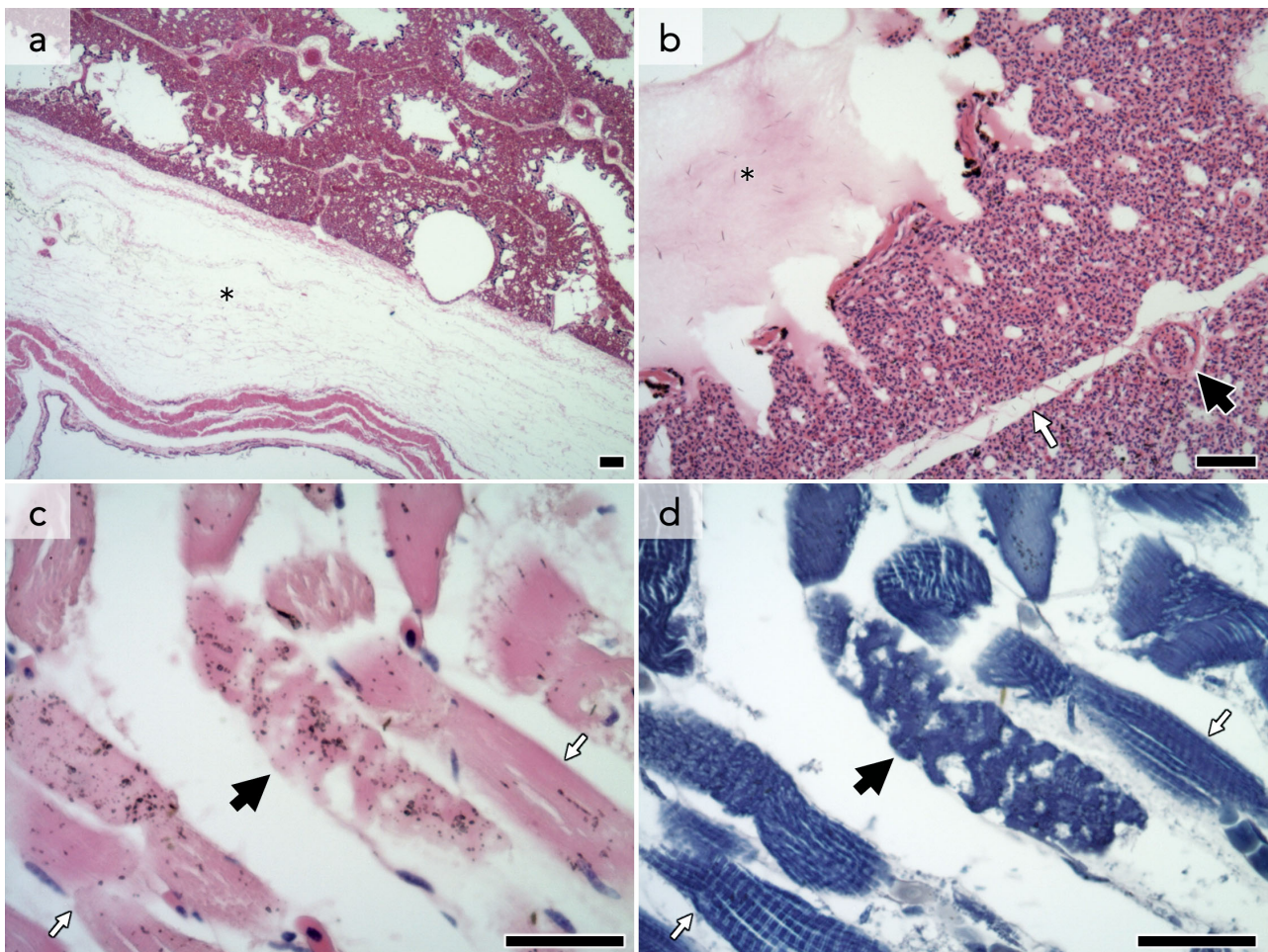


Fig. 2. Histopathological lesions in bycaught Magellanic penguins *Spheniscus magellanicus*. (a) Lung (H&E, scale bar = 100 µm): diffuse congestion (top section), the visceral pleura (asterisk) is markedly stretched due to oedema; (b) lung (H&E, scale bar = 50 µm): diffuse congestion and oedema, proteinaceous material is present in the parabronchial lumen (asterisk), interparabronchial septa are stretched due to the oedema (white arrow) and all blood vessels are markedly congested, including septal arterioles (black arrow); (c,d) neck muscle (H&E and PTAH, scale bars = 25 µm): consecutive histological sections illustrating how histochemistry can facilitate the differentiation between a muscle fibre undergoing rhabdomyolysis (black arrow) and fibres with preserved striation bands (white arrows)

The avian respiratory system has a number of characteristics that distinguish it from its mammalian counterpart and that may affect the pathophysiology of drowning, such as the tubular structure of the lungs in which air flow is controlled through aerodynamic valving (Fedde 1998, Powell 2000), the remarkably thin blood–air barrier (Maina & King 1982, West 2009) and the different composition and physical properties of avian surfactant (Bernhard et al. 2001, 2004). Furthermore, penguin lungs are different from those of other birds in that they lack neopulmonic parabronchi (Fedde 1998, Powell 2000) and have histological adaptations to resist the high hydrostatic pressures involved in diving (Welsch & Aschauer 1986).

The lesions that were observed in the lungs of drowned penguins parallel those described in wet drowning in humans, with marked distension of lungs with foamy liquid (Püschel et al. 1999, Salomez & Vincent 2004, Lunetta & Modell 2005). The studied penguins, however, did not have a substantial quantity of foam and frothy liquid in the upper airways nor multifocal subpleural haemorrhages (Paltauf's spots) that are often observed in humans (Salomez & Vincent 2004, Lunetta & Modell 2005). The overall volume of the lungs was markedly increased, however the presence of liquid in the pulmonary airways did not seem to be accompanied by significant loss of structure or rupture of the parabronchial septa, in contrast to the emphysema aquosum that is classically described in humans (Püschel et al. 1999, Salomez & Vincent 2004, Lunetta & Modell 2005). If inhaled into the lungs, the hyperosmotic nature of seawater is expected to lead to an intense outflow of intravascular fluids to the airways, resulting in haemoconcentration and severe pulmonary oedema (Timperman 1972, Modell 1993, Lunetta & Modell 2005). It was not possible to quantify the extent to which the fluid in the lungs corresponded to inhaled water, to passive extravasation of intravascular fluids due to osmotic forcing, to an increased vascular permeability due to an acute inflammatory response, to pulmonary oedema secondary to the increased hydrostatic pressure within blood vessels, or to a combination of 2 or more of these factors.

In contrast to the marked distension and oedema of the lungs and pleura, the air sacs contained only very small quantities of liquid. Similarly, previous studies did not find substantial quantities of liquid in the air sacs of drowned Canada geese *Branta canadensis* (Springer et al. 1989) or common loons *Gavia immer* (Daoust et al. 1998). A possible explanation is that as the birds inhale water there is a rapid increase in the

flow resistance within the intrapulmonary bronchi (caused by the greater viscosity of water in comparison to air) that surpasses the negative pressure provided by the expansion of the ribcage, limiting the flow of inhaled water into the caudal air sacs.

### External lesions and skeletal muscles

External lesions consistent with fishing net trauma are often used to diagnose bycatch in cetaceans (Read & Murray 2000, Dierauf & Gulland 2001, Boer et al. 2012, Mannocci et al. 2012). There is evidence to indicate that some (but not all) bycaught penguins may present lesions consistent with entanglement in fishing nets, such as external and subcutaneous bruising, internal haemorrhaging and crushing injuries (Darby & Dawson 2000, Schlatter et al. 2009). In this study, only 1 of the 2 cases had external bruising, and these did not present the characteristic linear or cross-linear pattern consistent with fishing nets.

On the other hand, muscle congestion and rhabdomyolysis were observed in both drowned penguins, particularly in the neck. The presence of haemorrhages in skeletal muscles, particularly in the neck and trunk, are frequently observed in drowned humans as a result of agonal convulsions, overexertion and hypercontraction (Püschel et al. 1999, Salomez & Vincent 2004, Lunetta & Modell 2005). The rhabdomyolysis and haemorrhage in the neck and pectoral muscles of drowned penguins, without lesions in the adjacent subcutaneous tissue, may have resulted from exertional myopathy or agonal movements. Previous studies demonstrated the value of histopathology and histochemistry to differentiate muscular lesions from autolysis on stranded cetaceans (Sierra et al. 2014), and in this study we found that PTAH histochemistry was particularly helpful to confirm the occurrence of rhabdomyolysis (see Fig. 2c,d).

### Other organs and tissues

Although the most prominent lesions were seen in the respiratory tract, other tissues and organs also presented pathological changes that may be related to drowning. The increased vascular resistance caused by the presence of liquid in the airways might explain the dilation of the right cardiac ventricle. The increased vascular resistance in the lungs, combined with cardiac anoxia and acidosis, presumably led to decreased cardiac output (Salomez & Vin-

cent 2004, Lunetta & Modell 2005), which explains the generalized congestion that was seen in most tissues.

Although early experiments on the pathophysiology of drowning hypothesized that cardiac impairment due to hypernatremia, hyperchloremia and haemoconcentration played a key role in seawater drownings, this was dismissed in later studies and it is now considered that anoxia plays a much more significant role regardless of the osmotic characteristics of the drowning medium (Orlowski et al. 1989, Salomez & Vincent 2004, Lunetta & Modell 2005). Respiratory acidosis is also thought to be involved in cardiac failure in drowned humans (Lunetta & Modell 2005), however this probably plays a lesser role in penguins considering they are exceptionally tolerant to acidosis as an adaption to diving (Williams 1995).

#### **Implications for the diagnosis of drowning in beach-cast carcasses**

The marked distension of the lungs with foamy red fluid and marked oedema of the dorsal visceral pleura of drowned penguins are prominent lesions consistent with those described in cases of wet drowning in humans, and may aid the diagnosis of drowning in beach-cast carcasses of penguins (and possibly other seabirds). However, lung congestion and oedema are not specific findings and may also occur in other pathological processes unrelated to drowning (see Schmidt et al. 2003). Interestingly, the air sacs of the penguins in this study contained remarkably small quantities of liquid, suggesting that absence of water in the air sacs might not be a reliable sign to exclude drowning. External or subcutaneous bruising and hematomas may be a strong indication of bycatch, especially if linear or cross-linear patterns consistent with fishing nets are present (Darby & Dawson 2000, Schlatter et al. 2009); however, these lesions might not always be discernible and their absence is not sufficient to exclude the possibility of entanglement in fishing nets. On the other hand, we found that overexertion muscular lesions, particularly of the neck, may provide additional clues to support the diagnosis of drowning in penguins.

While these lesions can provide circumstantial evidence to corroborate the diagnosis of drowning in beach-cast carcasses, none of them may be considered unequivocal signs as to whether or not entanglement and drowning occurred. Additionally, it is worth considering that infections and diseases oc-

curing concurrently with drowning might confuse the interpretation of the pathological findings; for instance, the cases herein examined presented a number of secondary findings (granulocytic pneumonia, lymphoplasmacytic hepatitis, splenic lymphoid hyperplasia, etc.) that are thought to be unrelated to drowning and instead probably reflect the health challenges inherent to the first-year migration of a Magellanic penguin (see Table A1 in the Appendix). Furthermore, these lesions might be masked by autolysis/putrefaction in beach-cast carcasses or by freezing artefacts if carcasses have to be temporarily frozen due to field logistics. If the carcasses have remained in the surf zone for an extended period of time, post-mortem penetration of water and sand in the respiratory system could be an additional confounding factor.

An alternative approach to the diagnosis of wet drowning is the detection of exogenous substances or microorganisms (especially diatoms and bacterioplankton) in the tissues and blood stream (Lunetta & Modell 2005, Uchiyama et al. 2012). However, because agonal aspiration of seawater could occur in birds dying at sea from unrelated illnesses, caution must be exercised in determining whether or not a drowned bird was bycaught. In this context, the diagnosis of entanglement and drowning will benefit from considering the broader circumstances in which the deaths occurred and the type of fisheries active in the region, testimony from the local community and fishermen, and the body condition and presence of fish in the stomach of the deceased birds (Simeone et al. 1999, Hocken 2002, Schlatter et al. 2009).

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## Appendix

Table A1. Histopathological lesions thought to be unrelated to drowning in bycaught Magellanic penguins *Spheniscus magellanicus*. Superscript letters indicate the case(s) (A or B) in which lesions were recorded

Organ/tissue	Histopathological findings
Lungs	Pneumonia, granulocytic, interstitial, diffuse, mild to moderate <sup>A,B</sup>
Cerebrum	Gliosis, diffuse, mild <sup>B</sup> Satelitosis, diffuse, mild <sup>B</sup>
Tongue	Glossitis, granulomatous, focal, with giant cells <sup>A</sup>
Oesophagus	Esophagitis, lymphoplasmacytic, diffuse, mild to moderate <sup>A</sup> Lymphocytolysis in the gut-associated lymphoid tissue, mild to moderate <sup>A</sup>
Stomach	Ventriculitis, predominantly mononuclear, multifocal to coalescing, mild, with occasional granulocytes <sup>A</sup>
Small intestine	Gut-associated lymphoid tissue, lymphocytolysis, mild <sup>A</sup>
Caecum	Typhlitis, granulomatous, focal, mild <sup>A</sup>
Liver	Hepatitis, lymphoplasmacytic, periportal, mild <sup>A</sup> Hepatitis, mixed, perivascular, moderate <sup>B</sup> Steatosis, diffuse, mild <sup>A</sup> Cellular oedema, diffuse, mild <sup>A</sup> Hemosiderosis, multifocal, mild <sup>B</sup> Bile duct hyperplasia, multifocal, mild <sup>A</sup>
Spleen	Histiocytic hyperplasia, diffuse, moderate to severe <sup>A</sup> Lymphoid hyperplasia, diffuse, moderate <sup>A</sup> Structural disorganization and obliteration of red pulp ('starry sky' pattern) <sup>A</sup>
Bursa	Lymphocytolysis, diffuse, mild <sup>A</sup>
Oviduct	Salpingitis, mixed, diffuse, mild, with occasional granulocytes in the <i>lamina propria</i> <sup>A</sup>
Oral mucosa	Ulcerative stomatitis, granulocytic, focal, mild <sup>A,B</sup>