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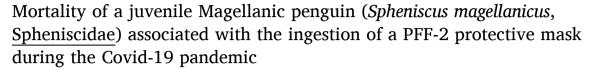
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ABSTRACT

We report the discovery of a dead Magellanic penguin (*Spheniscus magellanicus*) found on Juquehy Beach (23° 46′S 45° 44′W), municipality of São Sebastião, Brazil, on September 9th 2020. Following necropsy, we noted the presence of an adult size PFF-2 protective mask within the stomach of the penguin which we inferred as the cause of death. As far as we are aware, this is the first recorded instance of marine animal mortality by protective face mask ingestion. Whilst concerns have been raised relating microplastic contamination in marine environments from Covid-19 related waste, there has been relatively less attention paid to the potential risk of macroscale contaminants, such as protective face coverings. We suggest that Covid-19 related macro contaminants be considered in coastal marine risk assessments and urge further research on this topic.

The disposal of solid waste of anthropogenic origin in marine environments causes several environmental, socio-economic, aesthetic, cultural and safety impacts (Abt Associates, 2019; Leggett et al., 2014, Arthur et al., 2009). However, many countries have only recently started to draw attention and interact with this problem (McNicholas and Cotton, 2019; Williams and Rangel-Buitrago, 2019; UNEP, 2005; Derraik, 2002). This is despite several instances of entanglement, ingestion and suffocation of marine animals mainly related to plastic waste but also encompassing other litter (e.g. Villarrubia-Gómez et al., 2018; Rochman et al., 2016; Gall and Thompson, 2015; Kühn et al., 2015). Over the past 20 years, these impacts have increased significantly (e.g Galgani, 2014) and the number of marine species known to have been affected by solid waste has increased from 247 (Laist, 1987) to 680 (Gall and Thompson, 2015) during this period. The problem of marine litter persists and continues to increase in the world, due to the deficiency in the implementation and application of existing regulations and standards, whether international, regional or national, which if applied correctly could improve the situation. Marine waste is part of the broader problem of waste management, which has become a major public and environmental health concern in many countries (UNEP, 2021; Hahladakis,

2020)

In developing regions most of the impacts of marine litter on marine species involve entanglement in fishing equipment and ingestion of solid waste (Wilcox et al., 2015). The IUCN Red List highlights that at least 17% of species affected by entanglement and waste ingestion were listed as 'threatened' or 'near threatened'. Therefore, where marine litter combines with other anthropogenic stressors, these impacts can substantially affect populations, trophic interactions and assemblages (Gall and Thompson, 2015).

Since the appearance of the Sars-CoV-2 (Covid-19) coronavirus pandemic, in December 2019 (Nicola et al., 2020), the production of personal protective equipment (PPE) such as face masks and latex gloves has increased substantially, as has the incorrect environmental disposal of these items (e.g Canning-Clode et al., 2020). Historically, the production of surgical masks has been directed mainly towards the protection of health professionals, who receive the necessary guidelines for their proper use and disposal (Canning-Clode et al., 2020). In Brazil and worldwide, however, the general public was encouraged to wear protective or surgical masks during the recent global outbreak of Covid-19 (WHO, 2020), which led to an increase in their irregular disposal (e.g

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Canning-Clode et al., 2020). The consequences of the pandemic for marine life were initially considered to be predominantly related to the entry of microplastics into the marine system (Aragaw, 2020; Fadare and Okoffo, 2020). However, it has been estimated that during the pandemic period more than 200 million protective masks were being produced in China per day (Aragaw, 2020), which is likely a gross underestimate, since it considers only surgical masks. Prata et al. (2020) estimate the global use of protective masks by medical professionals at up to 130 billion per month. Almost inevitably the widespread use of protective masks, by the general population, has led to an increase in Covid-19 related waste in both land and water environments, indicating that the pandemic has, in some respects increased, not decreased, pollution in the global environment (e.g Fadare and Okoffo, 2020). This is in direct contrast to previous findings of reductions in global environmental pollution (Muhammad et al., 2020). Here, we present our results on the growth in the irregular disposal of face masks at the beaches monitored by the Argonauta Institute during the pandemic. We document the case of lethal interaction between a Magellanic Penguin and residue related to Covid-19 at the Southeastern coast of Brazil.

On the North coast of the State of São Paulo, the Argonauta Institute for Coastal and Marine Conservation, a non-governmental organization founded and linked to the Ubatuba Aquarium, has as its mission in conservation of the Environment, in particular the conservation of coastal and marine ecosystems. The organization supports and develops projects for research, rescue and rehabilitation of marine animals, environmental education and through the assessment of anthropogenic solid waste in coastal and marine environments. The Santos Basin Beach Monitoring Project (PMP-BS) is an example of these projects and was developed to meet the condition of the federal environmental licensing of Petrobras' activities in the production and disposal of oil and natural gas in the Santos Basin, conducted by IBAMA – Instituto Brasileiro do

Meio Ambiente e dos Recursos Naturais Renováveis. The project aims to assess the possible impacts of oil production and disposal on birds, turtles and marine mammals. It does this by monitoring the beaches and providing veterinary care to live animals and performing necropsy of the animals which are found deceased. The Institute monitors the occurrence of both live and deceased marine animals that are stranded on 235 beaches in the North coastal region of the State of São Paulo (PETRO-BRAS, 2014, 2019). During these monitoring activities, several teams also observe and record the anthropogenic waste discarded in an irregular way on the region's beaches.

All rescued animals are sent to the laboratories of the PMP-BS of the Argonauta Institute, at either the São Sebastião Stabilization Unit or the Ubatuba Rehabilitation and Depetrolization Center, where the live animals undergo treatment and rehabilitation and whenever possible are released into the natural environment. Those deceased animals with viable carcasses, i.e., with initial or moderate decomposition, undergo necropsy and evaluation that seeks to identify the cause of death.

Access to beaches along the North coast of the State of São Paulo, as in many other places worldwide, was restricted in March 2020 as a preventive measure to mitigate the Covid-19 pandemic (UBATUBA, 2020a; SÃO SEBASTIÃO, 2020a). Over time however, there was a gradual opening of beaches and the population returned to using the coast for touristic activities (UBATUBA, 2020b; SÃO SEBASTIÃO, 2020b). Consequently, individual protective masks have become an item often found in the strip of sand along 54 coastal beaches. In the period from April to October 2020 we registered 178 face masks at beaches in all municipalities. The most common occurrences were in the region of Caraguatatuba, representing 49% of the total (87/178). Of these, most occurred at Massaguaçu Beach (23° 34′S, 45° 19′W) where 24% (43/178) of all the masks recovered were found. In São Sebastião, the proportion of found masks was 21% (37/178), followed by Ubatuba,

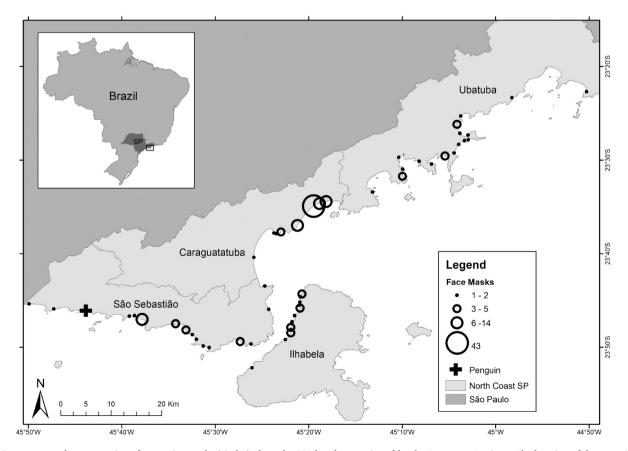


Fig. 1. Occurrence and concentration of protective masks (circles) along the 235 beaches monitored by the Argonauta Institute. The location of the penguin found deceased with the mask inside the stomach is given as a cross.

16% (29/178), and Ilhabela with 14% (25/178) (Fig. 1).

As the beaches opened and attracted greater numbers of visitors the disposal of masks along the beaches also increased significantly (Fig. 2). The occurrence of disposed masks peaked in the months of July and September which was also associated with the greatest number of non-resident visitors to the coast during national holidays (Fig. 2). Manufactured cloth masks were the most numerous, making up 60% (104/178) of those total masks found. We categorized the other type of masks as disposable which could be both surgical and/or protective.

During daily beach monitoring, between January and October 2020, we registered 613 stranded penguins, and of these, 347 were subject to a detailed assessment by necropsy, in which we identified interaction with solid waste in 17.6% (61/347) of the animals. Among all the solid waste registered this was the first time we had discovered a protective face mask.

On September 9, 2020, we were notified of the stranding of a deceased individual on the beach of Juquehy (23° 46′S 45° 44′W), municipality of São Sebastião. The animal was registered, collected by the field team and subsequently sent for necropsy at the São Sebastião Stabilization Unit. The specimen was a juvenile female individual, 58 cm long and 2085 g in weight. At the time of necropsy, we observed cadaverous changes in swelling, hypostasis and foul odor, and hence classified the individual as in a moderate stage of decomposition.

In the external exam, we noted the cachectic body condition score 1 (Vanstreels et al., 2012) with the pronation of the sternal keel and in the peripheral region of the cloaca we observed some greenish excreta with characteristics of biliverdin. In the internal examination, we observed changes in the skeletal muscle system related to generalized atrophy in the muscles of the pectoral region. Upon accessing the celomatic cavity we observed caseous plaques in the interclavicular and cranial thoracic air sacs, suggestive of infectious aerosaculitis. In the analysis of the digestive system, we noticed that the syntopy and the silhouette of the stomach showed changes and, after exploring the organ, we found traces of food content associated with a solid residue of intensely firm consistency, which we confirmed to be a full PFF-2 protective mask (3 M brand) (Fig. 3), with sufficient size and structure that made it impossible to pass through the transition lumen from the stomach to the small intestine. For this reason, we attribute the ingestion of the mask as the main cause of death.

Biliverdin is responsible for the greenish color of the urate that appears in cases of prolonged fasting and, when the body does not use it in

the digestion process, its excretion occurs, as observed in the individual's cloacal portion (e.g Capitelli and Crosta, 2013). Prolonged fasting predisposes the intake of options other than the preferred diet, and according to its degree of severity, accidental ingestion of anthropogenic solid waste. The condition of prolonged anorexia exerts a severe catabolism on the organism, one of the consequences of which is the autophagy of muscle tissue (e.g Capitelli and Crosta, 2013). Such changes in metabolic syndrome and cachexia are observed in free-living penguins. The severity of cases is related to migration, adverse climatic conditions and inadequate nutrition. Inadequate nutrition may relate to the inability of hunting in young animals or to the deficit in the availability of resources in large ranges and/or subsequent unhealthy ingestion of anthropogenic solid waste (Boersma et al., 2020).

The Magellanic Penguin is a migratory seabird found along the South coast of South America and which nests in Argentina, Chile and the Malvinas Islands (Sick, 2001; Boersma et al., 2013). Both IUCN and the Red Book of Threatened Species in Brazil classify the species as Almost Threatened (NT) and point to a sharp population decline (BirdLife International, 2020; ICMBIO, 2018). Gandini et al. (1996), Boersma (2008) and Gownaris and Boersma (2019) identified that the main sources of mortality in this species are related to human activities, such as oil exploration and production, reduction of the source of prey due to commercial fishing, disordered coastal development and effects of human-induced climate change (BirdLife International, 2020).

There is a high incidence of Magellanic Penguins along the Brazilian coast, mainly in Rio Grande do Sul and Santa Catarina (Sick, 2001). Mäder et al. (2010) estimate that 19,500 penguins can strand dead on the south coast of Brazil each year, and of these, nearly 7000 die due to anthropic and climatic factors. The interaction of marine animals with solid waste of anthropogenic origin is known by researchers and represents one of the greatest impacts that marine and coastal species face, both in Brazil and the rest of the world (Stelmack et al., 2018; Silva-Cavalcanti et al., 2017; Gall and Thompson, 2015; Mascarenhas et al., 2008; Derraik, 2002). Ewbank et al. (2016) identified foreign bodies in the gastric content of 24% (12/50) of penguins analyzed between May and July 2015 off the coast of Praia Grande, in the State of São Paulo, including remains of plastics, rubbers, wire threads and ropes. Almost all of the mortalities were in juvenile females, making up 82% (41/50) of the total stranded animals. Based on our data, in the period from January 2016 to October 2020, 899 penguins were stranded on the stretch of beaches that we monitor and 470 of these were necropsied. Of

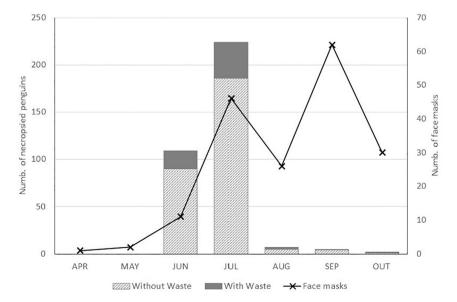


Fig. 2. - Number of necropsied penguins in 2020 (left axis) that exhibited evidence of being either with and without interaction with anthropogenic solid waste. The black line and crosses indicate the number of protective face masks (right axis) found on the monitored beaches.



Fig. 3. Penguin found dead in São Sebastião (A). Mask inside the open stomach during the necropsy (B). Detailed face protective mask (C).

these, 98.5% (463/470) were juveniles, with 74.5% (345/470) females and 20.3% (94/470) males. From the same group analyzed in this article, 15.3% (71/470) showed interaction with anthropogenic solid waste, with a proportion of 81.7% (58/71) of females, to 16.3% (9/71) of males (Argonauta Institute, unpublished data). In that period, it was only this one penguin, discussed in this study, that displayed evidence of interaction with a protective face mask.

The case we report here is perhaps the first scientific record of mortality of any marine species directly linked to the ingestion of a protective mask. However, if patterns globally are as we have observed along the Brazilian coast, the irregular disposal of protective masks will lead to a greater number marine animal fatalities related to anthropogenic waste of a Covid-19 nature. It is then essential that both the amount of litter and the litter's interaction with marine animals is correctly recorded. In terms of the Magellanic Penguin it is expected that more occurrences of mortality related to Covid-19 waste will be observed along both the Atlantic and Pacific coasts as research and data dissemination related to this topic increases. Information on ingestion of solid waste by this species has increased significantly in recent years (Brandão et al., 2011; Azevedo and Schiller, 1991; Mäder et al., 2010; Pinto et al., 2007; Tourinho et al., 2010) but more data is required. It is important to note that the ingestion of debris by penguins can also cause prolonged sub-lethal effects, such as reproductive failure and delayed ovulation, which can contribute to population decline of the species, since on average around 36% of Magellanic penguins arriving in Brazil display evidence of being affected by the ingestion of solid waste (Brandão et al., 2011).

It is essential that the occurrence of Covid-19 related waste is properly monitored by governmental and non-governmental institutions. As a result of COVID-19, more waste is being produced than normal, including masks, gloves, aprons and other personal protective equipment, as well as increased single use plastics (Canning-Clode et al., 2020). These residues, when not properly managed, may be subject to uncontrolled disposal, leading to public health risks. It is well known that such waste can also reach other water sources and increase river and marine pollution (UNEP, 2020). There are attempts to provide

guidance material, as released by UNEP, and we further recommend the dissemination of public practice guidelines for the correct disposal of protective face masks and other Covid-19-related waste generated during the pandemic. We also recommend that coastal risk assessments be reviewed in the light of the waste generated during the pandemic.

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CRediT authorship contribution statement

Hugo Gallo Neto: Conceptualization, Methodology, Writing – review & editing. Carla Gomes Bantel: Conceptualization, Methodology, Writing – review & editing. John Browning: Writing – original draft, Writing – review & editing. Natalia Della Fina: Methodology, Writing – review & editing. Tami Albuquerque Ballabio: Methodology, Writing – review & editing. Fabio Teles de Santana: Methodology, Writing – review & editing. Mariana de Karam e Britto: Methodology, Writing – review & editing. Carla Beatriz Barbosa: Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest with respect to the preparation and publication of the manuscript 'Mortality of a juvenile Magellanic Penguin (*Spheniscus magellanicus*, Spheniscidae) associated with the ingestion of a PFF-2 protective mask during the Covid-19 pandemic'.

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