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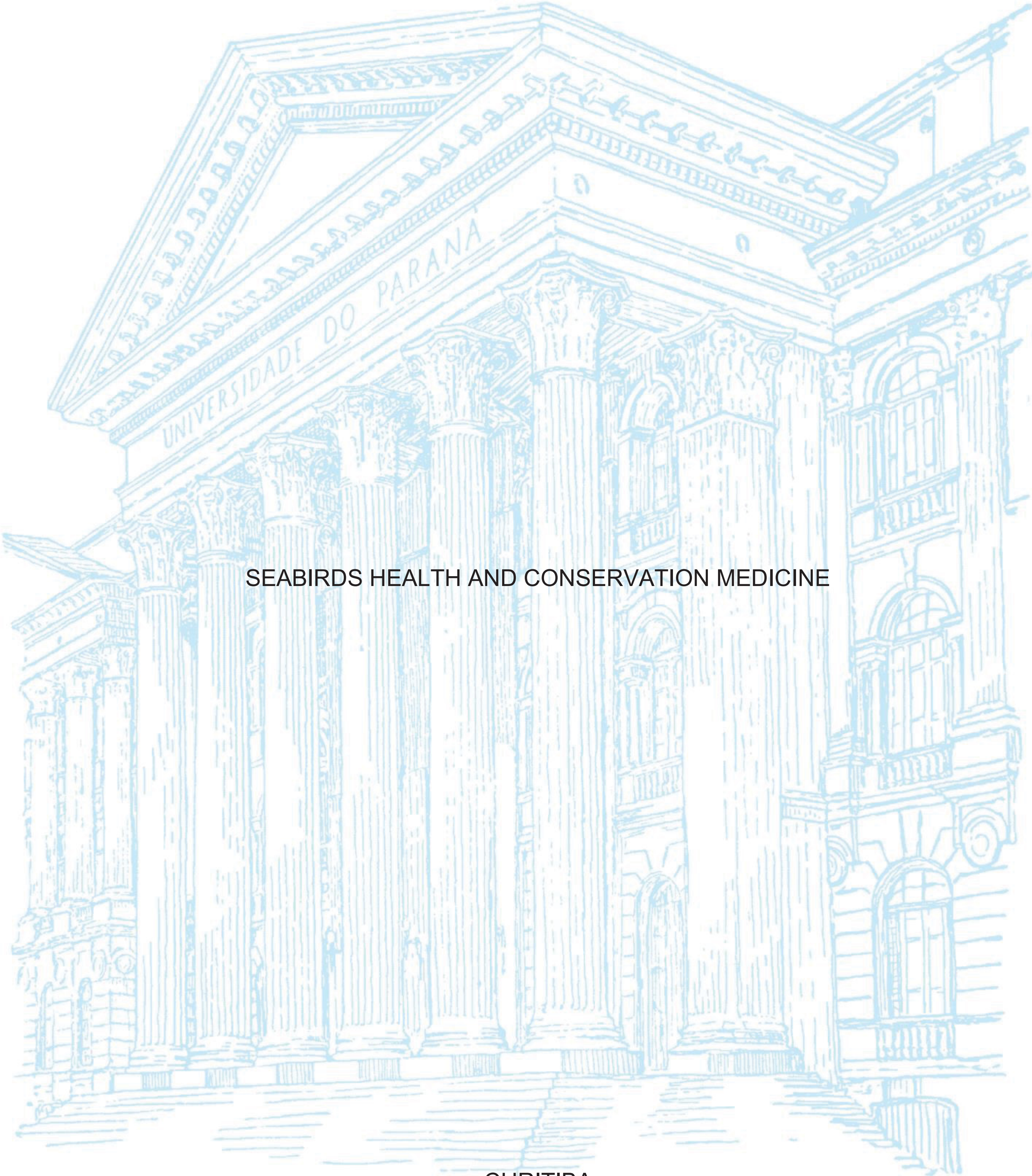
RAFAELLA MARTINI



SEABIRDS HEALTH AND CONSERVATION MEDICINE

CURITIBA
2022

RAFAELLA MARTINI

SEABIRDS HEALTH AND CONSERVATION MEDICINE

Dissertação apresentada ao curso de Pós-Graduação em Ciências Veterinárias. Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências Veterinárias.

Orientador: Prof. Dr. Rogério Ribas Lange

Coorientador: Prof. Dr. Paulo Rogerio Mangini

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **RAFAELLA MARTINI** intitulada: **Seabirds health and conservation medicine**, sob orientação do Prof. Dr. ROGERIO RIBAS LANGE, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
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ROGERIO RIBAS LANGE
Presidente da Banca Examinadora

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Aos que sempre me apoiaram e estavam junto, neste ou em outros universos possíveis e imagináveis.

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“Moça eu não vou precisar
Ler na sua mão
Pra saber que você não vai voltar
Pra vida maluca
Das pessoas, do mundo
Das formigas tentando
Se esconder da chuva”

Almir Sater

RESUMO

O monitoramento sanitário de aves marinhas é essencial para a avaliação da qualidade ambiental e entendimento de doenças importantes para as populações oceânicas e costeiras. A ação antrópica pode alterar os ecossistemas e afetar a saúde e dinâmica das populações animais. Com o desenvolvimento da pesquisa nessa área ao longo dos anos, mais estudos se dedicam a entender essa dinâmica a partir da "perspectiva das aves marinhas". O presente trabalho teve como objetivos fornecer um embasamento teórico sobre a saúde de aves marinhas no contexto da medicina da conservação em território brasileiro, bem como apresentar um estudo de monitoramento de aves marinhas e estuarinas realizado na Região do Banco de Abrolhos. Para tanto, foi elaborada uma revisão de literatura sobre as principais ameaças às aves marinhas no Brasil. A pesquisa foi realizada em diferentes bases de dados com as palavras-chave "seabirds" AND "conservation" AND "Brazil" e foram incluídos artigos com menção à saúde, doenças e conservação de aves marinhas no Brasil no título e resumo. Ao todo, foram selecionados 93 artigos relevantes que abordavam principalmente a indústria pesqueira, a poluição e a contaminação ambiental, mudanças climáticas e invasões biológicas entre as principais ameaças à sobrevivência de aves marinhas e estuarinas. Os impactos reais dessas ameaças à conservação desse grupo é uma lacuna do conhecimento, apesar do aumento de estudos dedicados ao entendimento da imunidade das aves, sua condição de saúde e das cadeias epidemiológicas a elas associadas. Dessa maneira, além de detalhar a problemática a partir da extensa revisão, foi realizado um estudo de monitoramento sanitário de aves marinhas que pode ser utilizado como referência para futuras pesquisas no contexto da medicina da conservação. Com o objetivo de verificar se era possível avaliar a saúde dos indivíduos utilizando diferentes parâmetros, foram capturadas 64 aves na Região do Banco de Abrolhos, sendo 33 exemplares de aves marinhas da espécie *Fregata magnificens* e 31 exemplares de aves estuarinas, compreendendo 14 *Nytanassa violacea* e 17 *Egretta caerulea*. Após a captura, as aves foram submetidas a exame físico, coleta de sangue e penas e *swab* cloacal. Além da análise dos dados do exame físico, as amostras foram encaminhadas para detecção de hidrocarbonetos policíclicos aromáticos, metais pesados e microorganismos. A maioria dos indivíduos foi considerada saudável, embora alguns indivíduos apresentassem alterações do estado geral. Apesar disso, foi possível considerar que as aves estariam aptas à sobrevivência a curto e médio prazo. Este estudo foi o primeiro a associar diferentes parâmetros na avaliação de saúde de aves marinhas e fornece dados de referência para estudos futuros na região e com as espécies abordadas, além de constituir uma base conceitual para novas pesquisas que procuram integrar a saúde das espécies e a saúde ecológica.

Palavras-chave: aves marinhas; aves estuarinas; poluição marinha, ameaças antropogênicas; parâmetros de saúde; medicina da conservação.

ABSTRACT

The monitoring of seabirds health status is essential for the evaluation of environmental quality and the understanding of important diseases for oceanic and coastal populations. Anthropogenic disorders can alter ecosystems and affect the health and dynamics of animal populations. With the development of research in this area over the years, more studies are dedicated to understanding these dynamics from the "seabird perspective". The present work aimed to provide a theoretical background on seabird health in the context of conservation medicine in Brazil, as well as to present a study on seabirds and estuarine birds performed in the Abrolhos Bank Region. For this purpose, a literature review on the main threats to seabird in Brazil was developed. The search was carried out in different databases with the keywords "seabirds" AND "conservation" AND "Brazil" and included articles with mention of health, diseases and conservation of seabirds in Brazil in the title and abstract. In all, 93 relevant articles were selected that addressed the fishing industry, environmental pollution and contamination, climate change, and biological invasions among the main threats to seabird and estuarine bird survival. The current impacts of these threats to the conservation of this group are a knowledge gap, despite the increase in studies devoted to understanding bird immunity, their health status, and the epidemiological chains associated with them. Thus, in addition to detailing the problem from the extensive review, a seabirds health monitoring study was carried out and can be used as a reference for future research in the context of conservation medicine. With the aim to to evaluate the health of individuals using different parameters, 64 birds were captured in the Abrolhos Bank Region, being 33 seabirds of the species *Fregata magnificens* and 31 estuarine birds, 14 *Nytanassa violacea* and 17 *Egretta caerulea*. After capture, physical examination, blood and feather collection, and cloacal swab were performed. In addition to the analysis of the physical examination data, the samples were forwarded to polycyclic aromatic hydrocarbons, heavy metals, and microorganisms detection. The majority of the individuals were considered healthy, although some individuals presented altered general condition. Despite this, it was possible to consider that the birds would be able to survive in the short to medium term. This study was the first to associate different parameters in the evaluation of seabirds health and provides reference data for future studies in the region and with the species addressed, as well as a conceptual basis for further research that aims to integrate species health and ecological health.

Keywords: seabirds; estuarine birds; marine pollution; anthropogenic threats; health parameters; conservation medicine.

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1 INTRODUÇÃO GERAL

O Brasil é mundialmente conhecido por sua vasta biodiversidade, abrigando em torno de 10% de todas as espécies conhecidas de seres vivos (LEWINSOHN; PRADO, 2005). A compreensão do estado de conservação das espécies do país é essencial para o planejamento de ações que contribuam para a proteção dos ecossistemas. A conservação da biodiversidade requer o entendimento da biologia e ecologia, por exemplo, aspectos intrínsecos de cada espécie, mas também requer uma análise e compreensão das principais ameaças às quais estão submetidas. Acessar as condições de saúde de determinado ecossistema envolve determinar as relações entre (a) as mudanças no habitat e uso do ambiente, (b) a emergência e reemergência de patógenos e (c) a manutenção da biodiversidade e das funções do ecossistema. O campo de estudo que apresenta essa abordagem transdisciplinar é a medicina da conservação, que desde o princípio propõe que a saúde ecológica depende da complexa interação entre saúde dos seres humanos, dos animais e do ambiente (AGUIRRE, 2002; AGUIRRE; OSTFELD; DASZAK, 2012; MANGINI; SILVA, 2006).

A saúde pode ser compreendida como um equilíbrio dinâmico na fisiologia de um indivíduo e pode ser avaliada entre outros fatores pela condição clínica, por parâmetros biológicos, sucesso reprodutivo e sobrevivência a longo prazo (OWEN; HAWLEY; HUYVAERT, 2021). A perda da homeostase com danos ao indivíduo é o conceito estabelecido de doença, que pode ser de causa infecciosa ou não (OWEN; ADELMAN; HENSCHEN, 2021). Devido à enorme diversidade e ocupação de diferentes nichos ecológicos, a interação entre as aves e possíveis patógenos é um desafio para a conservação desse grupo e para a compreensão dos riscos à saúde pública.

As aves marinhas são definidas por sua relação com o ambiente marinho, o que inclui ilhas, regiões costeiras, estuários, e áreas úmidas litorâneas e possuem características morfofisiológicas específicas que permitem sua adaptação ao meio ambiente aquático, terrestre e aéreo de maneira equivalente (SCHREIBER; BURGER, 2002). Também são reconhecidas por sua característica de deslocamento a longas distâncias e essa capacidade possibilita a ocupação em diversos ambientes naturais. Tal particularidade é relevante no que diz respeito às cadeias epidemiológicas de patógenos de interesse para a saúde pública e conservação. O litoral brasileiro abriga uma grande rota de aves migratórias e os ambientes marinhos e costeiros do país sofrem diferentes tipos e intensidades de impactos (SERAFINI; LUGARINI, 2014).

Durante anos, as espécies de aves marinhas foram ameaçadas pela caça, coleta de ovos e ninhos, degradação ambiental e introdução de espécies exóticas predadoras, quando suas áreas de ocorrência ainda não eram protegidas por lei (ANTAS, 1991; MANCINI; SERAFINI; BUGONI, 2016; SCHULZ-NETO, 2004). Ainda atualmente, as principais ameaças às populações de aves marinhas são a perda de habitat, exploração econômica, sobrepesca e outros impactos antrópicos (BURGER; GOCHFELD, 2001). O aparecimento e a invasão de animais exóticos, como ratos, gatos, porcos, ovelhas, cabras e lagartos, também são uma ameaça importante às colônias de aves marinhas. As espécies invasoras ameaçam a biodiversidade local ao destruir o ambiente de nidificação ou se alimentar de ovos, filhotes e indivíduos adultos das aves, provocando redução populacional desses animais e até mesmo sua extinção (SERAFINI; LUGARINI, 2014).

Outros distúrbios ao ambiente como vazamentos de óleo, poluição da água e mudanças climáticas afetam a disponibilidade de alimento e, consequentemente, colocam em risco a sobrevivência das espécies que deste dependem. Ainda, o aumento crescente da população humana costeira causa perturbações ao ecossistema e aproxima os seres humanos dos animais, aumentando o risco de exposição a patógenos de importância para a saúde pública (RAJPAR et al., 2018). Dessa forma, o monitoramento sanitário de aves marinhas é essencial para a avaliação da qualidade ambiental e entendimento de doenças importantes para a saúde pública. No Brasil, há poucos estudos epidemiológicos relacionados à ornitofauna oceânica e costeira e, quando eles existem, estão restritos a alguns poucos grupos de aves (SERAFINI; LUGARINI, 2014). Entretanto, nos últimos 10 anos, há mais oportunidades de pesquisa, principalmente após o advento do licenciamento ambiental para atividades como exploração de petróleo, a exemplo dos Projetos de Monitoramento de Fauna (PMP) na costa brasileira (ICMBIO, 2019).

Nesse contexto, o presente estudo propõe uma abordagem ampla no entendimento do papel das aves marinhas como sentinelas do ambiente, no que se refere tanto à saúde das espécies a serem estudadas, como também na relação dessas espécies com o seu ambiente natural, considerando os impactos causados pelas consequências das ações antrópicas.

2 OBJETIVOS GERAIS E ESPECÍFICOS

OBJETIVO GERAL

Reunir informações acerca do estado atual de conhecimento sobre a saúde de aves marinhas no Brasil, estabelecendo direções para futuras pesquisas que visem a conservação desse grupo.

OBJETIVOS ESPECÍFICOS

a) Trazer novas informações a respeito da saúde de aves marinhas no Brasil e assim contribuir com dados de referência para o conhecimento das espécies em questão.

b) Fornecer informações epidemiológicas relevantes para a saúde pública em âmbito local e nacional, por meio da pesquisa e vigilância ativa de doenças de interesse.

c) Contribuir com a conservação das espécies de aves marinhas e estuarinas estudadas em ambientes oceânicos.

3 REVIEW MANUSCRIPT

SEABIRDS HEALTH AND CONSERVATION MEDICINE

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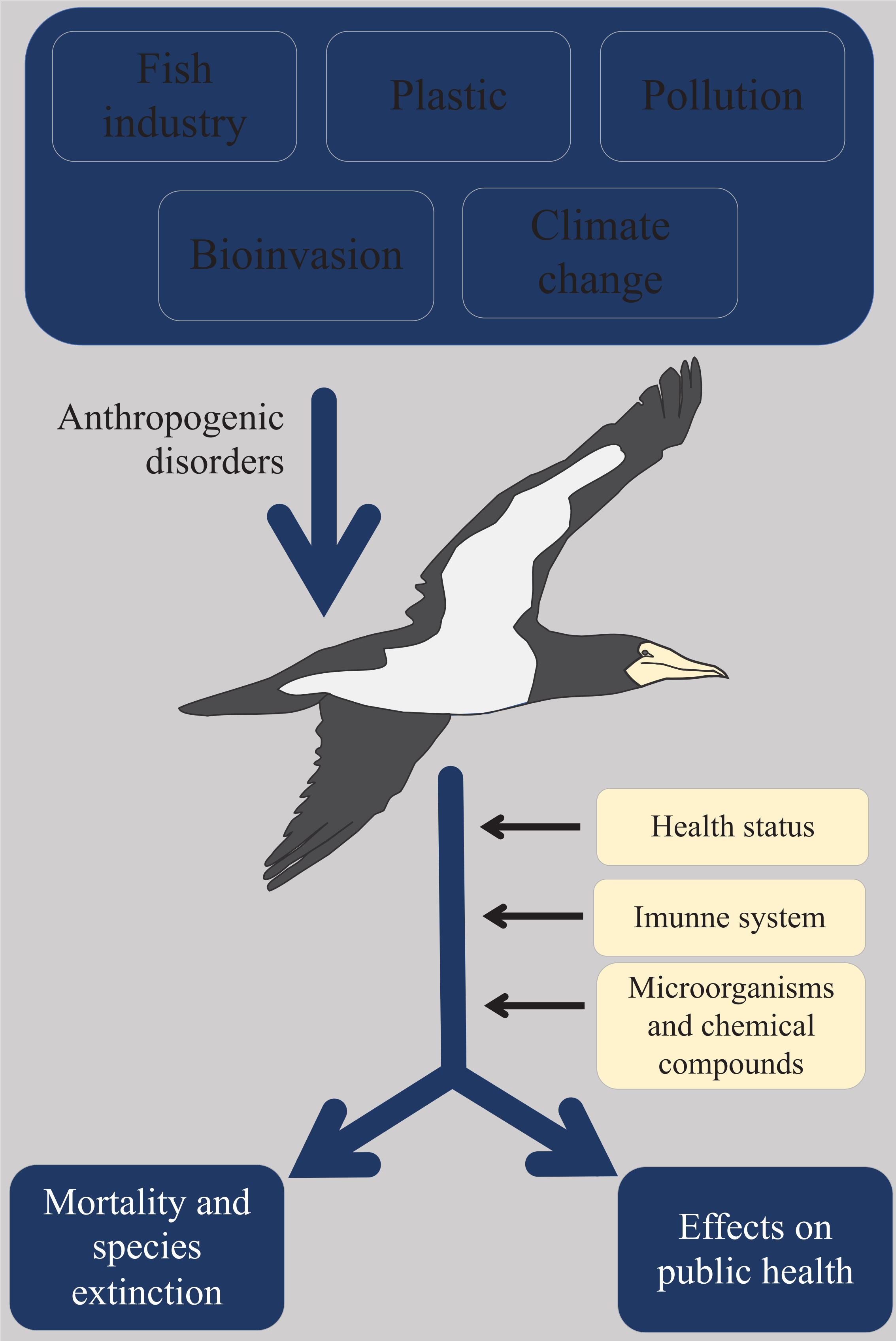
Abstract

Seabirds are considered good bioindicators for environmental issues, such as contamination, productivity, and health. Fish industry, climate change, bioinvasions, and pollution are the most reported threats to seabird populations. The aim of this review is to address those threats and discuss the health issues that impact seabirds in Brazil through the lens of conservation medicine. In total, 93 records were selected, regarding the previously mentioned threats, as well as the interaction between seabirds and etiologic agents and chemical compounds. The most studied species was the *Spheniscus magellanicus* and most of the records were published in the last 10 years, which indicates that although the health status of this group is still poorly addressed in Brazil, the studies have been increasing and created a background for the development of new research on this topic. This manuscript also highlights the urgency in performing transdisciplinary studies to better understand the health risks for seabirds and human populations associated with marine ecosystems.

Keywords: marine birds; anthropogenic threats; Brazilian coast; health status; marine ecosystem.

GRAPHIC ABSTRACT

Figure 1. Graphic abstract of the article "Seabirds health and conservation medicine".



3.1. INTRODUCTION

The marine ecosystem is rich in biodiversity and is an important source of oxygen, water, and biomass (DE MOURA et al., 2012). Although it is not always perceived as such, this environment benefits humans in several ways, from supporting fishery to providing human welfare (AGUIRRE; OSTFELD; DASZAK, 2012). Therefore, the ocean's health is crucial for an ecological balance and ultimately to promote human health (DE MOURA et al., 2012). However, anthropogenic impacts have been threatening the quality of this ecosystem and its consequences may be devastating for the whole world (ANDERSEN, 1997).

Seabirds or marine birds depend on the marine environment directly or indirectly (SCHREIBER; BURGER, 2002). They can be found in coastal areas, wetlands, estuaries, islands, from land to ocean, frequently crossing ecological and political boundaries on a dynamic scale (SCHREIBER; BURGER, 2002; TROMBULAK; BALDWIN, 2010; RAJPAR et al., 2018). Seabirds are unique in their biology, behavior, and ecology (TROMBULAK; BALDWIN, 2010). Along with the transboundary nature, their dependence on marine habitats make them vulnerable to environmental changes (JODICE; SURYAN, 2010) and, therefore, good bioindicators of their ecosystem (JODICE; SURYAN, 2010; RAJPAR et al., 2018). Habitat loss and fragmentation, introduction of invasive species, hunting, collection of eggs, tourism, climate change, pollution, and commercial fishing are the major threats to seabirds conservation worldwide (BOERSMA; CLARK; HILLGARTH, 2002; DIAS et al., 2019).

Conservation medicine is a field developed around the 2000s to research and understand ecosystem health as the complex interaction among human, animal and vegetal health (AGUIRRE, 2002; AGUIRRE; OSTFELD; DASZAK, 2012). In this context, studies with seabirds that address this relationship are critical to better understand what should be done on behalf of species conservation, which ultimately reflects the ecosystem's balance and human health protection.

Seabirds naturally found in Brazil are comprised of six orders: Sphenisciformes (penguins), Procellariiformes (albatrosses and petrels), Suliformes (frigatebirds and boobies), Pelecaniformes (pelicans), Phaethontiformes (tropicbirds), and Charadriiformes (sandpipers, gulls, and noodies) (PACHECO et al., 2021). Despite the large coastal area of Brazil, few studies address seabirds' ecology in the country. Even more scarce are information about the health status of these populations and its impacts on ecological health (SERAFINI; LUGARINI, 2014). One important strategy that has been contributing to research on this topic is the environmental licensing requirement. This can be seen especially in areas of research and exploration of hydrocarbons, which created several Coastal Monitoring Projects along the coast. Reports from these projects contribute to research on stranded animals or carcasses, and support the stakeholders decisions on conservation issues (ICMBIO, 2019).

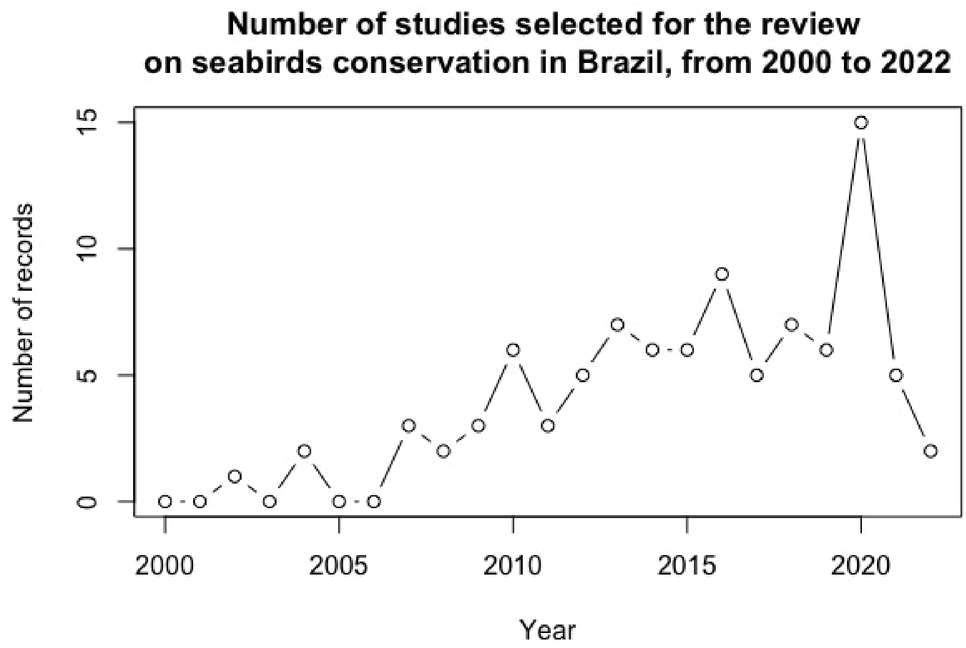
This review provides a comprehensive and extensive background of health issues, diseases, and threats to seabird populations in Brazil, based on a conservation medicine approach. Furthermore, our aim is to discuss knowledge gaps in this field and possible contributions to the future.

Literature review

The search for scientific publication was performed in *Google Scholar* and *Web of Science* as our main databases with the following keywords and boolean operators: "seabirds" AND "conservation" AND "brazil". The titles of peer-reviewed papers and relevant conference presentations, master's and PhD thesis published from 2000 onwards were screened with the aid of *Revtools* (RStudio) (WESTGATE, 2019). Mention of health and diseases of seabirds on the title, as well as location (studies in Brazil) were used as inclusion criteria. In total, 93 relevant records were selected and analyzed. From these, 72 were published in peer-reviewed journals, 10 in non-refereed journals or conference presentations, 5 master's thesis, 4 PhD thesis and 2 bachelor's thesis.

We also analyzed the evolution in time of records published in Brazil, and from 2010 onwards it is possible to observe an increasing trend in the numbers. This could be explained by a rising concern about seabirds conservation and by new opportunities from mitigation measures of environmental impacts, such as environmental license/permits.

Figure 2. Records selected for this review, showing their distribution in time. Each point indicates one year and the corresponding number of records. In: RStudio.



3.2. MAJOR THREATS TO SEABIRDS

Using the keywords in each study, a word cloud was generated to visualize the most common topics for the studies performed in Brazil with seabirds health. In a word cloud, the most frequent words are written at a larger size and in different colors.

Figure 3. Word cloud performed with the keywords of the papers selected, showing the most frequent topics, excluding seabird species. Here are displayed words found at least two times in the list of keywords. This word cloud was generated using Rstudio.

Overall, in the studies selected, the most common keywords addressed anthropogenic impacts on seabirds and mortality of this group. Plastic, pollution, and fish industry are an overwhelming concern to these species in Brazil and worldwide. The most common microorganisms studied are bacteria, a particular species of fungi, and parasites. References to diseases are not always addressed. Chemical contamination is also frequently reported. We selected, among the most relevant pointed threats, those that could affect population health status to the following discussion.

3.2.1. Plastic

The presence of plastic debris in the ocean has been well-documented after humanity started the intense consumption of plastic products (COZAR et al., 2014). This global behavior led to the accumulation of plastic even in the most remote places of the world (LEBRETON et al., 2018). The debris can be found in marine birds nests (BRENTANO et al., 2020) or can be ingested by fish, reptiles, mammals, and seabirds, possibly causing not only gastrointestinal obstruction, but also intoxication by the contaminants plastic may have (COZAR et al., 2014).

For a long time, scientists believed plastic pollution in the marine environment was represented mostly by microplastics - small particles originating from plastic objects fragmentation and photodegradation (COZAR et al., 2014). However, a recent study on the Pacific reported larger debris as the most common and remarkably estimated that at least 46% of the Great Pacific Garbage Patch (GPGP) consisted of fishing nets (LEBRETON et al., 2018), adding the responsibility of the fishing industry to the discussion. Other studies also support this finding and attribute to fishing equipment a major source of plastic in different regions, such as subarctic North Atlantic, Greenland, Norwegian, Barent and Kara Seas. Aquaculture, hydrocarbon exploration, ship traffic, and domestic use represent other sources (BERGMANN et al., 2022). In Brazil, there is a major gap in studies addressing plastic sources and pathways. However, isolated reports from different estuarine systems also indicate fishing activities and domestic sources as the main origins, with emphasis on illegal litter dumping and basic sewage system deficiency (LIMA et al., 2020).

In a study performed on the archipelago of Saint Peter and Saint Paul, located 1010 km off the Brazilian coast, Brentano et al. (2020) found anthropogenic items in 20,4% and 13,3% of *Sula leucogaster* nests, in 2015 and 2016, respectively.

Threadlike plastic were the most common items found in 2016. In the coastline of the state of Rio Grande do Sul (South Brazil), from 115 seabirds found dead on beaches and 78 caught in longline fisheries, 38,3% had plastic items in their digestive tract, mostly fragments, pellets and lines (COLABUONO et al., 2009). A similar study in the same state found that 40% of 35 seabirds encountered stranded had ingested debris, and 66% comprised the Procellariiformes (TOURINHO; IVAR DO SUL; FILLMANN, 2010). This order seems to be the most affected worldwide as well, probably due to their foraging behavior on the surface and their gastrointestinal tract anatomy that hinders regurgitation of foreign items (COLABUONO; TANIGUCHI; MONTONE, 2010; COLABUONO; VOOREN, 2007; PETRY; BENEMANN, 2017). A recent investigation in Eastern Brazil supports other reports by highlighting albatrosses, shearwaters, brown boobies and Magellanic penguins as the species that ingest plastic most frequently (n=126 carcasses of 19 different species) (VANSTREELS et al., 2021).

Petry & Benemann (2017) observed an increasing trend in debris ingestion by White-chinned Petrels (*Procellaria aequinoctialis*), although their size sample was small (n=114) in the three periods analyzed (1990, 1997-1998, 2007-2014). Di Beneditto & Siciliano (2017) also compared debris found in the stomachs of stranded *Spheniscus magellanicus* (collected from south-eastern Brazil) in 2000 and 2008. They observed an increase of more than 100% in debris ingestion over this period, being plastic the main item. This indicates a worsening trend in this scenario, if no measures are taken to reduce litter contamination in the ocean. They also inferred that the poor physical condition of the Magellanic penguins prevented diving and made them more vulnerable to ingestion of foreign items that likely occurred on surface waters (DI BENEDITTO; SICILIANO, 2017).

Plastic ingestion can be a direct cause of death to marine birds, but may also cause sub-lethal effects, such as lower food consumption or absorption, increasing time for growth and sexual maturation (TOURINHO; IVAR DO SUL; FILLMANN, 2010). Beside the physical effect, ingestion of plastic may have chemical effects on seabirds due to plastic contaminants added during its manufacture (plastic-additive), such as metal ions, phthalate, Bisphenol-A, flame retardants, and antibiotics, or plastic-adsorbed chemicals from seawater, such as Persistent Organic Pollutants (POPs) that include organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), for example (COZAR et al., 2014; LITHNER; LARSSON; DAVE, 2011; ROMAN et al., 2020; THUSHARI; SENEVIRATHNA, 2020). Despite the large number of studies on contaminants in seabirds in Brazil, only one has supported the evidence that plastic ingestion is another source of Persistent Organic Pollutants (POPs), by analyzing PCBs and OCPs in plastics ingested by eight species of Procellariiformes (COLABUONO; TANIGUCHI; MONTONE, 2010, p. 200). A discussion about contaminants is provided further in this review.

It is still unknown whether the ingestion of foreign items is a cause of mortality or a consequence of other injuries. Nevertheless, this cannot be measured unless researchers monitor the health status of the populations exposed. It was only in the last few years that researchers have tried to investigate plastic ingestion and its toxicity in live animals. The magnitude of plastic pollution in the ocean is a global threat and a major concern, its impact over seabird population status is hard to quantify and also hard to avoid, considering the nature of plastic origins and dispersion on the oceans.

3.2.2. Fishery

As previously mentioned, the fish industry is responsible for a relevant amount of the plastic found in the ocean (LEBRETON et al., 2018). Unfortunately, this is not the only impact the activity causes to the environment. Bycatch in fisheries is even more unsettling and seabirds are particularly threatened by activities such as netting and long-lining (BENEMANN et al., 2016; DIAS et al., 2019). Between 1997 and 1998, in South Brazil, Petry & Fonseca (2002) found 507 seabirds carcasses with signs of injuries by sharp objects and fishing nets. Magellanic penguins were the most impacted species, possibly due to their migration pattern and ecological niches, which make them interact more intensely with fishery. L. G. Cardoso et al. (2011) and Fogliarini et al., (2019) also confirmed that penguins are also susceptible to bycatch by bottom set or drift gillnet off southern Brazil and the latter showed a gender bias. According to this study, a female-biased bycatch was observed, and this feature could have a greater impact on population stability and future survival.

Although penguins are the most common victims, other seabird species are also threatened by interaction with fishery in several ways, and sex-bias is frequently observed (BUGONI; GRIFFITHS; FURNESS, 2011). From the seabirds perspective, the association with fishery can be an adaptive behavior, even though the outcome is mostly unfortunate. Albatrosses and petrels, for example, are mutilated, aggressively handled, and intentionally killed by fishermen that try to avoid bait depredation

(GIANUCA et al., 2020). In other situations, due to seabirds' boldness to get a fish under any circumstances, injuries such as mutilation and fractures in wings, feet, and mandible, are still observed, despite the tolerance or willingness to help displayed by some fishermen (SAZIMA; SAZIMA, 2008). Another study reported an unexpected negative effect of fishery discards and found dead seabirds with a species of shrimp stuck in their throat, a food item not always available to the birds in natural conditions (BENEMANN et al., 2016).

The real magnitude of this threat is still undocumented, since mortality due to fishery is underestimated and intentional harm to seabirds declines when observers are onboard (GIANUCA et al., 2020). Mitigation measures are mandatory to conservation success. The enforcement of these measures, as well as educational activities to fishermen or incentives are not enough. In addition, it should also be implemented means of monitoring illegal fishing practices that endanger the future of the oceans (GIANUCA et al., 2020; NEVES et al., 2007), provision of security to observers onboard, and reliable sustainability certificates.

Nevertheless, fishery discards can also model the shape, size and health of several seabirds‘ populations (CARNIEL; KRUL, 2012; MANGINI, 2010; SHERLEY et al., 2020). Mangini (2010) has highlighted the direct relationship between availability of fishery discards and seabirds health, observed from the register of a seasonality of seabirds rescued in the State of Paraná coast in relation to the shrimp production. From this point of view, fishery created a completely new environmental niche that seems to benefit some species, but also make them dependent on human actions. This aspect of fishery is still poorly addressed and understood.

3.2.3. Climate change

Climate is changing globally due to increased concentration of carbon dioxide (CO2) and greenhouse gasses in the atmosphere (BAHRI; BARANGE; MOUSTAHFID, 2018). This fact is accelerated each year by human activities, such as burning of fossil fuels, deforestation, industrial pollution, and livestock farming (AYANLADE et al., 2020). The changes include warming temperatures, variation in precipitation patterns, and increasing frequency and intensity of extreme climate conditions (HALL, 2021; PLOWRIGHT et al., 2012). The consequences for the oceans include a lower dissolved oxygen concentration due to temperature increase and acidification of the water due to the capacity of absorbing CO2 from the atmosphere. These may reflect in aquatic life as a whole (BAHRI; BARANGE; MOUSTAHFID, 2018). For seabirds, anomalies in sea surface temperature may interfere with availability of food and, therefore, delay reproduction, decrease growth rates, and increase mortality of chicks (QUILLFELDT; MASELLO, 2013).

Some well-documented events, such as the El Niño Southern Oscillation (ENSO), can cause failures in breeding and emigration of seabirds, or even massive mortality related to extreme and adverse climatic conditions (ANCONA; DRUMMOND, 2013; TAVARES et al., 2020; TAVARES; FULGENCIO DE MOURA; SICILIANO, 2016). Those effects have been supported by a study in a colony of blue-footed boobies on the Pacific coast of Mexico, where the authors observed impacts of ENSO conditions on recruitment time and reproduction were influenced by the moment in life-history they occurred (ANCONA; DRUMMOND, 2013). Along the Brazilian coast, Tavares et al. (2020) also evaluated the effects of ENSO in three migratory seabirds species. An increased mortality of Manx shearwaters related to this climate event was observed, while the mortality of Atlantic yellow-nosed albatrosses and Magellanic penguins could not be associated entirely with this condition. These findings reflect the great differences of impacts caused by weather changes worldwide, among and within species, and also the importance of timeline to establish nexus. Therefore, studies should be based on long-term monitoring, and ideally including new technologies available for this purpose, such as aerial photography by aircraft and drones, biotelemetry and bio-logging (BESTLEY et al., 2020).

Another concern of climate change is the impact on seabirds' physiology, behavior, distribution, and migration patterns, which are related to a major issue that is disease dynamics (HALL, 2021). In Brazil, Ruoppolo (2016) observed physiological changes during molt cycle interfering with immune parameters in captive *S. magellanicus*. Molting is usually influenced by seasons and climate (RUOPPOLO, 2016). If climate change somehow affects molting, maybe seabirds are facing physiological challenges beyond their survival capability. The results of the interaction between host and etiological agents depend on many factors that, influenced by climate change, could also increase or decline parasitism (HALL, 2021). However, few studies bring evidence on how and in which proportion these changes - as an indirect anthropogenic impact - pose a threat to biodiversity and human health. This is probably due to the complexity in analyzing those factors in the long-term and also because climate change is rarely the only threat affecting the populations (DIAS et al., 2019; HALL, 2021).

3.2.4. Bioinvasion

Introduction of alien species is considered one of the most significant threats to biodiversity (CLAVERO; GARCIABERTHOU, 2005). They can change the structure of the natural environment, increase predation, and compete with native species, as well as change disease dynamics (MICHELETTI et al., 2020). Particularly in seabird colonies, alien species can predate adult birds, eggs, and chicks, destroying their nests and its environment, therefore, resulting in population decline and species extinction (SERAFINI; LUGARINI, 2014).

M. P. Dias et al. (2019) emphasized that rats and cats jeopardize the survival of seabirds, and the management of these invasive animals should be a major priority in conservation strategies. This threat is even more noteworthy on oceanic islands and archipelagos (SARMENTO et al., 2014), where strategies should be carried out together, since control or eradication of just one invasive species could increase the impact of the other on native specimens (DIAS et al., 2017).

3.3. HEALTH STATUS AND DISEASES OF SEABIRDS

Several toxic contaminants and organisms interact with biodiversity: organic pollutants, heavy metals, bacteria, fungi, viruses, and helminths, for example (KHAN et al., 2019). Seabirds are transboundary and travel long distances in relatively short time. This may be important in spreading diseases or being more exposed to them (BOULINIER et al., 2016; TROMBULAK; BALDWIN, 2010). Also unique for these specimens is that they are long-lived, breed in large and dense colonies, and are faithful to their breeding sites, which may favor the etiological agent's maintenance and dissemination (BOULINIER et al., 2016). Ultimately, seabirds can be hosts to zoonotic pathogens, such as arboviruses, influenza virus, *Salmonella* spp., *Mycobacterium* spp., or disperse infected vectors, therefore menacing public health (KHAN et al., 2019; REED et al., 2003).

The threats discussed earlier may have direct or indirect effects on health and survival of seabirds worldwide. The interaction between these threats and potential pathogens, and its long-term consequences, remain unclear. The host's immune system is essential to prevent or limit disease. Stress status, for example, can decrease immune response and, consequently, increase susceptibility to infections (HOFER; EAST, 2012; OWEN; HAWLEY; HUYVAERT, 2021). Despite its importance, ecological studies usually overlook the impact of pathogens in wildlife. In some cases, host mortality could be underestimated, since infection may cause weakness and thus, increase susceptibility to predation (HOFER; EAST, 2012).

Although much is known about some species, others remain unstudied, despite their key role in the ecosystem. This is especially true for seabirds in regard to their health assessment, but also avian hosts in general, due to the complex diversity and interaction between them and etiological agents (OWEN; ADELMAN; HENSCHEN, 2021). While disease could be a disruption in homeostasis caused or not by etiological agents, it is complex to define the health of the hosts. Especially when records are based only in isolation and identification of organisms as potential pathogens or parasites, which are the majority of the studies found for this review. The concept of pathogen and parasite will be used here to refer to agents that are capable of causing disease, and microorganisms will be the general term and their specific features are provided in the text (OWEN; ADELMAN; HENSCHEN, 2021). In the following section, published material was selected and discussed regarding reports and research of organisms and contaminants in seabirds in the country for the last 20 years. A list of the microorganisms and summarized methodology used in each study is provided in the Appendix.

3.3.1. Microorganisms

a. Bacteria

Bacterial diseases are reported as the most common cause of mortality in wild birds, not only causing infection, but also toxin related diseases. Despite its acknowledged impact on birds, few studies aimed to identify bacterial agents in seabirds in Brazil. Most of them searched for microorganisms with zoonotic potential, such as *Aeromonas* sp. and *Vibrio* sp. *Salmonella* sp., *Escherichia coli*, *Staphylococcus* sp., *Streptococcus* sp, *Salmonella* sp., *Enterococcus* sp., *Citrobacter* sp., and *Shigella* sp. were also reported in seabirds studied along the Brazilian coast (CARDOSO, 2018; CARDOSO et al., 2018; EBERT et al., 2016; EWBANK et al., 2022;

ROGES et al., [s.d.]; SARAIVA et al., 2021; SAVIOLLI et al., 2016; ZAMPIERI; MARANHO; OLIVEIRA, 2014). Those were the most frequent and prevalent among the surveys. Other microorganisms were also isolated, but not considered relevant for the discussion and are outlined in the supplementary material. While worldwide studies also include microorganisms of great concern, such as avian cholera (*Pasteurella multocida*), Lyme disease (*Borrelia burgdorferi*), avian botulism (*Clostridium* sp.), and Chlamydiosis (*Chlamydia psittaci*) (KHAN et al., 2019), these were not investigated in the studies in Brazil.

The most common seabirds species included in the studies that surveyed bacteria were *Spheniscus magellanicus*, *Sula leucogaster*, *Larus dominicanus*, *Fregata magnificens*, *Puffinus puffinus*, *Phaethon lepturus*, and *Sterna hirundinacea*. The sampled seabirds were either free-ranging wild and live captured, or stranded/wrecked, leading to species determination by chance in the latter method. Although many species have been contemplated, most were represented by just one specimen, which is not enough for significant results, but act as descriptive studies. Even in designed studies, some species were represented by overly small samples, maybe due to challenging captures or population limitations. It was not possible to determine the prevalence of each bacteria for each species because some studies gathered together results from different species, segregating only by location, which narrows the interpretation of these results, as different species may play different roles in the epidemiology.

Aeromonas sp. is found mostly in aquatic birds, often associated with a piscivorous diet, as some of these microorganisms belong to fish microbioma (e.g. *Aeromonas sobria*) (STENKAT et al., 2014). Some species of *Aeromonas* can cause gastroenteritis, ocular and cutaneous manifestations, urinary infections and septicemia in humans and some cases of zoonotic infections have been reported (CARDOSO et al., 2018). Although in other aquatic birds enteritis and septicemia have been reported in association with *Aeromonas* sp., cases of diseases in seabirds related to this microorganism remain undocumented (FONTENELLE; BARROS, 2014).

On the coast of Rio de Janeiro, *Aeromonas* sp. were isolated from oral and cloacal swabs in 33% of 116 alive, wrecked and debilitated marine birds, with prevalence of *Aeromonas caviae* (25%) and *A. hydrophila* (21%) (CARDOSO et al., 2018). *Aeromonas* sp. was also identified in 11 Manx shearwater (*P. puffinus*) in the same location, and the prevalence was 18% (CARDOSO et al., 2014). Cardoso (2018) also described this genus in marine birds from São João da Barra (40%), Pontal do Paraná (40%), Niterói (12%), Marajó Island (4%), and Rio Grande (4%). The percentages here are the distribution of positive results, however, the research informs only the total number of samples (n=122) and not the respective quantity from each location, which makes the interpretation of the results difficult. All studies followed the same methodology – enrichment with Alcaline Peptone Water (1% NaCl) and culture onto thiosulfate citrate bile salts and sucrose (TCBS) agar for the isolation of *Vibrio* sp. or culture onto Glutamate Phenol Red Starch agar for the isolation of *Aeromonas* sp. – and, therefore, the prevalences are comparable between the species. Also, the samples' storage temperature and transport time followed the recommendation of the Brazilian Department of Health (CARDOSO et al., 2014; CARDOSO, 2018).

Regarding *Vibrio* sp., Cardoso et al. (2018) isolated 22 *Vibrio* species in 65% of the individuals (n=116), whereby 9% were considered pathogenic only to animals, 9% only to humans, and 21% were pathogenic to both. As for the marine birds from São João da Barra, Pontal do Paraná, Niterói, Marajó Island, and Rio Grande, Vibrio sp. was distributed in 8%, 50%, 32%, 0%, and 8%, respectively (CARDOSO, 2018). This microorganism is known to cause profuse diarrhea and is transmitted by contaminated water, or even fish (SERAFINI; LUGARINI, 2014). The most popular species is the *Vibrio cholerae*, which although in low prevalence, has also been identified in seabirds (CARDOSO et al., 2018). Thus, birds can be a source of contamination and spread of zoonotic microorganisms.

Salmonella sp. is relatively more studied than the previous microorganisms worldwide, but this may not be the truth for Brazil. Only three studies described this genus isolated from seabirds in the country, of which one in free-ranging apparently healthy specimens. Each study used a different culture method and only one characterized *Salmonella* by molecular methods. Zampieri et al. (2014) isolated *Salmonella* sp. from tracheal swabs in 5% of 63 seabirds in rehabilitation. This is a low prevalence, probably due to the collection method, since *Salmonella* sp. is most commonly found in the gastrointestinal tract (ZAMPIERI; MARANHO; OLIVEIRA, 2014). Ebert et al. (2016) captured immature individuals of kelp Gulls (*Larus dominicanus*) in three islands of Santa Catarina and obtained cloacal swabs for microbiological survey, in which the prevalence of *Salmonella enterica* ranged from 17,43% and 24,36%. Cardoso (2018) also isolated *S. enterica* in three species of wrecked estuarine and seabirds (n=122, 15 species), *Sula leucogaster* (8%, n=26), *Phalacrocorax brasilianus* (18%, n=28) and *Croicocephalus maculipennis* (11%, n=9), and identified three distinct serovars - *S. enterica* Panama (63%), *S. enterica* Typhimurium (25%), and *S. enterica* Newport (13%). The genus *Salmonella* is known to cause enteric diseases or septicemia in humans and some researchers suggest that this bacteria is found only in seabirds exposed to direct or indirect anthropogenic impacts (CARDOSO, 2018). From that, seabirds may be part of an epidemiological chain, also contaminating humans, domestic and wild animals (SERAFINI; LUGARINI, 2014).

Ebert et al. (2016) also found a higher prevalence of *Staphylococcus aureus* (25,69% n=13; 30,43% n=17; 35,9% n=9), *Citrobacter koseri* (14,10% n=13; 11,96% n=17; 10,09% n=9), and *Shigella* sp. (6,42% n=13; 7,61% n=17) among the kelp Gulls studied (Tamboretes, Moleques do Sul and Lobos Island). Bacteria of the genus *Staphylococcus* are commensal and naturally found in the skin and mucous membrane of respiratory and gastrointestinal tracts. When causing infection, primarily or secondarily, they are often associated with skin lesions, pneumonia, meningitis, endocarditis, and septicemia (SERAFINI; LUGARINI, 2014). *Staphylococcus* sp. was isolated from cloacal and tracheal swabs of 16 white-tailed tropicbirds (*P. lepturus*) and two Audubon's shearwater birds (*P. lherminieri*) captured in the archipelago of Fernando de Noronha (SARAIVA et al., 2021). From 18 birds, 15 samples were positive for *Staphylococcus*, distributed in five species: *S. sciuri* (22 isolates, 73.3%), *S. intermedius* (4; 13.3%), *S. saprophyticus* (2; 6.7%), *S. aureus* (1; 3.3%), and *S. haemolyticus* (1; 3.3%) (SARAIVA et al., 2021). Zampieri et al. (2014) found *Staphylococcus* sp. in 74,5% of the tracheal swabs from seabirds in rehabilitation.

Escherichia coli strains were surveyed in free-ranging frigates (*Fregata magnificens*) and the microorganism was found in 86,8% of the individuals (n=38), whereby in 88,4% of the strains, virulence genes were identified (SAVIOLLI et al., 2016). *E. coli* are also commensal organisms, but some strains may acquire virulence genes and become pathogenic. Saviolli et al. (2016) also detected antibiotic resistance in 62,7% of the *E. coli* strains and 11,6% were multidrug-resistant, even though no frigate was treated with antibiotics prior to this survey. Ewbank et al. (2022) identified extended-spectrum ß-lactamase - producing *Escherichia coli* (ESBL-EC) in 2,4% (n=204) of their sample, which included five seabirds species, but all isolates came from magnificent frigatebirds (5/35). Although little is known about the role of seabirds in the epidemiology of this bacteria, some serotypes found are associated with human and avian diseases, and seabirds can disseminate antibiotic resistant strains, which should be considered when treating those potential patients in rehabilitation centers (SAVIOLLI et al., 2016).

Ewbank, Esperón, et al., (2021) surveyed antimicrobial resistance genes (ARGs) in gastrointestinal samples of six species of wild birds in Fernando de Noronha and found at least one ARG in 84,8% of the individuals. The most prevalent resistance were to tetracyclines, quinolones and phenicols. Saraiva et al. (2021) also investigated antimicrobial resistance in an island away from the coast and found *mec*A genes in *S. sciuri,* cultured from non-migratory seabirds, highly homologous to the *mec*A gene associated with methicillin-resistant *S. aureus* (MRSA), often associated with hospital-acquired infections. Those new results show how important are researches focused on bacterial microorganisms associated with seabirds, not only for epidemiological studies, but also to investigate the origin and spread of antimicrobial resistance, especially in populations under low selection pressure by antibiotics. There is still a lack of knowledge in our territory that must be fulfilled to serve as a basis for further investigations.

b. Fungal

Data regarding fungal diseases and infection in seabirds are often related to *Aspergillus* and *Candida* (SERAFINI; LUGARINI, 2014; XAVIER; MARTINS-MADRID, 2014). A wide survey investigated fungus from tracheal swabs in 63 individuals of five seabird species (penguins, frigates, gulls, boobies, and petrels) in a rehabilitation center in Brazil. In addition to *Aspergillus* sp. identified in 9,5%, Zampieri et al. (2014) found *Candida* sp. (22%), *Penicillium* sp. (3%), and *Trichophyton* sp. (1,5%). The last one was isolated in only one individual of *Daption* sp. and *Penicillium* sp. was found in two boobies (*Sula* sp.) (ZAMPIERI; MARANHO; OLIVEIRA, 2014). *Candida* sp. is usually isolated from birds microbiota, and this fungus may cause disease in immunocompromised and juvenile birds, primarily or secondarily to other diseases (SERAFINI; LUGARINI, 2014). *Penicillium* and *Trichophyton* have already been described in other wild birds, but there is a lack in evaluating their pathogenic potential for seabirds (ZAMPIERI; MARANHO; OLIVEIRA, 2014).

Although highly prevalent in captive birds, the epidemiology of aspergillosis in free-living seabirds in Brazil is still uncertain and penguins are usually the most studied species (FILHO, 2012; MELO et al., 2020a). This could be related to the opportunistic feature of this microorganism. Aspergillosis in birds is caused mainly by *Aspergillus fumigatus*, among the 339 species of this genus already described (BEERNAERT et al., 2010). These are saprophytic and ubiquitous fungi that have the potential to affect plants, invertebrates, birds, reptiles and mammals through the inhalation of conidia (SEYEDMOUSAVI et al., 2015).

Birds are particularly susceptible due to anatomical features, such as lack of an epiglottis, lack of diaphragm which prevents coughing, and a respiratory tract deficient in ciliated and defense cells (TELL, 2005), as well as a physiologic higher body temperature (FILHO, 2012). Furthermore, *A. fumigatus* spores are smaller than the spores of the other species, which may predispose the infection, along with other factors such as poor ventilation and sanitation, warm and humid environment, and the bird's immunity, for example (BEERNAERT et al., 2010). Aspergillosis is considered the main cause of death in penguins in rehabilitation in Brazil, since debilitation and immunosuppression are predisposing factors, and the poor conditions of the environment enhance chances of infection (FILHO; RUOPPOLO, 2014). The susceptibility to *Aspergillus* of captive spheniciformes has been already documented worldwide (ALVAREZ-PEREZ et al., 2010). Furthermore, the high risk of infection in rehabilitation facilities has been investigated by Burco et al. (2014), that found an increased burden of *Aspergillus* conidia in water and air from wildlife centers compared to natural seabird environments.

The clinical manifestations can be acute or chronic related to infection, but also a result of mycotoxicosis. Acute signs in birds include emaciation, lethargy and respiratory distress (FILHO; RUOPPOLO, 2014). This form has a high morbidity and mortality and differs from the chronic aspergillosis, that is usually related to immunosuppression. Mycotoxicosis results from ingestion of mycotoxin contaminated food and contributes to the patogenesis of aspergillosis or can also cause sudden mortality (Seyedmousavi et al., 2015). The diagnosis is based on a combination of clinical signs, culture, histology and molecular techniques for example, as no single modality has 100% specificity or sensitivity. Clinicians should also consider the animal's susceptibility and environmental conditions that may predispose the disease (SEYEDMOUSAVI et al., 2015; XAVIER; MARTINS-MADRID, 2014).

Martins (2015) evaluated hematological parameters in Magellanic penguins in rehabilitation and compared the results between animals presenting aspergillosis and a control group. In infected penguins, it was observed a progressive decrease in the hematocrit, whereas total plasmatic protein increased significantly. This is expected, as well as leukocytosis, heterophilia in earlier stages, monocytosis and lymphopenia in later stages (FIORELLO, 2020; XAVIER; MARTINS-MADRID, 2014). Anemia is probably caused by hemolysins from the pathogen and has already been reported in other infected birds. Hyperproteinemia occurs mainly due to output of globulins in response to aspergillus and chronic inflammation response (MARTINS, 2015). This is the only study regarding health parameters related to infection by aspergillosis in seabirds in Brazil.

A cohort study performed in a rehabilitation center in Brazil identified aspergillosis by macroscopic lesions and mycologic culture in 66 out of 327 admitted penguins (*S. magellanicus*) between 2004 and 2009. The majority of cases occurred in 2008, juveniles were more susceptible than adults, and penguins originated from other rehabilitation centers presented a higher incidence of aspergillosis. Mortality rate due to aspergillosis was 48,5% within six years of study (FILHO, 2012). A recent study evaluated 61 carcasses from 325 Magellanic penguins found dead by the Coastal Monitoring Program in Cananéia (São Paulo) and diagnosed aspergillosis in two of them and *Candida palmioleophila* in one, by histopathologic and molecular analysis (EWBANK et al., 2021b). This was the first time *Aspergillus* was isolated in free-ranging Magellanic penguins. It stresses the importance of the monitoring programs along the Brazilian Coast, as well as related research, for a better understanding of the threats of these microorganisms to the species conservation.

Melo et al. (2020) reported *A. fumigatus* in two other species of free-ranging seabirds for the first time, white-chinned petrel (*Procellaria aequinoctialis*) and brown-hooded gull (*Chroicocephalus maculipennis*). The petrel was found emaciated when diagnosed, while the gull was apparently healthy, however both died during the transportation to a rehabilitation center. These two cases are relevant and enhance the need in investigating the epidemiology of aspergillosis in other seabirds' species. A. M. Melo, Silva Filho, et al., (2020) also investigated *Aspergillus* in albatrosses that died (n=14) during rehabilitation and identified *A. flavus* in one and *A. fumigatus* in two specimens of *Thalassarche melanophris*, which may indicate this disease as a limiting factor in their rehabilitation.

This study has also demonstrated that the fungi were susceptible to itraconazole, by determining the minimal inhibitory concentrations (MIC) of *A. flavus* (0,5µg/ml) and *A.*

fumigatus (0,25µg/ml) to this drug. An increasing concern on this topic has been the azole resistance, specially in *A. fumigatus*, which has also a great importance on human aspergillosis (SEYEDMOUSAVI et al., 2015). Changes in *cyp51A* gene, metabolism (e.g. overexpression of eflux pumps) and biofilm components may contribute to the emergence of *Aspergillus* resistance that has been linked to long-term treatments with azole compounds, as well as the indiscriminate use of pesticides containing fungicides (MELO et al., 2020c). Therefore, considering the seabirds' migration features, investigations on their role in *Aspergillus* and resistant strains dispersion are critical to preserve human and animal health.

c. Virus

Although some viruses have been monitored for scientists in Brazil, such as West Nile virus (WNV), eastern equine encephalitis virus (EEEV), avian influenza virus, and Newcastle Disease virus, little is known about the role of seabirds on its spread, as well as the consequences for these groups (SERAFINI; LUGARINI, 2014). Once more, Magellanic penguins are the most studied group, and strains of paramyxovirus, flavivirus, and poxvirus have already been reported in other countries (FILHO; RUOPPOLO, 2014).

In Brazil, a novel herpesvirus was identified causing an outbreak of respiratory disease in oiled Magellanic penguins undergoing rehabilitation (NIEMEYER et al., 2017). In this outbreak (winter season of 2011), 58,3% out of 168 penguins presented acute respiratory signs, and death occurred in 86,7% of the symptomatic birds. Presenting signs were anorexia, coughing, dyspnea, and serosanguinolent mucus; necropsy findings included lung congestion and edema, fibrinous airsacculitis, and necro-hemorrhagic tracheitis (FILHO; RUOPPOLO, 2014; NIEMEYER et al., 2017). Niemeyer et al. (2017) identified a novel virus as the responsible for this outbreak, Magellanic penguin herpesvirus 1 (MagHV-1).

Other species in the facility were sampled at the time of the outbreak. In addition, a cross-section study was further performed in asymptomatic birds from Abrolhos and Argentinian-Patagonia and other species undergoing rehabilitation. Two years after the outbreak, Niemeyer et al. (2017) identified Magellanic penguin herpesvirus 2 (MagHV-2) in nestling and adult penguins in Argentinian-Patagonia. In Abrolhos, two *S. dactylatra* (masked booby), one *S. leucogaster* (brown booby) and two *P. aethereus* (red-billed tropicbirds) presented sequences corresponding to Sulid Herpesvirus (SuHV). At last, from one *T. chlororhynchos* (yellow-nosed albatross), Thalassarchid herpesvirus (ThaHV) was sequenced (NIEMEYER et al., 2017).

Niemeyer (2015) also surveyed other viruses in Sphenicisdae, Sulidae, Procellaridea, and Phaetontidae, free-ranging or in rehabilitation. Besides, Herpesvirus, Poxvirus and Coronavirus were identified in this survey from tracheal and cloacal swabs, through DNA and RNA extraction, PCR (polymerase chain reaction) and further sequencing.

The *Avipoxvirus* genus is known to cause verrucous lesions in the skin of birds, as well as more severe lesions in gastrointestinal and respiratory tract. Its transmission occurs by direct contact with the lesions and viral particles, or indirectly by a mechanical vector (NIEMEYER, 2015). The *Avipoxvirus* has been already detected in 374 bird species worldwide (WILLIAMS; TRUCHADO; BENITEZ, 2021), and there are few reports in Brazilian avifauna (CATROXO et al., 2009; ESTEVES et al., 2017). Through phylogenetic analysis, Niemeyer (2015) identified six genotypes of two different groups of *Avipoxvirus* in penguins from distinct rehabilitation centers in Brazil, one isolated in Espírito Santo and the other five in Santa Catarina. The latter were considered the most virulent, causing lesions in the skin, esophagus and upper respiratory tract, while the former caused only skin lesions and esophagitis.

Outbreaks of this disease can be common in captivity, and sometimes, clinically ill specimens are euthanized to prevent transmission, due to the lack of knowledge about the epidemiology of Avipoxvirus Brazilian avifauna and the belief that infected birds can become reservoirs and disseminate the virus (NIEMEYER, 2015). However, preventive and control measures should be encouraged in captivity, such as preventing contact with contaminated vectors or objects (e.g. feeders and perches) and isolating infected birds (BOYLE, 2007). Further research to understand the real risks of this virus for the seabirds' conservation is mandatory.

Avian coronaviruses are known to cause infectious bronchitis, usually acute and highly contagious, resulting in respiratory, gastrointestinal, renal, and reproductive disorders in birds, mainly in poultry (CAVANAGH, 2007). Coronavirus was identified from cloacal swabs in one *M. giganteus* (giant petrel), two *S. leucogaster* (brown boobies), and 12 *S. magellanicus* (Magellanic penguins), as well as from kidney macerates in ten Magellanic penguins (NIEMEYER, 2015). None of the individuals presented clinical signs during the study. This was the only survey published about coronaviruses in seabirds in Brazil up to now. Surveillance on viruses is important to determine possible outbreak agents for seabirds population, poultry, and humans as well.

3.3.2. Parasites

a. Apicomplexa

Hemosporidia are microorganisms transmitted to birds by vectors (e.g. flies and mosquitoes). *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Hepatozoon,* and *Babesia* are potential hemoparasites for marine birds (VANSTREELS et al., 2017). The prevalence of blood parasites in seabirds depends on taxonomy, phylogenetic, ecology and life-history of the species (QUILLFELDT et al., 2011). Although some studies may indicate that the prevalence of hemosporidia is low in seabirds, the absence of records may be related to a failure in diagnostic methods or insufficient sample size (VANSTREELS et al., 2017), and not always related to absence of vectors in marine, arid, and cold environments, host-parasite specificity, and host-immunity capable of preventing infection - features used to explain the apparent low prevalence of these microorganisms (QUILLFELDT et al., 2011; SERAFINI; LUGARINI, 2014).

In Brazil, since 2000, only five studies were performed aiming to identify hemoparasites in seabirds. From these, two were designed to capture species of the family Suliformes and Charadriiformes on islands, two evaluated Magellanic penguins in rehabilitation centers, and one reports *Plasmodium* for the first time in Manx shearwater (*P. puffinus*) (VANSTREELS et al., 2020). Magellanic penguins are susceptible to avian malaria, caused by the Haemosporidia of the genera *Plasmodium* and transmitted by mosquitoes (Culicidae) or even sandflies (Psychodidae) (QUILLFELDT et al., 2010). Infection usually leads to acute mortality and post-mortem findings include hepatomegaly, splenomegaly, severe pulmonary congestion and hydropericardium (FILHO; RUOPPOLO, 2014).

Vanstreels et al. (2015) surveyed avian malaria in Magellanic penguins by morphological and molecular methods from six rehabilitation centers along the Brazilian coast and found an estimated prevalence of *Plasmodium* between 6,6% and 13,5% (n=774). In this study, five different species of *Plasmodium* were identified, as well as five distinct lineages not yet reported in penguins, which suggests a wide diversity of plasmodium in these species (VANSTREELS et al., 2015). During the previous study, there was an outbreak of avian malaria in 28 *S. magellanicus* from a rehabilitation center in Florianópolis, where haemosporidia was identified in 64% (18) of the specimens. *Plasmodium tejerai* was the most prevalent and the most pathogenic, isolated in 72% of the infected penguins and in 89% of those who died (VANSTREELS et al., 2014). An alarming fact is that most of the avian malaria episodes probably occurred during rehabilitation. The infection increases mortality and reduces rehabilitation success, although the disease is more frequent in the season that few penguins are found wrecked (VANSTREELS et al., 2015). Therefore, prevention should include efforts to reduce the time of rehabilitation and enable faster release of these seabirds.

Another kind of avian malaria caused by *Haemoproteus* is transmitted by louse flies (Hippoboscidae) and biting midges (Ceratopogonidae), and among the seabirds it is usually reported in gulls and frigatebirds (SERAFINI; LUGARINI, 2014). Mariano and Dantas (2020) investigated the prevalence of *Plasmodium* and *Haemoproteus* in blood samples of *S. dactylatra* (n=92), *S. sula* (n=42), *S. leucogaster* (n=69) and *F. magnificens* (n=13) in four archipelagos: Fernando de Noronha, Atol das Rocas, São Pedro e São Paulo, and Trindade e Martim Vaz. Those blood parasites were not identified in their sample by PCR method, probably due to absence of vectors in these environments and immune system efficiency of the birds (MARIANO; DANTAS, 2021). These negative results could also be related to the methodology, since they performed three independent PCR tests - which can fail to amplify DNA in low intensity of microorganisms -, instead of nested PCR protocol that has been used in the majority of the studies worldwide (CLARK; CLEGG; LIMA, 2014).

Quillfeldt et al. (2014) investigated by PCR in the same islands, plus Abrolhos archipelago, the prevalence of blood parasites in wild boobies and noodies, including adults and chicks. *Leucocytozoon* was not identified in any seabirds. No microorganisms were detected in *A. minutus* (black noddy) or *S. sula* (red-footed boobies). *Haemoproteus* was found in 8 of 98 adult birds and in none of the chicks. Although most of the birds are relatively asymptomatic for *Haemoproteus* infection, studies suggest that parasitemia may indicate conditions of stress, including underlying diseases, and other studies found negative effects on bird fitness (QUILLFELDT et al., 2014).

For *S. leucogaster* (brown boobies), *Babesia* was found only in juveniles in three out of four breeding sites. In *S. dactylatra* (masked boobies), prevalence of *Babesia* was ten times higher in juveniles, although also found in adults. In addition, juveniles infected were significantly lighter than the mean. In all samples, identification was only possible by PCR screening, since in blood smears parasites were not detected, probably due to low intensity of infection (QUILLFELDT et al., 2014). *Babesia* spp. is referred to as Piroplasmids, transmitted by ticks and may infect mammals and birds. Quillfeldt et al. (2014) found that infection of this protozoan may decrease or disappear in adults, probably due to acquired immunity. The epidemiology and effect of this parasite on seabirds is still understudied, and therefore, their features need to be further clarified, as well as abundance of ticks in seabird colonies.

For other protozoan species, such as *Toxoplasma* sp., *Sarcocystis* sp. and *Neospora* sp., birds in general may play a role as intermediate hosts, and become infected by ingestion of oocysts in contaminated water or food (SERAFINI; LUGARINI, 2014). Only four studies were found in Brazil referring to these microorganisms. Some authors have recently found *Neospora caninum* isolated from the heart tissue of one specimen of *Procellaria aequinoctialis* (SATO et al., 2020). Although this single report is relevant for the species, further studies should be carried out to better understand the epidemiologic role of seabirds in this context.

Gennari et al. (2016) surveyed *Toxoplasma gondii* antibodies in rescued and captive Magellanic penguins on the Brazilian coast. From 100 blood samples, seroprevalence was 28% based on a modified agglutination test (MAT). The authors did not establish an association between serology and origin, sex or age of the penguins (GENNARI et al., 2016a). Another serological study of *Toxoplasma gondii* was conducted by the prior author in two islands of Abrolhos Archipelago (located 65km off the Brazilian coast), where antibodies were found in 8/23 *Sula dactylatra*, 9/19 *Sula leucogaster* and 7/25 *Phaethon aethereus*. Seropositivity was not found in *Phaethon lepturus* (n=2) (GENNARI et al., 2016b). Although death related to infection by *T. gondii* has been reported in seabirds, in none of the previous studies illness was observed, which may indicate that the specimens can be sentinels for environmental contamination by this protozoan (GENNARI et al., 2016a, 2016b).

Acosta et al. (2018) detected anti-*T. gondii* antibodies in 18/145 rescued juvenile penguins by the modified agglutination test (MAT). Besides the serological evaluation, the author also surveyed *T. gondii* and *Sarcocystis* sp. in tissue samples (pectoral muscle, heart, and brain). Only *Sarcocystis* was identified in pectoral muscles (16/342) by polymerase chain reaction (PCR), with genetic sequence closely related to *Sarcocystis falcatula*, and in none of the anti-*T. gondii* seropositives (ACOSTA, 2018). The source of infection by these protozoans remains unclear, as well as prevalence in wild populations and its relation to the health profile of the seabirds.

b. Ectoparasites

Ectoparasites were reported in three studies in Brazil for three different species of seabirds. Labruna et al. (2020) captured and examined 13 Atlantic yellow-nosed albatrosses (*Thalassarche chlororhynchos*) and found 14 ticks on three individuals. Also, two individuals were examined in a rehabilitation facility and both were infested by ticks, 23 in total. The species were identified as *Ixodes percavatus* or *Ixodes kerguelenensis*, since they are extremely similar and both have been reported in *T. chlororhynchos* (LABRUNA et al., 2020). Around the world, hard ticks belonging to the genus *Ixodes* are the most common in seabirds (KHAN et al., 2019).

Brito (2018) evaluated 15 frigatebirds from a rehabilitation center and found ectoparasites in 14, however the health profile of the birds was not accessed due to lack of information in clinical records. The parasites found comprised only chewing lice distributed in three species and were classified as *Colpocephalum spineum*, *Fregatiella aurifasciata*, and *Pectinopygus fregatiphagus* (BRITO, 2018). Melo et al. (2012) evaluated 16 carcasses of *P. puffinus*, collected biting lice in five specimens, and identified *Halipeurus diversus*, *Trabeculus aviator, Austromeno paululum*, *Saemundssonia* sp., and *Naubates* sp., and multiple infestation was observed (DE MELO et al., 2012).

Louse species are usually host-specific and a greater infestation may be correlated with poor health conditions, such as stress, lack of food or nutrients, and underlying diseases. Not only a consequence, lice infestation can cause thermoregulation stress and increase of grooming, changing the bird’s balance (KHAN et al., 2019). Therefore, the evaluation of occurrence of these parasites should always be reported along with the clinical condition of the individuals and further studies should be carried out in living animals to better evaluate the correlation between ectoparasites and host’s health.

c. Endoparasites

Endoparasites were reported in five studies and only one was performed in live animals with evaluation of fecal samples. This reflects the lack of studies on this topic in Brazil, even though the occurrence of parasites is frequent in wildlife (SERAFINI; LUGARINI, 2014). Melo et al. (2012) also surveyed endoparasites in *P. puffinus* and identified two species of nematodes and one cestode in five out of 16 carcasses, respectively: *Seuratia shipleyi, Contracaecum* sp., and *Tetrabothrius* sp. The parasites were collected from the proventriculus and small intestine, and only one species of parasite was found in each carcass (DE MELO et al., 2012).

Wartchow (2017) evaluated the gastrointestinal tract of 34 marine birds found dead on beaches from Rio Grande do Sul, Brazil. Although the aim of this study was to identify oocysts of *Cryptosporidium* sp. in these birds, only helminths were found, though species identification was not performed. The 25 *S. magellanicus* found were infected by at least one helminth species, being nematodes observed in the esophagus (76%), stomach (100%), and intestines (40%). Trematodes and cestodes were present only in the intestines.

For *Procellaria aequinoctialis* (n=4), nematodes were found in the esophagus of one, and in the proventriculus and ventriculus of two others, and one was not parasitized. For *M. giganteus* (n=3), one had only nematodes in proventriculus and ventriculus, while another one presented nematodes also in the esophagus, and trematodes in the intestines. Wartchow (2017) also evaluated one specimen of *P. puffinus*, *Stercorarius antarctica*, and *F. magnificens*. Only in *P. puffinus*, nematodes were found in the proventriculus and ventriculus, and trematodes in the intestines (WARTCHOW; ALEGRE, 2017).

The other three studies comprising parasites used only *S. magellanicus*. Rezende et al. (2013) evaluated 237 juvenile Magellanic penguins wrecked in beaches from São Paulo and Rio de Janeiro. In necropsy, helminth fauna was present in 118 individuals, represented by *Contracaecum pelagicum* (nematode), *Cardiocephaloides physalis* (digenetic), and *Tetrabothrius lutzi* (cestode). The first one is found in the stomach, and the other two are found in the initial portion of the small intestine. In comparison with other studies on penguins' helminth fauna (performed during breeding season), this study demonstrated a lower prevalence and diversity of parasites in penguins during migratory season (REZENDE et al., 2013).

De Paula et al. (2020) collected carcasses of 63 Magellanic penguins found in beaches of the State of Rio Grande do Sul and evaluated age (juvenile or adult), body condition, helminth fauna, and preferable food items throughout necropsy. In total, 42 juveniles and 21 adults were examined, with at least one species of parasites found in all of them. Eight species of parasites were identified: *C. pelagicum* and species of Acuariidae (nematodes), *T. lutzi* (cestode), *C, physalis, Stephanoprora uruguayense*, *Ascocotyle longa (Phagicola)*, and *Ascocotyle (P.) sp.* (trematodes), and *Corynosoma* sp. (acanthocephalan). Acuariidae nematodes and *A. longa* were absent in adult specimens. *T. lutzi* was the most common parasite in juveniles, while *C. pelagicum* prevailed in adults. Adult lean penguins presented higher mean abundance and diversity of parasites, whereas juvenile lean penguins presented the highest richness and mean abundance (DE PAULA et al., 2020).

This difference observed in comparison to supposedly healthy individuals may show that parasite infection can debilitate the body condition of the hosts. This may happen by hindering the absorption of nutrients, for example, but even weakness and stress may compromise the immune system and predispose to parasitism (DE PAULA et al., 2020). However, more studies are necessary to fully understand this relationship, since the wrecked Magellanic penguins on the Brazilian coast are already debilitated and parasitism could be or not an intensification factor in the survival rate of this species.

Another study performed in Pontal do Paraná evaluated 31 carcasses and seven alive juvenile Magellanic penguins from the Marine Study Center of the Federal University of Paraná. Among the 31 carcasses, 29 were parasitized by at least one of the following species: *C. pelagicum* (nematode), *C. physalis* (digenetic), and *T. lutzi* (cestode), as Rezende et al. (2013) and de Paula (2020) also identified. For the seven alive penguins, fecal samples were examined through Willis-Mollay method and eggs from *C. pelagicum* were found in four individuals (VANHONI et al., 2018). To the best of our knowledge, this is the only coproparasitological study in living penguins in Brazil, and further studies in this direction should be conducted, including other fecal examination methods, other alive species and with a wide sample size, also evaluating the health status of the populations.

3.3.3. Chemical pollution

a. Heavy metal

Seabirds are considered good indicators of heavy metal pollution due to their long lifespan and feeding habits (BARBIERI et al., 2010). Six studies were found evaluating trace elements in samples from seabirds in Brazil, two in *L. dominicanus*, two in *S. magellanicus*, one in *F. magnificens* and *S. leucogaster*, and another one in *S. leucogaster*. Ebert et al. (2020) evaluated lead (Pb), chromium (Cr), mercury (Hg) and zinc (Zn) in feathers of young *L. dominicanus* from three islands in the State of Santa Catarina and found relevant Pb and Zn concentrations on two and three islands, respectively. Although the results found were not considered harmful, they may indicate bioaccumulation and environmental pollution of the region.

On another island, in the same state, Barbieri et al. (2010) compared cadmium (Cd), cobalt (Co), cupper (Cu), manganese (Mn), nickel (Ni), Cr, Zn and Pb concentrations in feather samples of adult, subadult and juvenile *L. dominicanus* and observed an increase in the concentration of all the elements with age, though it remained at acceptable levels.

Padilha et al. (2018) evaluated trace elements (Cd, Sn, Mn, Cu and Se) in feathers of *F. magnificens* and *S. leucogaster* in the Cagarras Archipelago (five kilometers away from Ipanema beach - Rio de Janeiro) and correlated those levels with biometric parameters. Although the levels found are not considered harmful to the species, a significant negative correlation between selenium (Se) concentration and tarsus length (TrL) was verified in both species.

In the Marine National Park of Currais Islands (10km from the coast of Pontal do Parana), levels of aluminum (Al), arsenic (As), iron (Fe), magnesium (Mg), Ni, Cd, Co, Cu and Zn were evaluated in feathers and eggshells of *S. leucogaster* and found that Ni and As were in higher concentration in eggshells - at levels that could be harmful (DOLCI et al., 2017).

The other two studies were performed in wrecked carcasses of *S. magellanicus* along the Brazilian coast, and, therefore, tissue samples such as kidney, muscle and liver could be assessed in addition to feather analysis. Silveira (2010) compared the concentration of Hg in the liver and pectoral muscle of three groups of penguins, based on the origin of the carcass (States of Sergipe, Rio de Janeiro and Rio Grande do Sul). The concentration of Hg found in the liver was higher than in the muscle and the levels observed increased over the years.

Kehrig et al. (2015) collected liver, kidney and feather samples of juvenile *S. magellanicus* found stranded in Southern Brazil to perform analysis of Se, total mercury (Hg), methylmercury (MeHg), Pb, Cd and metallothioneins (MTs). Probably due to their role in biotransformation, higher concentrations of those elements were found in the liver and kidney. Selenium and metallothionein were observed to play a role in detoxification of trace elements.

For the analysis of heavy metals, samples of kidney, liver, muscles, bone, egg and excrements can be used, however, feathers are a convenient sample method. However, their metal concentration can have two origins: metals deposited from the atmosphere or from the blood during the growing process (MARKOWSKI et al., 2013). Exposure of seabirds to heavy metals, especially mercury, lead, cadmium, and selenium, may have toxic effects not always perceived, but that could be alarming in terms of environmental contamination and bioaccumulation, which inevitably affect humans. Also relevant in this scenario are longitudinal studies that observe the evaluation in time of these compounds in living animals, as it was performed by Nunes et al., (2022) before and after the Fundão dam collapse, whose mud reached the Abrolhos Archipelago. This study monitored three species of seabirds: *Phaethon aethereus*, *Sula leucogaster*, and *Pterodroma arminjoniana*, and found an increased concentration of non-essential elements in feathers and blood, which may be correlated to the disaster consequences.

b. Industrial chemicals

Persistent organic pollutants (POPs) have features such as persistence, bioaccumulation and toxicity, and in the environment may be affecting wildlife (BALDASSIN et al., 2012). They are substances used in industrial and agricultural processes from 1940 until they were banned or regulated in the 1970s (CLARK; FRID; ATTRILL, 2001). Polychlorinated biphenyls (PCBs) are halogenated chemicals highly soluble in organic material and tend to bioaccumulate in organisms from high trophic levels, such as seabirds (WALKER, 1990). After the ban in PCBs production and use, polybrominated diphenyl ethers (PBDEs) came as substitutes, but they also happened to have some toxic effects in wildlife and biomagnification in human food (GRIM; FAIRBROTHER; RATTNER, 2012). Organochlorine pesticides (OCPs) are represented by dichloro-diphenyl-trichloroethane (DDTs), mirex, drins, hexachlorocyclohexane (HCHs), hexachlorobenzene (HCBs) (CLARK; FRID; ATTRILL, 2001). They were extensively used in agriculture until their ban, have long half-lives, biomagnify in the food chain, and may cause neurological effects in several species, as well as non-neural toxic effects (GRIM; FAIRBROTHER; RATTNER, 2012).

Stranded *S. magellanicus* were evaluated in three studies. In two of them, liver samples were collected for the analysis of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs: DDTs ~ HCB ~ Drins) and polybrominated diphenyl ethers (PBDEs). Baldassin et al. (2012) found a predominance of hexachlorobiphenyls and heptachlorobiphenyls among PCBs, DDT among organochlorine, and also a positive correlation between cardiovascular failure and HCB values. While Baldassin et al. (2016), from 2008 to 2012, observed a decrease in PCBs along the years, and found evidence of low concentrations of POPs in the southern portion of South America.

A recent study evaluated policyclic aromatic hidrocarbons in biliary metabolites of *S. magellanicus* from the State of São Paulo coast. In the analysis, the bioavailability of the total metabolites (naphthalene (NAP), phenanthrene (PHE) and benzo[a]pyrene (BaP)) was considered low, though data were compared with studies in fish bile and more studies are needed to investigate the impact of this compounds (BARRETO et al., 2020). Quinete et al. (2020) identified PCBs, OCPs and PAHs in liver (n=9) and muscle (n=13) of juvenile Magellanic penguins wrecked on the State Rio de Janeiro coast and although most of the compound levels were low or non-detectable, the concentration was higher than previous studies.

Ferreira (2015) investigated polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in the liver of dead or injured adult *F. magnificens* from Rio de Janeiro, and found concentrations below the level of concern. P. S. Dias et al. (2018) surveyed PCBs, OCPs and PBDEs in the liver of dead adult and nestling *S. dactylatra, S. leucogaster, A. stolidus, A. minutus* and *Onychoprion fuscatus* from Rocas Atoll. Although the levels found can be considered low, PCBs, DDTs and hexachlorobenzene were predominant, and the highest levels were found in adults, when compared to nestlings.

Colabuono (2011) also evaluated PCBs and OCPs in samples of liver, muscle and fat tissue of eight species of Procellariiformes. This study showed that diet is not the only factor that contributes to this bioaccumulation, and noted that PCBs and OCPs were present in pellets and plastic content found in the seabirds gastrointestinal tract. A single study surveyed POPs in blood of six species of seabirds (*P. arminjoniana*, *S. dactylatra*, *S. leucogaster*, *A. stolidus*, *A. minutus*, and *O. fuscatus*) from the Saint Peter and Saint Paul Archipelago and Trinidad Island (SILVA, 2019). Silva (2019) observed that PCBs had the highest concentration in the blood of all species, followed by DDTs, and Mirex; a migratory species also showed higher concentration when compared to non-migrants, and a negative correlation between body mass and POPs concentration was demonstrated in *P. arminjoniana*.

Before Silva (2019), P. S. Dias et al. (2013) investigated the presence and distribution of POPs in Saint Peter and Saint Paul Archipelago, and observed a greater mean concentration of higher chlorinated PCBs in *S. leucogaster*, compared with *Exocoetus volitans* (flying fish) samples, which can be an example of bioaccumulation. Silva (2019) evaluated the levels of POPs in blood samples, hence comparison was limited, since the majority of studies surveyed those compounds in tissue samples. However, it provides reference for future studies on live populations.

3.4. FINAL CONSIDERATIONS

This manuscript is the first review on the health of seabirds with emphasis on conservation medicine in Brazil and may be a reference for incoming research on this topic (Figure 3.). This review exposes the main threats to seabirds conservation worldwide and how this is addressed by studies in Brazil. We highlight that seabirds are essential for the health of the marine ecosystem and as bioindicators. However, few studies are conducted in Brazil to truly evaluate how this species interacts with anthropogenic impacts and microorganisms and how this affects their health, though the number has been steadily increasing since 2010. Moreover, few studies are performed in populations of non-wrecked/healthy seabirds, which does not provide enough evidence of the health status of the populations. Most studies provide data only on already debilitated animals and/or carcasses collected on seashore, which may be a result of the Costal Monitoring Programs over the last years, and despite its importance in providing data and increasing research in this field, may not reflect the real prevalence and may represent a biased sample. In addition, the majority of the studies comprised only or mainly Magellanic penguins, which are very particular seabirds in Brazil. Therefore, conservation priorities should also include threatened native species in the country, such as *Pterodroma arminjoniana* (trindade petrel), *Sula*

sula (red-footed booby), and *Fregata ariel trinitatis* (lesser frigatebird), for example, that live almost their entire life cycle in Brazilian jurisdictional waters. These gaps should be considered for future research, also regarding methodology, that limits comparison of some studies discussed in this review with the international literature. Accordingly, active search in *in situ* populations, as well as longitudinal studies should be encouraged and funded to provide a better understanding on what scientists, the public, and the government should be doing for the conservation of the seabirds in Brazil.

Declaration of competing interest

The authors have declared that no competing interests exist.

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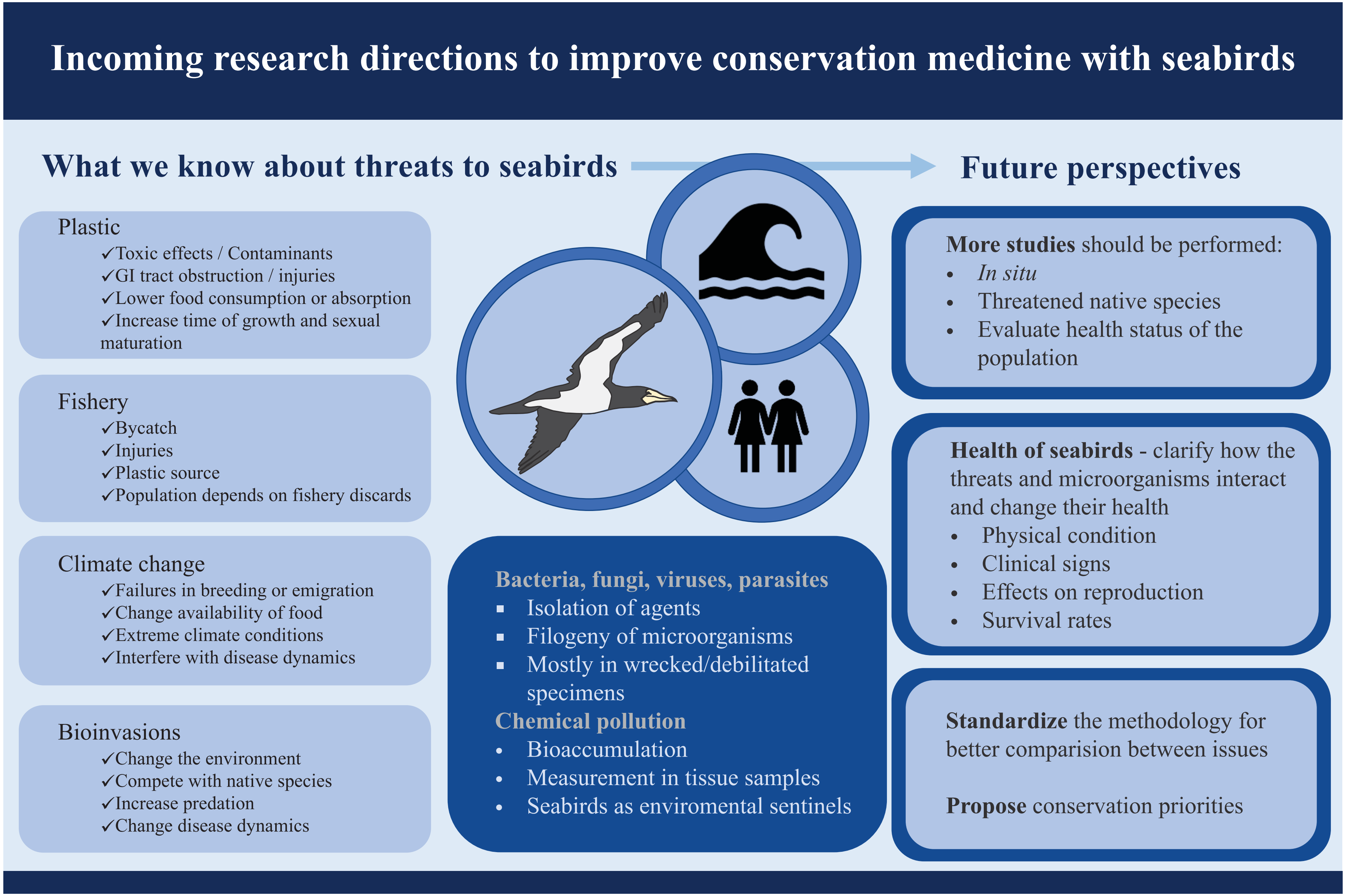
Figure 4. Organization chart about the state of knowledge and future perspectives on seabirds conservation in Brazil.



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4 SEABIRD POPULATION HEALTH ANALYSIS

Health assessment of seabird communities in Abrolhos, Bahia State, Brazil
A concept model for population health analysis

Abstract

Seabirds are considered as environmental quality sentinels. Consequently, many studies aim to identify pathogens or quantify contaminants in these species using carcasses, tissue samples, feathers, or even eggs. However, few studies evaluate those threats from the “birds’ perspective” and how these exposures jeopardize the sentinels' survival. Therefore, the present work brings a more complete assessment of the health status of birds associated with the marine environment, which gives a better understanding about risks they are exposed to and establishes a baseline for conservation efforts. In total, 64 specimens of marine and estuarine birds were captured in the Abrolhos Bank Region, Bahia State, Brazil: 33 *Fregata magnificens*, 14 *Nyctanassa violacea* and 17 *Egretta caerulea*, and submitted to physical exams, cloacal swabs, feather collection and blood drawing. Samples were forwarded for pathogen analysis by PCR, as well as polycyclic aromatic hydrocarbon (PAH) and heavy metals' analysis. Although the sampled birds were considered able to survive in nature in the short and medium term, the general health status of several individuals was altered. Our work is the first to associate different parameters in the evaluation of sea and waterbirds health in the Abrolhos Bank Region before 2016 and also provides a baseline for future reference.

Keywords: marine birds; estuarine birds; health status; microorganisms; heavy metals; PAH.

4.1. INTRODUCTION

Around 111 species of birds in Brazil are associated with or naturally occur in marine ecosystems. Notably, among these, 94% occur in the seashore of Bahia State (VOOREN; BRUSQUE, 1999). Due to their biological features and habits, seabirds are considered sentinels in the assessment of marine environmental health and quality (RAJPAR et al., 2018). Although many bird species are resident, a significant proportion are migratory and travel long distances to reproduce or feed. Therefore, the approach of these communities must be large-scale extended.

Anthropogenic disorders could be considered one of the major threats to these populations. Particularly, acute or chronic oil pollution is gaining attention due to their direct impact on the environment (BARROSO, 2010; DAHLMANN et al., 1994; DARBRA; CASAL, 2004; MCORIST; LENGHAUS, 1992; PROVENCHER et al., 2020; WASZAK et al., 2021; WIENS et al., 1996). The effects of this contaminant are usually perceived in wrecked animals or stranded carcasses and more recently in seabirds eggs (POWER et al., 2021). However, hidden effects caused by exposure to compounds such as polycyclic aromatic hydrocarbons and heavy metals may jeopardize the survival of this species and must be monitored (BOSTRÖM et al., 2002; DOUBEN, 2003). Sea and waterbirds may also host infectious pathogens that are relevant for poultry and human health, such as Paramyxovirus, Influenza A virus, *Salmonella* sp. and *Chlamydia* sp. (SERAFINI; LUGARINI, 2014). In addition to pathogen analysis, the assessment of the birds' physical condition and hematology can provide data on their clinical status and immunity (CORAIOLA et al., 2014). Despite its key role, little is known about the health status of birds associated with oceanic and coastal areas in Brazil.

This knowledge gap hinders the understanding of the conservation status of birds associated with these environments, as well as the evaluation of eventual impacts, since it is not possible to compare with local reference values. For example, in November 2015 tons of ore tailing were released after a dam from Samarco company collapsed, at the municipality of Mariana (Minas Gerais State). The disaster affected the Rio Doce River, progressed to the continental shelf and in June 2016, reached the Abrolhos Bank Region (FRANCINI-FILHO et al., 2019). This study is the only to determine heavy metal concentration in feathers of birds associated with the marine environment in the Abrolhos Bank Region prior to the Mariana environmental disaster. Therefore, the purpose of this article is to provide new insights on the health status of birds as sentinels of the marine environment, as well as to provide baseline values for incoming research in the area.

4.2. METHODS

4.2.1. Ethical issues

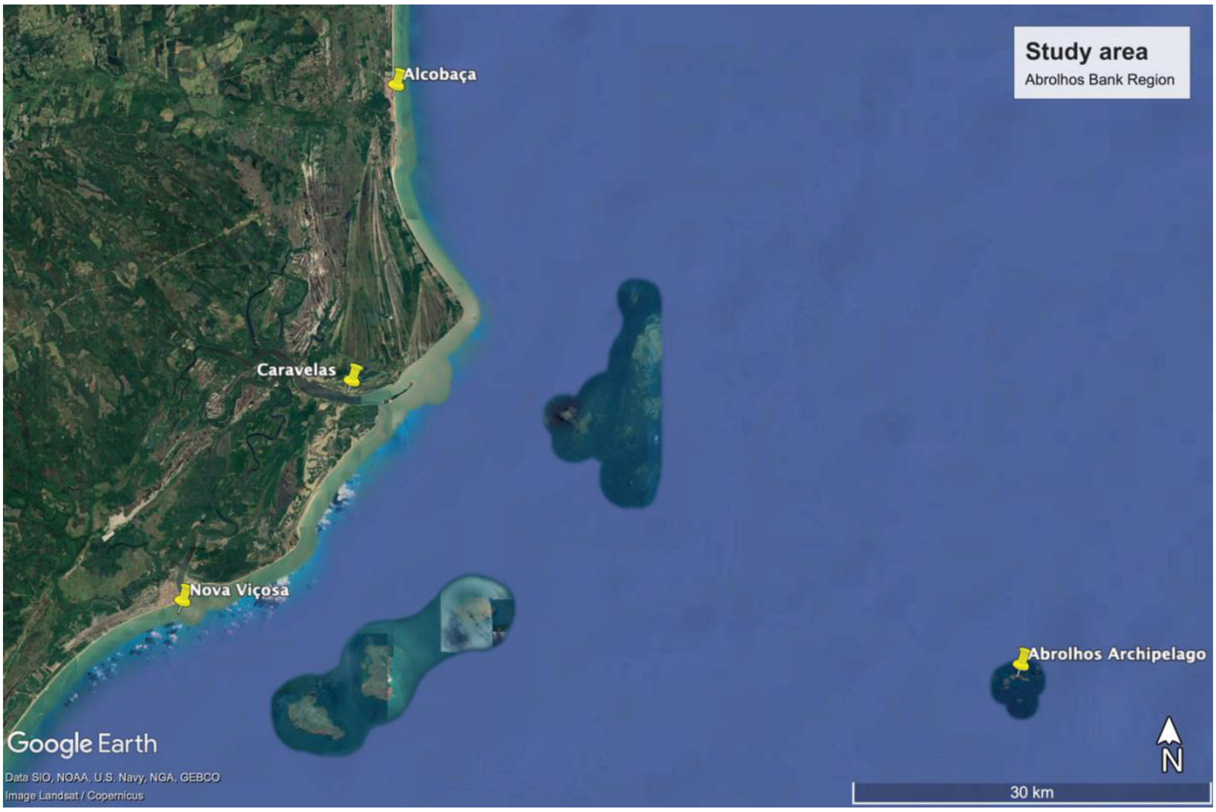
This study was approved by the PAR 02022.000682/2014-96 COEXP/IBAMA, received on January 29, 2015, along with Authorization for Capture, Collection and Transport of Biological Material (ACCTMB) n° 560/2014.

4.2.2. Study area

The Abrolhos Bank Region is located between the municipality of Prado, State of Bahia (BA) (16040'S), and the Rio Doce estuary, State of Espírito Santo (ES) (190 40'S). It comprises an enlargement of the Brazilian eastern continental shelf, with approximately 46000km2. This landscape holds the largest reef formations in Brazil, calcareous algae plains, important estuarine areas, and volcanic features that constitute the Abrolhos Archipelago (MARCHIORO; NUNES, 2003).

The study was conducted in three municipalities of this region: Nova Viçosa, Caravelas and Alcobaça, according to Figure 1, including areas of open seawater and protected waters. The open seawater is located between the continent and the Abrolhos Archipelago, bordering the limits of this Marine National Park. The protected waters are located between the mouth and continental portions of these water bodies, in the locations between Nova Viçosa and Alcobaça.

Figure 5. General overview of the study area. Google Earth.



4.2.3. Study species

Our study involved three species of birds, the magnificent frigatebird (*Fregata magnificens*) as a specimen associated with open sea (marine bird), and two other specimens from estuarine environment (estuarine birds), which included the yellow-crowned night heron (*Nyctanassa violacea*) and the little blue heron (*Egretta caerulea*).

The magnificent frigatebird (*F. magnificens*) belongs to the Fregatidae family, occurs in the tropical Atlantic and eastern Tropical Pacific, and has long pointed wings, which provides a very efficient energy use during foraging, allowing those birds to displace for long distances with minimal energy costs, as well as a long hooked beak. Their inadequate waterproof plumage prevents safe swimming, they usually feed on small fishes on the sea surface or may also piracy on boobies, seagulls or terns.(SCHREIBER; BURGER, 2002). They are distributed along the Brazilian coast, frequently with colonies offshore (“WikiAves”, 2016).

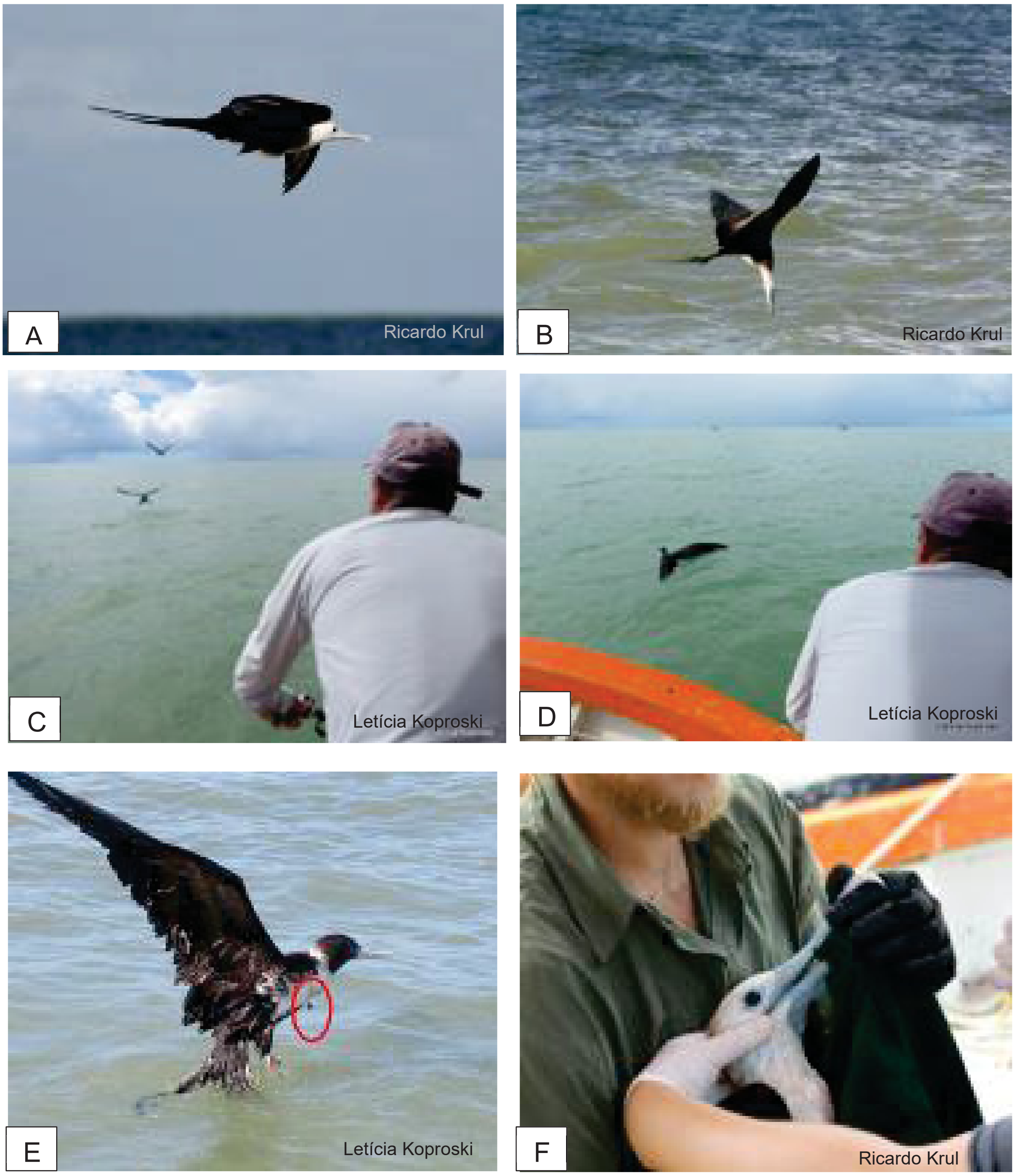
The yellow-crowned night heron (*N. violacea*) belongs to the Pelecaniformes order, is widely distributed in the Brazilian coast and can also be found from North America to northern Peru, especially common in coastal areas, but also inland, always associated with water (SOMENZARI et al., 2018). Previous studies in other locations found that *E. caerulea* feed on a limited variety of food (stenophagous), and the majority of food items are crustaceans (MARTÍNEZ, 2004).

Also a member of the Pelecaniformes order, the little blue heron (*E. caerulea*) can be found from North America to southern South America, particularly associated with mangrove forest. Generally, their diet can be diverse, including fish, crustaceans, amphibians and insects, but prey items depend on the occurrence region (KUSHLAN; HANCOCK, 2005).

4.2.4. Sample collection

Our study was performed in 2015, in four different periods of sample collection along the year (Campaigns Feb/May/Sep/Dec). The bird's handling was performed with personal safety equipment. In protected waters, capture of juveniles was performed manually or with the aid of dip nets in nests and branches nearby. In the open sea, the birds were attracted to the boat with fishery discards and capture was performed using a sport fishing rod without the hook. After being hit by the line with the sinker, the bird cannot fly properly, so the boat approaches and the bird can be manually captured or with the aid of dip nets (Figure 6).

Figure 6. Young individual of *Fregata magnificens*, approaching the boat (A) and catching discarded fish (B). Illustration of the capture technique from the moment the line is cast over the bird (C); Detail of the sinker on the bird (D); flight restriction (E) and immobilization of the bird in the boat (F).



After the capture, the birds were manually restrained for physical exams, cloacal swabs, feather collection and blood drawing (Figure 7). The physical examination comprised inspection of skin, eyes and oral cavity, as well as weight, body condition score, auscultation and integument inspection for ectoparasites. Blood was drawn from jugular, metatarsal or ulnar veins, with a volume range of 0,5 to 4,0 ml (according to their body mass) and stored refrigerated in specific containers. Feathers were collected from wings, tail and pectoral area, and stored in paper envelopes. The restraint time ranged from 10 to 30 minutes.

Figure 7. Nestlings of *E. caerulea* (A); Feather collection of *E. caerulea* (B); General examination of *N. violacea* (C); blood drawing (D), feather collection (E) and cloacal swab (F) in *Fregata magnificens*, captured at sea. Pictures: Ricardo Krul.

4.2.5. Sample analysis

a. Clinical evaluation and Blood parameters

Clinical observations were organized in tables. Data obtained through previously defined qualitative scales, such as the variables "Body Condition Score" and "Degree of infestation by ectoparasites" had their variation classes arranged in ordinal scales, from least to most favorable, to enable statistical analysis.

Total blood stored in EDTA tubes was used for microhematocrit and total plasma protein (TPP) analysis.

b. PCR

Cloacal swabs and total blood were forwarded to polymerase chain reaction (PCR) test for seven pathogens, including Avipoxvirus, *Chlamydophyla psittaci*, *Mycoplasma* spp., *Salmonella* spp., *Toxoplasma gondii*, Influenza A virus and Paramyxovirus.

c. Polycyclic aromatic hydrocarbon analysis

Blood plasma was used for polycyclic aromatic hydrocarbon analysis, measured by Gas Chromatography Coupled with Mass Spectrometry according to EPA method 8270D (modified), except for Naphthalene carried out by method 8260 B (modified). The compounds evaluated were: acenaphthene; acenaphthylene; anthracene; benzo(a)anthracene; benzo(a)pyrene; benzo(b)fluoranthene; benzo(g,h,i)perylene; benzo(k)fluoranthene; chrysene; dibenzo(a,h)anthracene; phenanthrene; indeno(1,2,3-c,d)pyrene; fluoranthene; fluorene; naphthalene; pyrene. The minimum measurable limit in the samples for each parameter was 0.01 µg/L.

d. Metal analysis in feathers

For metals and metalloids analysis, mass dilution calculations were used to quantify the final concentrations. For this purpose, sub aliquots of the sample extracts were prepared directly in the vials and mass measurements made for the calculation. The vials were prepared by diluting an aliquot of the final digestion extract in HNO32%, prepared with ultrapure water processed in a PURELAB Ultra (model Ultra an MKZ, from Elga) and distilled HNO3 65% (DistillAcidsub-boiling BSB-939-IR). A multi-element internal standard (Internal Standard Mix - Bi, Ge, In, Li, Sc, Tb and Y, Agilent Technologies) was used for corrections of possible fluctuations in the signals of the analyzed element measurements. Calibration curves were built ranged between 0.5 - 300 ng/g, based on the following standards: multi-element (ICP multi-element standard solution XXI for MS, CentiPUR® MERCK, Darmstadt - Germany) and single-element (Boron ICP standard, CentiPUR® MERCK, Darmstadt - Germany).

The digestion procedure of the samples followed EPA method 3052 (*Microwaveassistedaciddigestionofsiliceousandorganicallybasedmatrices*). Approximately 0.25 g of samples, previously lyophilized and macerated, were digested in Teflon® tubes with 9 ml of distilled HNO3 (65 %) and 2 ml of H2O2 using a microwave oven (Mars X-press CEM). Each sample was heated to 180 ± 5 º C for 5.5 minutes and remain at 180 ± 5 º C for 9.5 minutes. The watery extract was filtered on quantitative filter paper (Whatmann 40) and trace element analysis performed using Agilent ICP-MS (Inductively Coupled Plasma - Mass Spectroscopy) model 7500 cx. The validation of EPA method 3052, for the analysis of metals and metalloids, was performed by means of an accuracy test with DORM-3 (Fishproteincertifiedreference material for trace metals) and DOLT-4 (Dogfishlivercertifiedreference material for trace metals) certified reference material (MCR).

The quantification of the analyzed elements complies with the descriptions in the EPA 6020A method for multi-element determination of analytes using ICP-MS (Inductively Coupled Plasma - Mass Spectroscopy) equipment. The analytical quantification of the elements was determined through a calibration curve by linear regression ($y = ax + b$), using different readout modes (No Gas mode, H mode and He mode). The choice of the specific reading mode for each element was determined by the recovery values of the certified reference materials (CRM). The detection and quantification limits were calculated by measuring standard whites and their standard deviations.

4.2.6. Statistical analysis

Descriptive statistics were used for the presentation of percentages of abundance, frequency or prevalence of data in general. In order to evaluate normality of the data, Shapiro-Wilk tests were performed “a priori”. Unpaired T-tests were used for comparison of numeric variables within the same species. One-way analysis of variance (ANOVA) and Multivariate tests were used to verify the significance of the evaluated differences among species. Contingency tables were constructed for better comprehension of some groups of data. Analysis and graphical illustrations were made in Graph Prism and Jamovi Software.

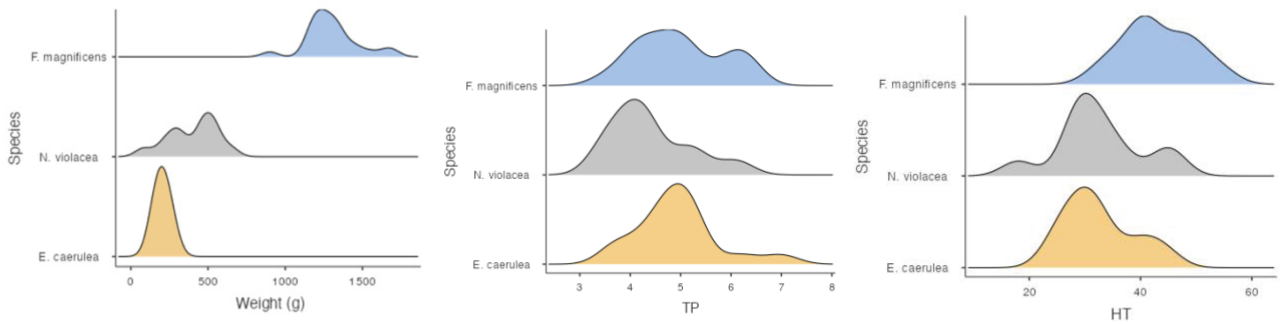
4.3. RESULTS

Over the four sampling campaigns, 64 individuals among the three species selected for the study were captured. Individuals of *Fregata magnificens* were captured in all campaigns, totaling 33 over the year and representing specimens of open seawater. Bird species from protected waters totaled 31, being 14 individuals of *N. violacea*, and 17 individuals of *E. caerulea*. It was not possible to evaluate all variables in all animals sampled in the study, due to field study limitations, thus, the data presented consider the missing values.

4.3.1. Clinical aspects

The mean values for body mass, total plasmatic protein and hematocrit for *F. magnificens* were 1.303kg ± 0.171 (n=24), 5mg/dL ± 0.861 (n=19), 41.8% ± 6.41 (n=16); for *N. violacea* was 0.402kg ± 0.160 (n=14), 4.2mg/dL ± 0.804 (n=11), 31.5% ± 8.055 (n=11); and for *E. caerulea* was 0.200kg ± 0.040 (n=17), 4.9mg/dL ± 0.797 (n=17), 32% ± 6.080 (n=17). In the following histograms, the difference between the bird species of this study regarding the quantitative variables can be better understood.

Graphic 1. Histogram showing the distribution of weight, total protein (TP) and hematocrit (HT) frequencies for each species (*F. magnificens*, n=24; *N. violacea*, n=14; *E. caerulea*, n=17). Jamovi.



The body condition score was evaluated in all sampled species and the classification in each score (good, moderate, emaciated, extremely emaciated) can be seen in the Table 1. Frequencies for "Degree of infestation by ectoparasites" and presence or absence of clinical signs in each species are also organized in the Tables 2 and 3.

Table 1. Number of individuals in each category of body condition score (BCS), according to the

Frequencies of BCS

		Species	
BCS	*F. magnificens*	*N. violacea*	*E. caerulea*
Good	13	8	3
Moderate	14	4	6
Emaciated	6	1	8
Extremely emaciated	0	1	0

Table 2. Number of individuals registered with or without clinical signs, according to the species.

Frequencies of clinical signs

	Species		
Signs	*F. magnificens*	*N. violacea*	*E. caerulea*
No	20	10	7
Yes	13	3	10

Table 3. Number of individuals in each category of ectoparasites infestation, according to the species.

Frequencies of Parasites

	Species		
Parasites	*F. magnificens*	*N. violacea*	*E. caerulea*
Null	6	13	13
Low	19	0	0
Signs	2	0	0
Medium	5	0	2
High	1	0	0

Ectoparasites found included feather lice and hippobocids. The *F. magnificens* showed mostly low parasite infestation, only two individuals of *E. caerulea* showed medium degree of infestation, while the others and all individuals of *N. violacea* had no signs of ectoparasites.

Most of the examined birds were considered clinically healthy, showing no relevant signs of any diseases that could affect their short-term survival, despite mild respiratory and digestive clinical signs. Except for a young *Nyctanassa violacea*, which was emaciated and dehydrated.

4.3.2. PCR

Frequencies of individuals that were positive or negative for any PCR analysis divided by species can be seen in the Table 4.

Table 4. Number of individuals that showed positive or negative results in PCR analysis for at least one agent, according to the species. Jamovi.

Frequencies of PCR

	Species		
PCR	*F. magnificens*	*N. violacea*	*E. caerulea*
Negative	19	2	13
Positive	14	11	4

In February, three out of eight sampled *F. magnificens* were positive, and genetic material for *Avipoxvirus*, *Mycoplasma* spp. and Influenza A virus was detected.

From eight *F. magnificens* sampled in May, one individual was positive for *Chlamydophyla psittaci*, one for *Mycoplasma* spp., and two were positive for both *Mycoplasma* spp. and *Salmonella* spp. In September, four out of nine *F. magnificens* were positive only for *Salmonella* spp., two only for *Mycoplasma* spp. and one for both *C. psittaci* and *Salmonella* spp. Also in September, genetic material of *Mycoplasma* spp. was found in six from seven *N. violacea* samples. In December, only one from eight *F. magnificens* was positive for *Mycoplasma* spp. As for *N. violacea*, five of six were positive for *Mycoplasma* spp. Regarding 17 *E. caerulea*, two were positive for *Salmonella* spp., one for *Mycoplasma* spp., and one for both *C. psittaci* and *Salmonella* spp.

4.3.3. PAH

No external visible contamination of the birds by petroleum products was detected, either in the individuals handled for biological collections or in other observations.

In the present study, 33 seabird samples obtained from February to December 2015 and 31 estuarine bird samples obtained from September and December 2015 were evaluated. None of the samples evaluated (n = 64) showed concentrations of PAHs higher than the quantifiable minimum (< 0.01 µg/L).

4.3.4. Metals

The quantification of heavy metals was performed in 23 feather samples of *F. magnificens,* nine *N. violacea* and 18 *E. caerulea* from the Abrolhos Bank Region. For interpretation purposes, ten feather samples of *F. magnificens* from Fernando de Noronha Archipelago were also analyzed and used here as a control group.

Values obtained for each sample are in the Tables 5, 6 and 7.

Table 5. Analysis results of heavy metals and arsenic in feathers of *Fregata magnificens*, collected from birds captured around fishing vessels on the inner continental shelf, opposite the town of Nova Viçosa, BA, between February and December 2015.

Sample	As	Cr	Cu	Fe	Mn	Ni	Zn	Pb	Cd	Co	Sr	Al
LD	0,009	0,001	0,004	0,001	0,000	0,002	0,001	0,006	0,001	0,003	0,006	0,003
LQ	0,029	0,002	0,012	0,004	0,000	0,006	0,004	0,019	0,003	0,015	0,019	0,011
Recovery (%)	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**Pb**	**Cd**	**Co**	**Sr**	**Al**
DORM 3	94,84	104,76	101,48	95,39	95,65	92,19	101,48	83,54	96,90	106,02	-	103,24
DOLT 4	92,30	114,29	100,52	95,25	-	90,72	100,52	118,75	90,67	-	-	101,00
µg/g	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**Pb**	**Cd**	**Co**	**Sr**	**Al**
AM BA 10	0,26	0,02	0,012	22,16	6,07	0,006	48,23	0,13	<LQ	<LQ	76,32	8,38
AM BA 11	0,67	0,002	2,98	150,87	22,00	0,07	59,89	0,14	<LQ	<LQ	59,61	71,61
AM BA 12	0,73	0,002	0,72	32,00	5,10	0,01	51,04	0,15	<LQ	<LQ	46,47	9,16
AM BA 13	0,46	0,002	0,012	28,59	7,43	0,006	43,16	0,15	<LQ	<LQ	64,85	8,26
AM BA 14	0,44	0,01	0,012	20,27	7,12	0,006	47,39	0,18	<LQ	<LQ	21,04	7,19
AM BA 15	0,71	0,002	0,12	15,60	3,61	0,08	53,17	0,18	<LQ	<LQ	33,17	3,58
AM BA 16	0,51	0,75	2,78	51,38	11,77	4,24	48,95	0,14	<LQ	<LQ	21,31	24,75
AM BA 19	0,49	0,18	5,88	21,07	3,29	0,006	36,16	0,18	<LQ	<LQ	12,95	18,75
AM BA 20	0,32	0,04	0,012	21,15	6,05	0,37	37,96	0,15	<LQ	<LQ	57,22	6,31
AM BA 21	0,88	0,73	1,90	54,70	11,78	3,24	55,89	0,21	<LQ	<LQ	34,36	12,08
AM BA 22	0,57	0,19	7,61	364,57	28,23	0,08	60,68	0,15	<LQ	<LQ	43,79	163,26
AM BA 24	0,55	0,002	1,51	34,01	6,40	0,006	57,46	0,13	<LQ	<LQ	76,25	12,96
AM BA 25	0,21	0,02	0,012	18,16	5,91	0,006	36,03	0,12	<LQ	<LQ	40,41	7,27
AM BA 26	0,44	0,002	0,012	7,60	2,40	0,006	47,81	0,11	<LQ	<LQ	32,75	1,82
AM BA 27	0,66	0,002	0,40	18,76	4,02	0,13	36,45	0,15	<LQ	<LQ	29,48	5,37
AM BA 28	0,24	0,13	1,71	7,25	0,84	0,57	42,07	0,15	<LQ	<LQ	0,72	27,23
AM BA 29	0,52	0,16	1,94	8,18	1,41	2,81	52,98	0,19	<LQ	<LQ	23,49	26,67
AM BA 30	0,53	0,15	2,01	17,54	2,66	1,54	50,73	0,16	<LQ	<LQ	31,00	23,69
AM BA 31	0,11	0,15	1,85	9,42	1,21	0,32	39,25	0,14	<LQ	<LQ	39,30	24,14
AM BA 32	0,59	0,08	1,04	21,88	3,66	0,006	55,08	0,19	<LQ	<LQ	45,36	7,23
AM BA 33	0,34	0,04	2,37	26,62	5,22	3,39	562,39	0,22	<LQ	<LQ	52,71	6,40
AM BA 34	0,39	0,12	4,66	17,47	5,54	0,96	43,94	0,18	<LQ	<LQ	36,18	21,95
AM BA 35	0,34	0,002	0,012	11,58	3,20	0,01	42,60	0,15	<LQ	<LQ	23,01	2,56
Mean	**0,477**	**0,1211**	**1,7202**	**42,64**	**6,736**	**0,777**	**69,97**	**0,1587**	**-**	**-**	**39,21**	**21,766**
SD	**0,187**	**0,2066**	**2,0247**	**76,1**	**6,518**	**1,312**	**107,61**	**0,028**	**-**	**-**	**19,12**	**34,214**
Min	**0,11**	**0,002**	**0,012**	**7,25**	**0,84**	**0,006**	**36,03**	**0,11**	**-**	**-**	**0,72**	**1,82**
Max	**0,88**	**0,75**	**7,61**	**364,6**	**28,23**	**4,24**	**562,39**	**0,22**	**-**	**-**	**76,32**	**163,26**

Table 6. Analysis results of heavy metals and arsenic in feathers of *Nyctanassa violacea* (samples 01 to 14) and *Egretta caerulea* (samples 15 to 32), collected from young birds captured in nests found in mangroves in the region of Nova Viçosa and Caravelas, BA, between February and December 2015.

Sample	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**Pb**	**Cd**	**Co**	**Sr**	**Al**
LD	0,009	0,001	0,004	0,001	0,000	0,002	0,001	0,006	0,001	0,003	0,006	0,003
LQ	0,029	0,002	0,012	0,004	0,000	0,006	0,004	0,019	0,003	0,015	0,019	0,011
Recovery (%)	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**Pb**	**Cd**	**Co**	**Sr**	**Al**
DORM 3	94,84	104,76	101,48	95,39	95,65	92,19	101,48	83,54	96,90	106,02		103,24
DOLT 4	92,30	114,29	100,52	95,25		90,72	100,52	118,75	90,67			101,00
µg/g	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**Pb**	**Cd**	**Co**	**Sr**	**Al**
AE BA 01	0,50	0,002	3,29	45,29	2,79	0,006	70,49	0,13	<LQ	<LQ	44,56	11,08
AE BA 02	1,03	0,31	4,84	78,52	6,10	0,006	60,87	0,22	<LQ	<LQ	28,47	4,44
AE BA 03	0,88	0,56	3,13	61,11	5,85	0,006	61,77	0,19	<LQ	<LQ	34,53	1,15
AE BA 08	3,01	0,20	13,23	74,99	8,86	0,006	80,65	0,87	<LQ	<LQ	51,74	19,48
AE BA 09	4,19	0,002	11,94	373,41	70,20	0,006	91,11	0,93	<LQ	<LQ	551,29	134,75
AE BA 11	0,72	0,002	2,09	24,49	5,43	0,006	52,33	0,14	<LQ	<LQ	28,00	8,92
AE BA 12	0,59	0,002	1,81	64,43	12,01	0,006	58,55	0,12	<LQ	<LQ	36,89	29,81
AE BA 13	0,35	0,002	4,50	34,00	3,35	0,006	51,63	0,13	<LQ	<LQ	30,37	3,34
AE BA 14	0,67	0,002	0,012	18,11	3,88	0,08	46,57	0,13	<LQ	<LQ	39,06	4,72
AE BA 15	1,03	0,46	10,78	34,54	3,40	0,006	55,98	0,26	<LQ	<LQ	7,14	32,58
AE BA 16	3,10	1,02	20,52	45,51	41,57	0,06	70,90	0,76	<LQ	<LQ	35,26	117,09
AE BA 17	1,40	0,17	8,69	92,74	12,01	0,006	70,30	0,36	<LQ	<LQ	17,01	19,96
AE BA 18	0,57	0,19	9,69	27,00	3,04	0,006	67,31	0,17	<LQ	<LQ	4,70	24,54
AE BA 19	2,25	0,67	22,09	54,52	7,09	1,07	66,73	0,62	<LQ	<LQ	37,74	98,29
AE BA 20	1,44	0,002	9,99	58,33	5,26	0,006	68,57	0,41	<LQ	<LQ	14,37	6,60
AE BA 21	1,61	0,51	12,08	23,66	3,05	0,006	64,88	0,37	<LQ	<LQ	14,15	57,91
AE BA 22	2,25	0,58	15,25	37,30	4,89	0,006	59,39	0,54	<LQ	<LQ	33,83	87,84
AE BA 23	1,64	1,30	11,72	33,86	8,04	0,03	51,50	0,33	<LQ	<LQ	0,21	36,38
AE BA 24	0,60	0,52	0,90	16,75	2,35	0,47	48,45	0,18	<LQ	<LQ	15,35	20,53
AE BA 25	1,13	0,16	6,85	28,81	6,48	0,006	52,92	0,25	<LQ	<LQ	7,53	0,28
AE BA 26	0,51	0,002	7,82	34,62	3,53	0,006	65,36	0,27	<LQ	<LQ	9,05	4,63
AE BA 27	<LQ	0,35	13,19	21,71	3,55	1,35	60,75	0,38	<LQ	<LQ	15,66	66,29
AE BA 28	0,66	0,18	9,15	22,29	1,99	0,08	48,11	0,16	<LQ	<LQ	9,26	30,79
AE BA 29	0,63	0,42	12,49	42,15	5,50	0,006	61,36	0,44	<LQ	<LQ	20,74	56,34
AE BA 30	1,49	0,05	7,16	67,02	17,48	0,006	64,92	0,50	<LQ	<LQ	11,57	15,48
AE BA 31	0,51	0,14	11,79	37,66	3,00	0,006	57,04	0,21	<LQ	<LQ	7,55	30,32
AE BA 32	0,54	0,05	7,39	25,60	4,26	0,006	55,98	0,14	<LQ	<LQ	8,71	4,28
Mean	**1,28**	**0,29**	**8,98**	**54,76**	**9,44**	**0,12**	**61,64**	**0,34**	**-**	**-**	**41,29**	**34,36**
SD	**0,97**	**0,33**	**5,52**	**66,79**	**14,39**	**0,33**	**10,12**	**0,23**	**-**	**-**	**102,87**	**37,11**
Mín	**0,35**	**0,00**	**0,01**	**16,75**	**1,99**	**0,01**	**46,57**	**0,12**	**-**	**-**	**0,21**	**0,28**
Max	**4,19**	**1,30**	**22,09**	**373,41**	**70,20**	**1,35**	**91,11**	**0,93**	**-**	**-**	**551,29**	**134,75**

Table 7. Analysis results of heavy metal and arsenic in feathers of *Fregata magnificens*, collected from birds captured on the beach around the largest island of the Fernando de Noronha Archipelago, PE, in November 2015.

Sample	As	Cr	Cu	Fe	Mn	Ni	Zn	Pb	Cd	Co	Sr	Al
LD	0,009	0,001	0,004	0,001	0,000	0,002	0,001	0,006	0,001	0,003	0,006	0,003
LQ	0,029	0,002	0,012	0,004	0,000	0,006	0,004	0,019	0,003	0,015	0,019	0,011
Recovery (%)	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**Pb**	**Cd**	**Co**	**Sr**	**Al**
DORM 3	94,84	104,76	101,48	95,39	95,65	92,19	101,48	83,54	96,90	106,02	-	103,24
DOLT 4	92,30	114,29	100,52	95,25	-	90,72	100,52	118,75	90,67	-	-	101,00
µg/g	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**Pb**	**Cd**	**Co**	**Sr**	**Al**
AM FN 01	0,69	0,32	2,72	11,19	1,46	0,41	32,60	0,17	<LQ	<LQ	16,02	22,95
AM FN 02	0,73	0,002	1,00	41,11	8,53	0,58	40,76	0,17	<LQ	<LQ	141,73	13,50
AM FN 03	0,029	0,09	2,78	5,58	1,46	0,17	35,06	0,15	<LQ	0,03	21,10	26,04
AM FN 04	0,30	0,002	0,012	18,54	4,55	0,17	39,76	0,16	<LQ	<LQ	64,31	4,03
AM FN 26B	1,90	0,03	1,06	67,52	13,56	0,23	41,50	0,39	<LQ	<LQ	67,64	27,69
AM FN 26	0,54	0,44	0,78	8,67	1,65	0,24	37,72	0,19	<LQ	<LQ	21,06	25,81
AM FN 27B	1,94	0,74	9,06	27,66	12,91	0,49	39,14	1,03	<LQ	<LQ	0,77	81,21
AM FN 27	0,64	0,002	3,69	37,35	5,53	0,35	40,26	0,16	<LQ	<LQ	39,50	7,58
AM FN 28B	2,31	0,04	1,24	16,30	14,99	0,10	43,99	0,44	<LQ	<LQ	29,83	0,54
AM FN 28	0,74	0,64	1,23	29,90	4,97	1,34	44,52	0,22	<LQ	<LQ	18,27	23,73
Mean	**0,98**	**0,23**	**2,36**	**26,38**	**6,96**	**0,41**	**39,53**	**0,31**	**-**	**0,03**	**42,02**	**23,31**
SD	**0,78**	**0,28**	**2,61**	**18,81**	**5,23**	**0,36**	**3,68**	**0,27**	**-**	**-**	**40,92**	**22,66**
Min	**0,03**	**0,00**	**0,01**	**5,58**	**1,46**	**0,10**	**32,60**	**0,15**	**-**	**0,03**	**0,77**	**0,54**
Max	**2,31**	**0,74**	**9,06**	**67,52**	**14,99**	**1,34**	**44,52**	**1,03**	**-**	**0,03**	**141,73**	**81,21**

4.4. DISCUSSION

The main objective of health assessment in birds as sentinels in the marine environment is to develop a database that allows a correlation between the health status, individual and population, and the levels of detectable hydrocarbons and/or heavy metals in these birds. This is the only way to confirm the negative influence or not of contamination on these birds, as indicators of the ecosystem quality.

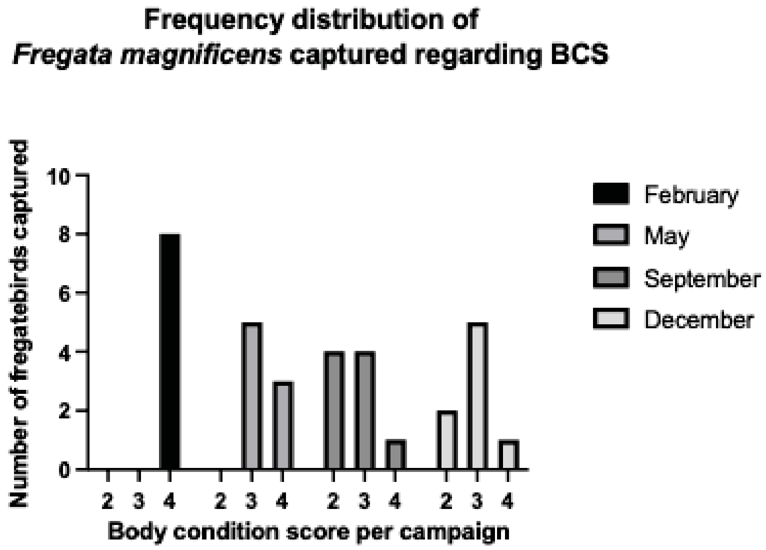
Body condition is a key parameter that is often related to behavior, reproduction and survival of birds, because it may reflect the amount of nutritional reserves and immunity against parasites and diseases (LABOCHA; HAYES, 2012; SUTHERLAND; NEWTON; GREEN, 2004). In the present study, two patterns were observed, according to the species and location. In protected waters, due to inadequacy of other forms of capture and collection restricted to non-destructive methods, efforts were directed at young/nestlings birds. Therefore, the information from this environment is limited to the period of spring and early summer. Also, these individuals were still fed by their parents, and had low muscle development scores, which is considered normal for this age (RIVERA, 2008). Thus, their body score depends mainly on the ability of their parents to provide food, which is influenced by food availability or brood size (CORNELIUS RUHS et al., 2020). However, several health problems, especially enteric infections, may also be responsible for poorer body development conditions of the chicks (JIMÉNEZ-PEÑUELA et al., 2019) .

For open ocean birds, body score changes during different seasons of the year are expected, as they depend on highly unpredictable food resources (STIENEN; BRENNINKMEIJER, 2002). For example, the interaction of *F. magnificens* with fishing is notable, especially with discards from shrimp trawling (KRUL, 2004). Thus, the seabirds tend to lose body mass in periods of low food supply, such as out of shrimp fishing season , which may generate a metabolic stress and predispose the emergence of diseases and clinical manifestation of latent infections, as observed by Mangini (2010) (MANGINI, 2010).

Despite the low range of body score variation recorded, which probably did not allow the observation of significant differences, there is a tendency for a different distribution of the *F. magnificens* body score according to the sample period, as demonstrated in the Graphic 2.

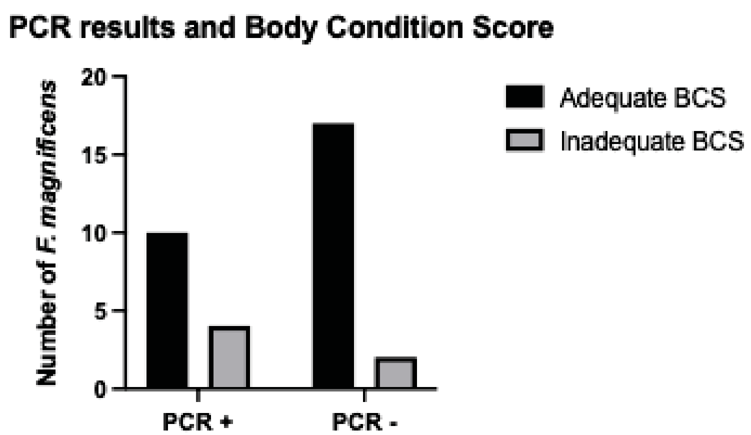
In February, there was a greater proportion of birds with good body scores. On the other hand, a greater number of birds with moderately low body score were observed in May, and with a more accentuated reduction in September, when more animals were considered lean or moderately lean. This drop could be expected, considering that the shrimp season has been closed, for three months, until the second half of May. The low body condition of the birds may be a consequence of the low supply of fishery discards, the parasite status and/or diseases indicated by PCR findings, since weak and debilitated birds usually present poorly developed pectoral muscles (DONELEY; HARRISON; LIGHTFOOT, 2006). However, other factors related to food supply or environmental changes may also have influenced the body score as well as incidence of pathogens and ectoparasites, since no association between those variables could be established with significance in this study. When compared to May, the variation in body score in December was not evident, indicating a tendency for the general body condition of the birds to return at the end of that year.

Graphic 2. Frequency of *F. magnificens* according to body condition score (1=extremely emaciated, 2=emaciated, 3=moderate, 4=good) and divided by campaigns. Graph Prism.



For our analysis, the Good and Moderate conditions were considered adequate and indicative of good health status, while the Emaciated and Extremely Emaciated conditions were considered inadequate. Therefore, for *F. magnificens* no significance was found when comparing TP, HT and body mass values between those two categories. In a contingency analysis, 66% of the *F. magnificens* with inadequate BCS were positive to at least one of the surveyed pathogens, while among the birds with adequate BCS this ratio was 37%.

Graphic 2. Frequency of *F. magnificens* with Adequate or Inadequate Body Condition Score that showed positive or negative results by PCR analysis. Graph Prism.



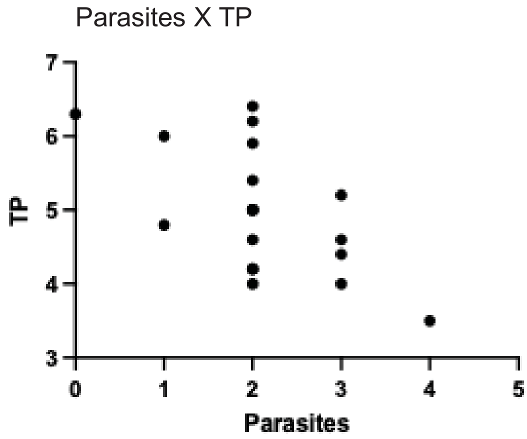
Regarding *E. caerulea*, 37.1% with adequate BCS were positive to at least one of the surveyed pathogens, and 12.5% of the positive birds presented inadequate BCS. This comparison was not possible with *N. violacea*, as only one bird sampled was categorized as inadequate BCS. For the reasons described before and the results we obtained, the BCS may be not reliable when evaluated alone, specially in nestlings, although low BCS are usually related to poor prognosis (DONELEY; HARRISON; LIGHTFOOT, 2006).

The clinical signs observed in the physical examination, when evaluated alone, are not sufficient to categorize the animals as sick. The results together, though, indicate that the general state of health of several individuals sampled is altered. Although this does not mean that these animals cannot survive in nature in the short and medium term, the results indicate that in May and September there was a greater sensitivity of the seabird population to health problems. In *F. magnificens*, when comparing clinical signs among positive and negative PCR, the birds appeared to have 20% more risk of developing clinical signs when positive for one of the pathogens surveyed.

The evaluation of the degree of infestation by ectoparasites also represents a way to access the general health conditions of birds, as well as the environments where they live, considering that less altered environments tend to have a less expressive load of external and hematologic parasites (MANGINI, 2010). However, the parasite load depends on seasonal factors beyond its relationship with individual and environmental health and may also be a consequence of poor conditioning instead of its cause (SUTHERLAND; NEWTON; GREEN, 2004). No clear differentiation in ectoparasite burden between the May, September, and December campaigns can be noted, with only a trend toward lower infestations in September and December. Only in February, a clearly lower load of ectoparasites in the sampled birds was observed. This is consistent with other health indicators that show a better general condition of the birds in February. In general, birds associated with protected water environments showed zero parasite load, which did not allow us to use this parameter as an inference to assess the health status of these specimens.

Also, for *F. magnificens* it was possible to observe an inverse relationship between the presence of parasites and the total plasma protein value. In the Grafic 4, the bird most parasitized, had the lowest plasma protein value, while the highest protein values have no or low infestation, although for the overall regression analysis, there was no significance.

Graphic 3. Scatter plot comparing the degree of parasite infestation and total plasmatic protein values, using scores from 0 to 4 (0=null, 1=signs, 2=low, 3=medium, 4=high) in *F. magnificens* captured along the year in the Abrolhos Bank Region (BA). Graph Prism.



The pathogen analysis by PCR searched for common infectious agents in birds (ANDERSEN; FRANSON, 2007; DAOUST; PRESCOTT, 2007; LEIGHTON; HECKERT, 2007; LUTTRELL; FISCHER, 2007; STALLKNECHT et al., 2007; VAN RIPER; FORRESTER, 2007). Thus, our goal was to evaluate the positive results and their epidemiological distribution within the sampled population with other health indicators and the presence of environmental contaminants in birds. As well as to determine an epidemiological pattern for these pathogens in the population of birds associated with marine ecosystems at this location, also providing a baseline parameter for latter comparison in the case of a marine oil spill event.

From the marine birds (group of open ocean birds), 42% (14/33) were positive for detection of genetic material from one or more of the infectious agents studied. *Salmonella* spp. was the most prevalent agent among the magnificent frigatebirds, with eight positives, followed by *Mycoplasma* spp. with six cases. Genetic material from *Chlamydophyla psittaci* was detected in two animals and Influenza A and *Avipoxvirus* appeared with one positive each.

Among the estuarine birds, 13 individuals of *Nyctanassa violacea* were evaluated by PCR. *Mycoplasma* spp. was the only infectious agent identified in this species, but it was present in high prevalence with 11 positives (84.6%). While high, this prevalence can indicate a normal pattern for the population at this development stage, since all animals were considered healthy by physical examination. Still within the estuarine birds, 16 specimens of *Egretta caerulea* were sampled. In this taxonomic group, the results were quite different from those observed in *N. violacea*: the prevalent agent was *Salmonella* spp. with only three positive cases, followed by *Chlamydophyla psittaci* and *Mycoplasma* spp. with only one positive case for each agent.

In the last three campaigns, only genetic material of *Mycoplasma* spp; *Chlamydophyla psittaci* and *Salmonella* spp. was detected, showing a high prevalence of these three infectious agents on the bird population evaluated. Paramyxovirus was not found in these bird samples, which shows that such pathogen, though relevant to the health of domestic birds and other wild bird groups, are not good health indicators for marine or estuarine birds. The prevalence of *Mycoplasma* spp. and *Salmonella* spp. in the other hand, proved to be good indicators of the health profile of these birds. Additionally, *Chlamydophyla psittaci*, Influenza A and *Avipoxvirus* were presented in this study as secondary indicators despite those are extremely relevant to the bird's health in general. Although their prevalence is naturally low under normal conditions, they can cause significant outbreaks, which has already been recorded in different species (PETERSON, 2012).

The predominance of positive results for *Mycoplasma* spp. (84.6% positive) in *Nyctanassa violacea*, a species that uses exclusively protected water environments, is noteworthy. Whereas the *Fregata magnificens*, a bird almost exclusive of the offshore environment and that interacts strongly with commercial fishing, had a predominance of positive results for *Salmonella* spp. This strong aggregate distribution for *Mycoplasma* spp. in *Nyctanassa violacea* and *Salmonella* spp. in *Fregata magnificens* represents the most evident factor of epidemiological segregation between the sampled bird groups. Considering that both species are equally susceptible to the diseases studied, our results show that these groups of birds use the local environment in different ways, which causes them to be exposed in different proportions to the pathogens studied. Thus, the different prevalence observed indicates that ecological and environmental factors, as well as taxonomic features, influence the epidemiological pattern of each species, as previous studies with other species have demonstrated (MCCOY; LÉGER; DIETRICH, 2013).

This fact justifies the use of more than one vertebrate species as biological indicators, especially when seeking to assess the health of an ecosystem and the impacts that certain events can cause on the health of these environmental quality indicators.

Also, in the evaluation of the data from the physical-clinical examination, combined with the positive PCR response for the presence of infectious agents, we can observe two distinct situations. Considering all sampled birds, including those from marine and estuarine environments, among the most present and relevant clinical signs two groups can be distinguished: 1) birds that presented signs of altered respiratory capacity, represented by light or moderate respiratory rales, although auscultation does not allow us to determine whether such audible respiratory lesions are a consequence of a current active infection, or sequel of pathological processes already resolved by the organism; and 2) birds that presented signs indicative of intestinal dysfunction such as diarrhea (evidenced by altered plumage integrity in the cloacal region), presence of pasty feces or gas accumulation. Four individuals, within the sample universe, showed signs of functional alterations in both the respiratory and digestive systems.

To evaluate data from PCR and physical-clinical exams together, it is necessary to group the positive results for *Chlamydophyla psittaci* and *Mycoplasma* spp. and consider separately the positive ones for *Salmonella* spp. The three pathogens mentioned above are infectious agents that can remain in birds without causing clinical signs, called subclinical or latent infection. However, in situations of immune suppression, these pathogens can benefit by intensifying their reproduction rate, thus producing clinical signs. *Mycoplasma* spp. can cause respiratory changes such as sinusitis, aerosaculitis or pneumonia. *Salmonella* spp. causes mainly gastrointestinal changes such as diarrhea. Although clinical signs compatible with active infection by these two pathogens were observed in many positive sampled birds. However, it should be noted that in this survey there were other individuals sampled with positive response to these and other pathogens, that showed no signs related, which proves asymptomatic infection in the population, a situation expected for free-living animals (PETERSON, 2012).

Among the results, 20 were positive for *Chlamydophyla psittaci* and *Mycoplasma* spp., among which 15 were positive for one or more of these pathogens, but without respiratory signs. While five showed mild or moderate rates and positive results for the presence of infectious agents. This represents 75% of asymptomatic birds in the sample. Additionally, nine birds were identified with respiratory signs and did not show positive responses for any of the infectious agents surveyed, which indicates the participation of other airway pathogens.

Salmonella spp. was isolated in 10 birds and four showed signs of gastroenteric changes, which could be considered as an asymptomatic rate of aproximatelly 66%. However, from 13 individuals sampled that showed signs of digestive dysfunction, nine had no positive results for this pathogen, indicating the presence of other relevant pathogens that can cause digestive tract changes. It is also noteworthy that despite the high prevalence of *Mycoplasma* spp. (respiratory tract infectious agent) in *N. violacea*, few individuals showed signs of altered air sac functionality. This is another indication that this pathogen is apparently distributed in the population in an asymptomatic way, i.e., without causing evident damage to the birds' health.

Polycyclic aromatic hydrocarbons are among the most toxic hydrocarbons to wildlife (PARUK et al., 2014). Sublethal effects due to chronic exposure to PAHs have only been addressed recently (ESLER et al., 2002; PÉREZ et al., 2008, 2010). Chronic exposure to PAHs may cause liver damage, hemolytic anemia, weight loss, developmental changes in salt-secreting glands, enteric changes, immunosuppression, as well as tumors (BHARDWAJ; JINDAL, 2019; BOSTRÖM et al., 2002; BRIGGS; YOSHIDA; GERSHWIN, 1996; LEIGHTON, 1993; TROISI; BEXTON; ROBINSON, 2006). Few studies have measured PAHs in seabirds, most of them in tissue samples from dead birds, or euthanizing them to allow for tissue collection (BROMAN et al., 1990; CUSTER et al., 2000; KAYAL; CONNELL, 1995; TROISI; BEXTON; ROBINSON, 2006). Pérez et al (2008) (PÉREZ et al., 2008) conducted a study measuring blood levels of PAHs in gulls (*Larus michahellis*) for monitoring oil spill impacts. The authors indicate that measurement of PAHs in blood plasma is a sensitive technique even for small amounts of oils ingested by birds.

Traditional chromatography used for PAHs requires large sample volumes to achieve the sensitivity needed to assess the presence of hydrocarbons in tissues, generally more than can be obtained from small seabird samples (PARUK et al., 2014). Thus, more advanced techniques, such as Gas Chromatography with Triple Quadrupole Mass Spectrometer, with splitless injection system, are necessary to process samples with lower volume and, consequently, obtain from bioindicators, such as seabirds, data for environmental monitoring without the need to slaughter the animals.

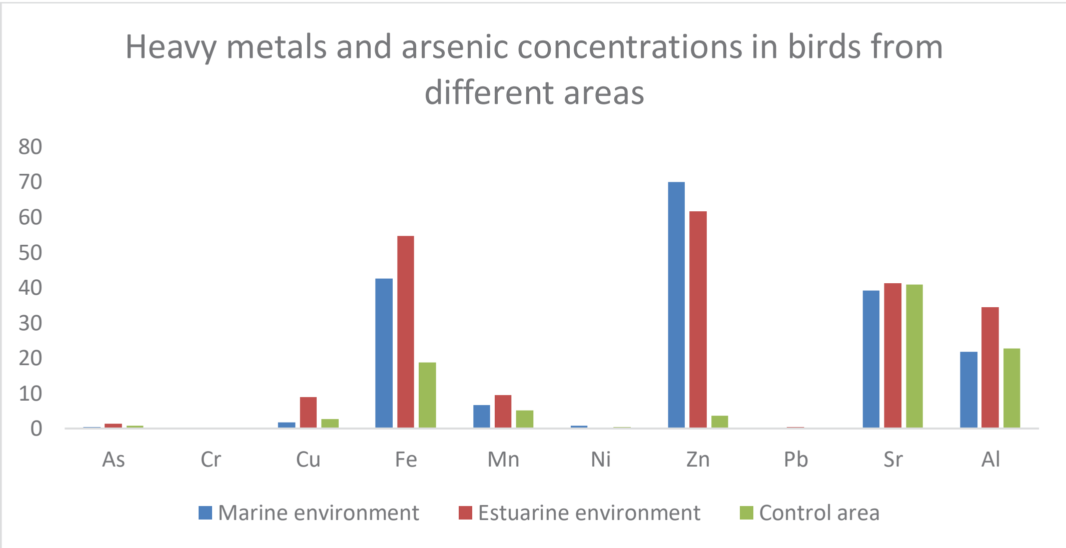
The evaluation of PAHs in the bloodstream, more precisely their concentration in plasma, is the best parameter to assess the impact of these pollutants on living organisms. The circulating levels represent the concentrations that are available to the tissues at the time the animals are evaluated, thus accessing PAH values that can be correlated with toxic effects on the organism (ALONSO-ALVAREZ; PÉREZ; VELANDO, 2007; PARUK et al., 2014; PÉREZ et al., 2008, 2010). Samples obtained from body tissues result in variable concentrations for different tissues and do not exactly reflect the bioavailability values of PAHs for the organism as a whole, considering that these compounds may be stored as "reserves" in tissues, such as feathers and muscle, or adipose tissue. Thus, PAHs are not widely available unless birds lose body mass. Additionally, obtaining blood samples from birds is a low invasive and non-destructive method. It can easily be repeated on the same individuals over time, allowing the degree of environmental decontamination to be monitored. A technique that can be considered the least invasive and most instructive method for monitoring environmental oil contamination (PARUK et al., 2014).

The lack of data on PAHs plasma concentration makes it impossible to directly interpret the concentrations obtained in a given species. As well, direct comparison of plasma values of HPAs for different species is difficult and should be avoided (PARUK et al., 2014). Thus, obtaining health data that contributes to the understanding of the effects of HPAs on birds are critical. Alonso-Alvarez et al. (2007) (ALONSO-ALVAREZ; PÉREZ; VELANDO, 2007) have already shown that differences in blood parameters (such as glucose; inorganic phosphorus levels) reflect sublethal effects of HPAs, with liver and kidney damage for *Larus michahellis*, reinforcing the need for studies that address various aspects of bird health, not just plasma levels of HPAs.

The PAHs tested in this study coincide with the 16 PAHs considered to be the most toxic by the U.S. Environmental Protection Agency (EPA) (KEITH, LARRY; TELLIARD, WILLIAM, [s.d.]). Among the birds sampled in our study, no measurable changes in blood PAHs were detected (< 0.01 µg/L), and it was not possible to correlate the health status of the birds and the pollutants assessed. The lack of PAHs values in the plasma of the birds evaluated in this study may only indicate that the birds did not have measurable circulating levels of PAHs at the time of sampling. This corroborates with other studies that have not detected elevated hydrocarbon levels in all sampled individuals, even when there were animals with measurable HPA levels detected in the study populations (PARUK et al., 2014). However, our results may indicate no environmental contamination in the study area, or even in low concentration or affecting a low percentage of the bird populations studied. Finally, the absence of measurable values for PAHs does not invalidate the methodology applied, but reinforces the urge for integrated health assessments along with the measurement of hydrocarbons. Especially considering that although it can be related to health damage to species common to the Brazilian coast, PAH reference values are not available.

Regarding heavy metal analysis, the mean concentrations of metals and arsenic were relatively close in the three environmental scenarios, which were: the protected or estuarine water environment, the open water environment of the inner continental shelf, and the control (Fernando de Noronha Archipelago). In the comparison with control, only Fe and Zn showed higher mean concentrations in both protected and open waters. In protected areas, Cu and Al showed higher mean concentrations than open waters and control.

Graphic 5. Comparison of mean metal and arsenic concentrations analyzed in bird feathers from coastal open sea (blue), protected or estuarine waters (red) and Control environments in Fernando de Noronha (green).



Cooper (Cu), iron (Fe), zinc (Zn), manganese (Mn), chromium (Cr) and nickel (Ni) are considered essential metals(loids), while arsenic (As) and lead (Pb) are considered non-essential and the role of aluminiun (Al) remains unclear. Some metals, such as zinc (Zn) and iron (Fe) are used as plastic additives and may accumulate in the food chain. Others are influenced by food items and nutritional condition. Therefore, the concentration of these elements in serum and tissue samples of seabirds should be associated with their general condition, as well as environmental features (ROMAN et al., 2020). In this study, the elevated concentrations of Fe and Zn in the birds from Abrolhos Bank Region may reflect some of these scenarios, when comparing to the control area, which is considered an area of less environmental impact.

Burger and Eichhorst (2007) and Bond and Diamond (2008) reported that the susceptibility of a bird to environmental contamination will depend on several variables related to their niche, physiology, age and life cycle (BOND; DIAMOND, 2008; BURGER; EICHHORST, 2007). Therefore, depending on the study design, it is preferable to sample nestling feathers instead of adult bird feathers (JASPERS et al., 2019). Feathers are widely used in environmental contamination assessment studies and monitoring projects worldwide (MARKOWSKI et al., 2013).

The determination of negative effects of contaminants on the sentinels' health requires knowledge of their concentration in target organisms. Therefore, experimental studies have been conducted to relate metal and arsenic contamination levels and changes in behavior, physiology, or reproductive success (BURGER et al., 2009; BURGER; GOCHFELD, 1997). However, many studies report only the doses and effects, without presenting tissue contamination levels.

The use of birds as sentinels of these contaminants is extremely advantageous due to their representative features, and ease of identification and sample collection. Also important, the sample collection can be performed in alive birds, is non-invasive or harmful and allows periodic monitoring (MARKOWSKI et al., 2013). The heavy metal analysis performed here occurred before the disaster of Mariana, and therefore, should be used as a reference baseline data by other studies.

4.5. CONCLUSIONS

The sampling method and parameters evaluated in this study to assess seabird health profiles were effective and results can be used for future predictive modeling, risk analysis and conservation efforts. However, some limitations could have interfered with the statistical analysis and prevented some associations between variables. These limitations include evaluation of subjective parameters, such as BCS, clinical signs and degree of ectoparasite infestation; challenges in capturing adult specimens of sea and waterbirds; and access to gold standard equipment for PAH and heavy metal analysis. The evaluation of the epidemiological profile of infectious agents in these bioindicators is justified by the fact that environmental stress events, acute and chronic, tend to provide changes in the prevalence rates of these pathogens, especially for the infectious agents studied, characterized by being opportunistic and widely present in estuarine and marine bird populations. Thus, the characterization of what can be determined as an expected health profile serves as a baseline standard and a parameter for comparison against environmental contamination events, such as Mariana dam rupture. This study brings an approach for marine environment conservation and contributes to a more accurate evaluation of the extent of environmental damage that may occur in this location. Further studies with this methodology should be encouraged along the Brazilian coast.

Declaration of Competing Interest

None of the authors declares any conflict of interest.

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5 CONSIDERAÇÕES FINAIS

A extensa revisão sobre estudos brasileiros em saúde de aves marinhas no contexto da medicina da conservação trouxe uma grande contribuição para identificação do estado da arte e quais lacunas precisam ser avaliadas em estudos futuros. O estudo experimental presente nesse trabalho buscou acessar a condição de saúde das aves marinhas a partir de uma abordagem mais ampla, frente às inúmeras ameaças às quais as aves estão expostas e possibilitou entender de que maneira tais ameaças refletem na saúde das populações de aves marinhas e na sua sobrevivência a longo prazo, embora esse continue sendo um dos principais desafios para a conservação desse grupo. Nesse contexto, dados oriundos dos estudos de licenciamento ambiental são extremamente importantes como oportunidades de pesquisa de populações arribadas e/ou populações *in situ*, e consequentemente contribuem para os esforços de conservação da biodiversidade brasileira.

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APÊNDICE 1

Chart 1. List of articles about bacteria found in seabirds in Brazil, divided by prevalence found in the study, the target species, age, where and when the study was performed.

Bacteria	Prevalence	Sampling	Storage and isolation method	Species studied			Location	Year of study	Reference
				Common name	Latin name	Taxonomic family			
Aeromonas	38,77% (n=49)	Oral, clocal, ocular, tracheal and skin wounds swabs	Cary-Blair transport medium; Samples processed within 3 days max.; Culture; PCR for virulence evaluation; Antimicrobial susceptibility testing	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	North-central coast of the state of Rio de Janeiro	Jan/2008 to Jun/2012	Cardoso et al. 2018
	25% (n=24)			Brown booby	*Sula leucogaster*	Sulidae			
	36% (n=11)			Kelp Gull	*Larus dominicanus*	Laridae			
	33% (n=9)			Manx shearwater	*Puffinus puffinus*	Procellariidae			
	67% (n=3)			Atlantic yellow-nosed albatross	*Thalassarche chlororhynchos*	Diomedeidae			
	100% (n=2)			South American tern	*Sterna hirundinacea*	Laridae			
	100% (n=1)			Grey-headed gull	*Chroicocephalus cirrocephalus*	Laridae			
	0% (n=3)			Frigatebird	*Fregata magnificens*	Fregatidae			
	0% (n=2)			Brown noody	*Anous stolidus*	Laridae			
	0% (n=2)			Common tern	*Sterna hirundo*	Laridae			
	0% (n=2)			Cory´s shearwater	*Calonectris borealis*	Procellariidae			
	0% (n=1)			Cape Petrel	*Daption capense*	Procellariidae			
	0% (n=1)			Southern Fulmar	*Fulmarus glacialoides*	Procellariidae			
	0% (n=1)			Southern Giant Petrel	*Macronectes giganteus*	Procellariidae			
	0% (n=1)			Atlantic Petrel	*Pterodroma incerta*	Procellariidae			
	0% (n=1)			Great shearwater	*Puffinus gravis*	Procellariidae			
	0% (n=1)			Parasitic jaeger	*Stercorarius parasiticus*	Stercorariidae			

	0% (n=1)			Cabot´s Tern	*Thalasseus acuflavidus*	Laridae			
	0% (n=1)			White-chinned petrel	*Procellaria aequinoctialis*	Procellariidae			
	31% (n=13)	Cloacal swab		Cabot´s Tern	*Thalasseus acuflavidus**	Laridae	Açu, São João da Barra-RJ	2015/2016	Cardoso 2018
	50% (n=12)			South American tern	*Sterna hirundinacea**	Laridae			
	11% (n=9)	Oral, clocal, ocular, tracheal and skin wounds swabs		Brown-hooded gull	*Chroicocephalus maculipennis*	Laridae	Pontal do Paraná (CEM-UFPR), Rio Grande do Sul (CRAM-FURG), Rio de Janeiro - (CRAS-UNESA)		
	33% (n=3)			Kelp Gull	*Larus dominicanus*	Laridae			
	100% (n=1)			Laughing gull	*Leucophaeus atricilla*	Laridae			
	7% (n=28)			Neotropical cormorant	*Phalacrocorax brasilianus*	Phalacrocoracidae			
	7% (n=14)			Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae			
	27% (n=26)			Brown booby	*Sula leucogaster*	Sulidae			
	18% (n=11) *Aeromonas sobria* (33%)			Manx shearwater	*Puffinus puffinus*	Procellariidae	North-central coast of the state of Rio de Janeiro	2009 to 2012	Cardoso et al. 2014
Citrobacter koseri	11,96% (n=17)	Cloacal swab	Cary-Blair transport medium; Samples processed within 2 days max.; Culture; Biochemical analysis	Kelp Gull	*Larus dominicanus**	Laridae	Moleques do Sul	May 2011 to November 2013	Ebert et al. 2016
	14,10% (n=13)			Kelp Gull	*Larus dominicanus**	Laridae	Tamboretes		
	10,09% (n=9)			Kelp Gull	*Larus dominicanus**	Laridae	Lobos		
Clostridium	8,33% (n=12)	Tracheal swab	Buffered peptone water; Culture Agar Nutrient; Gram stain; Biochemical analysis	Brown booby	Sula leucogaster	Sulidae	Baixada Santista Coastal Region - São Paulo	2012/13	Zampieri et al. 2014
Enterococcus	12,5% (n=40)			Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae			
	8,33% (n=12)			Brown booby	*Sula leucogaster*	Sulidae			

	50% (n=4)			Frigatebird	*Fregata magnificens*	Fregatidae			
ESBL-EC Extended-spectrum β-lactamase - *Escherichia coli*	0% (n=36)	Cloacal swab	Amies transport medium; Culture, antimicrobial susceptibility testing, genome sequence analysis	Sooty terns	*Onychoprion fuscatus**	Laridae	Rocas Atoll	-	Ewbank et al. 2022
	0% (n=34)			Brown noody	*Anous stolidus**	Laridae		-	
	0% (n=33)			Masked booby	*Sula dactylatra**	Sulidae		-	
	0% (n=33)			Brown booby	*Sula leucogaster**	Sulidae		-	
	0% (n=33)			Red-footed booby	*Sula sula**	Sulidae		-	
	15,3% (n=35)			Frigatebird	*Fregata magnificens**	Fregatidae		-	
Escherichia coli strains	86,84% (n=38)	Cloacal and choanal swab	Culture; virulence genotype; phylogenetic analysis	Frigatebird	*Fregata magnificens**	Fregatidae	São Paulo state coast	2008 to 2010	Savioli et al. 2016
Pseudomonas sp.	20% (n=40)	Tracheal swab	Buffered peptone water; Culture Agar Nutrient; Gram stain; Biochemical analysis	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Baixada Santista Coastal Region - São Paulo	2012/13	Zampieri et al. 2014
	16,66% (n=12)			Brown booby	*Sula leucogaster*	Sulidae			
	50% (n=4)			Frigatebird	*Fregata magnificens*	Fregatidae			
Salmonella sp.	2,5% (n=40)			Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae			
	16,66% (n=12)			Brown booby	*Sula leucogaster*	Sulidae			
	11% (n=9)	Oral, clocal, ocular, tracheal and skin wounds swabs	Cary-Blair transport medium; Samples processed within 3 days max.; Culture (OXOID); PCR for virulence evaluation; Antimicrobial susceptibility testing	Brown-hooded gull	*Chroicocephalus maculipennis*	Laridae	Pontal do Paraná (CEM-UFPR), Rio Grande do Sul (CRAM-FURG), Rio de Janeiro - (CRAS-UNESA)	2015/2016	Cardoso 2018
	18% (n=28)			Neotropical cormorant	*Phalacrocorax brasilianus*	Phalacrocoracidae			
	8% (n=26)			Brown booby	*Sula leucogaster*	Sulidae			

Salmonella enteritidis	20,65% (n=17)	Cloacal swab	Cary-Blair transport medium; Samples processed within 2 days max.; Culture (peptone water, tetrathionate broth/manitol salt agar, SS, XLD or BS agar); Biochemical analysis	Kelp Gull	*Larus dominicanus**	Laridae	Moleques do Sul	May 2011 to November 2013	Ebert et al. 2016
	24,36% (n=13)			Kelp Gull	*Larus dominicanus**	Laridae	Tamboretes		
	17,43% (n=9)			Kelp Gull	*Larus dominicanus**	Laridae	Lobos		
Shigella sp.	7,61% (n=17)			Kelp Gull	*Larus dominicanus**	Laridae	Moleques do Sul		
	6,42% (n=13)			Kelp Gull	*Larus dominicanus**	Laridae	Tamboretes		
Staphylococcus aureus	30,43% (n=17)			Kelp Gull	*Larus dominicanus**	Laridae	Moleques do Sul		
	25,69% (n=13)			Kelp Gull	*Larus dominicanus**	Laridae	Tamboretes		
	35,9% (n=9)			Kelp Gull	*Larus dominicanus**	Laridae	Lobos		
Staphylococcus sp.	80% (n=40)	Tracheal swab	Buffered peptone water; Culture Agar Nutrient; Gram stain; Biochemical analysis	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Baixada Santista Coastal Region - São Paulo	2012/13	Zampieri et al. 2014
	50% (n=12)			Brown booby	*Sula leucogaster*	Sulidae			
	50% (n=4)			Frigatebird	*Fregata magnificens*	Fregatidae			
	100% (n=6)			Kelp Gull	*Larus dominicanus*	Laridae			
	100% (n=1)			Cape Petrel	*Daption capense*	Procellariidae			
	n=16 n=2 75%**	Cloacal, oral and tracheal swab	Eagle's minimal essential medium; Culture; Biochemical analysis; Minimum inhibitory concentrations; Genome sequencing	White-tailed tropicbirds	*Phaethon lepturus**	Phaethontidae	Fernando de Noronha Archipelago	2021	Savioli et al. 2021
				Audubon's shearwater birds	*Puffinus lherminieri**	Procellariidae			
Streptococcus sp.	45% (n=40)	Tracheal swab		Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae		2012/13	Zampieri et al. 2014

	16,66% (n=12)		Buffered peptone water; Culture Agar Nutrient; Gram stain; Biochemical analysis	Brown booby	*Sula leucogaster*	Sulidae	Baixada Santista Coastal Region - São Paulo		
	25% (n=4)			Frigatebird	*Fregata magnificens*	Fregatidae			
	66,66% (n=6)			Kelp Gull	*Larus dominicanus*	Laridae			
Vibrio sp.	65% (n=49)	Oral, clocal, ocular, tracheal and skin wounds swabs	Cary-Blair transport medium; Samples processed within 3 days max.; Culture; PCR for virulence evaluation; Antimicrobial susceptibility testing	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	North-central coast of the state of Rio de Janeiro	Jan/2008 to Jun/2012	Cardoso et al. 2018
	58% (n=24)			Brown booby	*Sula leucogaster*	Sulidae			
	45% (n=11)			Kelp Gull	*Larus dominicanus*	Laridae			
	89% (n=9)			Manx shearwater	*Puffinus puffinus*	Procellariidae			
	33% (n=3)			Frigatebird	*Fregata magnificens*	Fregatidae			
	67% (n=3)			Atlantic yellow-nosed albatross	*Thalassarche chlororhynchos*	Diomedeidae			
	50% (n=2)			Brown noody	*Anous stolidus*	Laridae			
	100% (n=2)			Cory´s shearwater	*Calonectris borealis*	Procellariidae			
	50% (n=2)			South American tern	*Sterna hirundinacea*	Laridae			
	100% (n=1)			Grey-headed gull	*Chroicocephalus cirrocephalus*	Laridae			
	100% (n=1)			Cape Petrel	*Daption capense*	Procellariidae			
	100% (n=1)			Southern fulmar	*Fulmarus glacialoides*	Procellariidae			
	100% (n=1)			Giant Petrel	*Macronectes giganteus*	Procellariidae			
	100% (n=1)			White-chinned petrel	*Procellaria aequinoctialis*	Procellariidae			
	100% (n=1)			Atlantic Petrel	*Pterodroma incerta*	Procellariidae			
	100% (n=1)			Great shearwater	*Puffinus gravis*	Procellariidae			
	100% (n=1)			Parasitic jaeger	*Stercorarius parasiticus*	Stercorariidae			
	100% (n=1)			Cabot´s Tern	*Thalasseus acuflavidus*	Laridae			
	0% (n=2)			Common tern	*Sterna hirundo*	Laridae			

	33% (n=3)	Oral, clocal, ocular, tracheal and skin wounds swabs		Kelp Gull	*Larus dominicanus*	Laridae	Pontal do Paraná (CEM-UFPR), Rio Grande do Sul (CRAM-FURG), Rio de Janeiro - (CRAS-UNESA)	2015/2016	Cardoso 2018
	36% (n=28)			Neotropical cormorant	Phalacrocorax brasilianus	Phalacrocoracidae			
	100% (n=3)			White-chinned petrel	Procellaria aequinoctialis	Procellariidae			
	100% (n=1)			Atlantic Petrel	Pterodroma incerta	Procellariidae			
	93% (n=14)			Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae			
	42% (n=26)			Brown booby	*Sula leucogaster*	Sulidae			
	17% (n=12)	Cloacal swab		South American tern	*Sterna hirundinacea**	Laridae	Açu, São João da Barra-RJ		
	15% (n=13)			Cabot´s Tern	*Thalasseus acuflavidus**	Laridae			
	90% (n=11)	Cloacal, oral, ocular and tracheal swabs		Manx shearwater	*Puffinus puffinus*	Procellariidae	North-central coast of the state of Rio de Janeiro	2009 to 2012	Cardoso et al. 2014

* Birds captured in the wild, adults or nestlings

** Total percent, considering sample size from both species

Chart 2. List of articles about virus found in seabirds in Brazil, divided by prevalence found in the study, the target species, age, where and when the study was performed.

Virus	Prevalence	Sampling	Storage and isolation method	Species studied			Location	Year of study	Reference
				Common name	**Latin name**	**Taxonomic family**			
Avian Coronavirus	14,2% (n=84)	Cloacal swabs / kidney	Hemi Nested PCR; Phylogenetic analysis	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	CRAM - FURG - Rio Grande do Sul and R3 - Santa Catarina	2013	Niemeyer 2015
	50% (n=4)	Cloacal swabs		Brown booby	*Sula leucogaster*	Sulidae		2013	Niemeyer 2015
	50% (n=2)	Cloacal swabs		Giant Petrel	*Macronectes giganteus*	Procellariidae		2013	Niemeyer 2015
MagHV-1	38% (n=89)	Trachea tissue samples; tracheal swabs; PCR	Pathology; Electron microscopy; Immunohistochemistry	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	CRAM - FURG - Rio Grande do Sul	Winter 2011	Niemeyer el al. 2017
Poxvirus	15/17 (symptomatic)	Tissue samples from skin lesions	Clinical signs; Histopathology; Molecular detection	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Santa Catarina/Espírito Santo	2012/13	Niemeyer 2015
SuHV*	8,6% (n=23)	Tracheal swab	PCR; Phylogenetic analysis	White booby*	*Sula dactylatra*	Sulidae	Abrolhos (Island)	March 2013	Niemeyer el al. 2017
	5% (n=20)	Tracheal swab		Brown booby*	*Sula leucogaster*	Sulidae		March 2013	Niemeyer el al. 2017
	6.8% (n=29)	Tracheal swab		Red billed tropicbird*	*Phaeton aethereus*	Phaethontidae		March 2013	Niemeyer el al. 2017
ThaHV	8.3% (n=12)	Tracheal swab		Atlantic yellow-nosed albatross	*Thalassarche chlororhynchos*	Diomedeidae	CRAM - FURG - Rio Grande do Sul	Mar/2013	Niemeyer el al. 2017
* Birds captured in the wild, adults or nestlings									

Chart 3. List of articles about fungus found in seabirds in Brazil, divided by prevalence found in the study, the target species, age, where and when the study was performed.

Fungi	**Prevalence**	**Sampling**	**Storage and isolation method**	**Species studied**			**Location**	**Year of study**	**Reference**
				Common name	**Latin name**	**Taxonomic family**			
Aspergillus fumigatus	100% (n=1)	Necropsy and tissue samples	Culture; Molecular detection	White-chinned petrel	*Procellaria aequinoctialis*	Procellariidae	Cassino beach - Rio Grande do Sul	2017/18	Melo et al. 2020
	100% (n=1)			Brown-hooded gull	*Chroicocephalus maculipennis*	Laridae		2017/18	
	100% (n=1)			Neotropical cormorant	*Nannopterum brasilianus*	Phalacrocoracidae		2017/18	
Aspergillus fumigatus (1) Aspergillus flavus (2)	20,18% (n=327) 65 (1) e 1 (2)	Cohort study	Based on necropsy lesions and mycologic culture on the records	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	CRAM - FURG - Rio Grande do Sul	2004 to 2009	Filho 2012
Aspergillus fumigatus Aspergillus flavus	n=32 Mortality (n=14) 14,28% *A. fumigatus* and 7,14% *A. flavus*	Necropsy and tissue samples	Culture; Molecular detection; Itraconazole susceptibility	Black-browed albatross	*Thalassarche melanophris*	Diomedeidae	CRAM - FURG - Rio Grande do Sul	2015/17	Melo et al. 2019
Aspergillus sp. (3) *Candida palmioleophila* (4)	n=325 Necropsy=61 3,27% (n=61) (3) 1,63% (n=61) (4)	Necropsy and tissue samples	Histopathological evaluation; Molecular detection	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Cananéia county Souther Sao Paulo State IPeC and Coastal Monitoring Program	Jul/Aug 2018	Ewbank et al. 2021
Aspergillus sp.	12,5% (n=40)	Tracheal swab	Buffered peptone water; Culture Agar Mycosel	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Baixada Santista Coastal Region - São Paulo	2012/13	Zampieri et al. 2014
	8,33% (n=12)			Brown booby	*Sula leucogaster*	Sulidae			
Candida sp.	20% (n=40)			Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae			
	25% (n=12)			Brown booby	*Sula leucogaster*	Sulidae			
	25% (n=4)			Frigatebird	*Fregata magnificens*	Fregatidae			

	16,66 (n=6)			Kelp Gull	*Larus dominicanus*	Laridae			
	100% (n=1)			Cape Petrel	*Daption capense*	Procellariidae			
Penicillium sp.	16,66 (n=12)			Brown booby	*Sula leucogaster*	Sulidae			

Chart 4. List of articles about parasites found in seabirds in Brazil, divided by prevalence found in the study, the target species, age, where and when the study was performed.

Parasite	Prevalence	Collection	Species studied			Location	Year of study	Reference
			Common name	Latin name	Taxonomic family			
Fregatiella aurifasciata	40% (n=5)	Through clinical examination and collection using Carbaryl and Cipermetrina powder	Frigatebird	*Fregata magnificens*	Fregatidae	CRETA - São Paulo	Out/2016 to Out/2017	Brito 2018
	80% (n=10)		Frigatebird	*Fregata magnificens*	Fregatidae	CRAS - Rio de Janeiro	Out/2016 to Out/2017	Brito 2018
Pectinopygus fregatiphagus	100% (n=5)		Frigatebird	*Fregata magnificens*	Fregatidae	CRETA - São Paulo	Out/2016 to Out/2017	Brito 2018
	90% (n=10)		Frigatebird	*Fregata magnificens*	Fregatidae	CRAS - Rio de Janeiro	Out/2016 to Out/2017	Brito 2018
Colpocephalum spineum	60% (n=10)		Frigatebird	*Fregata magnificens*	Fregatidae	CRAS - Rio de Janeiro	Out/2016 to Out/2017	Brito 2018
Ixodes percavatus sensu lato	23% (n=13)* 2 juveniles 1 sub-adult	Through clinical examination	Atlantic yellow-nosed albatross	*Thalassarche chlororhynchos*	Diomedeidae	Rio de Janeiro (coast)	Sep/2019	Labruna et al. 2020
	100% (n=2)	Through clinical examination (1) or necropsy (1)	Atlantic yellow-nosed albatross	*Thalassarche chlororhynchos*	Diomedeidae	Rehabilitation Espírito Santo	17 May 2018 and 07 May 2019	Labruna et al. 2020

Halipeurus diversus (Ischnocera, Philopteridae), *Trabeculus aviator* (Ischno- cera, Philopteridae), *Austromenopon paululum* (Amblycera, Menoponidae), *Saedmundssonia* sp. (Ischnocera, Philopter- idae), and *Naubates* sp. (Ischnocera, Philopteridae).	Lice: 31,25% (n=16) 80% polyparasitism	Necropsy / Morphology	Manx shearwater	*Puffinus puffinus*	Procellariidae	CETAS - Paraíba	Jun/2011 to Dec/2011	De Melo et al. 2012
Nematodes *Seuratia shipleyi* and *Contracaecum* sp., and cestodes *Tetrabothrius* sp.	Helminths: 31,25% (n=16) From the proventiculus and small intestine	Necropsy / Morphology	Manx shearwater	*Puffinus puffinus*	Procellariidae	CETAS - Paraíba	Jun/2011 to Dec/2011	De Melo et al. 2012
Nematodes (esophagus and stomach); cestoda or trematoda (intestines)	100% (n=24)	GI tract of carcasses	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Beach monitoring CETAS Rio grande do Sul	Jun to Aug 2017	Wartchow 2017
Nematodes (esophagus and stomach)	75% (n=4)		White-chinned petrel	Procellaria aequinoctialis	Procellariidae			Wartchow 2017
Nematodes (esophagus and stomach); cestoda or trematoda (intestines)	66,66% (n=3)		Giant Petrel	Macronectes giganteus	Procellariidae			Wartchow 2017
Nematodes (stomach); cestoda or trematoda (intestines)	100% (n=1)		Frigatebird	Fregata magnificens	Fregatidae			Wartchow 2017
Contracaecum pelagicum	57,14% (n=7)	Coproparasitologic	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Marine Study Center of the Federal University of Parana, CEM/UFPR	Aug/2014 to Jul/2015	Vanhoni et al. 2018
	93,54% (n=31)	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Marine Study Center of the Federal University of Parana, CEM/UFPR	Aug/2014 to Jul/2015	Vanhoni et al. 2018
	100% juveniles 100% adults Lean	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020

	100% juveniles 90,9% adults Healthy	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
	49,4% (n=237)	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Beaches of northern shore of São Paulo and southern shore of Rio de Janeiro	2005 to 2009	Rezende et al. 2013
Cardiocephaloides physalis	20/31	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Marine Study Center of the Federal University of Parana, CEM/UFPR	Aug/2014 to Jul/2015	Vanhoni et al. 2018
	93,8% juveniles 30% adults Lean	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
	100% juveniles 27,3% adults Healthy	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
	22,4% (n=237)	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Beaches of northern shore of São Paulo and southern shore of Rio de Janeiro	2005 to 2009	Rezende et al. 2013
Tetrabothrius lutzi	16/31	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Marine Study Center of the Federal University of Parana, CEM/UFPR	Aug/2014 to Jul/2015	Vanhoni et al. 2018
	100% juveniles 90% adults Lean ***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
	100% juveniles 54,5% adults Healthy***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020

	26,6% (n=237)	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Beaches of northern shore of São Paulo and southern shore of Rio de Janeiro	2005 to 2009	Rezende et al. 2013
Acuariidae	9,4% juveniles Lean***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
Ascocotyle (Phagicola) longa	6,2% juveniles Lean***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
Ascocotyle (Phagicola) sp.	40,6% juveniles 10% adults Lean***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
	10% juveniles 9,1% adults Healthy***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
Corynosoma sp.	71,9% juveniles 80% adults Lean***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
	50% juveniles 72,7% adults Healthy***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
Stephanoprora uruguayense	62,5% juveniles 10% adults Lean***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
	20% juveniles 18,2% adults Healthy***	Necropsy / Morphology	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Coast of Rio Grande do Sul	Austral winter of 2013 and 2014	De Paula et al. 2020
*** Juvenile lean (n=32); juvenile healthy (n=10); adult lean (n=10); adult healthy (n=11)								

Chart 5. List of articles about Hemosporidia found in seabirds in Brazil, divided by prevalence found in the study, the target species, age, where and when the study was performed.

Hemosporidia	Prevalence	Sampling	Isolation method	Species studied: Common name	Latin name	Taxonomic family	Location	Year of study	Reference
Babesia sp.*	6,7% (n=30)	Blood sample	PCR	Masked booby	*Sula dactylatra*	Sulidae	Abrolhos	Feb/2011	Quillfeldt et al. 2014
	17,6% (n=17) juveniles			Masked booby	*Sula dactylatra*	Sulidae	Abrolhos	Aug/2011	
	47,4% (n=19) juveniles			Masked booby	*Sula dactylatra*	Sulidae	Atol das Rocas	Sep/2010	
	11,8% (n=17) adults 33,3% (n=18) juveniles			Masked booby	*Sula dactylatra*	Sulidae	Fernando de Noronha	Jul/2011	
	7,7% (n=13) juveniles			Brown booby	*Sula leucogaster*	Sulidae	Abrolhos	Aug/2011	
	7,1% (n=14) juveniles			Brown booby	*Sula leucogaster*	Sulidae	SPSPA	Aug/2010	
	66,7% (n=15) juveniles			Brown booby	*Sula leucogaster*	Sulidae	Atol das Rocas	Sep/2010	
Haemoproteus	30,4% (n=23) adults*			Brown noddy	*Anous stolidus*	Laridae	SPSPA	Aug/2010	
	2,9% (n=35) adult*			Brown noddy	*Anous stolidus*	Laridae	Atol das Rocas	Sep/2010	
	3,57% [*H. (Parahaemoproteus)* sp.] (n=28)			Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Santa Catarina	2008 to 2009	Vanstreels et al. 2014
Plasmodium	100% (n=1)	Blood sample	Blood smear + nested PCR						
	25% (n=8)		Blood smears + PCR (1) Blood smears (1)	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Bahia	1999 to 2008	Vanstreels et al. 2015
	9,52% (n=21)		Blood smears + PCR (2)	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Bahia	Jun/2009 to Dec/2012	
	5,26% (n=19)		Blood smears (1)	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Espírito Santo	1999 to 2008	
	7,89% (n=38)		Blood smears + PCR (1) PCR (1)	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Espírito Santo	Sep/2012 to Feb/2013	

	15,38% (n=13)		Histopatology (1) PCR (1)	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Rio de Janeiro	1999 to 2008	
	12,94% (n=224)		Blood smears + PCR (19) Blood smears (2) PCR (8)	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Santa Catarina	Mar/2009 to Feb/2013	
	15,38% (n=13)		Histopatology (2)	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Rio Grande do Sul	1999 to 2008	
	1,35% (n=221)		Blood smears + PCR (3)	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Rio Grande do Sul	Jan/2009 to Dec/2012	
	(n=28) 42,85% *P. tejerai* 3,57% *P. elongatum+P. tejerai* 3,57% [*P. (Haemamoeba)* sp.] 7,14% *P. elongatum+P.*(*Haemamoeba)*sp. 3,57% Undertermined	Blood sample	Blood smear + PCR + histopatology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Santa Catarina	2008 to 2009	Vanstreels et al. 2014
	(n=33) 18,18% *P. cathemerium* 15,15% *P. tejerai* 9% *P. elongatum* 3% *P. nucleophilum 3% P. unalis* 3% *Plasmodium* sp. E 6% Plasmodium sp. G 3% Plasmodium sp. H 12,12% Unidentified lineages of *Plasmodium*	Blood sample	Blood smear + PCR + histopatology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Rio Grande do Sul; Santa Catarina; Espírito Santo	Jan/2009 to Feb/2013	Ewbank 2019

Chart 6. List of articles about other Protozoa found in seabirds in Brazil, divided by prevalence found in the study, the target species, age, where and when the study was performed.

Protozoa	Prevalence	Sampling and identification method	Species studied				Year of study	Reference
			Common name	Latin name	Taxonomic family	Location		
Toxoplasma gondii	34,8% (n=23)	Serology	Masked booby	*Sula dactylatra*	Sulidae	Abrolhos	Mar/2013	Gennari et al 2016
	47,4% (n=19)	Serology	Brown booby	*Sula leucogaster*	Sulidae	Abrolhos	Mar/2013	
	28% (n=25)	Serology	Red-billed tropicbird	*Phaeton aethereus*	Phaethontidae	Abrolhos	Mar/2013	
	8,3% (n=24)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Bahia	2015	Acosta et al 2018
	18,5% (n=54)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Espírito Santo	2015	
	9,0% (n=67)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Rio de Janeiro	2015	
	60% (n=5)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Aquarium of Guarujá (SP)	2015	Gennari et al (2)
	14,3% (n=21)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Aquarium of Guarujá (SP)	2015	
	45,8% (n=24)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Aquarium of Santos (SP)	2015	
	19% (n=21)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Penguin Zoo of Santo André (SP)	2015	
	37,5% (n=16)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	R3 Animal, Florianópolis (SC)	2015	
	8,3% (n=12)	Serology	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	IPRAM, Cariacica (ES)	2015	
Sarcocystis sp. closely related to *S. falcatula*	16/514 pectoral muscle (3% n=330)	342 samples of pectoral muscle, 86 of heart and 86 of brain PCR-18S	Magellanic penguins	*Spheniscus magellanicus*	Spheniscidae	Espírito Santo (ES), Rio de Janeiro (RJ) and Bahia (BA)	2012 to 2015	Acosta et al 2018
Neospora caninum	100% (n=1)	Nested-PCR	White-chinned petrel	*Procellaria aequinoctialis*	Procellariidae	-	2020	Sato et al 2020

Chart 7. List of articles about chemical compounds found in seabirds in Brazil, divided by prevalence found in the study, the target species, age, where and when the study was performed.

Compounds	Concentration	Sampling and identification method	Species studied			Location	Year of study	Reference
			Common name	Latin name	Taxonomic family			
Cd	Adult 0.072 µg/g	Feathers	Kelp Gull	*Larus dominicanus*	Laridae	Florianópolis - SC	2005	Barbieri et al. 2010
	Juvenile 0.021 µg/g							
Cu	Adult 13.30 µg/g							
	Juvenile 13.76 µg/g							
Mn	Adult 11.36 µg/g							
	Juvenile 1.184 µg/g							
Ni	Adult 5.92 µg/g							
	Juvenile 2.23 µg/g							
Pb	Adult 7.53 µg/g							
	Juvenile 1.47 µg/g							
Zn	1.424 ± 0.360 µg/g	Feathers of young	Kelp Gull	*Larus dominicanus*	Laridae	Tamboretes, Moleques do Sul and Lobos Island	2011 - 2013	Ebert et al. 2020
Pb	0.308 ± 0.009 µg/g							
Cr	2.480 ± 0.080 µg/g							
Hg	0,240 ± 0.060 µg/g							
Cd	28.1 µg/g	Feathers	Brown booby	*Sula leucogaster*	Sulidae	Cagarras Archipelago - RJ	2017	Padilha et al. 2017
Sn	210.6 µg/g							
Cu	8233.4 µg/g							
Se	2343 µg/g							
Mn	1635.7 µg/g							
Cd	82.9 µg/g		Frigatebird	*Fregata magnificens*	Fregatidae			
Sn	319,6 µg/g							

Cu	8008.6 µg/g							
Se	3026 µg/g							
Mn	5143 µg/g							
Al	50.62 µg/g	Feathers (n=51 adults)	Brown booby	*Sula leucogaster*	Sulidae	Marine National Park of Currais Islands	2013 - 2014	Dolci et al. 2017
As	0.35 µg/g							
Cd	0.05 µg/g							
Cu	15.12 µg/g							
Co	0.38 µg/g							
Fe	15.12 µg/g							
Mg	815.71 µg/g							
Ni	0.29 µg/g							
Zn	94.16 µg/g							
Al	9.58 µg/g	Eggshells (n=47						
As	2.37 µg/g							
Cd	0.03 µg/g							
Cu	0.99 µg/g							
Co	2.1 µg/g							
Fe	22.92 µg/g							
Mg	11.16.92 µg/g							
Ni	11.85 µg/g							
Zn	1.98 µg/g							
Se (min–max)	0.64 ± 0.32 (0.24-1.44) µg/g	Feathers of juveniles (n=22)	Magellanic penguin	*Spheniscus magellanicus*	Spheniscidae	Southern Brazilian Coast (stranded)	-	Kehrig et al. 2015
Hg (min–max)	0.78 ± 0.44 (0.27-1.78) µg/g							
MeHg (min–max)	0.62 ± 0.32 (0.22-1.27) µg/g							
Cd (min–max)	0.13 ± 0.07 (0.04–0.27) µg/g							
Pb (min–max)	0.14 ± 0.08 (0.05-0.32) µg/g							

Se (min–max)	5.15 ± 2.82 (1.85-13.63) µg/g	Liver of juveniles (n=22)						
Hg (min–max)	5.70 ± 3.73 (1.98-18.07) µg/g							
MeHg (min–max)	1.93 ± 0.92 (0.69-3.76) µg/g							
Cd (min–max)	7.25 ± 4.71 (2.52-22.24) µg/g							
Pb (min–max)	0.58 ± 0.32 (0.20-1.23) µg/g							
Se (min–max)	8.03 ± 4.48 (2.87-21.94) µg/g	Kidney of juveniles (n=22)						
Hg (min–max)	2.47 ± 1.42 (0.87-6.78) µg/g							
MeHg (min–max)	1.31 ± 0.73 (0.46-3.26) µg/g							
Cd (min–max)	46.50 ± 33.55 (15.35-133.11) µg/g							
Pb (min–max)	0.55 ± 0.30 (0.19-1.16) µg/g							
Hg (min–max)	7.19 ± 3.37 (1.16 – 14.22) mg/kg	Liver of wrecked (n=20)	Manx shearwater (n=37)	*Puffinus puffinus*	Procellaridae	North-central coast of the state of Rio de Janeiro (n=35); Coast of the state of Espírito Santo (n=2)	2005 - 2011	Cardoso et al. 2014
Se (min–max)	34.66 ± 20.14 (10.56 – 75.20) mg/kg							
Cd (min–max)	22.33 ± 25.46 (2.31 – 113.01) mg/kg							
Pb (min–max)	0.1 ± 0.06 (0.036 – 0.28) mg/kg							
Hg (min–max)	1.23 ± 0.53 (0.47 – 2.31) mg/kg	Muscle of wrecked (n=37)						
Se (min–max)	7.98 ± 3.68 (3.17 – 19.01) mg/kg							
Cd (min–max)	1.11 ± 1.72 (<LQ* – 8.94) mg/kg							
Pb (min–max)	0.16 ± 0.09 (<LQ* – 0.43) mg/kg							

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Chart 8. List of *F. magnificens* captured along the year of 2015 in the Abrolhos Bank Region and the results of variables evaluated in the study.

ID	CAMPAIGN	SEX	WEIGHT(g)	BCS	CLINICAL SIGNS	PCR	TPP mg/dl	HT %	Parasites
AM01	FEB	F	-	Good	N	NEG	-	-	Null
AM02	FEB	M	-	Good	N	NEG	-	-	Low
AM03	FEB	I	-	Good	N	NEG	-	-	Low
AM05	FEB	F	-	Good	N	POS	-	-	Null
AM06	FEB	F	-	Good	N	POS	-	-	Null
AM07	FEB	I	-	Good	N	NEG	-	-	Low
AM08	FEB	I	-	Good	N	NEG	-	-	Null
AM09	FEB	F	-	Good	N	POS	-	-	Null
AM10	MAY	I	1450	Good	N	POS	-	-	Low
AM11	MAY	M	-	Moderate	N	POS	-	-	Low
AM12	MAY	M	1350	Moderate	Y	NEG	-	-	Low
AM13	MAY	M	1200	Moderate	N	NEG	-	-	Signs
AM14	MAY	I	1150	Moderate	Y	POS	4,8	-	Low
AM15	MAY	M	1300	Good	N	NEG	-	-	Low
AM16	MAY	I	1300	Good	Y	POS	4,6	-	Medium
AM17	MAY	I	1250	Moderate	N	NEG	3,5	-	High
AM19	SEP	I	1350	Moderate	Y	NEG	4	41,6	Medium
AM20	SEP	M	900	Moderate	Y	NEG	6	32	Signs
AM21	SEP	I	1250	Emaciated	N	POS	5	35	Low
AM22	SEP	M	1350	Moderate	Y	NEG	4,2	48	Low
AM23	SEP	M	1550	Good	Y	POS	5,4	52	Low
AM24	SEP	M	1250	Moderate	Y	POS	4,2	47	Low
AM25	SEP	M	1200	Emaciated	Y	POS	5	55	Low
AM26	SEP	I	1450	Emaciated	Y	POS	4,4	42	Medium
AM27	SEP	M	1300	Emaciated	N	POS	4,6	50	Low
AM28	DEC	F	1700	Moderate	N	NEG	5	40	Low
AM29	DEC	I	1250	Emaciated	Y	NEG	-	-	Medium
AM30	DEC	M	1350	Good	N	NEG	4	40	Low
AM31	DEC	I	1150	Moderate	N	POS	5,2	39	Medium
AM32	DEC	I	1650	Moderate	N	NEG	5,9	44,5	Low
AM33	DEC	M	1180	Moderate	Y	NEG	6,4	41	Low
AM34	DEC	M	1190	Moderate	N	NEG	6,2	48	Low
AM35	DEC	M	1210	Emaciated	Y	NEG	6,3	36	Null

Chart 9. List of *N. violacea* captured along the year of 2015 in the Abrolhos Bank Region and the results of variables evaluated in the study.

ID	CAMPAIGN	WEIGHT (g)	BCS	CLINICAL SIGNS	PCR	PTT mg/dl	HT %	Parasites
AE01	SEP	500	Emaciated	N	NEG	3,4	30	Null
AE02	SEP	450	Moderate	N	POS	4,4	34	Null
AE03	SEP	200	Good	Y	POS	_	_	Null
AE04	SEP	300	Good	N	POS	4	28	Null
AE05	SEP	350	Good	N	POS	3,8	30	Null
AE06	SEP	475	Good	N	POS	3,6	37	Null
AE07	SEP	525	Good	Y	POS	4,4	30	Null
AE08	DEC	300	Good	Y	POS	_	_	Null
AE09	DEC	250	Good	N	POS	5,1	18	Null
AE10	DEC	80	Ext. Emaciated	-	-	_	_	-
AE11	DEC	550	Moderate	N	NEG	5,3	44	Null
AE12	DEC	550	Moderate	N	POS	6,1	33	Null
AE13	DEC	650	Good	N	POS	4,2	27,7	Null
AE14	DEC	450	Moderate	N	POS	4,1	46	Null

Chart 10. List of *E. caerulea* captured along the year of 2015 in the Abrolhos Bank Region and the results of variables evaluated in the study.

ID	CAMPAIGN	WEIGHT(g)	BCS	Clinical signs	PCR	TPP mg/dl	HT %	Parasites
AE15	DEC	200	Moderate	N	NEG	4,8	30	Medium
AE16	DEC	160	Good	N	POS	5,2	29	Null
AE17	DEC	210	Good	N	POS	4,8	24	_
AE18	DEC	250	Emaciated	Y	POS	4,2	32	Null
AE19	DEC	200	Emaciated	Y	NEG	5,1	32	Null
AE20	DEC	150	Emaciated	N	NEG	5,3	32	Null
AE21	DEC	240	Good	Y	POS	5,5	32	Null
AE22	DEC	210	Good	N	NEG	7	40	Null
AE23	DEC	150	Moderate	Y	POS	4,6	26	Null
AE24	DEC	270	Moderate	N	NEG	5	43	Null
AE25	DEC	170	Emaciated	Y	NEG	4,6	33	Null
AE26	DEC	200	Emaciated	Y	NEG	4,3	36	Null
AE27	DEC	190	Emaciated	Y	NEG	3,7	28	Null
AE28	DEC	260	Moderate	Y	NEG	5,2	45	-
AE29	DEC	180	Emaciated	Y	NEG	3,7	30	Null
AE30	DEC	160	Moderate	Y	NEG	5,2	25	Null
AE31	DEC	250	Emaciated	N	NEG	4,8	40	Medium
AE32	DEC	250	Moderate	Y	NEG	6,2	28	Null