Using the year-round ecology of seabirds to monitor Antarctic and Subantarctic ecosystems

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Introduction

Human influence over marine ecosystems has been growing substantially to the point that almost the whole global oceans now faces multiple sources of environmental change factors (Halpern et al. 2019; Bowler et al. 2020). Such changes have triggered abrupt shifts in marine ecosystems (Beaugrand et al. 2019). Identifying efficient indicators for measuring and monitoring those changes is of utmost importance.

Seabirds are usually accepted as good indicators of ecosystems' state (Piatt et al. 2007; Hazen et al. 2019; Heerah et al. 2019; Krüger 2022); their distribution overlap with a wide diversity of other marine organisms (Kuletz et al. 2015; Raymond et al. 2015; Waggitt et al. 2020), and they exploit habitats also used by several species of marine mammals (Patterson et al. 2016; Hindell et al. 2020, Kuletz et al. 2015; Waggitt et al. 2020), and fishes (Clua and Grosvalet 2001; Morato et al. 2008; Miller et al. 2018), including species exploited by humans (Clua and Grosvalet 2001; Miller et al. 2018). Several seabird species are pelagic, presenting with wide year-round home ranges and migratory behaviors (Coulson 2001). Therefore, they are exposed to a wide variety of habitats, environmental conditions and environmental stressors.

Seabirds rely on certain spatially and temporally predictable oceanic features that are indicators of food availability, such as upwellings (Crawford 2007; Grecian et al. 2016), shelf break fronts, (Cox et al. 2016; Yamamoto et al. 2016; Serratosa et al. 2020), eddies (Assali et al. 2017; van der Boog et al. 2019), gyre edges (Clay et al. 2017; Hátún et al. 2017), freshwater discharge plumes (Urbanski et al. 2017; Daudt et al. 2019) and seamounts (Rogers et al. 2017; Rogers 2018), that usually are patchy zones of higher primary productivity and biodiversity (Condie and Condie 2016; Cox et al. 2018; Weidberg et al. 2020). Those features are important not only from a biodiversity point of view, but they also sustain ecosystem processes that are fundamental for the balance of the biosphere.

However, the levels of association with different habitats/ resources/ conditions varies among species, populations of a same species and even among individuals of the same population (Waggitt et al. 2013; Werner et al. 2014; Krüger et al. 2018; Krüger et al. 2019). Understanding why and how those differences occur is important to allow using seabirds as sentinels and monitors of environmental changes.

Objectives

Quantify differences in year-round spatial ecology (habitat use, movements, foraging behaviour, migratory behavior) of seabirds breeding in Antarctica and Sub Antarctica in different levels of biological organization (individuals, populations, species) and identify intrinsic and extrinsic factors responsible for such differences.

Target species

Four species of seabirds are targeted to be studied: Adelie (*Pygoscelis adeliae*), Chinstrap (*P. antarcticus*) and Gentoo (*P. papua*) penguins and Southern Giant Petrels (*Macronectes giganteus*). Those species have different levels of specialization: *Pygoscelis* penguins are Krill specialists, but the three species also vary in terms of specialization levels, as Chinstraps and Adelie penguins are more specialized than Gentoo penguins; Giant Petrels are highly opportunistic predators and scavengers with a wide variety of feeding strategies (Petry et al. 2010; Jones et al. 2019; Corá et al. 2020; Grohmann Finger et al. 2021), even being able to take advantage of human-origin stressors i.e. fishing discards, feeding on large seabird species under impact of stressors (Krüger et al. 2017a; Risi et al. 2021).

Methods

Tracking during breeding season

During the breeding season, breeding animals with active nests will be used for tracking, as the need to return to the nest allows for the recovery of the tracking devices.

Penguins

Penguins will be captured by hand on the nest during duty shifts in late December and early January, corresponding to late incubation and early chick-rearing. Individuals about to leave the nest for foraging will be captured when its pair arrive at the nest, therefore nests are always guarded by one adult. Each captured animal will have the head covered by a hood to reduce stress (Wilson 1997). Axy-trek marine loggers (40 x 20 x 8 mm, 14g, GPS logger, time depth recorder TDR and accelerometer) will be attached to the dorsal feathers using 3M Extreme Hold Duct Tape 2835-B (1.88 inches) and Loctite super glue. Tracking devices will never go over a 3% body mass threshold, as these levels of extra load do not produce detrimental effects for diving animals. The Axy-trek loggers are shaped to reduce drag, and the placement of the device on the central or lower back of the animal maintains the hydrodynamic and body balance (Bannasch et al. 1994; Ludynia et al. 2012). We will test whether the devices deployment have any effect on the breeding of the pairs by following (Beaulieu et al. 2010), comparing nesting behaviour and breeding success of nests of instrumented animals with nests of non-instrumented animals, making use of camera traps (see sessions below).

Instruments will be recovered after 5 to 10 days, after which the animal will be recaptured and the device removed. After the removal of the device, 2ml of blood will be collected from the leg and the tip of 5 back feathers will be cut off with a sterilized scissor. The blood will be used for genetic analysis (1ml) and fat acids analysis (1ml).

Animal handling will be limited to a maximum of 15 minutes after which all individuals will be returned immediately to their nests. During recovery one researcher will watch the eggs or chicks for predators until the adult is released back to the nest. In both handling events (deployment and recovery of GPSs), behavioural states of the released animals will be recorded as follows: animal went directly to the nest or animal remained away from the nest (in this case time to return will be recovered). During the chick rearing, the weight of the chicks of the tracked animals will be measured before and after tracking. Chicks will also be weighted in nests which adults were not tracked, in order to compare effects of tagging.

Flying seabirds

During the breeding period, flying seabirds will also be captured by hand, at the nest. Prior to the capture, breeding animals will be sampled for personality using a pre-established protocol (Réale et al. 2007; Dingemanse and Dochtermann 2013) sampling for shyness-boldness, exploration-avoidance, activity, aggressiveness and sociability (Patrick and Weimerskirch 2014; Krüger et al. 2019). Shyness-boldness will be measured by handling time, struggle attempts, breathing rate (Brommer and Kluen 2012). Exploration-avoidance will be measured using the “response to a new object” method, consisting of positioning a new object 1-3m of the nest. Behaviors will be recorded with a digital camera attached at the object during a 5 min period to obtain data on the behaviors of the animals towards the object (Grace and Anderson 2014; Patrick and Weimerskirch 2014; Krüger et al. 2019). Activity will be measured by positioning a camera trap by the nest and recorded during 2h without any interference from researchers. The response of the animals to a new object and the activity will be evaluated at least five times each season. Aggressiveness will be measured using a simulated territory intrusion by conspecific STI (Wingfield et al. 1987; Botero-Delgadillo et al. 2020) by placing a play-back tape-recorder that will reproduce vocalizations of conspecific neighbors. Reactions will be recorded and used to describe aggressiveness. Sociability will be measured using distance from the nearest neighbor, obtained from georeferenced aerial pictures made with drone flights (see next sessions).

Before capturing, eggs will be taken from the nest and placed on a protected and warmed recipient while the adult is manipulated. Captured animals will have the head covered by a hood to reduce stress. Handling time will be limited up to 20 minutes, during which animals will be ringed with a metal band, have bill and tarsus measured (proxies of body size, Krüger *et al.* 2018), biological samples (feathers and blood) will be collected,and a GPS will be deployed on the lower back. The animals will be released back into the nest, and three states of behavioral responses will be recorded: remained in the nest after release, left the nest but remained at the colony, left the colony. Previous fieldwork efforts (Krüger in prep.) showed that with a handling-time limited to 20 minutes around 38% of the animals leave the nest after releasing but stay in the colony and come back to the nest in less than 5 minutes after releasing, and a minority (2.6%) will leave the colony deserting breeding. Animals overgoing this procedure do not have a significantly different breeding success compared to the rest of the population (Krüger in prep). Egg will be returned after the bird is back at the nest. Those measures of behavior will be used for generating an individual profile of personality of every animal using the methods in Krüger et al. (2019).

Two types of devices will be used: CatLog-P Gen 2 epoxy cased GPSs loggers (15g to 50g) and XAIS Centurion MKII (70g). CatLog GPSs are devices designed to record geographical fixes in given intervals, while the XAIS Centurion MKII are solar-powered GPS receivers equipped with Automatic Identification System AIS reader and radar detection. Their maximum weight of 70g stands below the 3% of body weight recommended for large flying seabirds (Phillips et al. 2003). Southern Giant Petrels weigh from 4000 to 6000 g, therefore, reaching 1.75% of body weight for the smaller animals. Such devices will record animals' position on a pre-configured interval (i.e. one position every 5 minutes during breeding). The AIS and radar detection identifies when an animal approaches any ship and starts to record data in a short period of time (i.e. each minute), inclusively, recording ships data from the AIS, which includes ships name, flag, position and activity. Studies have used similar technology to quantify interaction of Cape Gannets (Grémillet et al. 2019) and Albatrosses (Weimerskirch et al. 2018; Weimerskirch et al. 2020) with fishing vessels in the Atlantic and Indian Oceans. The devices will be deployed using two methods (for comparison of animals’ responses): wing/body harness (Thaxter et al. 2014) and directly on the dorsal feathers using ultra-adhesive tapes for marine applications, Loctite glue and plastic belts.

Breeding success

Nest level

Camera traps will be used to measure the breeding success of the studied nests, following (Southwell and Emmerson 2015; Black 2018; Jones et al. 2018). One time-lapse trail camera will be placed in the vicinity of each of the studied colonies. Those will remain in the area throughout the period of the project execution, and will record two pictures per day. It will allow us to estimate with precision if and when a nest fails and estimate the reason for failure: abandonment, predation, and/or environmental conditions precluding desertion, i.e. heavy rain, blizzards or storms.

Population level

Small-sized Remotely Piloted Aircraft Systems (RPAS or drones) will be used to assess population level breeding success of penguins. We will follow the procedures in ATCM (2018) and Harris *et al.* (2019). In Antarctica, plane or helicopter flights above areas of seabird breeding colonies is prohibited, therefore the risks of collision of the RPAS with any other passing aircraft is minimal, but flying schedules will be communicated to the proper channels. RAPS will be flown a minimum of 80m above ground level over penguins breeding colonies in December (peak of the incubation when all breeding individuals are likely active) and in crèche (February), and a maximum of 150m. RPAS will be flown always within line-of-sight during periods of good conditions of visibility and within the limits capacity of wind speed endurance of the aircraft, in order to avoid crashes. Aircraft will be launched at a minimum of 150m distance from any wildlife in order to minimize disturbance. One observer will record the behaviour of the animals before and after the passage of the aircraft in order to assess and quantify possible disturbances, following methods and behavioural classification from Mustafa et al. (2018) and Kim et al. (2019).

Pictures taken during flight will be used to estimate the number of nests in December and number of chicks in February. A rate of chicks raised per nest will be used as a measure of breeding success. Using drone pictures for counting penguins allows for a more precise estimation of population numbers with reduced disturbance to the animals, when risk-preventing measures are taken (Mustafa et al. 2018; Hyun et al. 2020).

RPAS have the bias of not allowing to separate active from inactive nests, which, in the case of the giant petrels, whose colonies are less numerous than penguins, is a disadvantage. Therefore, the number of active nests (December) and number of chicks (late February) of Giant Petrels will be counted in a portion of the studied colonies following methods in Krüger (2019).

Non breeding tracking

Penguins will be captured after molting in the breeding colonies, in mid to late February, depending on the species and study site. Following the same procedure described on the previous sessions, ARGOS platform transmitters will be fixed in the mid / lower back of the animals. Devices will be recovered from the back of the animals in the posterior breeding season, when they return to breed. As ARGOS devices transmit data, therefore it is possible to estimate animal mortality and make estimations whether the devices are affecting animal survival (Hinke et al. 2020).

Adult Giant petrels will receive two types of devices: solar-powered XAIS Centurion MKII (70g), and leg-mounted light-level geolocators (5g) for tracking of non-breeding movements and distribution. The Centurion devices will be deployed using two methods (for comparison of animals' responses): wing/body harness (Thaxter et al. 2014) and directly on the dorsal feathers using ultra-adhesive tapes for marine applications, Loctite glue and plastic belts. Geolocators will be fixed in the metal ring placed on the leg with plastic belts and Loctite glue, following (Krüger et al. 2017b). Devices will be recovered in the next breeding season when animals return to nest. Recovery of devices within one year period is usually in the range of 70% and 80% for Giant Petrels (Krüger et al. 2017b).

For all the 4 species, animals returning back to the colony will be assessed for body condition following Labocha and Hayes (2012), which will be compared to non-tagged animals. For a detailed record of capture, handling and device deployment please see GiantPetrels_GPS_deployment_1080pp.mp4. Certification for that procedure in N° 069 Lucas Krüger.docx

Animal welfare guidelines

We will follow NC3Rs strategy for animal welfare (Prescott & Lindster 2017) and the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) following du Sert et al. (2020). Following those guidelines: (i) there are no replacements, as the study itself targets understanding the species at-sea behavior and breeding ecology; those are species whose responses to the type of study proposed are well known and described, therefore allowing for a minimization of the research impacts (see methods section); (ii) the number of animals being sampled corresponds to less than 5% of the breeding populations in the areas being proposed for study; with the evidence that it has a minor impact of breeding success, the numbers we are proposing are well balanced between maximizing sampling and avoiding impacting the population; (iii) it is very unlikely that we will need to go through euthanasia, as the methods being applied do not generate any risks of non-reversible impacts for the animals, however, in case of necessity, we will follow methods in the session below; (iv) animals who abandon breeding after releasing will be excluded from the study, as changes in behavior are a direct result from the study handling, in this case (final point criteria); such cases are not usual, corresponding to less than 2% of animals for giant petrels, and likely even less for penguins, however, we will keep track of those numbers for follow-up reports and control of our own impact over the animals (see methods). Finally, a follow-up report will be submitted yearly for the committee, including a report of the behavioral responses of the animals to the different sampling methods, compared to the behaviour of non-studied animals (camera traps), number of desertions (if any) and testing for differences of breeding success among handled and not-handled animals. We will video-record the procedures applied to some of the animals in order to provide it for the re-evaluation of the protocol and procedures. A preliminary example of that is found in GiantPetrels_GPS_deployment_1080pp.mp4.

Euthanasia protocol

In the unlikely case that an animal experiences a non-reversible impact from the methods (such as leg, wing or bill lesions that prevent the animal from feeding), the method for euthanasia will be as follow: (1) With the animal restrained and with eyes covered it will be sedated (general anaesthesia); (2) we proceed with the intramuscular administration of 10mg/kg of ketamine + 0.5 mg/kg of midazolam in the pectoral muscle; (3) we proceed with over-sedation by intravenous administration of 0.5 ml/kg of Thiopental diluted in sterile distilled water.

FIgure 1. Immobilization of a chinstrap penguin with the GPS device deployed (left) and detail of one tagged animal leaving the nest (right).

Table 1. Summary of intended sampling sites, species and individuals, taking in to account sex (F: female, M: male, NA: non-applicable), age (Ad: adult, Fl: fledging) and breeding stage (BR: active breeder, PBR: post-breeding, NBR: non-breeder). Quantity per season is the target sampling size.

Location	Species	Age	Stage	Sex	Quantity per season
Harmony point (Nelson Island)	*Pygoscelis antarcticus*	Ad	BR	F	10
				M	10
			PBR	NA	10
		Fl	NBR	NA	10
	Pygoscelis papua	Ad	BR	F	10
				M	10
			PBR	NA	10
		Fl	NBR	NA	10
	Macronectes giganteus	Ad	BR	F	10
				M	10
			PBR	F	10
				Male	10
		Fl	NBR	F	10
				M	10
Stinker point (Elephant island)	*Pygoscelis antarcticus*	Ad	BR	Female	10
				Male	10
			PBR	NA	10
		Fl	NBR	NA	10
	Pygoscelis papua	Ad	BR	Female	10
				Male	10
			PBR	NA	10
		Fl	NBR	NA	10
	Macronectes giganteus	Ad	BR	Female	10
				Male	10
			PBR	Female	10
				Male	10
		Fl	NBR	Female	10
				Male	10
Kopaitic Island (O´Higgins station)	*Pygoscelis antarcticus*	Ad	BR	Female	10
				Male	10
			PBR	NA	10
		Fl	NBR	NA	10
	Pygoscelis papua	Ad	BR	Female	10
				Male	10
			PBR	NA	10
		Fl	NBR	NA	10
	Pygoscelis adeliae	Ad	BR	Female	10
				Male	10
			PBR	NA	10
		Fl	NBR	NA	10
Albatross islet (Fildes Bay)	*Macronectes giganteus*	Ad	BR	Female	10
				Male	10
			PBR	Female	10
				Male	10
		Fl	NBR	Female	10
				Male	10

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