Tourism and stress hormone measures in Gentoo Penguins on the Antarctic Peninsula

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Abstract

The impacts of tourism on wildlife have long been a concern in areas where ecotourism is a major industry. The issue is especially pressing in Antarctica, which has a rapidly growing tourism industry largely concentrated around penguin colonies on the Antarctic Peninsula. Guidelines developed by both the Committee for Environmental Protection and the International Association of Antarctica Tour Operators include measures to minimize wildlife impacts. In this study, we examined the relationship between physiologic stress in Gentoo Penguins (*Pygoscelis papua*) and tourism. Corticosterone is an adrenal glucocorticoid that has been shown in previous studies to increase in response to stressors such as low food availability, environmental conditions, as well as human visitation and proximity. Fecal glucocorticoids (FGM; primarily corticosterone and metabolites) were measured in Gentoo Penguin guano collected at 19 breeding colonies ($n = 108$, 3–10 samples per site) on the Antarctic Peninsula and the South Shetland Islands, representing a wide range of tourism visitation. We found a large degree of variation in FGM concentrations, and no relationship between FGM concentrations and number of tourists landed at that site. These results suggest that current tourism management guidelines on the Antarctic Peninsula are effective at preventing increased stress in Gentoo Penguins as measured by hormonal markers, and demonstrate the use of guano as a non-invasive, low-impact methodology for monitoring Gentoo Penguin stress.

Keywords Stress · *Pygoscelis papua* · IAATO · Tourism · Visitor guidelines

Introduction

Tourism in the Antarctic has grown rapidly over the last five decades, from the first trip in 1969 to the more than 58,000 tourists who visited Antarctica in the austral summer of 2017/18 (IAATO 2018). Given this rapid increase, there is a concern that human activities may have a negative impact on wildlife, particularly on the Antarctic Peninsula where the overwhelming majority of all tourism activity takes place (Lynch et al. 2010; Bender et al. 2016). Due to the difficulty of landing tourists in the Antarctic, activity on the Antarctic Peninsula is highly spatially concentrated, with more than 76% of landings occurring on a total of 200 ha of land (Bender et al. 2016). Adding to concerns about wildlife impacts, the most heavily visited sites usually have penguins that are either incubating eggs or guarding chicks at the time of visitation.

In the context of human–wildlife interactions during tourism visits, the Antarctic Treaty has adopted general guidelines for tour ship operations and a series of site-specific visitor guidelines for key tourism locations. This was done in consultation with the International Association of Antarctica Tour Operators (IAATO), with all proposed guidelines reviewed by the Treaty's Committee for Environmental Protection (CEP). The first Visitor Guidelines were adopted at the 1994 Antarctic Treaty Meeting, following the 1991 Protocol on Environmental Protection to the Antarctic Treaty, which designated Antarctica as a natural reserve, and set out environmental obligations for all tourist or scientific operations. These guidelines include site-specific regulations as well as general practice guidelines, the latter of which

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include limiting the number of landed visitors at a location to 100 at any one time, and enforcing a 5 m minimum distance from nesting penguins and greater distances from other nesting seabirds (e.g., Southern Giant Petrels) and mammals (e.g., Elephant Seal wallows). These guidelines also require maintaining a 1:20 guide:tourist ratio, strict prohibitions against depositing garbage on land or at sea, and prohibitions against removing any biological or non-biological material from visitor sites. All visitor sites share these guidelines while certain sites of historical or ecological sensitivity have even tighter restrictions placed on the number of landed visitors at any given time, or are closed during certain parts of the season. However, even with these restrictions in place, some popular sites receive more than 21,000 visitors each year (IAATO 2018) and, as such, understanding both acute and cumulative wildlife impacts remain an area of active research (e.g., Coetzee and Chown 2016).

Previous work on the effects of human activities on penguins (in the Antarctic and elsewhere) has shown mixed results depending on the species and the response metric used (Coetzee and Chown 2016). Adélie Penguins (*Pygoscelis adeliae*) display increased heart rates when approached by humans (Culik et al. 1990), whereas heart rates in Gentoo Penguins (*Pygoscelis papua*) are not elevated when humans approach slowly and remain at a distance of at least 3 m (Nimon et al. 1996). Gentoo Penguins do, however, show increased threat displays towards humans and increased vigilance behaviors even after a human stimulus has been removed (Holmes 2007). Human disturbance increased nest desertion, resulting in increased nest predation, in African Penguins (*Spheniscus demersus*) (Hockey and Hallinan 1981). Conversely, no effect was seen on reproductive success and fledging success due to tourism in Adélie Penguins (Carlini et al. 2007) or Gentoo Penguins (Cobley and Shears 1999; Holmes et al. 2006). Elevated concentrations of stress hormones have been observed in areas with heavy tourism impact in Magellanic Penguins (*Spheniscus magellanicus*) (Walker et al. 2005) and Yellow-eyed Penguins (*Megadyptes antipodes*), resulting in decreased fledging weights and breeding success (Ellenberg et al. 2007). Indirect effects of tourism have also been examined, as Crosbie (1999) found no effect of tourist presence on Gentoo Penguin nest predation rates by Skuas (*Catharacta* spp.).

Another group of studies suggests habituation, in which repeated exposure eventually decreases the magnitude of the response to that stimulus, may play a role in mediating responses to human activity. Previous work on African Penguins (van Heezik and Seddon 1990) and Magellanic Penguins (Walker et al. 2005) have demonstrated decreased flight responses in areas of heavy tourism compared to those with low levels of human interaction. Likewise, Holmes

et al. (2006) found decreased threat displays and vigilance among Gentoo Penguins near a scientific station compared to those breeding farther away. While Yellow-eyed Penguins showed increased heart rate in response to human approach, short and consistent approaches allowed them to habituate and diminished the elevated heart rate response (Ellenberg et al. 2009). Habituation has also been seen in stress hormone responses, with lower and more consistent corticosterone concentrations in heavily visited areas in Magellanic Penguins (Fowler 1999; Walker et al. 2006) and in Gentoo Penguins (Barbosa et al. 2013). In the case of such habituation, we would expect a quadratic relationship between stressor and the measure of stress (Busch and Hayward 2009).

In this study, we examined the impacts of tourism on the Antarctic Peninsula on Gentoo Penguins using measurements of fecal corticosterone and its metabolites (FGM) as a measure of adrenal glucocorticoid stress response. When short-term stress is experienced, elevated concentrations of corticosterone promote physiological coping mechanisms (e.g., creation of glucose from energy stores, increased cerebral blood flow, immune system regulation, etc.). However, continued exposure to stressors accompanied by chronically heightened levels of corticosterone has been shown to be detrimental to reproductive success, including increased nest abandonment, decreased chick provisioning, or impaired reproductive system function (Groscolas et al. 2008; Busch and Hayward 2009; Spee et al.2010; Thierry et al. 2013a, b). As with other response metrics, chronic exposure to a stressor may result in habituation, resulting in the down-regulation of the adrenal system in which both circulating and levels of corticosterone are suppressed (Rich and Romero 2005; Busch and Hayward 2009).

Measuring FGM in penguin guano allows for a minimally invasive approach that does not require handling birds or taking biological samples from them, thus minimizing researcher impact. Sampling from guano (fecal samples) also provides a longer-term, more cumulative measure of stress than sampling from blood, reflecting stress within a 24 h window but excluding the pulsatile and short-term stress release patterns occurring with measures from blood samples. Guano is, therefore, a useful metric, especially for examining seabirds on a colony or at the regional scale (Cavigelli et al. 2005; Möstl et al. 2005; Young and Hallford 2013). We examined FGM concentrations in penguin guano at 19 breeding colonies along the Antarctic Peninsula, representing the largest geographic range of a study of physiologic stress in penguins (Fig. 1). We also used detailed tourism data on the number of tourists visiting each breeding colony to examine the relationship between visitation and stress hormone concentration.
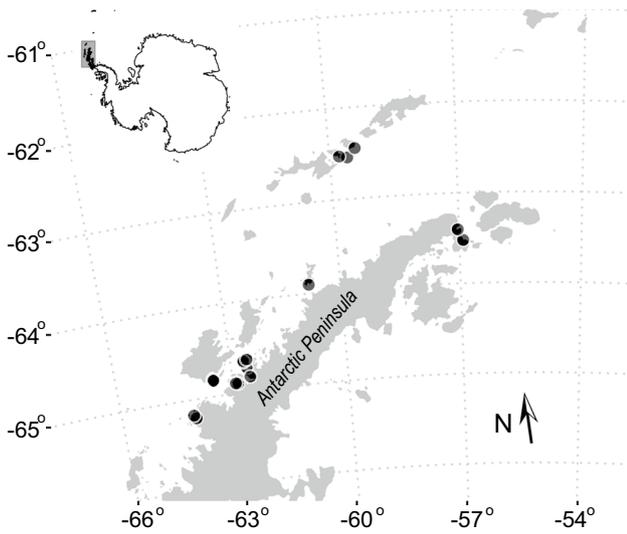
Fig. 1 Sample locations on the Antarctic Peninsula and the South Shetland Islands. The sample region is indicated on the inset map of Antarctica in gray

Materials and methods

Guano was collected from 19 Gentoo Penguin breeding colonies along the Antarctic Peninsula and the South Shetland Islands in 2017–2018 and 2018–2019 (Fig. 1, Table 1). In each season, samples were collected within 1 h of each other on a single day for each colony. Guano samples (~ 10 g, $n = 108$) were collected non-invasively from freshly excreted samples on the ground within the breeding colony. Given that samples were collected on the ground, and given the density of Gentoo Penguin nests within a colony, each sample has a possibility of including more than one excretion, potentially from more than one individual, and cannot be identified to an individual of known sex or age. Though corticosterone measures have been shown to vary by sex, age, reproductive status, and other individual factors (Cockrem 2013), our sampling was opportunistic and, though not randomized, is unlikely to be significantly different in terms of age or sex composition between colonies, which at any

Table 1 Full names and location of sampled Gentoo Penguin breeding colonies, with number of samples, mean, and standard error of FGM concentration at each colony as well as IAATO landings per season and Gentoo Penguin abundance

Code	Colony name	Latitude	Longitude	Samples	Mean (SE) FGM (µg/g)	IAATO season landings	Abundance (breeding pairs)
ALMI	Brown Station	− 64.896	− 62.870	8	0.6958 (0.1285)	10,078*	177*
BROW	Brown Bluff	− 63.522	− 56.905	3	0.5161 (0.0122)	11,229	711
BRYE	Bryde Island East	− 64.890	− 62.927	8	0.6346 (0.0369)	0*	520*
CUVE	Cuverville Island	− 64.682	− 62.621	9	0.4488 (0.0913)	16,311	6903
DAMO	Damoy Point	− 64.816	− 63.510	6	0.9212 (0.2309)	8432*	2129*
DANC	Danco Island	− 64.734	− 62.594	8	0.3028 (0.0329)	10,520*	2732*
FORT	Fort Point	− 62.543	− 59.579	6	0.4002 (0.0670)	1060	1006
GEOR	Georges Point	− 64.669	− 62.670	3	0.4684 (0.1203)	1074	3949
HOPE	Hope Bay/Esperanza Station	− 63.404	− 57.026	6	0.8870 (0.1520)	769	519
JOUG	Jougla Point	− 64.828	− 63.492	6	0.5368 (0.1350)	8200*	1111*
LOCK	Port Lockroy/Goudier Island	− 64.825	− 63.494	6	0.6615 (0.1660)	16,653*	548*
MIKK	Mikkleson Island	− 63.902	− 60.792	3	0.7329 (0.2591)	8820	1262
MOOT	Moot Point	− 65.206	− 64.078	6	0.7243 (0.0496)	0	693
NEKO	Neko Harbour	− 64.838	− 62.533	10	0.8105 (0.0591)	21,504	973
ORNE	Orne Islands	− 64.659	− 62.663	3	0.6575 (0.2370)	793	483
PETE	Petermann Island	− 65.173	− 64.135	5	0.8429 (0.1664)	11,584	3516
ROBE	Robert Point	− 62.448	− 59.386	3	0.6197 (0.0568)	2496	917
SELV	Selvick Cove	− 64.647	− 62.571	6	0.6522 (0.1473)	133*	616*
YANK	Yankee Harbour	− 62.526	− 59.769	3	0.6900 (0.1212)	5233	5466

For colonies with 2 years of sampling, the mean of both years' season landings and Gentoo Penguin abundance are reported here with an asterisk, however, landing data specific to each sampling year were used for analysis

point contain both sexes and a range of ages. Samples were dried in a food dehydrator set on low (approximately 110 C) for 8–12 h and stored at room temperature until processing.

Dried guano samples were crushed, and 0.2 g were weighed out and placed into a 16-mL borosilicate glass vial. 5 mL of 100% ethanol was added to each tube and then briefly vortexed, before being placed on a plate shaker for 1 h at 650 revolutions minute^{-1} and finally centrifuged at $650 \times g$ for 20 min. Supernatant was carefully removed to avoid collecting any fecal sample and placed into a clean borosilicate vial. Guano tubes were rinsed with another 5 mL of 100% ethanol, briefly vortexed and spun down ($650 \times g$ for 20 min) and supernatant was again collected and combined into appropriate vials and evaporated under air. Dried extracts were reconstituted with 2 mL of assay buffer and stored at -80 °C until analysis. 100 µL of each sample was analyzed using a commercially available kit (Corticosterone ELISA kit, Enzo Life Sciences Inc. USA). Spike recovery was performed using a known standard, recovery was consistently above 90%, and therefore no further adjustment of concentrations was made as there was low variability among samples. Assay characteristics included parallelism of standard curve with a serial dilution of extract; this was also used to determine the dilution of extract measurable on the standard curve. Assay sensitivity was 27 pg mL^{-1}. Antibody cross-reactivity was corticosterone (100%), deoxycorticosterone (21.3%), desoxycorticosterone (21.0%), progesterone (0.46%), testosterone (0.31%), tetrahydrocorticosterone (0.28%), aldosterone (0.18%), cortisol (0.046%) and < 0.03%: pregnenolone, estradiol, cortisone, 11-dehydrocorticosterone acetate. As such, the assay measured primarily corticosterone and two primary metabolites. The coefficient of variation for intra-assay variability was 3.56% and 2.09% for inter-assay variability. The final concentration of FGM was expressed as micrograms of FGM per gram of guano.

Several population-level variables were recorded for each sampled colony, including Gentoo Penguin abundance and annual growth rate for the colony (extracted from www.penguinmap.com; Humphries et al. 2017), as well as tourism visitation on time scales ranging from 1 day prior to sampling to the total landings for the season (D. Stanwell-Smith, IAATO, personal communication). The measure of tourist landings was "small boat landings" as defined by IAATO, in which passengers were physically on shore at the colony. Wild birds were not identified to individual so no individual-level parameters were recorded.

We fit a linear regression model with both linear and quadratic terms to examine the relationships between log-FGM (in sample j at site i in year t) and the annual growth rate of the colony (averaged over 6–10 years, depending on data availability) ($GrowthRate_i$), the abundance of Gentoo Penguin breeding pairs (averaged over both years

of sampling if applicable) ($Abundance_i$), the number of landed passengers in the 24-h period prior to sampling ($1DayLandings_{it}$), and a linear and quadratic interaction term to capture the potentially non-linear influence of longer-term visitation (the total number of landed passengers that season[$SeasonLandings_{it}$]). Abundance and growth rate are included as proxies for unmeasured demographic factors such as reproductive success in the colony. The interaction terms are included because ongoing exposure to landed passengers may habituate penguins and thereby affect the influence of visitation as captured in $1DayLandings_{it}$. Specifically, mean log-FGM for colony i in year t (μ_{it}) was modeled as:

$$
\begin{aligned}
\mu_{it} = \alpha &+ \left(\gamma_1 * GrowthRate_i\right) + \left(\gamma_2 * Abundance_i\right) \\
&+ \left(\beta_1 * 1DayLandings_{it}\right) \\
&+ \left(\beta_2 * 1DayLandings_{it} * SeasonLandings_{it}\right) \\
&+ \left(\beta3 * 1DayLandings_{it} * SeasonLandings_{it}^2\right)
\end{aligned}
$$

We assumed normally distributed residuals, so that log-FGM for sample j at site i in year t (Y_{itj}) is given by.

$$
Y_{itj} \sim N\left(\mu_{it}, \sigma^2\right)
$$

where μ_{it} is the mean log-FGM at colony i in year t, and σ^2 represents intra-sample variation within the colony. We estimated model parameters in a Bayesian framework, using broad prior distributions for all parameters, i.e., a normal distribution for the α, γ, and β parameters [N($\mu = 0$, $\sigma = 0.001$)] and a Gamma distribution for the variance parameter σ^2 [Gamma($k = 0.001$, $\theta = 0.001$)]. Models were run for 100,000 iterations with a burn-in of 1000 iterations.

While the above model is the one that best captures the key covariates of interest for our analysis, we used a series of t tests to explore the inclusion of additional covariates that might have explained some of the residual site-level variation, including the presence/absence of a research station at the breeding colony, sampling year, time of day, and whether the colony was a mixed-species breeding colony.

All statistical analyses were run in R (R Core Team 2018) using base R for t tests, and R2jags (Su and Yajima 2015) to interface with JAGS (Plummer 2003) for the Bayesian analysis.

Results

FGM concentrations in this study ranged from 0.127 to 1.959 µg g^{-1}. We found large within-colony variation in log-FGM concentrations ($\sigma^2 = 4.006, n = 108$), which was larger than that between colonies ($var(\mu_{it}) = 0.017$, $n = 26$). There was also large variation in annual tourism
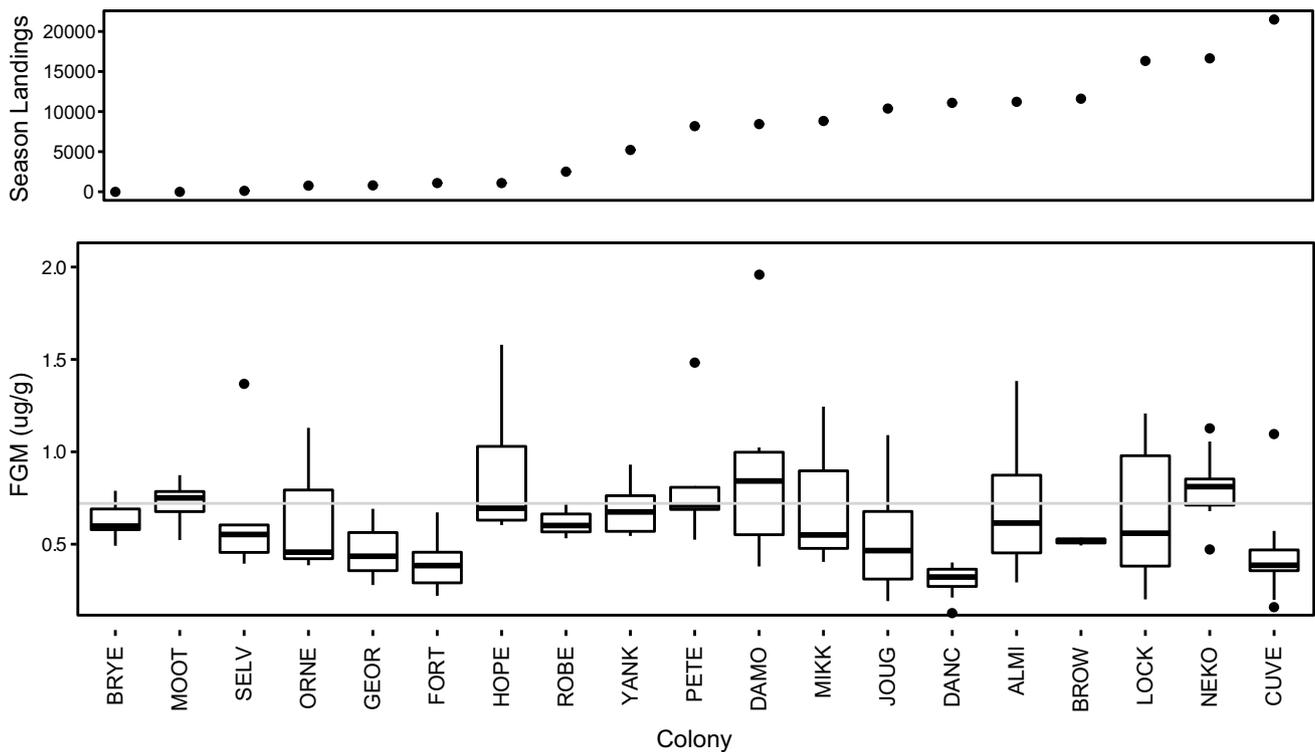
Fig. 2 Scatterplot showing season landings for each site (top) paired with boxplots demonstrating the variation in FGM concentrations in guano samples at each colony (bottom). The gray horizontal line indicates the grand median for all sample values. For colonies with 2 years of sampling, the mean of both years' IAATO small boat landings per season is reported here, as in Table 1. Full colony names are listed in Table 1

visitation at sampled colonies, ranging from 0 (Bryde Island, Moot Point, Selvick Cove) to 21,504 landings year^{-1} (Neko Harbour), with a median of 8420 landings year^{-1} (Jougla Point) (Fig. 2). We found no significant relationships between log-FGM and the presence of a research station (t test, $t_{26.85} = 1.303$, $p = 0.445$), the sampling year (t test, $t_{100.29} = 0.785$, $p = 0.434$), the time of day (t test, $t_{78.738} = -0.026$, $p = 0.980$), or whether the colony was a mix-species breeding colony (t test, $t_{78.987} = 0.441$, $p = 0.241$).

Model results indicate that FGM concentrations are not affected by tourism, including the interaction between landings 1 day prior to sampling and landings throughout the entire season (Table 2). We summarize our parameter estimates using the 95th percentile credible intervals (bounded by the 2.5th and 97.5th quantiles of the posterior distribution), which are analogous to 95th percentile confidence intervals but with a more direct interpretation in terms of the probability distribution of the model parameter. Although a 24 h period is a priori the most

Table 2 Parameter estimates and credible intervals from a Bayesian model relating log-FGM concentrations to tourism, population growth, and population abundance

Parameter	Mean estimate	95% credible interval	Description of parameter
α	− 0.597	(− 0.707, − 0.487)	Mean FGM concentration for colony i in year t given mean values for all covariates
γ_1	0.046	(− 0.052, 0.144)	Slope parameter for colony growth rate for colony i
γ_2	− 0.112	(− 0.210, − 0.014)	Slope parameter for colony abundance for colony i
β_1	0.034	(− 0.225, 0.157)	Slope parameter for 1 day landings for colony i in year t
β_2	0.014	(− 0.113, 0.140)	Slope parameter for the interaction between 1 day landings and season landings for colony i in year t
β_3	0.041	(− 0.070, 0.153)	Slope parameter for the interaction between 1 day landings and the square of season landings for colony i in year t
σ^2	3.978	(2.952, 5.154)	Variance between samples within colony i in year t

physiologically meaningful time period to consider given the approximate guano evacuation rates of Gentoo Penguins, FGM concentrations were also unrelated to visitation over the previous 7 or 14 day windows or the entire season. These results are robust to removing the six colonies with the lowest sample sizes from the model. The only parameter whose credible interval did not contain 0 was that related to colony abundance.

Discussion

Given the continuing increase in tourism and the unique nature of environmental management in Antarctica, understanding the impact of tourism on wildlife is an area of substantial concern. Prior studies in a variety of environments have provided a mixed picture of human impacts on penguin populations. Here, we use FGM in penguin guano to assess whether tourism activities are affecting physiological stress in Gentoo Penguins, whose colonies are a major draw for the industry.

We found no relationship between FGM concentration and the number of passengers recently landed at a site, nor do we find any indication of an interaction between recent visitors and total season visitors. This suggests that either Gentoo Penguins do not experience stress related to tourism presence at current levels and under current visitor management regimes, or that other sources of individual and colony variation in hormonal stress are greater than those due to tourism landings alone. However, another possible explanation is that Gentoo Penguins have become habituated to human presence in and around their colonies and accordingly do not have elevated FGM concentrations in response to tourism operations. While we cannot definitively rule out the effect of habituation given this sampling design, it is worth noting that Gentoo Penguins at sites with little or no tourism activity do not have higher FGM concentrations than those with heavy tourism, and we found no quadratic relationship between tourism and FGM concentration. Both of these findings are, therefore, inconsistent with the hypothesis of hormonal habituation at heavily visited sites. Rather, these data suggest that the current IAATO Visitor Guidelines are sufficient to minimize tourism impacts on Gentoo Penguins compared to other sources in variation, to the extent that impacts are reflected in stress hormone markers.

Our results are specific to Gentoo Penguins and species with overlapping breeding ranges, including Chinstrap Penguins (*Pygoscelis antarcticus*) and Adélie Penguins, may display different responses to tourism in the same region. Our results agree with other studies that indicate no impact of tourism on reproductive success (e.g., Stonehouse 1970; Cobley and Shears 1999) but contrast with the findings of Barbosa et al. (2013), who found significantly higher FGM levels in a Gentoo Penguin colony with high tourist visitation as compared to a nearby colony with little to no tourism impact. However, the study by Barbosa et al. (2013) measured corticosterone in feathers, which could reflect a different time scale than that represented in guano. It is also worth noting that our study over a large number of sites finds high inter-site variation that is not explained by patterns of visitation, which suggests that differences between a single pair of sites may be difficult to interpret.

Dunn et al. (2019) recently found a negative relationship between trends in Gentoo Penguin abundance and tourism at Port Lockroy (one of the sites sampled in this study), and cited stress as one potential mechanism for that relationship. While all of our samples from Port Lockroy were from the tourist visited (rather than the closed) area and we cannot directly assess whether the penguins on the visited side of the island are more or less stressed than those in the closed area, we find no evidence for a link between visitation and Gentoo Penguin FGM levels excreted in guano. The relationship that we find between FGM and colony abundance, though not strong, does suggest that stress responses may be driven by population-level processes, as in previous studies that have linked increased stress hormone levels to disappearance of individuals from decreasing populations (Kitaysky et al. 2007). We concur with Dunn et al. (2019) that these processes are likely affected by complex interactions between both intrinsic and extrinsic factors.

We focused our study specifically on tourist visitation from IAATO member companies, yet there are likely to be many factors that contribute to hormonal stress measures. We have also examined the effect of some of these variables such as time of day, year, and presence of a research base as an indication of increased exposure to human activity, as well as those related to colony abundance and growth (which can be associated with food availability, habitat quality, or predator interactions). However, there are multiple intrinsic and extrinsic factors not directly measured here for any given individual at any colony that may affect stress hormone concentration, such as age (Angelier et al. 2007), body condition (Goutte et al. 2014), or food availability (Kitaysky et al. 2007). Our results do not attempt to explain the ultimate drivers of the variation we see between colonies, but do emphasize that this variation does not appear to be driven by tourism or the other factors that we have explored here. It is likely that other individual factors (e.g. age, body condition, frequency of aggression from predators and conspecifics) and colony factors (e.g. seasonal weather changes, distance to foraging grounds) affect FGM levels to a larger degree than tourism visitation.

Given the large amount of variation found both within and between colonies, continued monitoring is important

as Antarctic wildlife management moves forward. Guano sampling, being non-invasive, provides a relatively easy and inexpensive method for doing so regularly. Repeated measures from the same group of individuals may help minimize the inter-sample variation and control for changing demographics over time, while increased sample size within a colony as well increased number of colonies sampled will help clarify these relationships. Adding such physiological markers to current work on population monitoring will help us to better understand both the short- and long-term effects of tourism on Gentoo Penguins in the Antarctic.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Stony Brook University Institutional Animal Care and Use Committee permit # 2011-1881-R1-6.19.20-BI, Antarctic Conservation Act permit #2016-013, United States Department of Agriculture permit #126,132).

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