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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ABSTRACT

Alert Distance (AD) and Flight Initiation Distance (FID) are popular measures used to explore the reaction of prey to approaching predators, and thus the economics that underlie optimal escape strategies. AD likely mirrors the effort invested into vigilance, while FID provides an estimate of the perceived risk of an approaching threat. Although individual variation in AD and FID is influenced by environmental factors such as variation in predation pressure and human disturbance, the repeatabilities of these traits (especially AD), and therefore their designation as a

personality trait, remain under investigated. Here, we studied the repeatability of AD, FID, and the decision to flee in a flightless, ground-breeding seabird, the king penguin (*Aptenodytes patagonicus*). A single experimenter approached three times over three consecutive days 47 incubating king penguins from two different colonies with varying human disturbance levels. We explored the effects of weather, time of day, and approach speed of the experimenter on these behaviors. Weather had an effect on all three behaviors. In warm, sunny weather AD increased, while in rainy, windy weather birds were more likely to flee yet waited longer before initiating flight. Faster approach speeds between AD and FID increased FID. Weather conditions and speed of approach affected repeatability estimates, highlighting the need to consider external sources of variation when refining such estimates. FID and the decision to flee were significantly and moderately repeatable (r = 0.26 and 0.57 respectively), while AD was not. There was no evidence of habituation or sensitization due to colony.

Key words: *Aptenodytes patagonicus*, personality, human disturbance, optimal escape decisions, predation risk,

INTRODUCTION

Understanding how wild animals react to threats - especially predators - and the economics underlying (optimal) escape decisions, is a central topic in behavioral ecology that has been widely studied by evaluating Alert (AD) and Flight Initiation (FID) Distances (Tätte et al., 2018; Blumstein, 2019; reviewed in Frid & Dill, 2002). AD and FID can be defined as the distances at which a focal animal interrupts its normal behaviour to become alert (AD) or begin to flee (FID) from an approaching predator. AD provides us with a surrogate of the amount of time, and possibly energy, invested by the focal individual into surveying its surrounding; i.e., the degree of vigilance (Fernández-Juricic and Schroeder, 2003; Beauchamp, 2015; Uchida et al., 2019; but see Tätte et al., 2019). Meanwhile, variation in FID informs us on the perceived predation-risk of the focal individual (Blumstein, 2006, 2019; Møller et al., 2008).

In practice, the decisions that prey undertake when approached by a predator (AD and FID) can be simulated by performing non-lethal approaches by human experimenters (Frid and Dill, 2002; Beale and Monaghan, 2004a). Animals are expected to respond in much the same way to human approach stimuli as they would to actual predators since (1) both divert time and energy that could be otherwise invested in fitness enhancing activities; and (2) animals should be selected to overestimate rather than underestimate risk due to the cost of miscalculation, *i.e.*, injury or death (Frid and Dill, 2002). While AD can be hard to observe in some species or contexts, FID has the advantage of being easily measured and quantified (Tarlow and Blumstein, 2007). Both can be standardized within and across studies (Blumstein, 2006; Møller et al., 2008), and are species-specific (Blumstein et al., 2003; Møller, 2008; Carette and Tella, 2011; Piratelli et al., 2015). As a consequence, FID in particular and AD when available, have become popular measures in conservation biology used in establishing set-back distances and buffer zones to

minimize stress-related disturbances on wildlife (Rodgers and Schwikert, 2002; Fernández-Juricic et al., 2005).

Although AD and FID are species-specific, there is fast accumulating evidence that FID (and to a lesser extent AD) can vary among populations of the same species and between individuals within the same populations (Edwards et al., 2013). For instance, FID is often shorter in urban versus rural populations (Piratelli et al., 2015; Carette and Tella, 2017), suggesting either that these animals have habituated (or developed tolerance) to human disturbance (Burger and Gochfeld, 1981, 1990; Walker et al., 2006), or that less tolerant individuals have migrated away from human presence (Ellenberg et al., 2009; Carette and Tella, 2010, 2011). Further, upon repeated approaches, FID has been shown either to decrease (habituation: Carter et al., 2012; Petelle et al., 2013; Arroyo et al., 2017), or increase (sensitization: Dill, 1974; Wheeler et al., 2009), demonstrating within-individual plasticity. FID can display consistent inter-individual (repeatable) variation over time (Carette and Tella, 2010; Carter et al., 2012; van Dongen et al., 2015). The same cannot be said of AD, which remains to be explored; however, vigilance behavior has often been found to be mildly repeatable (Couchoux and Cresswell, 2012; Roche and Brown, 2013). Repeatable behavioral traits measured over time are a prerequisite for the characterization of individual personality or temperament traits (Dingemanse & Wright, 2020), and might suggest genetic or early environmental constraints shaping individual risk-taking behavior. In fact, due to its repeatability, FID is frequently used as a metric to explore boldness (Atwell et al., 2012; Petelle et al., 2013; Highcock and Carter, 2014; Young et al., 2015), one of the five main personality traits defined by Réale et al., (2007), which is associated with risk-assessment and risk-taking, particularly in the context of predation and disturbance.

AD and FID should be strongly selected in prey species, in a way that balances the survival benefits of escaping approaching predators with the costs of abandonning other fitness-

enhancing activities such as reproduction (Ydenberg and Dill, 1986). Consequently FID is known to vary with the perceived risk of predator approaches (Frid and Dill, 2002; reviewed in Stankowich and Blumstein, 2005) including the size or number of predators (Beale and Monaghan, 2004a; Geist et al., 2005), directness of approach (Smith-Castro and Rodewald, 2010; Sreekar and Quader, 2013; but see Fernández-Juricic et al., 2005), speed of approach (Bateman and Fleming, 2011; Samia et al., 2016; but see Lafferty, 2001), and predator intent (*i.e.*, predator suddenly turning towards the prey or maintaining a purposeful gaze; Cooper, 2003; Bateman and Fleming, 2011; Sreekar and Quader, 2013). FID has also been shown to vary with the time of day (Patelle et al., 2013; Piratelli et al., 2015, Ferguson et al., 2019), can be reduced when prey have low energy reserves (Beale and Monaghan, 2004b; Piratelli et al., 2015), are in a large group (dilution effect; Ydenberg and Dill, 1986; Burger and Gochfeld, 1991, Santoyo-Brito et al., 2020), or are in close proximity to refuge (Cooper and Whiting, 2007). AD has not been explored to the same extent as FID, but evidence suggests that vigilance behaviour is also modified by time of day (Edwards et al., 2013), weather conditions (Couchoux and Cresswell, 2012), conspecific interactions (Hess et al., 2016), sex and reproductive status (Burger and Gochfeld, 1994), and group size (Díaz and Asensio, 1991; Carter et al., 2009; Boujja-Miljour et al., 2018).

The cost-benefit fitness trade-off of fleeing a tentative predator should be particularly strong for ground-laying birds tied to vulnerable nesting sites, such as penguins. Penguins commit a high amount of time and energy to reproduction (obligate bi-parental care, long-term fasting, prolonged breeding cycle and chick development; Williams, 1995), and face a strong fitness trade-off between the survival costs of defending their brood against predators (*i.e.*, injuries and potential death for the adult) and the reproductive costs of abandoning their current reproduction but surviving to breed another year (Montgomerie and Weatherhead, 1988; Frid and Dill, 2002; Dowling and Bonier, 2018). Accordingly, penguins are usually highly territorial and defensive of

their brood (Viñuela et al., 1995; Amat et al., 1996; Côté, 2000), though within species there is clear variation on the degree of territoriality and aggression depending on brood value (Amat et al., 1996; Côté, 2000), the type of threat considered (Lee et al., 2017), and bird personality (Traisnel & Pichegru, 2018). Surprisingly, few studies have investigated how variable AD and FID to approaching predators are in penguins, nor the factors affecting those traits, despite their importance in shaping breeding decisions, and despite the fact that several studies have documented marked effects of disturbance (e.g. ecotourism) on penguin behavior (vigilance and locomotory behavior, time budgets; Holmes et al., 2005, 2006; Burger and Gochfeld, 2007), physiological stress (Ellenberg et al., 2006, 2012, 2013; Viblanc et al., 2012; Carroll et al., 2016), or reproduction (Giese, 1996; McClung et al., 2004; Ellenberg et al., 2006; reviewed in Bateman and Fleming, 2017).

Here, we investigated the variability of both AD and FID in breeding king penguins (*Aptenodytes patagonicus*). King penguins are large, ground-laying, flightless birds that form extensive colonies on the subantarctic shorelines. Breeding pairs incubate their single egg or young chick on top of their feet (Stonehouse, 1960), therefore limiting their mobility. They are subject to on-land predation mostly by giant petrels, *Macronectes halli* and *Macronectes giganteus*, and brown skuas, *Catharacta lonnbergi* (Hunter, 1991; Descamps et al., 2005). Giant petrels and skuas especially target eggs and chicks, but petrels are also known to prey on injured adults (Hunter, 1991). These predators will harass incubating and brooding adults sometimes causing them to flee and abandon their eggs. Breeding adults have three choices: first to stay and fight, risking potentially fatal injuries; second to flee slowly cumbersomely with the egg on top of their feet clustering closer to neighbouring breeders; or finally, to flee entirely, guaranteeing survival but abandoning their current reproduction.

We calculated individual repeatabilities for AD and FID which were measured several times on incubating adults, and assessed the potential influences of approach speed, weather conditions, and time of day on AD and FID as these may impact the levels of vigilance and responsiveness to a threat. Specifically, we expected AD and FID to vary in a quadratic function, increasing at dawn and dusk, and coinciding with highest predation risk for chicks (giant petrel activity is highest at early and late hours of the night; Le Bohec et al., 2003; Descamps et al., 2005). We further tested whether individuals sensitized or habituated to repeated approaches by a human experimenter by examining bird responses in two different colonies; one close to human settlements and one relatively unexposed to human disturbance. King penguins have been shown to express significantly lower heart rate stress responses to human approaches in disturbed areas vs. undisturbed colony areas (Viblanc et al., 2012). Thus, we expected individuals measured in the colony close to human settlements to show habituation to approaching experimenters (decreased AD and FID compared to the undisturbed colony).

METHODS

Study sites

This study was conducted in mid-to-late January during the Austral summer of 2010-2011. Birds were selected from two colonies of king penguins located on Possession Island in the Crozet Archipelago, namely the colonies “La Baie du Marin” (BDM, 46°25’S – 51°52’E) and “Jardin Japonais" (JJ, 46°21’S – 51°43’E). BDM is home to ca. 22 000 breeding pairs (Barbraud et al., 2020) and is located on the east side of Possession Island in close proximity to a permanently inhabited research station built in 1961. This colony has been exposed throughout the year for the past 50 years to the daily presence of scientists and non-scientists in or close to

the colony (Viblanc et al., 2012). During this time, birds in BDM have been regularly exposed to humans including tourist visits, censuses, population counts, and systematic monitoring. Meanwhile, JJ is home to ca. 39 000 breeding pairs (Barbraud et al., 2020) and is located on the north side of the island some 13 km from BDM. JJ is a relatively undisturbed colony, far from the research station and visited by scientists only a few times every year for population count and census information.

Approach protocol

We repeatedly approached a total of 47 incubating birds in the two colonies (23 birds from JJ and 24 birds in BDM). Each individual was approached once per day over three consecutive days between the hours of 8:00 and 19:00 (from Jan. 11 - 13 in JJ, and from Jan. 30 - Feb. 1 in BDM). It was not possible to find all birds on the subsequent days and so two birds in BDM were only approached once, and one bird each in JJ and BDM were only approached twice. Following egg-laying, king penguin partners alternate incubation duties, allowing their partner to forage at sea while they fast on land (Stonehouse, 1960). The male takes the first incubation shift, and the egg hatches some 53 days later, usually during shift four when the female has possession of the egg (Weimerskirch et al., 1992).

Following the STRANGE guidelines (Webster & Rutz 2020), we provide hereafter details on how incubating birds were selected and highlight potential biases related to the selection of our study subjects. Birds were selected haphazardly while incubating their eggs, and therefore we had no individual information on their sex, age, incubation shift, or previous experience with humans. Chosen individuals visually appeared in good physical condition, based on their plumage (shiny) and morphological (fat) appearance, and thus had not been fasting for

196 extended periods of time. Individuals were selected at distance, and not after being approached or
197 captured. Therefore, we expected no strong initial sampling bias in relation to how individuals
198 were responding to human approaches (but see discussion).

199 Selected individuals were marked from a distance (roughly 1 m) at the end of the first
200 approach using a dot of non-permanent pressurized spray animal dye (Porcimark®, Kruuse,
201 Lageskov; Denmark) on the belly for identification during subsequent approaches. All approaches
202 were performed by the same observer (BG) dressed in the same clothing. Individuals were only
203 approached if they were resting upon arrival of the observer (*i.e.*, not engaged in aggressive,
204 preening, or sleeping behaviours). The approach started after having observed the bird resting for
205 at least one full minute before approaching it. During this time, the experimenter hid out of line of
206 sight. Each approach had a set starting distance of 18 m, as measured with a laser telemeter
207 (Leica DISTO^{TM} D5 Lasermeter, Leica Geosystems AG, Hexagon, Sweden), which we
208 standardized due to the known influence of starting distance on FID (Blumstein, 2003, 2010;
209 Dumont et al., 2012). We chose a starting distance of 18 m based on preliminary observations of
210 59 king penguins, including courting (paired) and incubating birds (a sample representative of the
211 various life-history stages in the colony, excluding moulting and chick-brooding birds), that
212 showed the maximal distance at which birds exhibited signs of vigilance towards an experimenter
213 was 12.45 m and the minimal distance was 3.03 m (mean ± SD = 6.85 ± 1.87 m). We used a
214 starting distance 1.5 times greater than the maximal detection distance recorded in the preliminary
215 study to ensure starting distance far exceeded maximum alert distance (see also Fleming and
216 Bateman, 2017).

217 The approaching observer followed a direct trajectory toward the focal individual, in
218 plain line of sight, always starting from outside the colony. The experimenter walked until the

first sign of alert was detected (the bird tilting its head or stretching its neck in the direction of the experimenter). At this distance, termed the Alert Distance (AD), the experimenter took a standardized one-minute pause to record AD and time. The time from the start of the approach until the bird became vigilant was recorded with a stopwatch and the remaining distance to the selected bird measured with a laser telemeter (closest cm). The approach was then resumed until the bird showed the first signs of fleeing (slowly walking away with its egg resting on its feet) – termed Flight Initiation Distance, FID. Some birds never showed signs of flight while being approached, right up until close (FID = 0 m) (see also Bateman and Fleming, 2011, for similar results in ibises). FID had therefore a zero-inflated distribution (see supporting information S1), suggesting this measure reflected two different processes: *i*) the decision to flee or not (0/1); and *ii*) the distance at which flight (escape) should occur if birds decide to flee. Hereafter, we analysed these two processes separately. We calculated the speed of approach (m/s) prior to the occurrence of AD and FID as the distance walked (m) divided by the duration of approach (s). The mean speed ± SE of approach prior to AD was 0.61 ± 0.09 m/s (range = 0.44 – 0.86 m/sec) and the mean ± SE speed of approach between AD and FID was 0.57 ± 0.10 m/s (range = 0.35 – 0.90 m/s). Walking speed varied slightly due to topography, entering the colony, and breeder density.

At the start of each approach, we recorded air temperature to the nearest 1°C, and we scored wind speed, solar levels, and rain on a scale from 0 to 2, with half levels (i.e., 0.5) being allowed. A value of 0 indicated no wind or rain, or full cloud cover (no sun). A value of 2 indicated heavy wind or rain or full sun. Mean temperature was 10 °C (range = 7 – 13 °C). As air temperature, wind speed, rain, and solar levels were naturally correlated, we used a Principal Components Analysis (PCA) to summarize all weather variables (package "FactoMineR", Lê et al., 2008). This approach had the advantage of capturing climatic variation on a continuous scale

through two independent and orthogonal axes, and avoided strong collinearity (VIFs >30) issues if 'raw' meteorological variables are used in the analyses. We kept the first two principal component (PC1 and PC2) which together explained over 84% of the variation in climate data. Increasing PC1 (64.86% of variation) values were mainly associated with higher sun scores (correlation = +0.939) and higher temperature (+0.918), and to a lesser extent higher wind scores (+0.639), and lower rain scores (-0.686). In contrast, increasing PC2 (19.77% of variation) values were mainly associated with higher wind scores (+0.702), higher rain scores (+0.543), but not with sun scores (-0.010) or temperature (-0.061). Thus, increasing PC1 values described sunnier, warmer days while increasing PC2 values described windier, rainier days. On average, the climate conditions varied during our three approaches in both colonies (see supporting information S2), highlighting the need to control for climatic variation in further analyses.

Statistical analyses

All statistical analyses were performed in R 3.6.1. (R Development Core Team, 2020). Results are presented as means ± SE. We investigated the sources of variation in AD (gaussian distribution), the decision to flee or not (FID: 0/1, binomial distribution), and the distance at which flight was taken for birds that did decide to flee (FID > 0; gaussian distribution) using linear mixed models (LMM) and generalized linear mixed models (GLMM) with the appropriate error distribution. In all the models, we entered individual ID as a random factor to control for repeated measures, and we included as fixed effects the time of day, weather (PC1 and PC2), approach speed, approach order (three levels: first, second, or third approach), and the colony (two levels: BDM vs JJ). To test whether birds differed in their behavior at different times of day, and whether habituation or sensitisation to human approaches occurred differently between

colonies, we also included second order interaction *colony* x *approach order* and *time of day x time of day* (viz., time^2) in our starting models. Second order interactions were dropped from the final model if not significant. Models were run using the *lmer* and *glmer* functions in the package "lme4" (Bates et al., 2015). The significance of the fixed effects was tested with the *anova* function (type 3) from the package "lmerTest" (Kuznetsova et al., 2017) using F tests with Satterthwaite estimation for the denominator degree of freedom for models with a gaussian distribution. Pseudo-R^2 for the models were calculated using methods developed in Nakagawa and Schielzeth (2013). Prior to analysis, time of approach was converted to decimal time, and non-zero FIDs were log-transformed (natural log) to normalize the distribution of the residuals. Independent variables were checked for collinearity using Variance Inflation Factors (VIF) (Zuur et al., 2010). A few VIFs were above 3 (see results), but those were considered acceptable as they represented different categorical levels of the same factor (*e.g.* approach order). All variables were scaled and centered prior to inclusion in the models to compare effect sizes (Schielzeth, 2010). Where appropriate, we insured model residuals were normally distributed by visual inspection of density distributions, Q-Q plots, cumulative distribution functions, and P-P plots using the "fitdistrplus" package in R (Delignette-Muller and Dutang, 2015).

Repeatabilities and their confidence intervals were calculated using the functions *rpt* for AD, and FID and *rptBinary* for the decision to flee or not, from the "rptR" package in R (Stoffel et al., 2017). Repeatability was calculated as the ratio of among-individual variance in AD or FID (σ^2_α) over total phenotypic variance (equal to the sum of among-individual variance and within-individual variance, σ^2_ε), so $r = \sigma^2_\alpha / \sigma^2_\alpha + \sigma^2_\varepsilon$ (Nakagawa and Schielzeth, 2010). We first calculated agreement repeatability, also called uncorrected repeatability, *r*, which is simply based on the among- and within-individual variance in AD or FID. Afterwards, significant effects from

289 the above models, which influenced the expression of AD and FID, were controlled for when
290 calculating adjusted repeatability r_{adj}, (also called corrected repeatability) (Wilson, 2018).

291

292 **Ethics statement**

293 No animal was caught or handled over the course of this study. The research was
294 approved by the Ethical Committee of the Institut Polaire Français – Paul-Emile Victor.
295 Authorization to enter the colony and approach birds was obtained from Terres Australes et
296 Antarctiques Françaises. The observations complied with the current laws of France. No eggs or
297 chicks were abandoned during the course of this study.

298

299 **RESULTS**

300 **Alert Distance (AD)**

301 On average, focal individuals became alert when the experimenter came to a distance of
302 6.94 ± 0.18 m; (range = 1.89 – 13.14 m) (Fig. 1A and 1B). The interaction between approach x
303 colony and time of day^2 did not have a significant effect influencing AD ($F_{2,111.0}$=0.22, P=0.802
304 and $F_{1,90.9}$=0.02, P=0.896, respectively), were sequentially removed from the model in that order
305 (least significant term removed first). The final model with time of day, weather PC1, weather
306 PC2, speed of approach prior to AD, approach order and colony, as fixed effects explained 13%
307 (marginal R^2) of the total variation, and including individual as a random effect explained 22%
308 (conditional R^2) of the total variation in AD (LMM, n=133 observations, N=47 individuals, 1.63
309 < VIFs < 3.68, among-individual σ^2 = 0.37; residual σ^2 = 3.63). We found no evidence that AD
310 was significantly affected by the time of day ($F_{1,92.2}$=2.00, P=0.160), speed of approach

311 ($F_{1,120.0}$=1.25, p=0.267), weather PC2 ($F_{1,98.8}$=2.90, p=0.092), colony ($F_{1,84.0}$=0.00, P =0.982), or
312 approach order ($F_{2,106.0}$=2.14, P=0.123) (Fig 2A). However, AD was significantly influenced by
313 weather PC1 ($F_{1,102.1}$=7.29, P=0.008) with individuals showing lower AD during warm and sunny
314 conditions (Fig. 2B).

315 AD was not significantly repeatable when analysed on its own (r=0.103; CI_{95}=[0, 0.290];
316 P=0.146), but repeatability was close to significant after adjusting for weather (PC1) as a fixed
317 effect (r_{adj}=0.145; CI_{95}=[0, 0.313]; P=0.062). Although some individuals appeared to be
318 repeatable, many others displayed large variability in their AD (Fig. 1A and 1B). Post-hoc power
319 analyses (see Online Supplementary Material OSM 3) revealed that significant repeatability
320 (power 0.8) for AD may be achieved for a sample size of 6 approaches on 47 birds – or 3
321 approaches on 140 birds.

322

323 **Flight Initiation**

324 Out of the 47 selected individuals, 41 (87.2%) decided to flee (i.e., move away with their
325 egg on their feet) during at least one of their approaches (20 birds in JJ, and 21 birds in BDM): 23
326 individuals always fled, 12 individuals fled twice in three approaches, 6 fled once in three
327 approaches, and 6 did not flee at all. This corresponded to 42 approaches out of a total of 135
328 (31%) that resulted in no FID. The FID of the individuals that decided to flee at least once was
329 2.83 ± 0.16 m (range = 0.68 – 8.58 m) (Fig. 1C and 1D).

330 Time of day^2 and the interaction between approach x colony did not significantly
331 influence the odds of fleeing (1) or not (0) during the approach (GLMMs; binomial, χ^2=2.35,
332 P=0.125 and χ^2=4.97, P=0.083), and were sequentially removed from the model in that order
333 (least significant term removed first). The final model with time of day, speed of approach

between AD and FID, weather PC1 and weather PC2 explained 23% (marginal R^2) of the total variation, and including individual as a random effect explained 73% (conditional R^2) of the total variation in the odds of fleeing (GLMM, n=133 observations, N=47 individuals, 1.18 < VIFs < 5.36, among-individual σ^2 = 3.29; residual σ^2 = 6.11). The odds of fleeing were not significantly influenced by weather PC1 (χ^2=0.24, P=0.626), time of day (χ^2=1.19, P=0.275), speed of approach (χ^2=1.86, P=0.172), approach order (χ^2=4.98, P=0.083) or colony (χ^2=3.09, P=0.079). (Fig. 3A). However, the probability of birds to flee increased significantly with increasing weather PC2 (χ^2=4.51, P=0.034), birds being more likely to flee from the approaching experimenter in windy and rainy conditions (Fig. 3A and 3B). The decision to flee was significantly repeatable before (original-scale: r=0.504; CI_{95}= [0.084, 1.724]; P<0.001) adjusting for weather, and increased slightly (r=0.573; CI_{95}= [0.110, 1.686]; P<0.001) after accounting for the significant effect of weather PC2.

For those birds that decided to flee, time of day^2 and the interaction between approach x colony did not significantly influence FID during the approach (LMMs; $F_{1,66.5}$=0.15, P=0.704 and $F_{2,67.8}$=0.48, P=0.619, respectively), and were sequentially removed from the model in that order (least significant term removed first). The final model with time of day, speed of approach between AD and FID, weather PC1 and weather PC2 explained 37% (marginal R^2) of the total variation, and including individual as a random effect explained 62% (conditional R^2) of the total variation in FID (LMM, n=91 observations, N=41 individuals, 1.19 < VIFs < 5.56, among-individual σ^2 = 0.13; residual σ^2 = 0.09). We found no evidence that FID was significantly affected by the time of day ($F_{1,79.3}$=0.122, P=0.727), weather PC1 ($F_{1,67.5}$=2.16, p=0.146), colony ($F_{1,47.2}$=0.77, P =0.384), or approach order ($F_{2,63.8}$=2.17, P=0.123) (Fig 4A). However, FID increased significantly with the speed of the approach ($F_{1,71.8}$=31.64, P<0.001, Fig. 4A and 4B) and decreased significantly with increasing weather PC2 ($F_{1,60.6}$=5.17, P=0.027, Fig. 4A and 4C).

In other words, FID decreased in windy and rainy conditions. Agreement FID repeatability was low and not significant (r=0.102; CI_{95} = [0.00, 0.339]; P=0.248), but increased significantly when approach speed and weather PC2 were adjusted for in the model (r_{adj}=0.258; CI_{95}=[0.010, 0.511]; P<0.022).

DISCUSSION

Our study showed that, in king penguins, both the decision to flee and FID were significantly and moderately repeatable, whereas AD was not. Climatic conditions had marked effects on AD, FID and the probability for birds to flee, affecting repeatability estimates, and highlighting the need to consider external sources of variation in refining such estimates in the wild (discussed below).

Between- and within-individual variation in flight initiation

Incubating king penguins showed significant (adjusted) repeatability both in the decision to flee from an approaching observer (0.57), and in the distance at which they initiated flight (0.26). In both cases, it is important to note that repeatability values increased when accounting for weather effects on behavior, which is not surprising since birds experienced different local climate conditions when they were approached. Notwithstanding, this underlines the importance of considering heterogeneity in individual habitats and timing of measures when establishing repeatability estimates from behavioral measures in the wild. Our repeatability values are comparable, albeit slightly lower, to those reported on FID in other studies: black swans, *Cygnus atratus* (r=0.61, van Dongen et al., 2015), juvenile yellow-bellied marmots, *Marmota flaviventris*

(r=0.40, Petelle et al., 2013), Namibian rock agama, *Agama planiceps* (r=0.71, Carter et al., 2012), and burrowing owl, *Athene cunicularia* (r=0.88, Carrete and Tella, 2010), and generally moderate as would be expected for behavioral traits (Bell et al., 2009). The repeatability of both the decision to flee, and the distance at which flight was initiated, suggest that these behavioural traits could constitute good candidates for personality traits in breeding king penguins. This remains to be further explored however, since our power to detect behavioral plasticity in AD and FID occurring over longer periods was limited by the fact that birds in our study were only measured over a short period of time (3 consecutive days), and were not previously known individuals. Separating the repeatable and plastic aspects of alert and flight behavior to approaching predators in king penguins would benefit from further studies on individually monitored population where simple measures such as AD and FID are incorporated into monitoring schemes allowing to test for repeatability and plasticity over the lifetime of individuals (Dingemanse et al., 2010; Dingemanse & Wright, 2020). Logistically, this would require permanent marking of individuals in order to follow them through a longer period of time and through different contexts. In addition, FID (and AD, see below) are likely to vary according to factors such as individual age, sex, stress responsiveness, or body condition (e.g. Seltman et al., 2012; Kalb et al., 2019). Testing for such factors in future studies should allow refining repeatability estimates, with repeatability increasing as more residual variation is accounted.

Repeatability estimates of individual propensity to flee and FID increased as extrinsic factors were accounted for in the models. This is not surprising, but highlights contrasting climate effects acting both on the propensity to flee (1/0) and on the distance at which birds initiated flight. Interestingly, weather PC2 (wind and rain) had opposite effects on these behavioral traits. As conditions were windier and rainier, birds were more likely to flee, but those that fled had lower FID. This suggests that birds that fled waited until the last moment before they did so,

404 which could be explained by the fact that they were less likely to detect the incoming threat in
405 windy and rainy conditions. Wind speed has been shown to have contrasting effects on animal
406 vigilance and escape behavior, from no effect to marked effects depending on the species and
407 taxa (e.g., no effect: birds; Clucas and Marzluff, 2011, Nordell et al., 2017, Petrelli et al., 2017;
408 reptiles; McGowan et al., 2014; wind effects: birds; Reynolds et al., 2020; mammals; Wolf &
409 Croft, 2010). Increased wind speed has been shown in 17 of 18 studies to decrease an animal's
410 ability to detect an approaching predator by masking sounds, smells and visual cues (Cherry and
411 Barton, 2017). Wind may also be a source of distraction (Chan et al., 2010; Tätte et al., 2019),
412 leading to delayed detection of approaching predators. It has been suggested that the magnitude
413 of FID responses should decrease under harsh weather conditions, as the energy cost of fleeing
414 increases (Collop et al., 2016). For endotherms wind chill increases thermoregulatory costs, and a
415 trade-off might exist between investing energy into flight and that into thermoregulation (Collop
416 et al., 2016, but see also Reynolds et al., 2020). Because penguins rely essentially on fat stores
417 during incubation on-land (Groscolas & Robin, 2001), and because energy-depleted birds will
418 abandon reproduction (Groscolas et al., 2000, Gauthier-Clerc et al., 2001), any energy savings –
419 however small – is critical. Thus, energy savings in harsh climate conditions may contribute in
420 explaining why birds waited for the last moment to flee. FID also increased with increasing
421 approach speed of the experimenter. Faster approach speeds after alert may be interpreted as
422 greater threat by the targeted prey, and thus generating longer FID (Stankowich and Blumstein,
423 2005; Cooper and Whiting, 2007; Smith-Castro and Rodewald, 2010; Bateman and Fleming,
424 2011).

425 It is interesting to note that it was not uncommon for the incubating penguins to stay and
426 defend their eggs aggressively instead of attempting to flee (there was an FID of 0 m in 42
427 approaches of 135). For these birds either the perceived risk of the approaching threat (a human)

was not high enough to elicit a flight response, or the motivation to breed and defend the territory was stronger than that of attempting to flee. FIDs of 0 m have been observed in other ground laying birds: yellow-eyed penguins *Megadyptes antipodes* (Ellenberg et al., 2007, 2009), humboldt penguin *Spheniscus humboldti* (Ellenberg et al., 2006), magellanic penguins *Spheniscus magellanicus* (Fowler, 1999; Villanueva et al., 2014), and African penguins *Spheniscus demersus* (Pichegru et al., 2016). King penguins are special in that they incubate their egg on their feet in a specialized brood pouch. Thus, fleeing is at any rate limited, and essentially amounts to clustering close to neighbours in the hope to benefit from confusion, dilution or selfish herd effects. In addition, fleeing may risk damaging or losing the egg, losing the breeding territory, suffering from increased aggression by territorial neighbours (Côté, 2000), or disturbing the thermal incubation environment required for chick development and survival. For instance, exposed eggs during transitory breeding abandonment in king penguins have been found to lose heat at a rate of 0.19 °C per minute (Groscolas et al., 2000).

Between- and within-individual variation in AD

In contrast to FID, the repeatability of AD was low (0.10) and not significant, but improved slightly (0.15) and was close to significant ($P = 0.062$) when accounting for the effects of climate (Weather PC1). As far as we are aware, there is no report of the repeatability of AD in the literature. Rather, researchers have considered vigilance behaviour when aiming to understand how animals respond to threats. More vigilant individuals are expected to detect predators earlier and thus become alert at longer distances (Fernández-Juricic and Schroeder, 2003; Beauchamp, 2015; Uchida et al. 2019; but see Tätte et al., 2019). Studies that measured repeatability in vigilance behaviours also reported low, but significant, repeatability: eastern grey kangaroos,

Macropus giganteus, (*r*=0.07-0.14, Edwards et al., 2013); redshanks, *Tringa totanus*, (*r*=0.21, Couchoux and Cresswell, 2012); house sparrows, *Passer domesticus*, (*r*=0.13-0.22, Boujja-Miljour et al., 2018); cliff swallows, *Petrocheldion pyrrhonota*, (*r*=0.089, Roche and Brown, 2013). As mentioned above, repeatability estimates are likely to increase as some residual variation is accounted for by including potential factors such as age, sex, or condition, that were unfortunately not available in this study as individuals were not followed or handled. Dingemanse and Dochtermannn (2013) determined that for lower repeatabilities, >4 samples per individual were required if the total number of individuals is <100. Similarly, Wolak et al. (2012) found fewer samples per individual were required for higher r values (3 samples per individual for an *r* of 0.8), but for an *r* of 0.2, the precision of the estimate continues to increase until up to 10 samples per individual. Because of time and field constraints we were only able to repeatedly approach 45 individuals 3 times. Yet, post-hoc power analyses revealed that doubling our sample size to 6 repeated measures per bird would have been sufficient to detect significant repeatability. At any rate, repeatability was low, indicating that individuals were more flexible in this behavioral trait than in their decision to flee. Similarly, vigilance levels (and presumably AD) have been found to be highly flexible (Couchoux and Cresswell, 2012; Edwards et al., 2013) and sensitive to numerous biological factors such as group position and group size, distance to cover, predation pressure, season, weather, and time of day (reviewed in Elgar, 1989). In our study, AD was significantly lower in warm and sunny conditions, suggesting that birds were less vigilant in good weather conditions.

Colony disturbance, predation pressure and STRANGE animals

We expected AD and FID to vary depending on colony and with changes in predation pressure throughout the day. However, our analyses suggests that neither were major factors influencing escape decisions in the king penguin. First, on-land predation in king penguin colonies is highest at dawn and dusk (Le Bohec et al., 2003; Descamps et al., 2005), whereas most of our measures were done between 8 AM and 6 PM. Extending measurements to early morning and late evening when predators are most active may allow to better capture potential effects of predation pressure on penguin behavior. Second, given that the two colonies surveyed in this study differ markedly in their exposure to human presence throughout the year (BDM is located next to a research station and breeders have seen humans almost every day for the past 50 years, whereas birds in JJ are rarely exposed to human visitors), we had expected birds in the BDM (vs. JJ) colony to habituate to approaching humans. However, the lack of difference in behavioral responses between the colonies is perhaps not so surprising. Previous findings indicate that birds breeding in more disturbed areas of the BDM colony show lower heart rate stress responses to an experimenter approaching to 10 m distance than birds in less disturbed areas – suggesting a potential for habituation to the presence of humans in chronically disturbed areas (Viblanc et al., 2012). Yet, heart rate responses were similar between the disturbed and undisturbed areas when birds were approached up to contact and captured (Viblanc et al., 2012). Although birds were not captured in the present study, they were approached up close (to a few cm), and the risk assessed was presumably more comparable to a capture than to an observer standing some 10 m distant.

Previous studies in similar seabirds have shown contrasted responses to human approaches. For instance, whereas Magellanic (*Spheniscus magellanicus*), African (*S. demersus*), and Gentoo penguins (*Pygoscelis papua)* show reduced behavioural and/or physiological responses (heart rate or corticosterone concentrations) to human visitation in high disturbance

areas (van Heezik and Seddon, 1990; Fowler, 1999; Walker et al., 2005, 2006; Holmes et al., 2006; Villanueva et al., 2012; Pichegru et al., 2016), yellow-eyed penguins (*Megadyptes antipodes*) appear to sensitize to human exposure through higher stress responses (Ellenberg et al., 2007). Taken together, these results highlight two important points: (1) behavioural and physiological responses to approaching predators (or humans) may tell seemingly different stories. A proper understanding of prey responses to approaching predators requires the integrative assessment of both physiological stress responses and behavioural reactions. (2) both behavioural and physiological responses are fine-tuned mechanisms integrating risk assessment into optimal escape decisions.

Finally, we must consider how STRANGE were the animals in our study (Webster & Rutz 2020). We selected birds in the colonies haphazardly, with no knowledge of their sex, age or past experience. Nonetheless we did not capture and measure bird morphometrics in our study, but visually targeted birds that appeared in overall good physical condition in order to minimize the risk of breeding abandonment for energy-depleted birds (Groscolas et al., 2000, Gauthier-Clerc et al., 2001) in this protected species. Our measures on individual birds were done over a few days during which changes in body condition were likely relatively minor. However, we might not have captured the full range of behavioral expression of AD and FID, particularly for birds of low body condition. This is important to consider since individuals in poor body condition may have enhanced stress and FID responses to approaching experimenters (Seltmann et al., 2012), and emphasizes the importance of controlling for body condition – where feasible – in performing repeated measures on wild animals.

As this study only tested the response to repeated approaches in two colonies, which will have obvious differences in colony size, density, and topography, replicating this study over additional colonies would help in a better characterisation of the factors shaping escape decisions

in penguins. In addition to landscape and social differences between colonies, there are also possible differences in predation pressure and genetic differentiation. Furthermore, the sampling in the two colonies took place 20 days apart, allowing for possible differences in the reproductive cycle to manifest. Lastly, sampling only occurred over a span of three days. Further studies should investigate these factors in more depth, as well as test repeated approaches over a longer time period – and over the lifetime of individual birds. In particular, these would help in capturing a greater range of weather conditions, and better understanding the extent of repeatability and plasticity of these behavioral traits (Dingemanse & Wright, 2020).

The response of a wild population to human disturbance creates special considerations in a world that is increasingly accessible to humans. Ecotourism and wildlife tourism, or travel to natural areas to engage in shared experiences with wildlife and whose aim is to conserve the environment through education and local spending, are increasingly common activities. As a charismatic anthropomorphic animal, penguins have garnered much attention. In many species, habituation occurs in response to disturbances caused by tourists (van Heezik and Seddon, 1990; Fowler, 1999, Holmes et al., 2006; Villanueva et al., 2014). However, even in species that have habituated to disturbance, human presence can impact natural behaviours, time budgets, heart rates and stress levels (Holmes et al., 2005; Walker et al., 2005, 2006; Burger and Gochfeld, 2007; Viblanc et al. 2012; Villanueva et al., 2012; Pichegru et al., 2016). For species that cannot habituate or which have sensitized to disturbance, individuals may experience negative impacts on their reproductive success ultimately endangering the population as a whole (Giese, 1996; McClung et al., 2004; Ellenberg et al., 2006, 2007, 2012, 2013; Carroll et al., 2016). Knowledge of AD and FID may help inform policy makers when deciding appropriate viewing guidelines for tourists (Ellenberg et al., 2006, French et al., 2019).

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