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Rovers minimize human disturbance in research on wild animals

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Investigating wild animals while minimizing human disturbance remains an important methodological challenge. When approached by a remote-operated vehicle (rover) able to make radio-frequency identifications, wild penguins had significantly lower and shorter stress responses (determined by heart rate and behavior) than when approached by humans. Upon immobilization, the rover—unlike humans—did not disorganize colony structure, and stress rapidly ceased. Thus, rovers can reduce human disturbance and the resulting scientific bias.

Approaching wild animals to collect data on their phenotypic traits induces stress, escape behavior and, potentially, breeding failure[1–3] and therefore jeopardizes the quality of the collected data. Even after habituation, human approaches and manipulations induce near-instantaneous, large and long-lasting increases in stress hormones[4]. In colonial species, disturbance is also likely to affect neighbors of targeted individuals. Bio-logging allows remote data collection, thereby reducing disturbance, but animals generally must still be captured to attach and recover devices. Moreover, the impacts of animal-borne instruments and tags on animal behavior and energetics, such as the drag costs on instrument-bearing sea turtles[5] or flipper-banded penguins[6], can have dramatic effects that lead to scientific biases[7,8]. Such methods that have a detrimental impact on wild animals are also unethical. Yet, wild animals are exposed to increasing disturbance, partly due to the need for larger data sets to rigorously study population trends—and for multiple investigations and study sites to gain a better understanding of the adaptive capacity of populations in response to environmental pressures. A key issue for field research, and the goal of this study, is to develop methods that reduce human-induced disturbances while opening new research perspectives.

Radio-frequency identification (RFID) enables individuals to be monitored with subcutaneous passive integrated transponders (PIT tags) at a population scale[9–11] while avoiding both the recapture stress and detrimental effects associated with conventional marking[7,8,12]. However, the current maximum reading distance for PIT tags is only around 60 cm (for the largest tag at 3.85 mm × 32 mm and 0.8 g). Thus, animals can be identified only when they are very close to RFID antennas, such as those buried or hidden along natural transit pathways[9,13,14]. Moreover, being invisible, PIT tags do not allow the localization and observation of individuals within a group.

Because rovers may be useful for approaching wild animals[15], we tested them as remote-controlled RFID antennas to identify and localize breeding king penguins (*Aptenodytes patagonicus*). Unlike handheld readers ('hand-readers'), which can make only one RFID detection at a time, our rover allowed bilateral identification, with large detection fields on each side that operate even while it moves. The newer version of the rover has a total detection field eightfold larger than that of a hand-reader (Online Methods). It can sequentially record identification and localization information without immobilization at a rate of three identifications per second, therefore enabling the quasi-simultaneous identification of all PIT-tagged individuals within 0.4 m of the antennas carried by the rover. Whenever a PIT-tagged individual is identified, its ID and corresponding Global Positioning System (GPS) coordinates are sent by wireless signal to a laptop carried by the person controlling the rover.

It is fundamental to know whether a rover disturbs animals less than a human observer does. We thus first examined physiological and behavioral stress responses of breeding king penguins exposed to the two methods (Online Methods and **Fig. 1a**). King penguins incubating eggs are stationary, generally maintaining a minimum distance from neighbors and birds in transit (**Supplementary Video 1**) that is determined by the reach of their beaks and flippers (~50 cm). As a sensitive indicator of stress[3], heart rate (HR) is used to study social interactions[16] and disturbance[3,17–19] in colonial animals. We equipped 34 incubating king penguins with external

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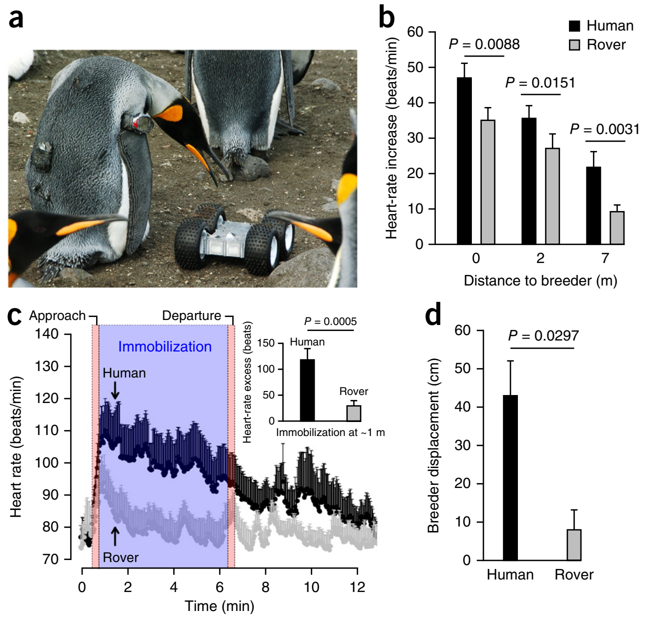

Figure 1 | Stress responses of wild king penguins when approached by a human or a rover. (**a**) Rover approaching an incubating king penguin equipped with a heart rate (HR) logger. (**b**) Increase in HR of 34 incubating king penguins when approached by a human (n = 100) or a rover (n = 101) at the indicated distances (linear mixed model: $Y \approx$ Intruder + Distance + (1 | Individual)). A distance of 0 m corresponds to an approach to the point of contact with the breeder, i.e., when the breeder is able to reach the intruder with its beak or flippers. (**c**) HR of 9 incubating king penguins during approach, immobilization at a distance of ~1 m, and departure of either a human (n = 9) or a rover (n = 9). Inset, number of heart beats produced in excess of resting HR (HR excess (in beats) during human and rover immobilization (paired-samples t-test). (**d**) Displacements of 10 incubating king penguins induced by the approach and immobilization of a human (n = 10) or a rover (n = 10) at a distance of ~1 m (paired-samples t-test). All results are given as mean ± s.e.m.

cardio-frequency meters as previously described[20,21]. The eyes of incubating birds were masked from behind, and the two units of equipment—a sensor-transmitter (30–40 g) attached to the back of the bird and a receiver-logger (a recording watch, 30 g) placed on a flipper—were attached to the individuals by a human kneeling inside their territory in a 5- to 10-min procedure. These birds were left to recover for one night before the experimental approaches were performed. The study was conducted in a part of the colony where the birds were naive to scientific studies and were not PIT tagged. Accordingly, we did not actually make PIT-tag identifications in this study.

The birds were first approached, by either a human or rover, at a mean speed of 0.5–0.8 m/s to a distance of 0 m ('contact') for no longer than 2–3 s to mimic PIT-tag detection and data collection. Approaches to distances of 2 m and 7 m also allowed evaluation of the potential impact on adjacent and distant neighbors of the target individual. Closer approaches led to larger HR increases (linear mixed model (LMM): 0 m vs. 2 m, $t = -3.33$, $P = 0.0011$; 0 m vs. 7 m, $t = -10.34$, $P < 0.0001$; 2 m vs. 7 m, $t = -6.93$, $P < 0.0001$; number of observations (n) = 201, number of birds (N) = 34; **Fig. 1b**). However, whatever the distance between the intruder (rover or human) and the breeder, the HR increase above the baseline value was significantly lower when approached by a rover than by a human (23.90 ± 2.11 vs. 35.04 ± 2.49 beats/min; LMM: $t = -5.15$, $P < 0.0001$, n = 201; N = 34). At contact, breeders attacked both the rover and the human, but the maximal HR was significantly lower for the rover (109.94 ± 4.19 vs. 115.71 ± 3.90 beats/min, respectively; generalized LMM: $z = -2.24$, $P = 0.0253$, n = 68; N = 34). From the 72-beats/min baseline, the maximal HR of 110 beats/min observed when breeders were approached to contact by the rover corresponded to a 1.53-fold increase similar to the 1.56-fold increase seen when these penguins defend their territory from their neighbors[21].

To discriminate between the increase in HR due to movements associated with classical territory defense and that due to stress or fear, we tested the response of ten breeders to a rapid approach (within 10–15 s) of the rover or human, followed by an immobilization for 6–7 min at a 1-m distance, followed by a retreat over 10–15 s. At every distance between the rover and a breeder (for example, even when at 1 m from the focal bird with the HR logger, the rover was located less than 20 cm from a neighbor), all behavioral reactions immediately ceased as soon as the rover stopped (**Supplementary Video 2**), and HRs rapidly returned to baseline levels (within 3.57 ± 0.73 min; **Fig. 1c**). In contrast, after human immobilization, HRs decreased progressively but remained higher than with the rover for the entire duration of the human presence (above 86.89 ± 7.05 beats/min until 6.33 ± 1.45 min after human immobilization; **Fig. 1c**). Human approaches led to an excess in HR approximately four times larger than that due to rover approaches (paired-samples t-test: $t = 5.60$, degrees of freedom (d.f.) = 8, $P = 0.0005$; **Fig. 1c**).

As a king penguin can move with its egg or small chick on its feet[22], especially when stressed, we estimated breeder displacements from their initial location at the approach and immobilization of the intruder. The rover induced very limited displacements (in any direction) of the targeted breeders, which remained clearly within their territories (**Fig. 1d**). Humans induced a displacement significantly greater (43.00 ± 9.43 cm vs. 8.00 ± 5.39 cm, respectively; paired-samples t-test: $t = 2.58$, d.f. = 9, $P = 0.0297$; **Fig. 1d**), as breeders all retreated with their eggs. Their approach, or even trespassing, into the territories of neighbors resulted in fights and disorganization of colony structure that extended for several rows beyond the territory of the focal bird. The HRs of the target penguins returned to baseline 7.50 ± 1.08 min after human departure—significantly later than with the rover approach

Table 1 | Observed response (retreat, defense or interest) and nonresponse (disinterest) to approaches at ≤5 m

	Behavioral response			
	Retreat	Defense	Interest	Disinterest
Adult king penguin				
With egg or chick (1-m^2 territory)	H	Rm, C	–	Rs
Without egg or chick (no set territory)	H, Rm	–	Rs	C
Adult emperor penguin				
With egg or chick (no set territory)	H, Rm	–	C, CR, Hs, R	R
Without egg or chick (no set territory)	H, Rm	–	CR, Hs, R	C
Southern elephant seal				
Resting (compact groups)	–	H	C	R

Observations are for approaches by a human (H), rover (R), camouflaged rover (CR) or conspecific (C). Stationary (s) or mobile (m) are indicated where the status of rover or human movement was explicitly tested. The CR condition (camouflaged with a fake chick) was tested in only the emperor penguin colony.

Figure 2 | Brooding emperor penguin with its chick approached by a rover camouflaged with a fake chick.

(paired-samples *t*-test: $t = 2.94$, d.f. = 8, $P = 0.0186$; **Fig. 1c**)—which corresponds to the amount of time it takes for the colony to recover to a standard equilibrium spacing of approximately 50 cm. We note that king penguin breeding pairs newly settled inside the colony (not yet incubating) systematically retreated in front of the approaching rover (**Table 1**) and returned to their selected breeding location only after it moved away.

To more broadly assess the utility of such rovers in colonial breeding birds, we tested the effect of rover approaches on emperor penguins (*Aptenodytes forsteri*), which are nonterritorial inside their colonies[23]. Of the 158 birds tested, 44 individuals (28%) reacted with alertness, and the tests were immediately halted; 75 (47%) displayed no reaction at all; and 39 (25%) demonstrated curiosity toward the rover by approaching and investigating it. But when the rover was camouflaged with a penguin model, all adult and chick emperor penguins allowed it to approach close enough for an electronic identification. Chicks and adults were even heard vocalizing at the camouflaged rover (**Fig. 2** and **Table 1**), and it was able to infiltrate a crèche without disturbance (**Supplementary Fig. 1**). Finally, rovers may be useful not only for penguins. In pilot studies with southern elephant seals (*Mirounga leonina*) on beaches (**Table 1**), the seals let the rover approach within RFID distances of their heads and also their tails, where they are usually tagged, with no visible disturbance (**Supplementary Video 3**). This is notable, as elephant seals generally react strongly when humans approach their tails (data not shown).

In conclusion, approaching animals with a rover can reduce impact, as measured by HR and behavior of king penguins, thus allowing such animals to be considered as undisturbed control individuals whose behavior and life history traits can be compared to those that must be captured for experimental investigations (for example, blood sampling or further equipment with loggers). The relevance of this technology extends beyond terrestrial populations of seabirds or mammals, as rovers could be adapted for use in aquatic or aerial environments as well as for many purposes beyond electronic identification. Indeed, rovers can be mounted with equipment to collect high-quality (very close) animal recordings for studies of vocalizations; or they may carry receivers to collect data from loggers mounted within a territory or directly on individuals[15]. Alternatives to buggy-type rovers will have to be developed for areas that preclude the circulation of wheeled vehicles. Further modifications, such as our use of camouflage for the shy emperor penguins, can overcome challenges that arise from approaching different animal species with a rover for scientific data collection.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.L.M., team leader of the IPEV project, designed and performed the study in the field, worked on the analyses and cowrote the paper; C.L.B., project co-leader, and J.D.W. worked on the analyses and cowrote the paper; N.H. and L.P. worked on the analyses and provided useful comments; technical developments pertaining to rover conception, development and construction were performed by M. Brucker, N.C., J.C., F.C. and B.F.; M. Boureau, L.K., E.G. and N.V. performed the study in the field and provided useful comments; R.G. suggested the use of HR monitoring to test the impact of approaching rovers, proposed a field test, performed some analyses and helped in revising the paper; B.T. helped in interpreting data within an ethological framework and in revising the paper; F.O. added useful modifications to the manuscript; V.A.V. and C.S. ran some preanalyses and greatly contributed in the paper's revision; P.T. participated in a prestudy on the field and added useful modifications to the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Study area and permits. Experimental procedures were approved by an independent ethical committee consulted by the French Polar Institute (Institut Polaire Français Paul-Emile Victor, IPEV). Authorizations to enter the 'Baie du Marin' king penguin colony at Possession Island (46° 25′ S, 51° 45′ E; Crozet archipelago) and the 'Pointe Géologie' emperor penguin colony, a specially protected area within Adélie Land (67° 40′ S, 140° 01′ E), to manipulate a limited number of king penguins ($N \leq 40$), and to test the behavioral responses of the latter and of emperor penguins to approaching rovers, were obtained from the Terres Australes et Antarctiques Françaises (TAAF).

Rover specifications and validation tests. For the study, the tests were initiated in November 2008 and were continued through December 2012. They were conducted over two summer seasons at Possession Island, i.e., at the time king penguins incubate their eggs, and during five winter breeding cycles of emperor penguins in Adélie Land.

During the first season (2009–2010) at the Baie du Marin colony, we compared the use of commercially available hand-readers (for example, Datamars reader GES3S or Bluestick) and of a remote-controlled rover for the RFID of PIT-tagged breeding king penguins. A RFID hand-reader requires a few seconds of immobilization by the human operator. Because its detection surface is only 0.03 m^2, identification must be focused on one specific bird at a time. The RFID TIRIS (Texas Instrument identification and registration systems) equipment was integrated into the body of a four-wheeled rover, 40 cm × 40 cm × 40 cm in size and 40 kg in weight, to protect it from sand and moisture. Owing to electromagnetic interference between its engine and the TIRIS equipment, the identification procedure also required a few seconds of immobilization. However, the rover had two antennas, which allowed animal identification over 0.06 m^2 of area on each side of the rover. Thus, the field of detection of the rover was twice as large as that of the hand-reader, enabling the identification of two PIT-tagged breeders from a single location. Nonetheless, because of ground topography (with many small stones) and increasing colony density as the breeding season advanced, this first rover model was found to be too large to circulate easily within the penguin colony. A more optimal rover size was therefore used during the next season (2010–2011), i.e., the present study: dimensions were 22 cm × 22 cm × 11 cm with a weight of 1.3 kg. This version was built to carry a removable white or black cap that fully covered the rover, i.e., masking the rover wheels. During movement, the rover produced noise at 30–35 dB at 1 m distance during operation. It could operate up to 40 min on a single battery charge. A Futaba 2.4-GHz FASST series remote-controlled system was used to control the rover. With this, it could be driven from more than 200 m away, but, owing to the visual occlusion of the small rover by penguins, the human driver was typically 30–40 m away during tests. A newer version of the remote-controlled RFID rover was tested in 2013–2014. Its overall size was 30 cm × 30 cm × 30 cm, and the field of detection, covering the front and sides of the rover, has been enlarged to 0.24 m^2. The behavioral response of the breeders to this model rover was similar to that shown for the previous generations. However, a major improvement is that there is no further interference between the engine and the TIRIS equipment, and the rover can record identification and localization information without immobilization at a rate of three identifications per second, therefore enabling the quasi-simultaneous identification of several individuals on each side of the rover while moving among penguins. Whenever a PIT-tagged individual is identified and localized, its ID and corresponding GPS coordinates are sent by Wi-Fi around 5.8 GHz to a laptop carried by the person controlling the rover.

The rover design and utilization are still actively being improved. The main limitation for the rovers has been the ability to navigate the steep, rocky slopes found in some colonies. Progressive updates in tread or wheel designs have enabled the rovers to access increasingly larger proportions of the colony. Another condition for a rover to be used inside a dense colony, especially with smaller rover models, is to have an elevated viewpoint for the person in charge of the controller.

King penguin trials. During the 2010–2011 season, 34 randomly selected king penguins of unknown age and sex were fitted with externally mounted heart rate (HR) loggers (Polar model RS800, Polar Electro Oy Kempele). Each system included two units: a sensor-transmitter (30–40 g) attached to the back of the bird and a receiver-logger (a recording watch, 30 g) placed on its flipper (see **Fig. 1a**) as described by Groscolas *et al.*[20] and Viera *et al.*[21]. Penguin HR and its changes have been well studied[3,16,20,21] and provide a sensitive measure of individual stress levels[3]. Incubating birds were handled after their heads were quickly covered from behind to mask their eyes, and handlers kneeled inside the penguins' territory for approximately 5–10 min to attach the equipment.

For practical reasons, the rover-versus-human approaches were not performed on PIT-tagged breeding birds. Our long-term monitoring of king penguins since 1998 is based on the PIT-tagging each year of ~450 fledgling chicks inside a section of the colony surrounded by fixed underground antennas on the passageways used by the birds to travel between the colony and the sea[9]. Because of the large disturbance that would have been induced by human circulation within the colony, the comparison between the rover and human approaches could not be performed inside the area where the PIT-tagged birds are monitored for long-term studies. We therefore chose an adjacent sector of the colony where most birds had not been captured and handled before their equipment with HR loggers for this study. Yet, there may be a few exceptions among our 34 studied breeders, and it is possible that our study includes the responses of some birds that have been captured before or of birds that have observed other birds being captured. In fact, it is not possible for any study of penguins anywhere, even within newly discovered colonies, to assume that a study population consists only of naive individuals. Furthermore, it would not have been appropriate to use birds entirely naive to humans in the present study. Most investigations require individual identification through tagging. For this, all study animals are typically captured at least once, usually before leaving the nest, and have likely observed other individuals being captured. The breeders could be equipped with the HR loggers while remaining in their natural breeding posture, as indicated above, but we did not PIT-tag them, as it would have required a much more disruptive handling procedure. It would also not have properly mimicked the standard handling conditions in our population because the birds are tagged as chicks. Finally, a comparison of

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HRs of king penguins that were either naive or experienced with regards to human disturbance found that birds in more disturbed areas exhibited attenuated HR responses to acute anthropogenic stressors of low intensity (i.e., human approaches) to which they had been subjected over the years[3]. Therefore, the differences we found in the present study between human and rover approaches may be underestimated.

HR analysis followed the procedure described by Viblanc *et al.*[3]. Following the equipment of the HR loggers, birds were left to recover for one night (at least 12 h)[3] before we performed the experimental approaches. To identify the optimum speed for the rover to navigate through the colony, we observed that penguins circulating inside the colony typically alternate between a rapid movement for several meters at 0.5–0.8 m/s followed by an immobilization for a few seconds (**Supplementary Video 1**). The HR logger–equipped king penguins were therefore approached by the human or a rover at about this speed. To obtain a standard baseline HR, we initiated the approaches when the breeders were neither vigorously preening nor defending their territory. On the basis of previous findings, king penguins recover within 15–30 min following a stress event[4], but to minimize the risk of a cumulative effect, we left at least 1 h between two consecutive approaches. To estimate changes in HR parameters (increase, maximum, duration and excess; see Viblanc *et al.*[3] for calculation details), human or rover approaches at 0 m, 2 m or 7 m were made in a random order following this procedure: approach in generally 10–15 s, 2–3 s at the targeted distance not including real immobilization, exit from the colony in 10–15 s. Because color might influence birds' responses[24], the rover approaches were also tested when fitted with a black or white cover to assess whether breeders' responses differed when approached with a color similar to that of the lesser sheathbill *Chionis minor*, a small scavenger, common inside the colony. Moreover, although pilot tests had shown that breeders react the same way regardless of the color of coat worn by experimenters, to avoid variability in aspect, color and behavior, the human investigator was always the same person wearing the same yellow raincoat, carrying an RFID hand-reader. Each of the 34 monitored incubating birds was therefore approached 12 times in randomly assigned tests, i.e., three distances for four types of intruder (human, rover without a cap, or with a white or black cap). We found that the initial HR of breeders was not significantly different for human versus rover approaches before starting each test (70.08 ± 1.79 beats/min vs. 72.18 ± 1.84 beats/min; linear mixed model (LMM): $t = 1.25$, $P = 0.2148$; number of observations (n) = 201, number of birds (N) = 34). Regardless of the distance, the increase in HR was not significantly different according to the color of the rover (LMM: all P values > 0.1459, $n = 294$; $N = 34$), suggesting that breeders reacted mainly against the intrusion into their territory, whereas the appearance of the intruder at such a small size and shape appeared to be less important. Also, their HR responses did not reveal any significant habituation, i.e., whatever the approach distance, the HR increase, duration and excess did not progressively decline over the three rover tests (LMMs: $Y \approx \mathrm{Order}_{\mathrm{Approaches}} + (1 \mid \mathrm{individual})$: all P values > 0.0940). Some HR-logger recordings did not work properly or stopped during the experiment, or the HR profile during the recovery period was affected by unrelated behavioral and physical interference. We discarded such cases, so that the actual total sample size was 394 approaches instead of 408. Moreover, after testing that birds react similarly regardless of the color of the rover, we retained the one type of rover for which we had the greatest sample size in order to have a comparable sample to the human approaches. Thus 100 human versus 101 rover approaches were used for the HR analyses.

Further, ten of the above birds were later tested to evaluate the effect of intruder immobilization (movement was halted and the intruder remained immobile throughout the test) on HR parameters and bird's displacement. The procedure was as follows: approach and immobilization of the intruder (human or rover without cap) at a distance of 0.995 ± 0.230 m within 10-15 s, immobilization for 6.40 ± 0.23 min, intruder retreat out of the colony in 10–15 s. The human and rover were positioned in the same spot for each trial. The HR logger failed for one breeder during the trial: this bird was thus removed from the HR analysis but conserved for the displacement analysis. Breeder displacements were estimated from a distance by the same observer to minimize variability. Preliminary validation using measurement of the breeders' tracks in the sand allowed us to calculate the precision of our estimations to within 5–10 cm. None of the human or rover approaches or immobilizations led to breeding failure.

Emperor penguin trials. Complementary studies were conducted on emperor penguins at the Pointe Géologie colony, close to the French scientific station in Adélie Land, Antarctica. Pilot studies were performed with a modified treaded mine-clearing rover in November–December 2008 (at the end of the breeding cycle) and with a small buggy-rover during the 2009, 2010 and 2011 winter breeding cycles. They showed that the birds reacted similarly regardless of the color of the rover, i.e., gray, white, red or orange. During the 2012 winter breeding cycle, the same behavioral responses of randomly selected birds incubating an egg or with a young chick ($N = 158$) were quoted as either stress behaviors (retreat or alertness) or interest (approaching or touching the rover) faced with the approach to within 5-m distance by the same rover used for the king penguin experiments, but either without camouflage or outfitted with a fake chick to hide the appearance of the rover (**Fig. 2**). The approach was immediately halted any time a breeding individual was seen to retreat.

Statistical analyses. All statistics were computed using R 3.1.1 statistical environment (R Development Core Team, 2013). Data were analyzed using a mixed-effect model approach (linear mixed models (LMM) with the "lme" function of the "nlme" package in R (http://cran.r-project.org/web/packages/nlme/nlme.pdf) or generalized linear mixed models (GLMM fitted with Poisson distribution) with the "lmer" function of the "lme4" package in R (http://cran.r-project.org/web/packages/lme4/lme4.pdf), and fitted models were selected through a stepwise Akaike's information criterion (AIC) procedure. Bird identity was included as a random term, enabling us to account for repeated measures on the same individual. Time since the equipment and time since the last approach were first included into each model to control for any confounding effects (all P values > 0.1135 and $\Delta\mathrm{AIC} \geq 2$ compared with models without these variables, and thus with fewer parameters). N and n represent the number of birds and approaches, respectively. Residual normality and homoscedasticity between groups were asserted using the Shapiro-Wilk normality test and Levene's test, respectively, before using

LMM or Student's t-test. Homogeneity of variance was satisfied in all tests, and, wherever necessary, data were transformed before analysis using Box-Cox power transformations[25] to ensure normality of residuals was satisfied. Student's t-test for paired samples was performed to compare the means of two sets of paired. We reported all values as mean ± standard error (s.e.), the α level of significance was set at 0.05, and Bonferroni's correction was applied whenever multiple comparisons were tested (differences were then considered as significant for $P < 0.05/n$ with n the number of comparisons).

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