Conservation Physiology

Volume 3 • 2015 10.1093/conphys/cov041

SEB Society for Experimental Biology

Research article

Effects of human disturbance on cave-nesting seabirds: the case of the storm petrel

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Human disturbance is an important stress factor with potentially strong impact on breeding activity in animals. The consequences can be extinction of the breeding population, because disturbed animals might desert their breeding area and find no suitable substitute area. In this study, we investigated the effects of anthropogenic disturbance on a breeding population of Mediterranean storm petrels. Seabirds are increasingly used as bio-indicators for sea environmental parameters, because they are very sensitive to changing conditions. Burrowing or cave-nesting species may be particularly susceptible to human disturbance because their direct contact with humans is usually minimal or absent. First, we compared two different populations (exposed or not exposed to human disturbance) for their individual stress response to a standardized stressor (handling and keeping in a cloth bag). Second, we compared the two sub-colonies for their population-level stress response. Third, we tested experimentally whether sub-colonies of storm petrels exposed to tourism have physiological adaptations to anthropogenic disturbances. Our results indicate that storm petrels may be habituated to moderate disturbance associated with boat traffic close to the colony.

Key words: Breeding period, habituation, human disturbance, seabird, stress hormones

Editor: Steven Cooke

Received 3 April 2015; Revised 30 July 2015; accepted 1 August 2015

Cite as: Soldatini C, Albores-Barajas YV, Tagliavia M, Massa B, Fusani L, Canoine V (2015) Effects of human disturbance on cave-nesting seabirds: the case of the storm petrel. *Conserv Physiol* 3: doi:10.1093/conphys/cov041.

Introduction

Many seabirds, especially ground-nesting species, are vulnerable to predation, for which reason they have developed specialized behavioural strategies. Typically, they breed in inaccessible areas, such as on cliffs or remote islands (Schreiber *et al.*, 2002), and visit their colonies only at night to avoid contact with other species, including predators (Miles *et al.*, 2013). This behaviour has also prevented contact with humans; thus, their presence is often unknown even to the people living close to the breeding colonies (Albores-Barajas *et al.*, 2008, 2012; Massa, 2009). However, increasing human activity linked either to urban development or to tourism is having an enormous impact on breeding populations, with potentially catastrophic consequences for threatened and endangered species (Nisbet, 1981, 2000; Lishman, 1985; Culik *et al.*, 1990; Culik and Wilson, 1991; Ellenberg *et al.*, 2007; Seddon and Ellenberg, 2008; see also review by Carney and Sydeman, 1999).

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So far, the impact of human disturbance on wild bird populations has been measured using behavioural parameters, such as nest abandonment (Vleck and Vleck, 2002; Spee *et al.*, 2010) or flight initiation (Beale and Monaghan, 2004). While early studies resulted in apparently contradictory results (see Duffy, 1979; Anderson and Keith, 1980; Parsons and Burger, 1982), it became evident later that identical stressors can cause opposite reactions depending on the individual's life-history stage. For example, during the early breeding season human presence causes nest abandonment in penguins (Hockey and Hallinan, 1981), but once breeding has started, most penguins will not respond behaviourally to human disturbance (Culik *et al.*, 1990; Culik and Wilson, 1991). The latter behaviour is a result of the strong commitment to complete reproduction once breeding has started. Nevertheless, it is likely that these breeding penguins perceive human disturbance as a stress stimulus even though they do not show behavioural responses. Indeed, more recent studies show that penguins exposed to human presence have increased heart rates compared with undisturbed penguins (Viblanc *et al.*, 2015).

The mechanisms that underlie physiologically mediated responses to anthropogenic disturbance have been studied only recently. Human disturbance can act as a generic stress factor and lead to a physiological stress response by activating the hypothalamic–pituitary–adrenal (HPA) axis, with resulting increased secretion of glucocorticoids [GCs; in birds, corticosterone (CORT)] within a few minutes and peaking after 30–60 min. Circulating GCs act on different target tissues to induce an appropriate response to a given stressor. This stress response is critical for survival because it suppresses all 'unnecessary' ongoing activities and allocates energetic resources to functions that are essential for immediate survival (Sapolsky, 1992; Sapolsky *et al.*, 2000; Rodrigues *et al.*, 2009). Physiological stress responses thus have the beneficial function of adjusting physiological and behavioural reactions to sudden environmental changes (Gross and Siegel, 1988; Wingfield and Ramenofsky, 2011; Cohen *et al.*, 2012). For instance, all reproductive functions will be shut down in order to favour immediate energy requirements of other systems and increase awareness (Goymann and Wingfield, 2004; Bonier, 2012). Different stressors will induce different stress responses and thus might induce different behaviours (Vos *et al.*, 1985; Canoine *et al.*, 2002). As soon as the stressor disappears, the stress response goes back to baseline levels, thanks to negative feedback on the HPA axis via GC receptors in different brain regions. However, if a stressor persists and an individual becomes chronically stressed, the physiological alterations will become noxious, causing irreversible damage or dysfunction of the HPA axis (McEwen and Sapolsky, 1995). Most studies on chronic stress and its physiological consequences have been carried out in captivity, and little is known about chronic stress in free-living animals. Physiological studies in the field present limitations because experimental conditions cannot be controlled fully (see Fusani *et al.*, 2005), but are essential to gain a better understanding of what happens in nature. Captivity and laboratory conditions, particularly in wild animals, can have non-negligible effects on chronic stress (Boonstra, 2013). For instance, dysfunctions of the HPA axis discovered in laboratory animals are rare in free-living animals, because natural selection will act immediately on them (see Boonstra, 2013).

To what extent is chronic stress visible in nature? Elevated GC levels have often been associated with chronic stress, but this is not always the case, and it is generally difficult to define the endocrine profile of chronic stress in wild animals (Dickens and Romero, 2013). The few studies conducted in the field have shown that chronic stress causes inhibition of the immune response, reduction of reproductive activity and/or fitness and poor body condition (Silverin, 1986; Wingfield and Silverin, 1986; Creel *et al.*, 2002, 2013; Tarlow and Blumstein, 2007; Ouyang *et al.*, 2012).

Recently, a number of ecological studies have started to use measurements of GCs as a means to investigate whether anthropogenic disturbance might cause chronic stress in animal populations (Partecke *et al.*, 2006; Romero and Wikelski, 2010). But to interpret GC levels as biomarkers of the stressed condition of individuals and/or populations exposed to a specific stressor (Wingfield *et al.*, 2008; Hau *et al.*, 2010) often requires additional experiments (see Cyr and Romero, 2009). For example, an individual might have normally low GC baseline levels and a reduced stress response only because its adrenals suffer from exhaustion of CORT production or a habituation to GCs in higher brain areas (Sapolsky *et al.*, 2000; Cyr and Romero, 2009; Dickens and Romero, 2013). In this case, specific physiological tests can be helpful to evaluate HPA axis responsiveness.

The adrenocorticotrophic hormone (ACTH) challenge test simulates the brain response to a stressor, which sends a specific signal (ACTH) to the adrenals. If the adrenals are fully functional, we expect to see an increase of GC release. If the adrenals are exhausted, we expect to see a reduced GC response. The dexamethasone (DEX) challenge test simulates the negative feedback resulting after a stress response, because DEX mimics GC action. In this case, the brain should show a negative feedback response dampen the stress response. An intact HPA axis should thus reduce GC release, whereas in the case of dysfunction GC production remains high.

Here, we investigated the impact of anthropogenic stress on a breeding population of the Mediterranean subspecies of the European storm petrel (*Hydrobates pelagicus melitensis*). The Mediterranean population is morphologically and behaviourally different from the Atlantic populations and is endemic to the Mediterranean (Cagnon *et al.*, 2004); although taxons are not yet considered as separated by IUCN, and the species, facing a generally decreasing trend, is still considered of least concern (IUCN, 2015). Threats to the Mediterranean population are disturbance and catastrophic events affecting the colonies, and climate change, with peculiar constraints owing to the geographical characteristics of the basin that does not allow expansion northward. In this study, we investigated whether storm petrels breeding in areas exposed to tourism

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are chronically stressed and whether they show any dysfunction of the HPA axis, in an attempt to apply a physiological approach to conservation biology, following the suggestions of Cyr and Romero (2009).

Given that this species has one single-egg brood per year, the costs of interrupting breeding activity are very high, and nest abandonment would have serious consequences for the breeding population. Given the extremely high value of the single clutch, we hypothesized that birds should show habituation or show low sensitivity to moderate anthropogenic stress, such as boat traffic. To test this hypothesis, we compared two groups of birds, i.e. exposed or not exposed to human disturbance. Besides the classical stress response to handling stress, following Wingfield *et al.* (1998), we also conducted a 'population stress response', in which we sampled different individuals of the same group every 5 min over a period of 40 min. In this way, we obtained the stress response of a population to human disturbance. Furthermore, we conducted both ACTH and DEX challenge tests, following Cyr and Romero (2009), to investigate whether the birds were chronically stressed because of the boat traffic or showed signs of habituation or reduced sensitivity.

Our results suggest that breeding storm petrels are not chronically stressed by a mild stressor, such as boat visits, or alternatively, that there exist individuals within the species that show a reduced sensitivity to this stressor and can therefore breed in tourist-exposed areas without showing physiological signs of chronic stress. However, our data indicate that this type of adaptation might also have negative consequences, because any further stressors might elicit in an habituated/less sensitive bird an even stronger stress response, with possible dramatic consequences.

Materials and methods

Study site and species

We studied a colony of ~2500 breeding pairs of the Mediterranean subspecies of the European storm petrel on Marettimo Island, Italy (37°58′20″N, 12°3′20″E; Albores-Barajas *et al.*, 2012). The study population is composed of two main sub-colonies: a small (about 150 nests) outer colony, more exposed to external disturbances, and a larger colony in the inner part of the cave. The outer chamber of the cave has open access to the sea and is exposed to human disturbance, i.e. noise from boats accessing the entrance of the cave during the tourist season, which coincides with the breeding season. The second (inner) chamber is further inside, and no noise can be heard from there; furthermore, it is more difficult to access and can thus be considered free from human disturbance. This provides an excellent model to compare stress responses between 'human-disturbed' and 'non-disturbed' breeding sub-colonies. Storm petrels lay single-egg clutches asynchronously, starting in May–June. Most hatching occurs during the first half of July; our fieldwork was conducted during the brooding period. The breeding season coincides with the tourist season on the island, when caves are visited daily by boats. Tourists are not allowed to land, but the outer part of the colony is nevertheless exposed to noise from boat engines and voices, with an average of 10–15 boats approaching the entrance on days with calm seas, while the inner sub-colony is completely silent and not exposed to any disturbance.

Both sub-colonies have been visited for ringing every summer since 1991, while breeding success and parental behaviour have been monitored more intensely since 2007 (Albores-Barajas *et al.*, 2015) with 1 month of fieldwork in July every year. Birds caught during the present study where ringed in previous years, so they were not handled for the first time. Fieldwork was carried out under permission from the Marine Protected Area no. 3/2011 and 1721/2012, and Regione Siciliana.

Experimental procedure

Experiments were carried out during two field seasons in June 2011 and 2012, when most breeding pairs were brooding. Field work always started at ~10.00 h and was carried out only in conditions of good weather, with no relevant differences in environmental conditions between the 2 years. We approached the cave by rowing a small rubber boat or swimming, then climbed up to the nest sites of the outer or inner chamber of the cave, avoiding any kind of noise. This approach usually took 5 min, and we started sampling immediately afterwards. We sampled the outer chamber first and then moved to the inner chamber, from where noise produced in the outer one cannot be heard. Every time, we sampled different nest clusters in order to reduce disturbance to a minimum. In the outer chamber there are three nest clusters separated by rock walls, and in the inner chamber there are four nest clusters on different terraces, allowing us to work at the lower level while leaving the upper ones undisturbed.

We quantified the physiological stress response of adult storm petrels by measuring the plasma levels of CORT (see '*Radioimmunoassay procedure and biochemical validations*' below). Blood samples (~100 µl) were collected from the wing vein with heparinized capillaries and centrifuged. The plasma was separated and kept on ice until returning to the station, and then stored at −20°C. After obtaining the blood sample, we measured wing length, tarsal length, bill depth and width and head size in order to estimate body condition.

Experiments

Experiment 1: individual stress response

During the breeding seasons 2011 and 2012, we compared the stress response between disturbed and non-disturbed groups ($n = 4$ from each group in 2011; and $n = 10$ non-disturbed and $n = 11$ disturbed in 2012). A baseline blood sample was collected within 5 min of entering the cave chamber. Thus, in 2011 only one bird per colony was sampled each day on our arrival and then after 30 min, while in 2012 using the same procedure, we used the control and saline-injected groups of experiment 3 (for more details see '*Experiment 3:*

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adrenocorticotrophic hormone and dexamethasone challenge test'). The bird was placed in a cloth bag after the first sample, and after 30 min a second blood sample was taken to measure stress-induced CORT levels. Handling and placing in a cloth bag is a standardized and well-established method to induce stress responses in birds.

Experiment 2: population stress response

In this experiment, we collected a blood sample consecutively in nine birds nesting in the same area (2 m diameter) at 5 min intervals, i.e. at 0, 5, 10, 15, 20, 25, 30, 35 and 40 min after the onset of the stressor, which was our entrance into the chamber. This reflects the stress response of birds to human disturbance and not to handling as in experiment 1. The procedure was repeated on different days until four groups of the inner sub-colony and three groups of the outer sub-colony were sampled. Immediately after blood sampling the birds were measured and put back into their nests.

Experiment 3: adrenocorticotrophic hormone and dexamethasone challenge test

In 46 individuals (23 of the inner colony and 23 of the outer colony) a baseline sample was obtained within 1 min after capture. Immediately after taking the first blood sample, birds were injected intramuscularly with ACTH (Sigma; $n = 6$ inner colony and $n = 6$ outer colony), dexamethasone (DEX, Soldesam; $n = 6$ inner colony and $n = 6$ outer olony) or saline (SAL; $n = 3$ inner colony and $n = 3$ outer colony). A further eight individuals were not injected and were used as control birds (CTL; $n = 8$ inner colony and $n = 8$ outer colony). Birds were then placed in cloth bags, and a second blood sample was taken after 30 min. In this experimental test, we used biometrics in order to discriminate gender (Albores-Barajas *et al.*, 2010) and balance sexes between treatments.

Radioimmunoassay procedure and biochemical validations

We measured plasma CORT concentration using a commercial available CORT ^{125}I radioimmunoassay kit (catalogue no. 07-120102; ICN Biomedicals/MP Biomedicals, Solon, OH, USA). We followed the protocol of the company with the following modifications as described by Washburn *et al.* (2002): the volume of all reagents was halved; the dilution of the samples was 1:50 instead of 1:200; and the standard curve was extended by two points, resulting in eight standard points to increase the sensitivity. Even though this protocol had been validated for different bird species, we repeated the validation for our species and could confirm the parallelisms. All samples were analysed in duplicate, with an interassay coefficient of variation <15% and intra-assay coefficient of variation between 12 and 20%. The detection limit was 3 ng/ml plasma.

Statistics

We calculated body condition by entering bird biometrics (wing length, tarsal length, bill depth and width and head size) into a principal components analysis, and factor one was used as a measure of body size. Factor one was plotted against the weight of the individual, and the residuals were used as an indicator of body condition (Catry and Furness, 1999).

We used generalized linear models (GLM) and two-way repeated-measures analysis of variance to determine differences between disturbed and non-disturbed sub-colonies and serial samples (baseline and 30 min after capture). We used Spearman's test in order to identify correlations between variables. Data were analysed using SPSS (SPSS, 2004) and R version 2.15.1 (R Development Core Team, 2014), with significance defined as $P < 0.05$ for all analyses.

Results

We first tested for difference in body condition between individuals of the inside and outside sub-colonies, finding no significant differences (sub-colony, $F_{1,23} = 1.028$, $P = 0.321$; and sub-colony + year, $F_{2,22} = 0.985$, $P = 0.390$).

Experiment 1: individual stress response, 0–30 min ($n = 8$)

Storm petrels had a flat stress response in 2011 (time, $F_{1,6} = 0.004$, $P = 0.949$; Fig. 1a) that did not differ between disturbed ($n = 4$) and non-disturbed birds ($n = 4$; sub-colony, $F_{1,6} = 3.185$, $P = 0.125$; and time × sub-colony, $F_{1,6} = 0.17$, $P = 0.899$). Similar results were obtained in 2012 with the birds of the control group of experiment 3 (time, $F_{1,19} = 0.025$, $P = 0.877$; Fig. 1b). In fact, to increase the sample size, we also analysed the individual stress response of control birds of experiment 3 (see below in Experiment 3 paragraph), which, like the birds of experiment 1, were handled to induce a stress response but were not treated with any hormone or drug. However, baseline values in 2012 were, although not significantly, lower than in 2011 ($F_{1,27} = 3.812$, $P = 0.061$). There was no significant difference between disturbed and non-disturbed birds (sub-colony, $F_{1,19} = 0.007$, $P = 0.934$; and time × sub-colony, $F_{1,19} = 0.281$, $P = 0.602$).

Considering all individuals sampled at 0 and at 30 min in 2011 ($n = 8$, experiment 1) and 2012 ($n = 21$, control group of experiment 3), we found no correlation between body condition and both baseline CORT levels ($S = 2152$, $\rho = 0.172$, $P = 0.408$) and stress response, namely change between 0 and 30 min ($S = 259$, $\rho = 0.002$, $P = 0.995$). We then fitted a linear regression analysis to establish whether body condition alone or additively with year or population had an effect on the individual stress response. In all cases, we observed no significant effects (body condition, $F_{1,23} = 0.450$, $P = 0.125$; body condition + year, $F_{2,22} = 1.422$, $P = 0.262$; and body condition + sub-colony, $F_{2,22} = 0.250$, $P = 0.782$). We then tested for the effect of the sampling year on individual stress response and observed a tendency for year effects (year, $F_{1,27} = 3.401$, $P = 0.076$).

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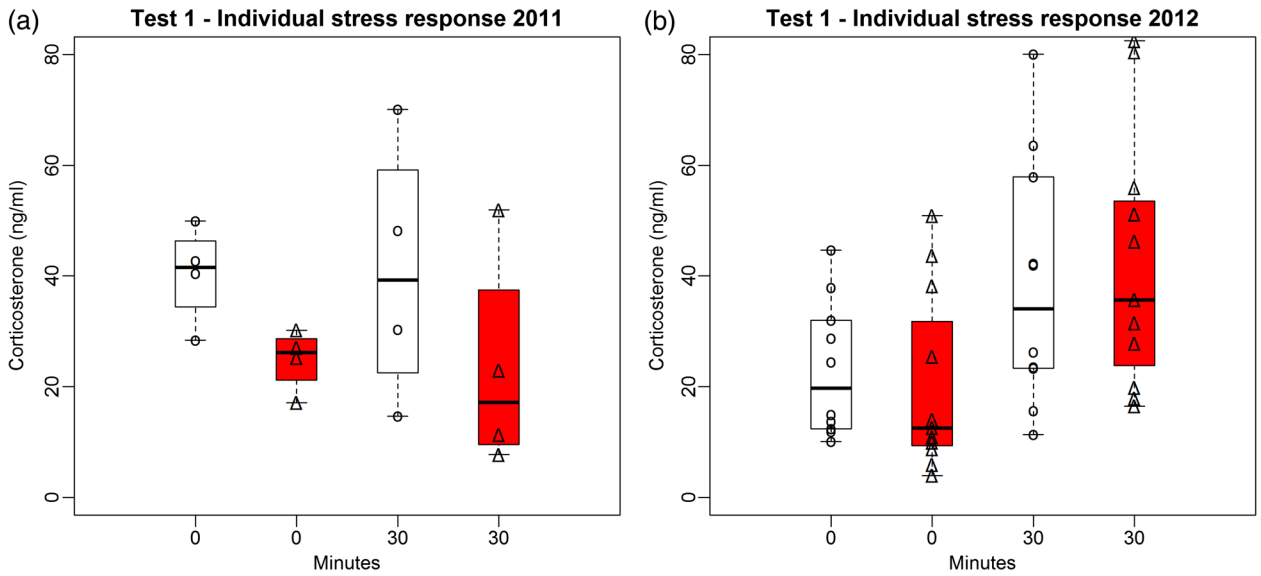

Figure 1: Experiment 1. Individual stress response: comparison of corticosterone (CORT) concentrations in the same individual of disturbed and not-disturbed groups at 0 and 30 min in 2011 (**a**) and in 2012 (**b**), considering the control group of experiment 3. The white boxplots and circles refer to the inner colony, while red boxplots and triangles refer to the outer colony.

Experiment 2: population stress response

In both the outer and the inner sub-colonies, the stress response did not change over time (time, $F_{8,53} = 0.669$, $P = 0.072$; Fig. 2), but the curve was lower in the disturbed sub-colony (sub-colony, $F_{1,53} = 13.4$, $P < 0.001$), while the interaction between time and sub-colony was not significant (time × sub-colony, $F_{8,53} = 0.909$, $P = 0.52$).

The non-disturbed sub-colony showed an increasing trend in the stress response after 25 min of human disturbance, with a peak of 50.49 ng/ml CORT concentration. In contrast to the non-disturbed sub-colony, the disturbed sub-colony did not show any fluctuation in stress response across time.

Experiment 3: adrenocorticotrophic hormone and dexamethasone challenge test

We first ran a global full-factorial GLM analysis, including time as a within-subjects factor and treatment, sub-colony and sex as between-subjects factors, and included all interaction effects. Only time ($F_{1,33} = 23.092$, $P < 0.001$), treatment ($F_{2,33} = 5.502$, $P < 0.01$) and treatment × sub-colony ($F_{2,33} = 3.303$, $P < 0.05$) provided significant results. In addition, Student's paired *t*-test showed no difference for CORT at time 0 ($t_{29} = 1.8$, $P = 0.075$) and 30 min ($t_{19} = 1.1$, $P = 0.28$) between uninjected birds and those injected with saline; therefore, we pooled the latter two groups. As already reported above, we observed no significant differences in the individual stress response of control and saline-injected birds between disturbed and non-disturbed colonies. We ran a second analysis for CORT at time 30 min including only the factors treatment and sub-colony. This analysis showed no effect of either treatment ($F_{2,39} = 1.567$, $P = 0.390$) or sub-colony ($F_{1,39} = 0.113$, $P = 0.768$) but a significant interaction effect ($F_{2,39} = 3.963$, $P = 0.027$), showing that the effect of treatment was different between the two sub-colonies (Fig. 3). Therefore, we ran an analysis of variance for the two sub-colonies separately, with treatment as the only factor. There was no significant effect of treatment on CORT in the non-disturbed sub-colony (treatment, $F_{2,19} = 0.161$, $P = 0.852$; Fig. 3a), whereas there was a highly significant effect of treatment in the sub-colony exposed to tourists (treatment, $F_{2,20} = 9.53$, $P = 0.001$). A *post hoc* analysis with Dunnet's test showed that ACTH-treated birds tended to have a higher CORT compared with control birds ($P = 0.06$; Fig. 3b), whereas DEX-treated animals had lower CORT than control birds ($P = 0.029$; Fig. 3b). Overall, DEX-treated birds showed lower levels of CORT, but in the non-disturbed group there was no suppression of the stress response, whereas this is evident in the disturbed group.

Discussion

Human presence can be stressful to breeding populations because it can be perceived as potential predation, which in turn may have dramatic consequences (Lishman, 1985; Culik *et al.* 1990; Culik and Wilson, 1991; Ellenberg *et al.*, 2007; Seddon and Ellenberg, 2008). However, storm petrels (Hydrobatidae) apparently lack anti-predator behaviour, making them particularly defenceless to predators (Burger and Gochfeld, 1993; Sirot, 2006). In this study, we investigated whether storm petrels breeding in a cave that is regularly disturbed by tourist boats during the breeding season are chronically stressed.

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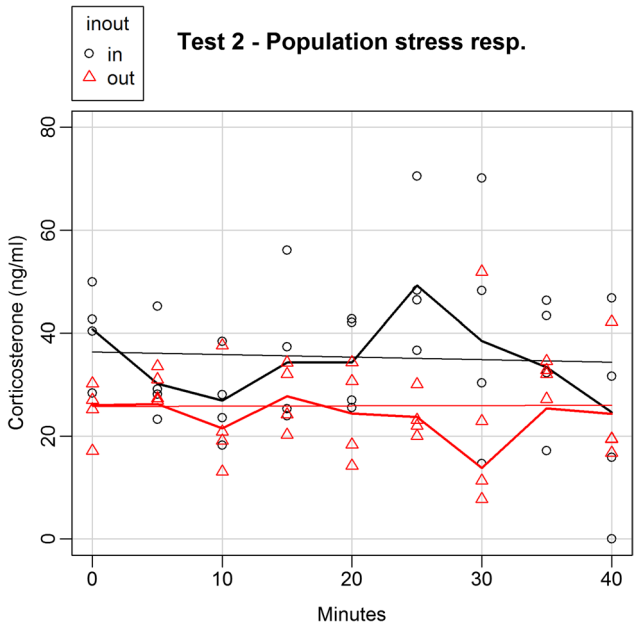

Figure 2: Experiment 2. Population stress response: comparison of CORT concentrations in different individuals of disturbed and not-disturbed groups every 5 min from 0 to 40 min. The terms 'in' and 'out' refer to the inner and outer colony, respectively.

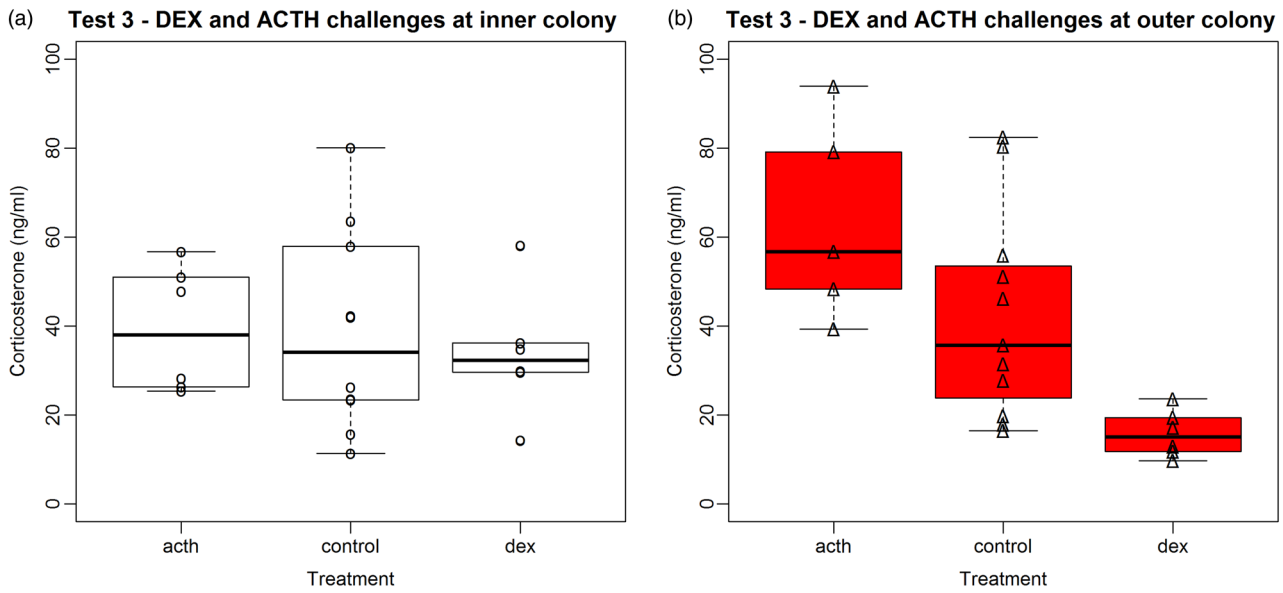

Figure 3: Experiment 3. Adrenocorticotrophic hormone (ACTH) and dexamethasone (DEX) challenges: comparison of CORT levels in ACTH-injected, DEX-injected and control individuals (pooled individuals, saline injected and not injected) at 30 min from a non-disturbed population, the inner colony (**a**), and a disturbed population, the outer colony (**b**).

Interestingly, in hormonally untreated birds, i.e. birds that were simply kept in a bag between the first and the second sampling (experiment 1, Fig. 1a; and the control group of the third experiment, Fig. 1b), we did not find an increase in CORT levels after 30 min in both disturbed and undisturbed birds. We were surprised that the non-disturbed sub-colony did not mount a stress response to handling stress, because we had expected that a perceived predation event, such as capture and handling, would elicit a clear hormonal response. These results were further supported by the results of the second experiment, in which we measured CORT levels of different individuals sampled at 5 min intervals over a period

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of 40 min, which is likely to provide a better representation of the real stress response towards a human intrusion than handling stress as in experiment 1. The stress response differed significantly between the two sub-colonies (Fig. 2), but again CORT levels did not change significantly over time. One possible explanation is that birds were already stressed when the first sample (i.e. time 0) was taken. However, this would not explain the flat response because, if the first sample was representing the peak, we would have expected a decrease over the next 30 min. Alternatively, the weak responsiveness found in both sub-colonies could be an adaptation to the reproductive phase/life-history stage to maintain breeding activity. Flat stress responses have been reported for other species in specific life-history stages (Romero, 2002, 2004; Partecke *et al.*, 2006; Romero and Wikelski, 2010). It should be remembered that our experiments were conducted when storm petrels were brooding. Generally, birds adapt their stress response according to their life-history stage and they do so particularly when breeding, in order to avoid overreaction to mild stressors which could have serious consequences on fitness (Wingfield *et al.*, 1994; Romero *et al.*, 1997; Walker *et al.*, 2002).

The life-history stage might explain the dampened stress response, but it does not explain why tourist-exposed birds had lower CORT concentrations than undisturbed ones. So far, reduced stress responses in free-living animals have been explained by habituation or adaptation to human disturbance (Romero, 2004; Partecke *et al.*, 2006; Romero and Wikelski, 2010; Dickens and Romero, 2013). Exposure to a repetitive stressor modulates the stress response, which becomes less sensitive to this particular stressor. In our study, boat traffic might have modulated the stress response to this specific disturbance. Our results are in line with studies conducted on other species, such as tourist-exposed Galapagos iguanas, which have lower baseline and stress-response CORT levels than undisturbed iguanas (Romero and Wikelski, 2010). Likewise, blackbirds living in an urban environment have comparable baseline levels with, but lower stress-response levels to acute stress than, those living in the forest (Partecke *et al.*, 2006). One could conclude that tourist-disturbed birds in our study have a lower stress response than the non-disturbed birds because they had reached a physiological exhaustion, i.e. their adrenals were not able to produce CORT any longer. This possibility, however, is ruled out by the results of the ACTH and DEX challenges. We obtained a clear increase of CORT 30 min after ACTH injection in both sub-colonies, with tourist-exposed storm petrels showing a significantly stronger response than the control group. This indicates that the adrenals of tourist-exposed storm petrels are fully functional and not exhausted. These results are in agreement with those of previous studies showing that in animals habituated to a specific stressor, novel stressors will elicit a stronger response than in non-habituated animals, a phenomenon called 'facilitation' (Romero and Sapolsky, 1996; Romero, 2004; Cyr and Romero, 2009). An alternative interpretation of our results is that birds breeding in tourist-exposed areas were not habituated in a physiological sense but instead showed a lowered responsiveness to stressors and had therefore chosen to breed in disturbed areas. If this was the case, our results would indicate that there exist in the population individual differences in the response to stressors, with birds that are little responsive to human disturbance but are still able to mount a stress response to other types of stressors.

The DEX test should induce the negative feedback that leads to the termination of the stress response (Cyr and Romero, 2009). As expected, we obtained a reduction of CORT release 30 min after the DEX injection, but only in the disturbed sub-colony. The absence of a reduced CORT response in undisturbed individuals does not exclude the possibility that a negative feedback response would have occurred later than 30 min. We are aware that the second blood sample in our study was taken earlier after injection compared with other studies (60–90 min; see Rich and Romero, 2005). However, we had to limit the time in captivity because our study birds were brooding and we could not keep them away from their nests any longer. Nevertheless, our results suggest that the disturbed group is more sensitive and reactive towards stress stimuli, in line with the finding of a facilitated stress response after ACTH challenge.

Body condition is often used as an indicator of chronic stress (Bonier *et al.*, 2009; D'Alba *et al.*, 2011; Dickens and Romero, 2013). In the present study, we did not find any difference in body condition between birds of the two sub-colonies, and the samples were similarly composed of individuals of both sexes.

In summary, our results indicate strongly that the human-disturbed storm petrels are not chronically stressed, either because they are physiologically habituated or because they have a reduced sensitivity specifically to humans. It remains unclear why tourist-disturbed birds sampled in 2011 had a lower maximal response than all others birds sampled in the following year (see Fig. 1a and b). A possibility is that the birds breeding in the outer cave became habituated to disturbance from researchers first in 2012. However, this is unlikely because these caves have been visited by researchers since the 1980s (Massa and Catalisano, 1986; Massa and Sultana, 1991; Soldatini *et al.*, 2014). Moreover, this hypothesis would not explain why non-disturbed birds had higher CORT concentrations than disturbed birds in 2012. It is more likely that small differences in the life-history stage during the experiments affected the results. In the first year (experiments 1 and 2), storm petrels started their breeding activity about 15–20 days later than in the following year (experiment 3) and were incubating eggs during the experiments, whereas in the following year some already had hatchlings.

Overall, our results show that storm petrels breeding in a tourist-exposed area are not chronically stressed. This might mean that the birds have become habituated to mild disturbance, such as frequent boat and human noises, or alternatively, that birds breeding in the outer cave have a lower responsiveness to human disturbance and can therefore breed

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in the tourist-exposed cave without being stressed. According to the latter interpretation, only individuals which can 'cope' better with tourist exposure will nest in the outer cave, while shyer or more sensitive individuals will breed inside, in the protected cave. Nevertheless, our data indicate that birds breeding in the outer cave are more susceptible to novel stressors, which could elicit a much stronger and potentially catastrophic response. Clearly, long-term studies on anthropogenic stress are needed in order to understand the extent of consequences of human activities on animal populations.

Acknowledgements

We thank two anonymous referees for stimulating discussions that resulted in improvements to the manuscript. We are grateful to Michael Romero for advice on the experimental design. We are particularly grateful to Gabriella Soldatini for making the cloth bags and to Caterina Tuci, Emanuela Canale and Paolo Lucido for helping in the field. Our thanks goes to to Dimitri Giunchi and Ana Marichal, whose advice and comments greatly improved the manuscript. This study was conducted under research permits issued by the Marine Protected Area nos 3/2011 and 1721/2012 and the Regione Siciliana, Assessorato Risorse Agricole e Alimentari.

Funding

Cecilia Soldatini was involved in the project CICESE 691111. This study was partially funded by Regione Siciliana, Assessorato Risorse Agricole e Alimentari.

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