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Corticosterone administration leads to a transient alteration of foraging behaviour and complexity in a diving seabird

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ABSTRACT: Hormones link environmental stimuli to the behavioural and/or physiological responses of organisms. The release of corticosterone has major effects on both energy mobilization and its allocation among the various requirements of an individual, especially regarding survival and reproduction. We therefore examined the effects of experimentally elevated baseline corticosterone levels on the foraging behaviour of Adélie penguins *Pygoscelis adeliae* during chick-rearing. We monitored the at-sea behaviour of corticosterone-implanted and control male birds using time-depth recorders, and monitored the effects of corticosterone treatment on their body conditions as well as their chicks' body masses and survival. Bio-logged data were examined via traditional measures of diving behaviour as well as fractal analysis as an index of behavioural complexity. Corticosterone administration caused a transient decrease in both overall foraging effort (i.e. reductions in the duration of at-sea trips, the time spent diving and the number of dives performed) and foraging complexity. In contrast, per-dive performance indices suggested an increase in both efficiency and prey pursuit rates. Ultimately, however, we observed no short-term effects of treatment on adult body condition and chick body mass and survival. We conclude that under higher corticosterone levels, sequences of behaviour may become more structured and periodic, as observed in treated birds. The increased energy allocation to dive-scale behaviours observed in treated birds might then reflect an adjustment to intrinsic constraints allowing reductions in energy expenditure at the trip-scale. This study highlights the utility of using both traditional and fractal analyses to better understand scale-dependent responses of animals to energetic and various other environmental challenges.

KEY WORDS: Adélie penguins · Allocation of energy · Bio-logging · Fractal analysis · Stress hormone

INTRODUCTION

Limitations to energy acquisition *in natura* form the basis for ecological and physiological trade-offs occurring throughout an animal's life (Stearns 1992), as has been demonstrated in numerous correlative studies (see review by Zera & Harshman 2001). During breeding periods, for example, individuals must allocate the resources available to them to maintain their own body condition while at the same time sustain the energy necessary for reproductive behaviours and the growth and development of their offspring. Among the hormones, corticosterone (hereafter CORT), the main glucocorticoid in birds, plays a major role in parental care and foraging behaviour and generally promotes survival through a variety of

mechanisms (reviewed in Landys et al. 2006). Within species and within individuals, however, the effects of CORT level modulation are context-dependent. CORT secretion depends on both extrinsic (e.g. food availability, predation risk) and intrinsic (e.g. body condition, energy requirements) factors. For instance, Kitaysky et al. (1999) found an increase in baseline CORT levels of black-legged kittiwakes *Rissa tridactyla* under low food availability. These authors also showed that the deterioration of an adult's body condition with the progression of the breeding season was associated with an increase in CORT levels. The influence of CORT on foraging behaviour has therefore been extensively studied in many bird species (e.g. Koch et al. 2002, 2004, Löhmus et al. 2006, Angelier et al. 2007, 2008, Miller et al. 2009).

Recently, experimental studies using CORT administration have attempted to understand the complex relationships between baseline CORT levels, foraging and fitness in wild seabirds (Angelier et al. 2007, Cottin et al. 2011, Spée et al. 2011a, Crossin et al. 2012). It is expected that increasing CORT levels during the breeding period should allow seabirds to cope with any additional energy requirements imposed by reproduction (Romero 2002), especially through an increase in the effort devoted to foraging. However, despite this positive effect on energy mobilisation during challenging periods, elevated CORT levels are also known to disrupt and/or interrupt parental behaviour since they can cause the complete abandonment of reproduction in seabirds (Silverin 1986, Wingfield & Sapolsky 2003, Groscolas et al. 2008, Spée et al. 2010). The effects of corticosterone depend largely on its concentration in the blood (basal, modulated or stress levels) as well as the life history stage of the individual (Bonier et al. 2009, Busch & Hayward 2009). These complex effects call for further investigations into the influence of elevated baseline CORT levels on foraging effort, and consequently on the trade-off between self-maintenance and reproduction regarding energy allocation.

To this end, the ability to link hormone manipulation with fine-scale behaviour recording through miniaturized data-recording devices attached to free-ranging seabirds (sensu bio-logging, cf. Ropert-Coudert & Wilson 2005, Ropert-Coudert et al. 2012) represents a major step forward. Bio-logging allows for the quasi-continuous monitoring of individual behaviour *in natura*, and therefore helps to determine the effects of perturbations such as hormone implantation on animal behaviour. Traditional methods analysing foraging patterns in diving seabirds include measurements of dive depth, duration or frequency.

Some indices of efficiency have also been created in order to estimate the effort invested in foraging behaviour. For instance, the index developed by Ydenberg & Clark (1989) assesses air management during a dive cycle, with the expectation that penguins should minimize recovery time spent at the surface after each dive. The number of undulations performed at the bottom phase of the dive is also known to be a good index of foraging effort as it correlates well with the number of prey pursued (e.g. Ropert-Coudert et al. 2001, Bost et al. 2007). These traditional methods provide invaluable information about certain quantitative behavioural parameters, but it remains difficult to interpret results with regards to optimal patterns. For example, an elevated number of prey pursuits could signify increased foraging success, provided that prey pursuits translate linearly into prey caught. Alternatively, an increasing number of prey pursuits may also represent poor foraging success if birds are forced to pursue more prey because of high failed-capture rates (but see Watanabe & Takahashi 2013). A further confounding factor is that the expected relationship between this index and optimal behaviour must also depend heavily on the quantity of prey available in the environment.

A more recent and novel approach to investigating animal behaviour has arisen with the realization that fractal (a.k.a. Lévy) movements may represent an optimal search pattern in animal behaviour (e.g. during foraging). Typically, animal movement consists of clusters of small-scale tortuous movements interspersed with periodic large-scale displacements of varying lengths (Bartumeus et al. 2005). Statistically, such patterns produce step-length distributions with a heavy tail (i.e. power laws) and can thus be described by their fractal geometry (Mandelbrot 1983). Fractal movement patterns are super-diffusive, i.e. have a greater capacity to cover ground than normally diffusive processes such as Brownian (random) motion, and have thus been considered an optimal foraging strategy — particularly in highly heterogeneous environments in which no *a priori* information exists regarding the nature of the resource being sought (Bartumeus et al. 2005, Bartumeus 2007, Sims et al. 2008, Viswanathan et al. 1999, 2008).

An additional insight is that fractal patterns are considered to be more robust to both internal and external perturbations, a pattern which holds over a wide range of biological systems (Goldberger et al. 1990, West 1990). Under this framework, the application of fractal tools has shown that alterations occur in the complexity (here the correlation structure in time series rather than spatial data) of a diverse array

of biological systems when operating under pathological conditions. For example, complexity loss is associated with various forms of physiological impairment in heart rhythms (Peng et al. 1995), stride patterns (Hausdorff et al. 1995), and even animal behaviour (Alados et al. 1996, Rutherford et al. 2004, MacIntosh et al. 2011, Seuront & Cribb 2011). Complexity loss may thus pose a long-term performance constraint with potential fitness costs if individuals can no longer cope with heterogeneity in their natural environments or achieve theoretically optimal foraging patterns. However, the link between search complexity and true foraging success (e.g. prey capture) remains largely untested, and a decrease in foraging behaviour complexity may simply represent an alternative strategy whereby individuals target different prey types to maximize energy acquisition.

In this context, we aimed to assess the effect of an experimental physiological alteration on the foraging behaviour of a diving seabird, the Adélie penguin *Pygoscelis adeliae*, monitored with time-depth recorders across several at-sea foraging trips during the chick-rearing period. We artificially increased baseline CORT levels and investigated subsequent changes in the foraging behaviour and dive sequence complexity of free-living male Adélie penguins. We predicted in the latter case that treated birds should show reduced complexity (i.e. greater periodicity) in their foraging sequences compared to control birds due to their altered physiological condition. While complexity loss is commonly suggested to be associated with increased 'stress', only one study has tested the relationship between a physiological indicator of stress (cortisol, the major circulating glucocorticoid in pigs) and fractal patterns in animal behaviour (Rutherford et al. 2006). Ours is the first study of fractal dynamics in animal behaviour to have manipulated physiological stress directly. Fractal analysis, encompassing both the tool and the theoretical framework, in addition to more common methods of behavioural investigation, can therefore provide a broader evaluation of the effects of perturbations on the behaviours of free-living animals.

MATERIALS AND METHODS

Study site and breeding cycle of subjects

We conducted fieldwork at the French polar station Dumont d'Urville in Adélie Land, Antarctica (66° 40′ S, 140° 01′ E), during the 2009–2010 breeding season. At the end of the courtship period, female Adélie penguins generally lay 2 eggs in approximately mid-November, after which both partners alternate between caring for the eggs/chicks at the nest and feeding at sea. The guard stage begins after the eggs hatch in approximately mid-December, during which time chicks are highly dependent on their parents for food and protection against cold and predation. To facilitate the experimental protocol used in this study (see next section), we randomly marked 40 penguin pairs with a Nyanzol-D (a commonly-used marker containing a mix of gum arabic, p-phenylene, sodium sulphite, ethanol and oxygen peroxide) number painted on their chests at the end of the courtship period (mid-November). Penguins were sexed by a combination of parameters, including cloacal inspection before egg-laying and observations of incubation behaviour (Beaulieu et al. 2010).

Experimental protocol

At the beginning of the guard stage, from 26 to 31 December 2009, 20 marked male penguins were captured at their nests. At this time, all individuals had 2 chicks that were between 2 and 10 d old. Each bird's head was covered with a hood (Cockrem et al. 2008) and chicks were kept safe and warm. We collected blood samples from the flipper or the tarsus vein within 5 min of capture. The levels of CORT measured within this period can be considered baseline in Adélie penguins (Vleck et al. 2000). Each sample was transferred into 2 pre-treated tubes with anticoagulants, one with EDTA and the other with heparin. All samples were centrifuged and plasma was subsequently stored in aliquots at −20°C until assays were conducted. We weighed each penguin using an electronic balance (Ohaus, ±2 g) and measured their flipper lengths using a ruler (±1 mm). Subjects were then equipped with temperature-depth recorders (see below) and half of them (hereafter CORT-birds) were implanted with a corticosterone pellet (see below). We implanted the pellet under the skin through a small incision (ca. 1 to 2 cm), which was then closed with a sterile stitch and sprayed with Alumisol® (aluminium powder, healing external suspension, CEVA). The other 10 birds (control group) underwent the same procedure including incision but without implantation. Overall, manipulation lasted for 22.2 ± 2.8 (SD) min (range: 20 to 28 min) for controls and 24.8 ± 2.0 (SD) min (range: 22 to 29) for CORT-implanted birds. After releasing birds near their nest, we observed these nests from a distance every 2 to 3 h (except from 02:00 to 07:00 h) to deter-

mine which individuals were present on the nest. In addition, the number of chicks per pair was carefully monitored at 6 different times over the study (at each capture as well as January 2, 5, 11, and between January 6 and 9).

All study subjects were recaptured at the colony after several foraging trips, between 17 and 19 d after deployment (from 12 to 17 January 2010). Another blood sample was immediately collected (again within 5 min of capture), and their loggers removed. Before releasing penguins, we also measured their body masses. Unfortunately, 3 penguins could not be weighed (1 CORT and 2 controls). An index of body condition (BCI) was calculated for the adults at the beginning and at the end of the experiment as follows: BCI = $bm/l^3 \times 10^7$, where bm is the body mass in kg, and l the flipper length in mm (Cockrem et al. 2006).

Corticosterone assays and implant characteristics

We used biodegradable CORT implants in pellet form (5 mm) containing 100 mg of corticosterone (G-111, Innovative Research of America). These pellets are designed for a 21 d release in rodents and have been previously used in studies of Adélie penguins (Cottin et al. 2011, Spée et al. 2011a,b). For instance, an increase of 3.3 times the amount of circulating CORT has already been shown in captive/ fasting male Adélie penguins within 3 d of treatment with these pellets (reaching on average ca. 65 ng ml^{-1}; Spée et al. 2011b). The CORT values in that study (Spée et al. 2011b) were lower than those reached during capture stress (Cockrem et al. 2008), and were therefore within the physiological range of this species. In our study, the CORT levels should have been lower since we were working with free-living and non-long-term fasting birds. Spée et al. (2011b) also indicated the maintenance of this elevated CORT level through 7 to 11 d post-implantation, corresponding therefore to less than the half of our study period.

We determined total plasma corticosterone concentrations in our laboratory at DEPE-IPHC, France by enzyme-immunoassay (AssayPro, AssayMax Corticosterone ELISA Kit). The concentration of corticosterone in plasma samples was calculated using a standard curve run in duplicate. The evaluation of intra-assay variations, by running some samples in triplicate, led to a coefficient of 10.7 %. There was no inter-assay variation as all samples were measured on a single plate. One CORT value (for a control bird

at first capture) was out of the physiological range for this species (>150 ng ml^{-1}). This value, as well as the CORT value at recapture, were removed from the analyses.

Recording of diving behaviour

To determine the dive profiles of the 20 study subjects, temperature-depth recording data loggers (M190-DT: 49 × 15 mm, 14 g; M190L-DT: 52 × 15 mm 16 g; Little Leonardo) were attached with mastic and strips of waterproof black Tesa® tape (Beiersdorf) (Wilson et al. 1997) along the median line of the penguin's lower back (Bannasch et al. 1994). These loggers recorded depth to 190 m at 1 s intervals with a 5 cm resolution. Data were stored on a 32 MB memory.

Because loggers may disrupt the behaviour of monitored birds (Ropert-Coudert et al. 2007), assessing instrumentation effects is essential for interpretation of our results. A recent study conducted by Beaulieu et al. (2009) showed that Adélie penguins handicapped by back-mounted, dummy Plexiglas devices performed longer foraging trips. Here, to examine such instrumentation effects, we monitored (via visual observations of the nest every 2h) the durations of foraging trips of 6 unequipped male control birds and then compared them with control birds equipped with loggers.

Diving data analysis

Diving data were analyzed with IGOR Pro software (Wavemetrics v.6.1). We conducted data surfacing (dive depth adjustments according to the sea surface) using the 'WaterSurface D2GT' program in the 'Ethographer' application (Sakamoto et al. 2009). This program allowed an automated procedure to correct depth using a linear regression between depth and temperature at the surface. Diving parameters (dive depth, dive duration, time spent at the bottom of the dive, number of undulations per dive, and post-dive interval duration) were extracted automatically for each dive using a purpose-written macro in IGOR Pro (see Ropert-Coudert et al. 2007 for parameter definitions). Only dives >1 m were included in the analyses. Diving efficiency was calculated as the ratio between the bottom duration and the duration of the complete dive cycle (dive duration + post-dive interval duration) (Ydenberg & Clark 1989). The number of undulations per dive was used as an index

of prey pursuits (Ropert-Coudert et al. 2001). The diving efficiency and number of undulations per dive were used in this study as 'traditional measures' to assess CORT-treatment effects on the diving performance of birds.

In parallel to these traditional measures, we used fractal analysis to measure the temporal complexity of dive sequences in relation to treatment effects as a third indicator of diving performance. While there are many approaches that fall within the rubric of fractal analysis, we examine here binary sequences of diving behaviour collected during penguin foraging trips (described in MacIntosh et al. 2013). First, we coded dive sequences as binary time series ($z(i)$) in wave form containing diving (denoted by 1) and lags between diving events (denoted by −1) at 1 s intervals to length N (Alados et al. 1996, Alados & Weber 1999). Series were then integrated (cumulatively summed), such that

$$y(t) = \sum_{i=1}^{t} z(i) \tag{1}$$

where $y(t)$ is the integrated time series, to create behavioural 'walks'. We then estimated the scaling exponents of these sequences using detrended fluctuation analysis (DFA) as an indicator of sequential complexity.

DFA was introduced by Peng et al. (1992) to identify long-range dependence in nucleotide sequences and has since become the method of choice for researchers studying fractal dynamics in a diverse array of systems ranging from temperature to heart rate to animal behaviour (Peng et al. 1995, Rutherford et al. 2004, Király & Jánosi 2005, Asher et al. 2009). The scaling exponent calculated via DFA (αDFA) provides a relatively robust estimate of the Hurst exponent, which measures the degree to which time series are long-range dependent and statistically self-similar or self-affine (Taqqu et al. 1995, Cannon et al. 1997). Briefly, after integration, sequences are divided into non-overlapping boxes of length n, a least-squares regression line is fit to the data in each box to remove local linear trends ($\hat{y}_n(t)$), and this process is repeated over all box sizes such that

$$F(n) = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left(y_n(t) - \hat{y}_n(t) \right)^2} \tag{2}$$

where $F(n)$ is the average fluctuation of the modified root-mean-square equation across all scales (2^2, 2^3, … 2^n). The relationship between F and n is of the form $F(n) \sim n^\alpha$ where α is the slope of the line on a double logarithmic plot of average fluctuation as a function of scale; $\alpha = 0.5$ indicates a non-correlated, random sequence (white noise), $\alpha < 0.5$ indicates negative autocorrelation (anti-persistent long-range dependence), and $\alpha > 0.5$ indicates positive autocorrelation (persistent long-range dependence) (Peng et al. 1995). Theoretically, αDFA is inversely related to the fractal dimension, and thus smaller values reflect greater complexity. In addition to identifying the scaling behaviour of self-affine sequences, DFA can also distinguish the class of signal being examined: $\alpha \, \epsilon \, (0, 1)$ indicates fractional Gaussian noise (fGn) while $\alpha \, \epsilon \, (1, 2)$ indicates fractional Brownian motion (fBm), which is critical for the accurate interpretation of observed scaling exponents (Delignieres et al. 2005, Seuront 2010). We performed DFA using the package 'fractal' (Constantine & Percival 2011) in R statistical software 2.11.1 (R Development Core Team 2008).

In order to avoid spurious results that can arise when relying on any single fractal analytical method (Gao et al. 2006, Stroe-Kunold et al. 2009), we supplement our analysis by also estimating the scaling exponents of these sequences using 2 other fractal methods: power spectral density (PSD), which is one of the more commonly used methods to identify the presence of scaling behaviour (Eke et al. 2000), and the madogram, which provides a robust estimate of fractal dimension (Bez & Bertrand 2011). Like αDFA, β_{PSD} also provides information about the nature of the signal under investigation, with $\beta \, \epsilon \, (-1, 1)$ and $\beta \, \epsilon \, (1, 3)$ indicating fGn and fBm, respectively (Cannon et al. 1997). Details of these methods are provided in the Appendix. We present results based on the scaling exponents of these methods (β_{PSD} and β_M, respectively), which, like αDFA, are inversely related to complexity (i.e. fractal dimension).

Statistics

Statistical analyses were conducted in R 2.11.1 (R Development Core Team 2008). We constructed General Linear Mixed effects Models (GLMM, 'nlme' package in R, Pinheiro et al. 2011) to investigate variation in dive performance between treated and control birds across time. When required, we nested individual identity with trip rank and set it as a random factor in the models to avoid pseudoreplication caused by repeatedly measuring behaviour of the same birds over successive dives during several foraging trips. The trip rank refers to the sequence of trips (1 through 4) post-implantation. For fractal analysis, in addition to the treatment, the foraging trip

rank, and their interaction, trip duration was added as a covariate to control for the effects of sequence length on scaling exponents (MacIntosh et al. 2013). ANOVAs were performed following mixed models in order to determine whether the interaction between treatment and trip rank was significant by comparing models with and without this interaction term. Two-sample Kolmogorov-Smirnov tests were used to compare the distribution of maximum dive depths between control and CORT-implanted birds according to the foraging trip rank. Values are presented as means ±1 SE.

RESULTS

At the beginning of the experiment, none of the parameters differed significantly between the control (n = 10) and CORT (n = 10) groups (Table 1). Comparisons between control birds that were equipped with loggers (n = 10) and those that were not (n = 6) also showed that the equipment had no effect on foraging trip durations (ANOVA: F = 1.2, df1 = 5, df2 = 57, p = 0.3). Of the 20 equipped birds, our last recapture attempts toward the end of the chick-rearing phase failed for 5 birds (2 controls and 3 CORT). In addition, 6 loggers (3 controls and 3 CORT) did not work properly (i.e. ≤1 trip was recorded because of problems with the batteries), so data from these individuals were removed from the diving analysis.

The number of trips performed during the experiment (range: 4 to 7) did not significantly differ between control (n = 5) and CORT-implanted (n = 4) birds (W = 14, p = 0.4). As penguins did not perform the same number of trips during the experiment, and there were no systematic differences between treatment groups, we considered only the first 4 trips after pellet implantation in the following analyses.

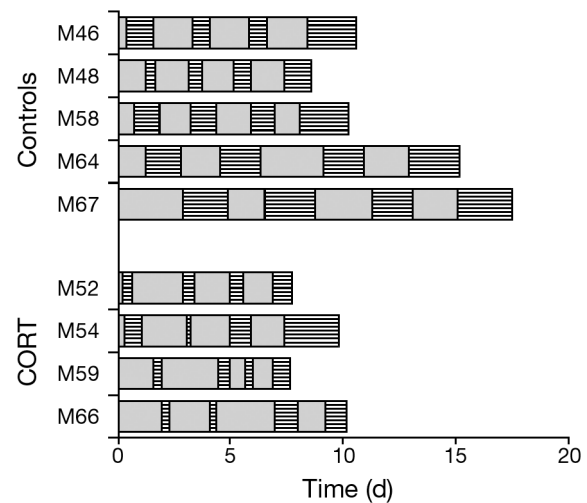Following implantation, CORT treatment had a significant negative effect on trip duration (ANOVA:

Fig. 1. Alternation between nesting bouts (grey bars) and at-sea foraging trips (hatched bars) of controls (n = 5) and CORT-implanted (n = 4) male Adélie penguins (ID tags) during the first 4 trips following CORT implantation

F = 6.7, df1 = 1, df2 = 7, p = 0.04) (Fig. 1). Trips lasted 1.5 ± 0.2 d for controls and less than half that for CORT birds (0.7 ± 0.1 d) (Fig. 1). There was no interaction between treatment and trip rank (ANOVA: F = 0.65, df1 = 3, df2 = 21, p = 0.6).

CORT treatment and trip rank had a significant interactive effect on time spent diving (ANOVA: F = 8.7, df1 = 3, df2 = 21, p < 0.001) (Fig. 2). CORT-implanted birds tended to spend less time diving during the first trip, although there was strong variation observed between individuals (range for Trip 1: 9 to 47% of time spent diving). CORT-birds showed an increase in the percentage of time spent diving with successive foraging trips, to the extent that during Trip 4, their time spent diving was higher than that of controls. The same trend was observed for the number of dives per trip (ANOVA: F = 6.0, df1 = 1, df2 = 25, p = 0.02). Control birds performed a constant number of dives across trips, reaching on average 1160 ± 90 dives per trip. However, CORT-birds showed significantly lower values after implantation (Trip 1 = 110 ± 9 dives, t = 3.5, p = 0.03) which increased with trip rank (Trip 2 = 220 ± 35 dives, t = 2.7, p = 0.05; Trip 3 = 624 ± 94 dives, t = 1.6, p = 0.2) to reach similar values to that of control birds in Trip 4 (1184 ± 174 dives, t = 0.7, p = 0.5).

For each trip, the distribution of maximum dive depths differed between groups (Kolmogorov-Smirnov tests: Trip 1: D = 0.8, p < 0.001; Trip 2: D =
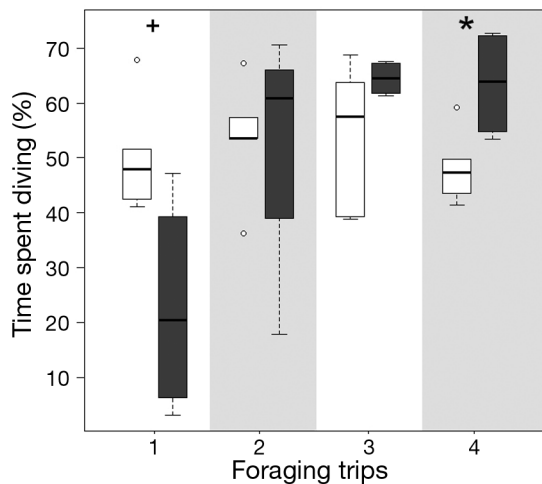
Table 1. Comparisons of morphological, physiological and breeding parameters (mean ± SE) at the beginning of the experiment between controls and CORT-implanted male Adélie penguins, using Student t-tests

Parameters	Controls (n = 10)	CORT (n = 10)	t	p
Body condition index	7.0 ± 0.2	6.8 ± 0.3	0.4	0.7
Brood mass (g)	492 ± 44	414 ± 71	1.4	0.2
Brood age (d)	6.3 ± 0.5	5.8 ± 0.5	0.7	0.5
Corticosterone levels (ng ml^{-1})	13 ± 3[a]	11 ± 2	−0.1	0.9

[a]One value (outlier) was removed

Fig. 2. Percent time (mean ± SE) spent diving during a trip according to the trip rank in control (open, n = 5) and CORT-implanted (filled, n = 4) male Adélie penguins. + indicates a tendency (p < 0.1) and * indicates a significant (p < 0.05) difference between treatment and control groups

0.7, p < 0.001; Trip 3: D = 0.7, p < 0.001; Trip 4: D = 0.7, p < 0.001). CORT birds used a greater depth range than controls as trip rank progressed, with

average maximum depths being 71 ± 25 vs. 99 ± 6 m (CORT vs. control) for Trip 1, 105 ± 19 vs. 92 ± 7 m for Trip 2, 100 ± 5 vs. 91 ± 2 m for Trip 3, and 111 ± 6 vs. 98 ± 5 m for Trip 4.

Because dive efficiency strongly depends on the depths used by individuals, maximum depth categories were added to the statistical models as a covariate. There was considerable variation in dive efficiency across individuals, particularly during Trips 1 and 2 (Fig. 3). Regardless, CORT-implanted birds had higher overall dive efficiencies than controls (ANOVA: F = 19.5, df1 = 1, df2 = 31719, p < 0.001). The interaction between the maximum depth categories and the treatment showed major differences between groups for Trips 1 and 4, but the difference during Trip 2 was reduced to a tendency only (Fig. 3). In addition, the number of undulations per dive, which strongly depends on the bottom phase duration, was significantly higher for CORT-implanted birds than controls for all trips (ANOVA: F = 61.3, df1 = 1, df2 = 31720, p < 0.001).

Fractal analyses show that dive sequences from foraging male Adélie penguins exhibited long-range and persistent autocorrelation. Values of αDFA aver-
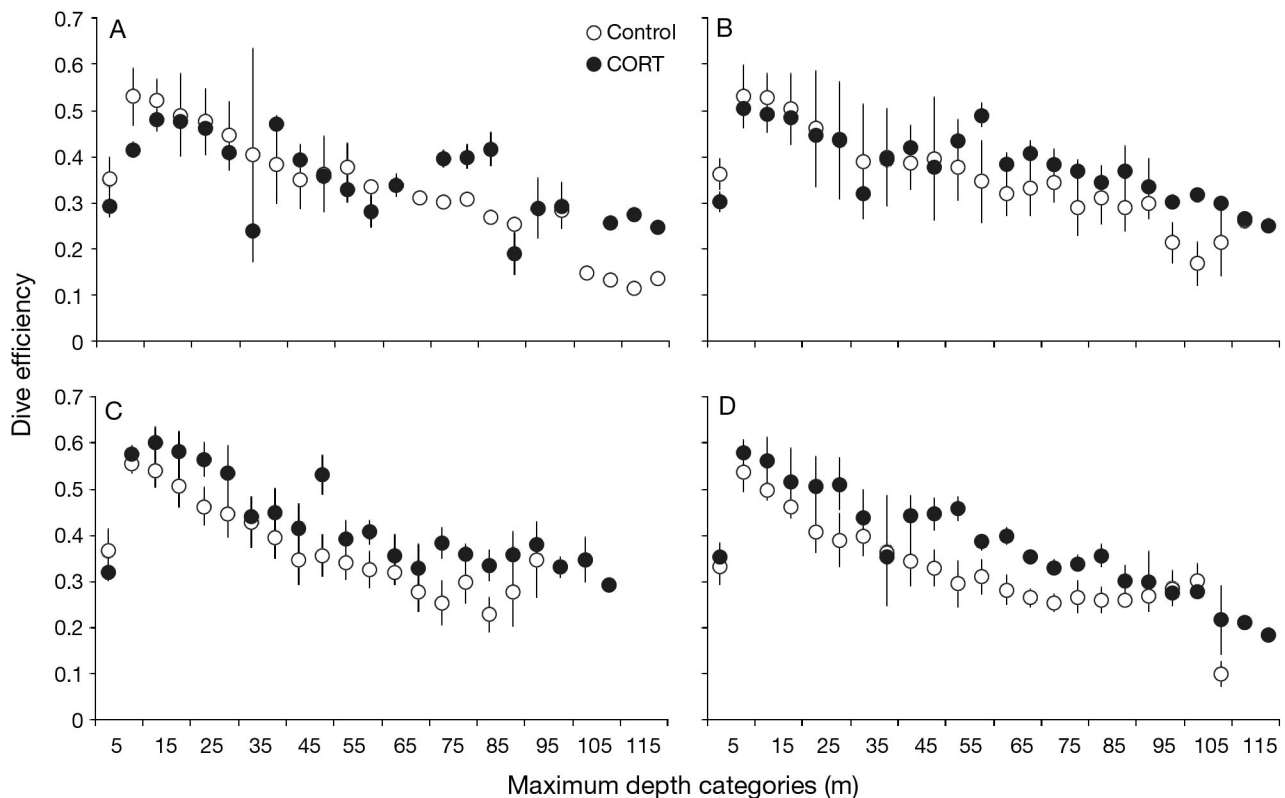

Fig. 3. Dive efficiency (mean ± SE) according to maximum dive depths in control (n = 5) and CORT-implanted (n = 4) male Adélie penguins during the first 4 trips following CORT implantation (from A to D). The absence of error bars for some of the deeper dives indicates that dive efficiencies at certain depth categories were calculated over single individuals

aged 0.920 ± 0.004, suggesting that the original sequences resembled persistent fractional Gaussian noise (fGn) before integration. This was supported by the β_{PSD} estimates, which averaged 2.896 ± 0.018 and therefore suggest persistent fractional Brownian motion (fBm) for the sequences following integration. However, we observed significant differences between sequences produced by control versus CORT birds. We illustrate the process of DFA by examining a representative pair of α-exponents from dive sequences of one control and one CORT-implanted male Adélie penguin (Fig. 4). The lower α-exponent characterizing the control bird's foraging sequence (Fig. 4C) is reflective of its greater dive sequence complexity than that of the treated bird (Fig. 4F). Indeed, the treatment had a significant effect on the complexity of diving behaviour across all subjects (ANOVA: αDFA1, $F = 3.5$, df1 = 3, df2 = 20, p = 0.03), and these results were supported by the other 2 fractal methods employed (ANOVA: β_{PSD}, $F = 5.5$, df1 = 3, df2 = 18, p = 0.007; β_M, $F = 3.5$, df1 = 3, df2 = 20, p = 0.02). The scaling exponents produced by all methods were higher in CORT-implanted birds, particularly during the first trip following implantation, sug-

gesting that treated birds exhibited lower complexity than controls, at least in the short term (Fig. 5). The differences between the groups declined during subsequent foraging trips as the scaling exponents produced by CORT-implanted birds decreased with trip rank to match those of control birds, which remained relatively stable.

At the end of the experiment, the corticosterone treatment showed no effect on the body conditions of the adult males or their chicks (Table 2). Penguins had on average 1.3 ± 0.2 chicks per pair, and this number did not differ significantly between groups ($n_{control} = 8$, $n_{CORT} = 7$; W = 34, p = 0.5). On average 18 d after CORT administration, both groups exhibited similar CORT levels (ca. 10 ng ml^{-1}). Although the treatment was designed to release CORT over 21 d in rodents, our results suggest that these implants only did so for fewer than 18 d in our avian study subjects.

DISCUSSION

In this study, we observed clear but transient modifications in the foraging behaviour of male Adélie
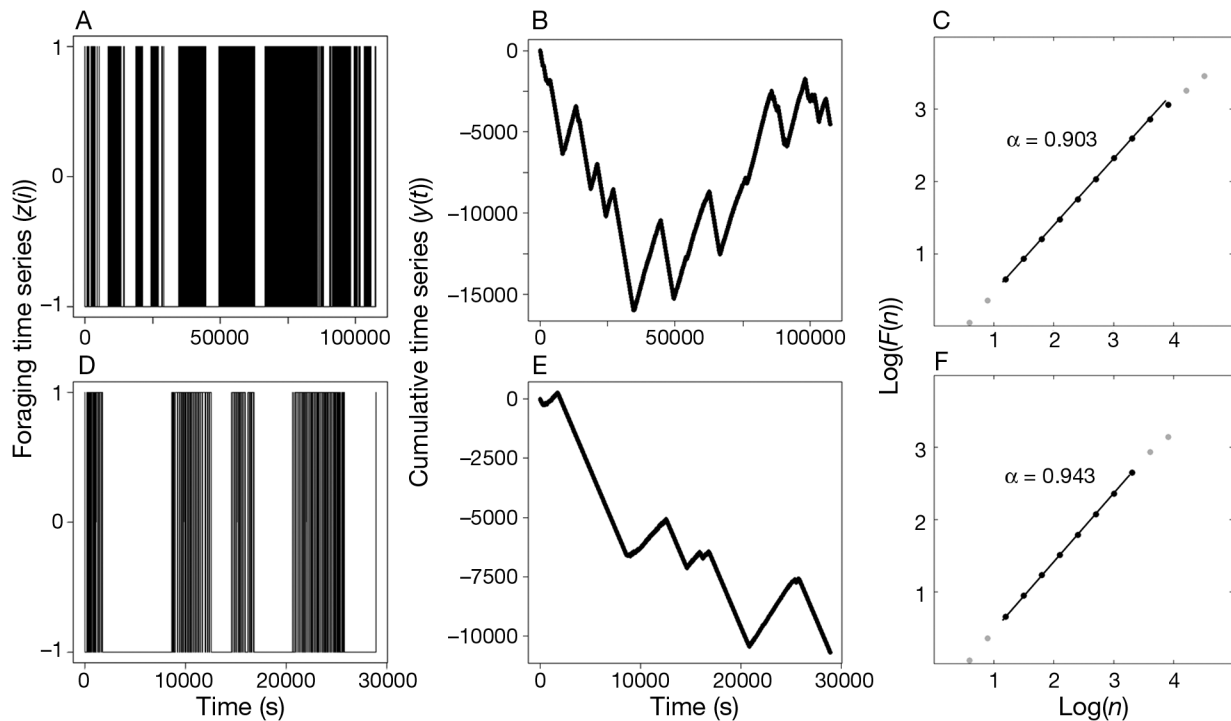

Fig. 4. Detrended Fluctuation Analysis (DFA) of foraging sequences from one control (top row) and one CORT-implanted (bottom row) male Adélie penguin. (A, D) Binary sequences ($z(i)$) generated from the diving (black bars) and not diving behaviour at 1 s intervals. The maximum value of the x-axis is higher for the control bird (A) since it spent more time diving compared to the CORT-implanted bird (D). (B, E) Integrated sequences ($y(t)$) generated by the accumulation of $z(i)$. (C, F) Log-log plots of the average fluctuation $F(n)$ on the y-axes as a function of scale (n) on the x-axes. The α-exponent is the slope of the regression line; the lower α-exponent reflects greater complexity. Note that only the points in black were used to fit the regression line to avoid biases introduced at small (<10 s) and large (sequence length/10) scales
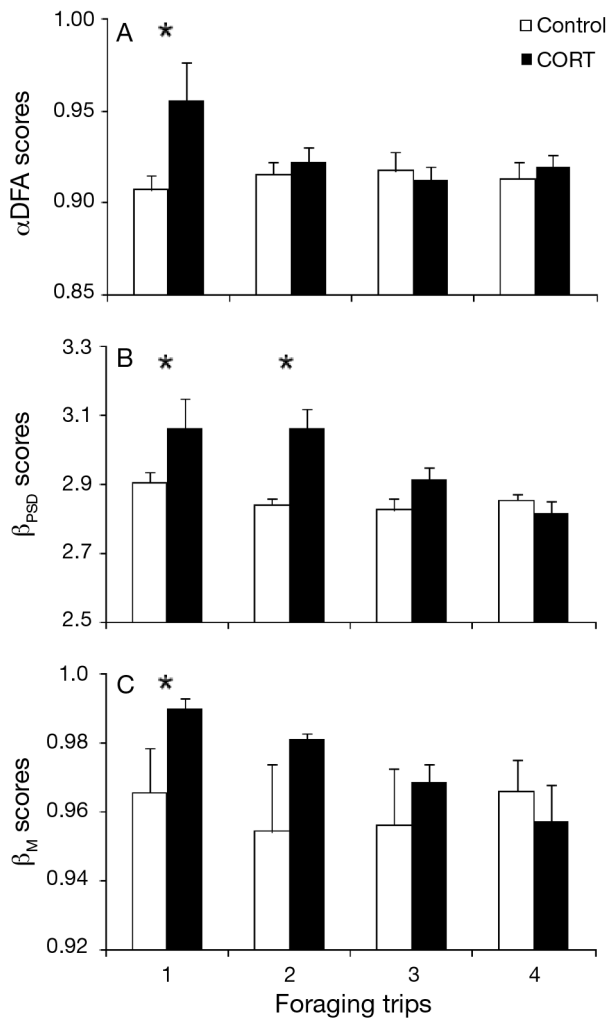
Fig. 5. Fractal analysis of binary sequences of diving behaviour showing scaling exponents. (A) αDFA (Detrended Fluctuation Analysis), (B) β_{PSD} (Power Spectral Density) and (C) β_M (madogram) scores (mean ± SE) in control (n = 5) and CORT-implanted (n = 4) male Adélie penguins during the first 4 trips following CORT implantation. See Appendix for details on the fractal analyses used. All 3 scaling exponents follow the same trend with the exponents of CORT birds converging towards those of the controls with increasing trip ranks. *indicates a significant difference (p < 0.05) between treatment and control groups

Table 2. Comparison of morphological, physiological and breeding parameters (mean ± SE) at the end of the experiment between control and CORT-implanted male Adélie penguins, using Student t-tests

Parameters	Controls (n = 8)	CORT (n = 7)	t	p
Body mass (kg)	4.5 ± 0.1	4.4 ± 0.1	0.3	0.7
Body condition index	6.4 ± 0.2	6.5 ± 0.4	−0.2	0.9
Brood mass (kg)	2.3 ± 0.2	1.9 ± 0.2	1.2	0.2
Corticosterone levels (ng ml^{-1})	10 ± 2	12 ± 3[a]	−0.8	0.5

[a]One value (outlier) was removed

penguins in response to CORT implants. Specifically, a number of diving parameters differed between treated and control birds, but these differences were only clearly observed during the first foraging trip following CORT implantation. Foraging effort, for example, was lower in treated birds, evidenced by decreases in trip duration, time spent diving and the number of dives performed per trip. Furthermore, fractal analysis indicated that, as predicted, treated birds exhibited less complex dive sequences than control birds, but this was again largely restricted to the first foraging trips. Interestingly, however, our frequency-based indices of diving performance, i.e. dive efficiency and the number of undulations per dive, increased in treated relative to control birds. These contrasting effects of CORT treatment on foraging behaviour resembled those observed previously by Cottin et al. (2011), who suggested the importance of scale-dependence (trip vs. dive scale) in assessing foraging behaviour. In this respect, the increased dive-scale effort observed in CORT-birds could be seen as a strategy allowing a reduction in trip-scale effort. Further supporting this hypothesis, we observed no differences in physiological parameters (body mass, body condition, CORT levels) or in the number of chicks per pair following the experiment.

Corticosterone and diving behaviour

According to several studies, high CORT levels can exert a positive effect on feeding activity in birds (Kitaysky et al. 2001, Koch et al. 2002, Löhmus et al. 2006, Angelier et al. 2008, Miller et al. 2009, Crossin et al. 2012). For instance, CORT administration in black-legged kittiwakes *Rissa tridactyla* led treated birds to increase their foraging activities at the expense of guarding chicks on the nest (Kitaysky et al. 2001). Furthermore, Crossin et al. (2012) recently showed that female macaroni penguins *Eudyptes chrysolophus* implanted with CORT exhibited a higher foraging effort than controls. As a result, females increased their mass gain as well as that of their chicks. In contrast, our results suggest that treatment altered the overall foraging behaviour at the trip scale, constraining both the amount of time the birds spent foraging and the complexity with which they did so, although such behavioural changes were not associated

with any changes in the condition of our study subjects or their chicks (see below).

High CORT levels were found to be a major component of the behaviour of reproductive abandonment in Adélie penguins, stimulating them to go to sea to feed when critical body masses were reached during their long incubation fast (Spée et al. 2010). The increase in CORT levels experienced by our study subjects might have been perceived as a situation of high energetic constraint, particularly in addition to the fact that the chick-rearing period is already energetically demanding for the parent that must fulfill its own energy requirements as well as those of the chicks. Variation in the magnitude of the CORT increase, the condition of individuals and their environment are therefore likely to underlie the different patterns observed across studies.

Analyses of the complexity of dive sequences indicate that all study subjects produced foraging sequences that fit the pattern expected for a persistent long-memory process characterized by temporal scaling, i.e. sequences had fractal structure. The applicability of fractal tools to time-series data, especially the long series that can be obtained through bio-logging approaches, was recently demonstrated using diving sequences of little penguins *Eudyptula minor* (MacIntosh et al. 2013). Together, these studies indicate a search strategy in penguins that approximates theoretically optimal behaviour (the Lévy flight foraging hypothesis), allowing an organism to maximize its encounter rates with resources under heterogeneous environmental conditions (Sims et al. 2008, 2012, Viswanathan et al. 2008, Humphries et al. 2010). There is debate about whether such statistical patterns of animal search represent an underlying intrinsic process or simply arise as an artefact of the underlying resource distributions. For example, Sims et al. (2008) suggest that observed search patterns of an immature basking shark *Cetorhinus maximus* differed from Lévy-like behaviour because the animal had yet to develop or adapt to the structure of its prey distributions, illustrating the role of an intrinsic component. The Lévy flight foraging hypothesis thus provides an elegant framework through which to understand animal search patterns, and how and why they may deviate from theoretically optimal behaviour.

We have demonstrated a temporal scale deviation in the present study, because as predicted, dive sequences of CORT-birds showed altered (reduced) complexity relative to those of controls (i.e. complexity loss) but only in the first foraging trips following implantation. Complexity loss in animal behaviour has been demonstrated in individuals under various challenging conditions such as health impairment (MacIntosh et al. 2011), parasitic infection (Alados et al. 1996, MacIntosh et al. 2011), advanced reproductive state (Alados et al. 1996, MacIntosh et al. 2011), toxic substance exposure (Motohashi et al. 1993, Alados & Weber 1999, Seuront & Leterme 2007), feed restriction (Maria et al. 2004), aggressive social interactions (Rutherford et al. 2006) and increased exposure to anthropogenic disturbance (Seuront & Cribb 2011). What our study adds to this increasing body of work is an investigation of the effects of a direct increase in a physiological indicator of stress: the glucocorticoid corticosterone, which is the main stress hormone in birds. The observed loss of complexity may reflect the incentive for treated birds to engage in less energy-intensive foraging. Indeed, it seems that the production of complex behavioural sequences is dependent upon having adequate levels of available energy (e.g. Kembro et al. 2009, Seuront & Cribb 2011). Under conditions of energy constraint, such as those likely produced by our experimental paradigm, sequences of behaviour may become more structured and periodic, further indicating an intrinsic component in the production of Lévy-like behaviour.

What is further compelling about our study is the contrast between the results of traditional measures of diving performance and fractal analysis. Given the theoretical link between fractal patterns in behaviour and optimal foraging, we might have expected that complexity loss would be associated with reductions in other measures of dive performance. Here, individuals may be forced to adopt a strategy that optimizes the time and energy allocated to diving behaviour given the energetic constraints imposed by treatment by engaging in a more efficient, but less complex and thus energy demanding, foraging pattern. In such a case, CORT-birds may have been able to decrease energy consumption by foraging for shorter durations and therefore probably closer to the colony than controls (Angelier et al. 2008). Additionally, by increasing their efficiency, they may have increased their energy gains with a minimum increase in effort. Exploiting different dive depths may also reflect a strategy to maximize prey encounters by searching in more predicable resource locations within the water column. While we cannot confirm such speculation with our current data, treated birds apparently engage in a less energetically demanding foraging pattern, resulting in increased efficiency at the individual dive scale but at the expense of dive quantity and complexity across the entire foraging trip. Finally, we can hypothesize that

complexity loss would be associated with a reduction in other measures of dive performance in situations where the increase in CORT levels and/or the decrease in resource availability is prolonged.

Stress, foraging and body condition

Ultimately, however, this reduced foraging effort did not seem to affect the males in terms of body mass and condition or baseline CORT levels at the end of the experiment, nor did we observe any differences in the body mass or survival of chicks sired by treated versus control birds. This may not be entirely surprising given that the observed effects of CORT treatment on foraging behaviour seemed to decrease across time in all parameters examined. Since no difference in CORT levels was observed ca. 18 d following implantation, we can assume that our pellets released CORT for a shorter duration than expected. This is in agreement with previous data from Adélie penguins showing that CORT values in C100-implanted birds were still high 15 to 16 d following treatment, and close to control levels thereafter (Spée et al. 2011a). It is likely that both the non-homogeneous diffusion of CORT by the pellets as well as the CORT regulation carried out by the birds themselves can explain the transient behavioural changes observed in this study.

Regardless of the exact duration of CORT release, however, our results do suggest that short-term increases in CORT and modifications in foraging behaviour may not have clear, short-term effects on the fitness-related parameters examined here. We propose 3, non-mutually exclusive hypotheses that might explain this phenomenon. The first hypothesis is that the effects of CORT administration are too transient to be able to affect the body condition or the reproductive output of treated birds. The second hypothesis is that the increased dive performance observed in CORT-implanted birds, disregarding complexity for the moment, might relate to an increase in prey capture rates. This hypothesis is supported by the fact that CORT-birds exploited different dive depths and might as a consequence have encountered different prey species (such as krill, fish or amphipods; Wienecke et al. 2000) than control birds, possibly of differing nutritional quality. Moreover, the increase in time spent diving observed during Trip 4 for CORT-birds may also reflect a compensation phenomenon once the hormone returned to baseline levels. Finally, a third hypothesis is that although treated males spent less time at sea, it is possible that their mates performed longer foraging trips and/or increased their own trip frequencies. Such increased foraging efforts by mates of treated males may also have provided a compensatory mechanism, as has been shown in little penguins *Eudyptula minor* (Saraux et al. 2011). In this regard, females may have increased their own contribution in the amount of food delivered to their chicks, highlighting the necessity of assessing the behaviour of both members of a pair in which one mate is implanted with CORT in future experiments.

Conclusions

Whether our study will ultimately result in long-term effects (see Goutte et al. 2010) remains to be seen. The current results suggest only a transient modification in foraging behaviour with CORT implantation, which may allow for the reallocation of energy to certain behaviours to adjust to individual intrinsic constraints. Further short-term examinations are also needed since changes in the body condition of treated birds and their chicks may have occurred shortly after CORT implantation. This study adds to the growing list of studies showing a negative relationship between various challenging conditions and fractal complexity, although the first to use an experimental approach with free-living animals. This is a testament to the robustness of this tool in detecting such altered behavioural states. This study provided novel insights into the behaviour of free-living animals: the application of fractal analysis to bio-logged data was discussed within the framework of optimal search strategies, compared and contrasted with other commonly-used measures, and combined with an experimental approach *in natura* (see also MacIntosh et al. 2013). We therefore encourage further investigation into fractal patterns in animal behaviour to allow for a more comprehensive understanding of the responses of organisms to various environmental constraints.

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Power Spectral Density (PSD)

Time series can be represented as the sum of their component cosine waves of different frequencies, and the resultant power spectrum for a fractal process is proportional to the reciprocal of the frequency such that

$$S(f) \propto cf^{-\beta}$$

where $S(f)$ is the variance or amplitude of frequency f, c is a constant, and β is the power exponent or spectral index (β_{PSD}). Here, we use the software package Benoit® v.1.3.1 (Trusoft) to estimate β_{PSD} via the fast Fourier Transform (FFT). PSD has often been used to estimate fractal dimension (D) via the theoretical relationship

$$D_{PSD} = \frac{5 - \beta_{PSD}}{2}$$

where D_{PSD} is the fractal dimension estimated via PSD. However, estimating D via spectral regression is problematic, so in order to reduce bias we pre-processed sequences by removing linear trends, smoothing the data via windowing at $N/100$, and applying a taper function to 'endmatch' the sequence before calculating β_{PSD} only from the low-frequency end of the spectrum in a method known as $^{low}PSD_{we}$ (Eke et al. 2000).

Fractal dimension estimation via the madogram

The fractal dimension is a much celebrated metric often used to represent both local roughness and global self-similarity, although its ability to measure the latter is true in only a limited number of cases (Gneiting & Schlather 2004, Bez & Bertrand 2011). We therefore focus on the roughness of penguin dive sequences characterized by D. There exist many methods for estimating D, but we use the madogram, which has been shown to provide a robust estimate of the fractal dimension in both spatial and time series data (Bez & Bertrand 2011, Gneiting et al. 2012). The madogram uses a generalized formula based on the classical variogram but with a power variation (p) of order 1, such that for a 1-dimensional time series

$$y_1(l/n) = \frac{1}{2(n-1)} \sum_{i=1}^{n} \left| X_{i/n} - X_{(i-1)/n} \right|^1$$

where $y_1(l/n)$ is the slope of the madogram near its origin on the double logarithmic plot of variation as a function of scale (lag) (Gneiting et al. 2012). The slope of the madogram is related to D via the linear relationship

$$D_M = 2 - \beta_M$$

where D_M is the fractal dimension estimated by the madogram and β_M is the slope of the madogram (Bez & Bertrand 2011). To avoid confusion, we have denoted the scaling exponents of PSD and the madogram as β_{PSD} and β_M, respectively. We use the madogram rather than the more commonly used variogram because of its suitability for various data distributions. When the data are Gaussian, the variogram and madogram produce equal results, but in other cases, the results of the madogram are more robust (Bez & Bertrand 2011).

A note about theoretical relationships between measures

The scaling exponent calculated via DFA (αDFA) provides a robust estimate of the Hurst exponent (H) (Taqqu et al. 1995, Cannon et al. 1997).

$$H = \alpha DFA \text{ for fGn, and } H = \alpha DFA + 1 \text{ for fBm.}$$

H can also be estimated via PSD, such that

$$H = \frac{\beta_{PSD} + 1}{2} \text{ for fGn, and } H = \frac{\beta_{PSD} - 1}{2} \text{ for fBm.}$$

In addition, H itself is inversely related to D, such that for 1-dimensional time series like those examined here,

$$D = 2 - H$$

Therefore, H can be related to the scaling exponent of the madogram β_M through their equivalent theoretical relationships with D.

While these measures are theoretically related, in practice the various methods can lead to different results. Stochastic time series need not exhibit a linear relationship between H, which is a measure of long-range dependence, and D, which is a measure of roughness (Gneiting & Schlather 2004). Therefore, we estimate each of these parameters separately using the 3 methods described in this study (DFA, PSD, and the madogram).