



**Cite this article:** Fairhurst GD *et al.* 2015

Assessing costs of carrying geolocators using feather corticosterone in two species of aerial insectivore. *R. Soc. open sci.* **2**: 150004.  
<http://dx.doi.org/10.1098/rsos.150004>

Received: 6 January 2015

Accepted: 7 April 2015

**Subject Category:**

Biology (whole organism)

**Subject Areas:**

physiology/ecology/behaviour

**Keywords:**

Energetic Expenditure Hypothesis, feather corticosterone, hormone biomarkers, light-level geolocators, migration physiology, swallows

**Author for correspondence:**

Graham D. Fairhurst

e-mail: [graham.fairhurst@usask.ca](mailto:graham.fairhurst@usask.ca)

# Assessing costs of carrying geolocators using feather corticosterone in two species of aerial insectivore

Graham D. Fairhurst<sup>1,2</sup>, Lisha L. Berzins<sup>3</sup>, David W. Bradley<sup>4,5</sup>, Andrew J. Laughlin<sup>6</sup>, Andrea Romano<sup>7</sup>, Maria Romano<sup>7</sup>, Chiara Scandolaro<sup>7,8</sup>, Roberto Ambrosini<sup>9</sup>, Russell D. Dawson<sup>3</sup>, Peter O. Dunn<sup>10</sup>, Keith A. Hobson<sup>11</sup>, Felix Liechti<sup>8</sup>, Tracy A. Marchant<sup>1</sup>, D. Ryan Norris<sup>4</sup>, Diego Rubolini<sup>7</sup>, Nicola Saino<sup>7</sup>, Caz M. Taylor<sup>6</sup>, Linda A. Whittingham<sup>10</sup> and Robert G. Clark<sup>1,2</sup>

<sup>1</sup>Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan, Canada S7N 5E2

<sup>2</sup>Environment Canada, 115 Perimeter Road, Saskatoon, Saskatchewan, Canada S7N 0X4

<sup>3</sup>Ecosystem Science and Management, University of Northern British Columbia, 3333 University Way, Prince George, British Columbia, Canada V2N 4Z9

<sup>4</sup>Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 1E4

<sup>5</sup>Bird Studies Canada, 115 Front Street, Port Rowan, Ontario, Canada N0E 1M0

<sup>6</sup>Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118, USA

<sup>7</sup>Department of Biosciences, University of Milan, via Celoria 26, Milan 20133, Italy

<sup>8</sup>Swiss Ornithological Institute, Seerose 1, Sempach 6204, Switzerland

<sup>9</sup>Department of Biotechnology and Biosciences, University of Milano Bicocca, Piazza della Scienza 2, Milan 20126, Italy

<sup>10</sup>Department of Biological Sciences, University of Wisconsin—Milwaukee, 3209 North Maryland Avenue, Milwaukee, WI 53201-0413, USA

<sup>11</sup>Environment Canada, 11 Innovation Boulevard, Saskatoon, Saskatchewan, Canada S7N 3H5

GDF, 0000-0001-8731-1298

## 1. Summary

Despite benefits of using light-sensitive geolocators to track animal movements and describe patterns of migratory connectivity, concerns have been raised about negative effects of these devices, particularly in small species of aerial insectivore.

Geolocators may act as handicaps that increase energetic expenditure, which could explain reported effects of geolocators on survival. We tested this ‘Energetic Expenditure Hypothesis’ in 12 populations of tree swallows (*Tachycineta bicolor*) and barn swallows (*Hirundo rustica*) from North America and Europe, using measurements of corticosterone from feathers ( $CORT_f$ ) grown after deployment of geolocators as a measure of physiology relevant to energetics. Contrary to predictions, neither among- (both species) nor within-individual (tree swallows only) levels of  $CORT_f$  differed with respect to instrumentation. Thus, to the extent that  $CORT_f$  reflects energetic expenditure, geolocators apparently were not a strong handicap for birds that returned post-deployment. While this physiological evidence suggests that information about migration obtained from returning geocator-equipped swallows is unbiased with regard to levels of stress, we cannot discount the possibility that corticosterone played a role in reported effects of geolocators on survival in birds, and suggest that future studies relate corticosterone to antecedent factors, such as reproductive history, and to downstream fitness costs.

## 2. Introduction

Understanding the ecological and population processes affecting migratory birds requires knowledge of habitat use and individual movements throughout the annual cycle [1–3]. Recent insights have been facilitated by advances in techniques for tracking animal movements and describing patterns of migratory connectivity [4–7]. The use of light-sensitive geolocators has become especially popular because the devices now weigh less than 1 g and, therefore, can be used on many species of small-bodied migratory passerines [8]. Indeed, the recent rapid increase in research using geolocators has revealed previously unknown information about breeding areas [9], migratory routes and stopover areas [10,11], non-breeding areas [12–14] and migratory connectivity [9,12,15] for a variety of small bird species [8].

Despite obvious benefits of using geolocators to track migration, concerns have been raised about negative effects of these devices and the potential biases in data derived from them [16–19]. A recent meta-analysis provided evidence that geolocators can reduce survival, particularly for aerial foragers and migratory species [17]. Effects of geolocators on flight mechanics can help explain these findings and include increased wing loading and drag owing to altered aerodynamic profiles [20,21]. To compensate for these effects, individuals carrying geolocators would be expected to increase energetic expenditure [16,22,23]. This added workload could be particularly taxing during migration, which is a period of high energetic demand [22,24] and high mortality [25]. Thus, geolocators have the potential to detrimentally influence the energetic balance of migrants.

Although this ‘Energetic Expenditure Hypothesis’ may provide a reasonable mechanism for reported effects of geolocators, testing it requires measuring the energetics of free-living birds following deployment. Unlike other tracking technologies [26], current geolocators suitable for use with small birds (i.e. devices < 1.0 g) cannot collect any biotelemetry data other than location. Moreover, most small migrant passerines cannot be recaptured until they return to the breeding grounds. These issues make it difficult to assess differences in *en route* physiology of individuals with and without geolocators, which is critical for establishing or refuting a physiological link between geolocators and variation in performance measures affecting fitness.

The hormone corticosterone (CORT) may be a useful proxy for measuring the effect of geolocators on the energetics of migratory birds. CORT is a metabolic hormone well known for its role in energy management [27,28], and CORT levels rise in response to increased energetic demands and facilitate the conversion (and thus depletion) of energy stores into usable forms [29–33]. In migratory passerines, CORT levels are elevated seasonally to meet the physiological demands of migration, but birds still respond to stressors during this period [33–35] and during winter [36,37]. Thus, if instrumentation with a geocator acts as a handicap that unpredictably increases energetic demands, CORT levels could rise to a point where costs, such as increased catabolism of energy stores, degradation of muscle and immunosuppression occur [28,38]. Short-term effects of geolocators and other tracking devices on CORT physiology are either ephemeral (e.g. [39,40]) or not detectable (e.g. [41]), but studies of seabirds show that baseline and handling-induced CORT levels are significantly elevated the year following geocator deployment [23,42]. All of these previous studies measured CORT during the breeding season (or in captivity) so we lack any assessment of the effects of geolocators on energetics outside of this period in wild populations.

Feathers may provide a retrospective ‘remote sensing’ of avian energetics because they contain a record of CORT during the period of feather growth [43]. The CORT in feathers ( $CORT_f$ ) has been shown experimentally to reflect levels of plasma CORT [44,45] and a variety of stressors [46–50] during

feather growth. For birds that moult after the deployment of geolocators,  $CORT_f$  could quantify a physiologically relevant proxy of energetic costs arising from instrumentation. In migratory passerines, assessing energetics during the pre-basic moult, which itself is energetically expensive [51,52], may be particularly pertinent because this moult is preceded by physiologically demanding activities (e.g. breeding, migration or both), the energetic costs of which could carry over into the moulting period. Therefore,  $CORT_f$  may reflect cumulative energetic costs that could be more pronounced in birds carrying geolocators.

Using  $CORT_f$  as a measure of physiological response to geolocators, we tested two predictions of the Energetic Expenditure Hypothesis: (i) compared with returning adults without geolocators from the same population (controls), individuals returning with geolocators (geocator birds) should have higher levels of  $CORT_f$ , reflecting their increased energetic expenditure; and (ii) within individuals carrying a geocator, post-deployment levels of  $CORT_f$  should be higher than pre-deployment levels. As a group, small aerial insectivores should be particularly susceptible to the effects of geolocators [17], making them appropriate models for this type of study. Thus, we tested our predictions in four populations of tree swallows (*Tachycineta bicolor*) and eight populations of barn swallows (*Hirundo rustica*) from North America and Europe. By studying how physiology of migratory passerines varies in response to geolocators, this study also provides data useful for resolving potential ethical and scientific issues facing researchers tracking small birds over long distances.

### 3. Material and methods

#### 3.1. Fieldwork

Complete details of field methods, including geocator instrumentation, for the birds in our study have been presented elsewhere (tree swallows [11,18]; barn swallows [19,53]). For tree swallows, fieldwork was conducted during May–July of 2011–2013 at three breeding sites in Canada (Prince George, British Columbia: 53°50' N, 122°57' W; St Denis National Wildlife Area, Saskatchewan: 52°13' N, 106°04' W; Long Point, Ontario: 42°39' N, 80°26' W) and one in the USA (Saukville, Wisconsin: 43°24' N, 88°0' W). Adults were captured at their nest-boxes during the brood-rearing period and individuals were banded, sexed, measured and dorsal contour feathers were collected from the upper back using forceps and stored in paper envelopes until subsequent  $CORT$  analyses. Geolocators (0.67 g; Lotek Wireless model MK12-S in 2011, MK5-S in 2012) were attached using a modified leg-loop backpack harness [10], composed of 1 mm diameter solid ethylenepropylene-diene rubber tubing, that had a combined mass of less than or equal to 1.0 g (less than 5% of body mass). The geocator, which sat just anterior to the tail, was secured to the contour feathers on the bird's back using a small amount of cyanoacrylate adhesive and did not directly impede movement of the wings. Different adult tree swallows were marked with geolocators in 2011 and 2012.

For barn swallows, fieldwork in North America was conducted during May–July of 2012 and 2013 at two breeding sites in Canada (Prince Albert National Park, Saskatchewan: 53°42' N, 106°3' W; near Sackville, New Brunswick: 45°58' N, 64°13' W) and three in the USA (Auburn, Alabama: 32°33' N, 85°21' W; Greenville, Mississippi: 33°17' N, 91°2' W; Seattle, Washington: 47°39' N, 122°21' W). Fieldwork in Europe took place during April–July of 2010–2012 at one breeding site in southern Switzerland (Magadino: 46°09' N, 8°55' W) and two in northern Italy (Piedmont: 45°33' N, 8°44' E; Lombardy: 45°19' N, 9°40' E). Adults were captured with mist-nets, individually marked with coloured leg bands, sexed, measured and the fourth outermost tail feather (R4) was plucked and stored for  $CORT$  analysis. For North American breeding sites, geolocators (0.7 g; Migrate Technology model Intigeo-P55B1–7) were deployed at this time and were attached using a leg-loop harness composed of elastic cord (Stretch Magic, Pepperell, MA, USA). The combined mass was less than 0.8 g (approx. 4.5% of body mass). For European breeding sites, adults were recaptured at the end of the breeding season and geolocators (Swiss Ornithological Institute model SOI-GDL2.10 in 2010, SOI-GDL2.11 in 2011) were deployed. Geolocators (2010: approx. 0.77 g; 2011: approx. 0.68 g) were attached using a leg-loop harness composed of an elastic silicone rubber tubing, and the combined mass was less than 0.8 g (less than 4% of body mass).

#### 3.2. Nomenclature and sample sizes of feathers

Feathers from geocator birds were either grown the autumn before (pre-deployment) or after (post-deployment) deployment. The moulting of tree swallow back feathers occurs from mid-July to early November, corresponding to the beginning of autumn migration for the majority of individuals, and is

probably completed within North America ([11,54,55] and references therein). Barn swallow tail feathers are moulted at the end of autumn migration on wintering grounds in Africa and South America ([55–57] and references therein).

As not all geolocator birds returned the year following deployment, for among-individual analyses of tree swallows we had four categories of feathers that comprised treatment groups: (i) feathers from controls reflecting the general population of returning individuals, (ii) post-deployment feathers from geolocator birds that returned the subsequent year, (iii) pre-deployment feathers from returning geolocator birds, and (iv) pre-deployment feathers from geolocator birds that did not return. Groups (iii) and (iv) are analogous to controls; analysing them separately enabled us to determine if differences in CORT physiology existed in these treatments prior to deployment (see Statistical analyses section). We had feathers from 40 tree swallows recaptured the year subsequent to their original sampling (control:  $n = 12$  birds; geolocator:  $n = 28$  birds). We did not have any pre-deployment feathers for barn swallows, and thus only had feathers in two treatment groups: (i) feathers from controls reflecting the general population of returning individuals and (ii) post-deployment feathers from geolocator birds that returned the subsequent year. Sample sizes for each treatment described above are presented in table 1.

For tree swallows, geolocator birds were randomly selected from previously banded adults. Control birds were selected as the next banded adult captured, which was generally the same day or shortly after deployment of a geolocator, so control and geolocator birds were well matched in their timing of breeding. For barn swallows, in 2010, birds were assigned alternately to control or geolocator groups within each colony of each breeding site. In 2011, this procedure was maintained at the Magadino and Piedmont breeding sites, but at the Lombardy site different breeding colonies were assigned to different treatment groups for practical reasons. Regardless, in both years and at all sites, birds in the two treatment groups were well matched in their timing of breeding.

### 3.3. Analysis of corticosterone from feathers

Analyses of  $CORT_f$  were conducted as in previous studies of tree swallows [44,58]. We first processed feathers by removing the calamus, weighing and measuring the length of the remaining portion of feather, placing each sample into a separate glass vial, and cutting the samples into small pieces with scissors. We then added 10 ml of HPLC-grade methanol (VWR International, Mississauga, Ontario, Canada) to each sample, sonicated all samples at room temperature for 30 min, followed by incubation at 50°C overnight in a water bath. A vacuum filtration system consisting of a plug of polyester wool in a glass filtration funnel was used to separate the methanol extract from the feather material. The original sample vial, remnant feather pieces and filtration material were washed twice with approximately 2.5 ml of additional methanol that was then added to the original methanol extract. Methanol extracts were placed in a 50°C water bath and subsequently evaporated in a fume hood. Samples were extracted in six batches. Recovery efficiency of the methanol extraction was assessed by including feather samples spiked with approximately 5000 CPM of  $^3H$ -labelled CORT, and an average of 93.4% (s.d. = 6.1) of the radioactivity was recoverable in the reconstituted samples. Samples were adjusted for recoveries. Extract residues were reconstituted in a small volume of phosphate buffer (0.05 mol l<sup>-1</sup>, pH 7.6) and analysed by radioimmunoassay in duplicate following [59]. Serial dilutions of sample extracts of both species were parallel to the standard curve, indicating no interference with the antibody. All samples were run blind with regard to individual identity. Samples from all populations except Saukville, WI, were randomly distributed throughout five assays, and the average intra-assay variability, computed using three aliquots per assay of the same standard, was 8.8% (s.d. = 5.4), inter-assay variability was 9.1%, and all samples were above the limit of detection ( $ED_{80}$ ; average  $\pm$  s.d.: 16.08  $\pm$  2.42 pg 100  $\mu$ l<sup>-1</sup>). Saukville samples were obtained 1 year later and randomized throughout a single assay run with a different internal standard but same antiserum as all previous samples. Our statistical analyses do not compare  $CORT_f$  values among populations (we intentionally control for this using population as a random effect; see Statistical analyses section) and are instead tested for differences among treatments within sites. This single assay had an intra-assay variability of 5.7% (i.e. was internally valid) and all samples were above its limit of detection ( $ED_{80}$ ) of 12.99 pg 100  $\mu$ l<sup>-1</sup> (similar to the other assays).  $CORT_f$  values were standardized by feather length (i.e.  $CORT$  mm<sup>-1</sup>) to best represent the time-dependent deposition of CORT [43,60,61].

### 3.4. Statistical analyses

$CORT_f$  values were log-transformed to improve normality. We used mixed models to analyse the effect of geolocators on  $CORT_f$ , using PROC MIXED in SAS v. 9.2 (SAS Institute, Cary, NC, USA), including

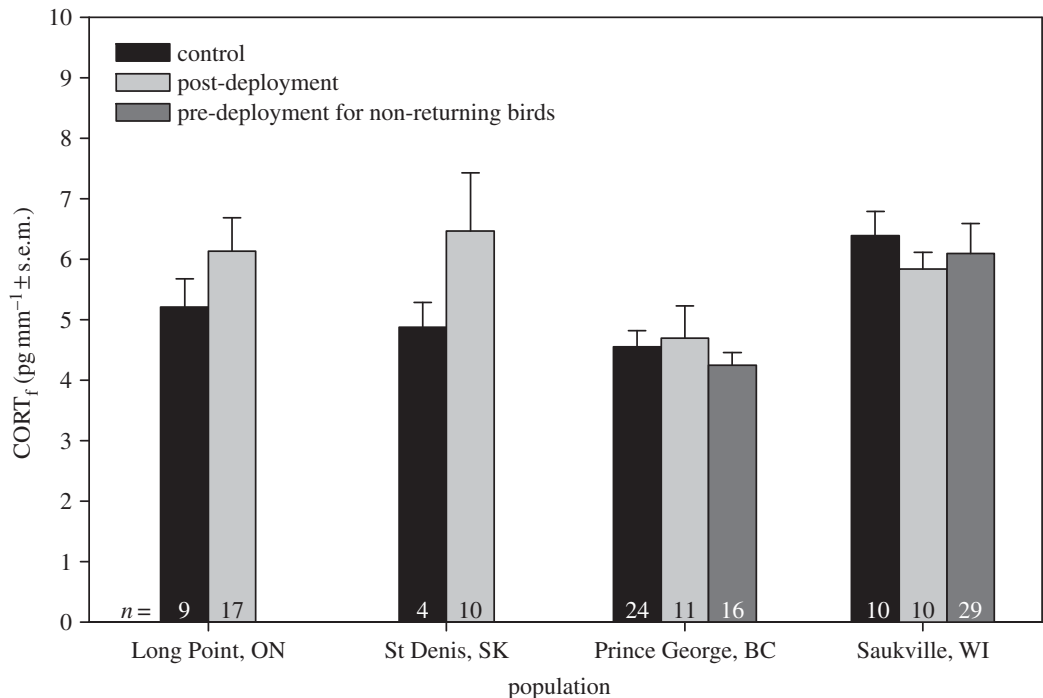
**Table 1.** Sample sizes of feathers from each species, population, year and sex (male/female) in each treatment group. (See text for explanation of treatments.)

population	year	control	pre-deployment from non-returning geolocator birds	pre-deployment from returning geolocator birds	post-deployment from geolocator birds	population total
<b>tree swallows</b>						
Long Point, ON	2011	0	0	2/6	0	8
	2012	0/1	0	0	6/7	14
	2013	3/5	0	0	2/2	12
	total	3/6	0	2/6	8/9	34
St Denis, SK	2011	0	0	0	0	0
	2012	4/0	0	0	8/2	14
	2013	0	0	0	0	0
	total	4/0	0	0	8/2	14
Prince George, BC	2011	0/2	0	0/2	0	4
	2012	5/7	5/11	5/4	0/1	38
	2013	5/5	0	0	5/5	20
	total	10/14	5/11	5/6	5/6	62
Saukville, WI	2011	0/0	13/16	9/1	0	39
	2012	2/8	0	0	9/1	20
	total	2/8	13/16	9/1	9/1	59
<b>barn swallows</b>						
Auburn, AL	2013	1/8/3 <sup>a</sup>	0	0	2/0	14
Greenville, MS	2013	7/9	0	0	0/1	17
Seattle, WA	2013	6/6	0	0	1/1	14
Sackville, NB	2013	6/9	0	0	0/2	17
Prince Albert NP, SK	2013	7/6	0	0	1/1	15
Lombardy, IT	2012	6/3	0	0	12/4	25
	2013	2/0	0	0	2/0	4
	total	8/3	0	0	14/4	29
Piedmont, IT	2012	11/3	0	0	11/3	28
	2013	8/0	0	0	8/0	16
	total	19/3	0	0	19/3	44
Magadino, CH	2012	13/3	0	0	13/3	32

<sup>a</sup>unknown sex.

population and year as random effects to account for clustered data and annual effects. Owing to the unbalanced sample sizes among treatment, sex, year and population, all mixed models used the Kenward-Rogers method for approximating degrees of freedom. Non-significant interaction terms ( $p > 0.05$ ) were eliminated from models. Because different types of feathers were used for tree swallows and barn swallows, we analysed each species separately.

For both species, we first examined the variation in  $CORT_f$  among treatments. These models started with fixed effects of treatment (for definitions see Nomenclature and sample sizes section), minimum age (youngest reliably estimable age) and sex, and included a treatment  $\times$  sex interaction. Only known-sex birds were used in analyses that included sex. Second, for tree swallows alone, we addressed within-individual effects of instrumentation with a geolocator using the 40 individuals sampled in two consecutive years. Each bird was used as its own control by subtracting pre-deployment (year 1)



**Figure 1.** Levels of corticosterone in feathers (CORT<sub>f</sub>) from tree swallows instrumented with a geolocator compared to non-instrumented (control) birds. All feathers were grown post-breeding, and pre-deployment feathers were grown the year prior to deployment of geolocators. See text for complete descriptions of treatments. Note that some populations contain multiple years of data (table 1).

values from post-deployment (year 2) values. This created a single variable that reflected the within-individual change in CORT physiology from one year to the next. We compared this variable between geolocator and control birds in a model that also included the fixed effects of minimum age and sex, and a treatment × sex interaction.

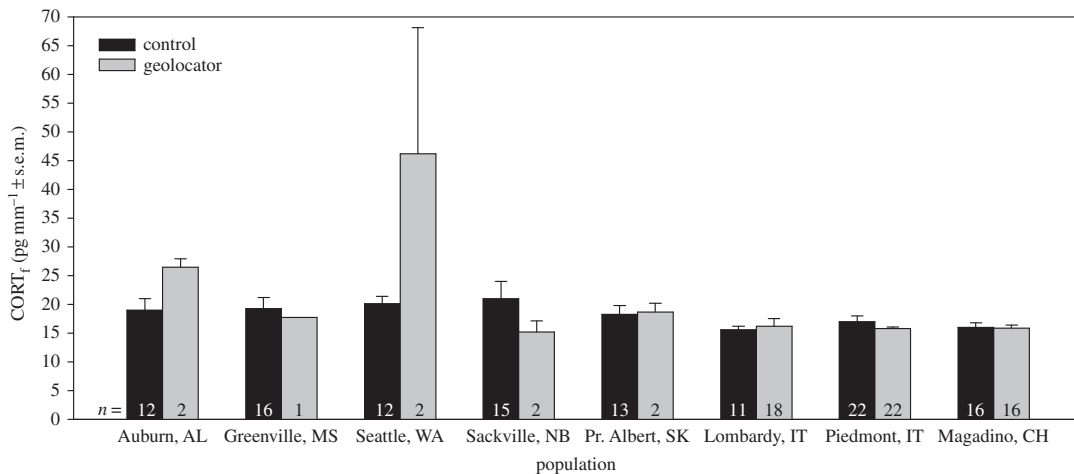
## 4. Results

In tree swallows, there was no interaction between treatment and sex on CORT<sub>f</sub> ( $F_{3,152} = 0.22$ ,  $p = 0.88$ ), so the interaction term was removed from the model. The final model revealed no effect of geolocators on CORT<sub>f</sub> ( $F_{3,149} = 0.69$ ,  $p = 0.56$ ; figure 1), and no effect of sex ( $F_{1,154} = 1.14$ ,  $p = 0.29$ ) or minimum age ( $F_{8,153} = 0.45$ ,  $p = 0.89$ ). In barn swallows, there was no interaction between treatment and sex on CORT<sub>f</sub> ( $F_{1,169} = 0.07$ ,  $p = 0.79$ ), so this term was also removed from the model. The final model showed no effect of geolocators on CORT<sub>f</sub> ( $F_{1,171} = 0.47$ ,  $p = 0.49$ ; figure 2), and no effect of sex ( $F_{1,170} = 2.45$ ,  $p = 0.12$ ) or age ( $F_{5,170} = 0.47$ ,  $p = 0.80$ ).

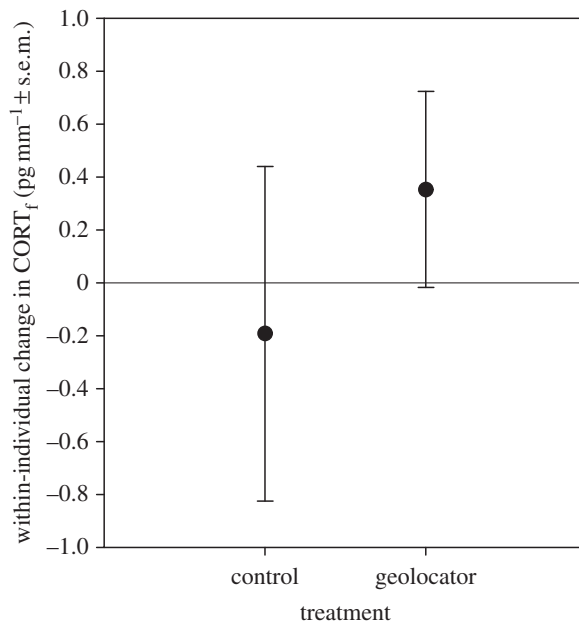
When we considered the 40 cases where tree swallows were sampled in two consecutive years, we found that within-individual changes in CORT<sub>f</sub> were not related to the interaction of treatment with sex ( $F_{1,30} = 1.80$ ,  $p = 0.19$ ) so this term was removed from the model. The final model revealed no effect of geolocators on within-individual changes in CORT<sub>f</sub> from one year to the next ( $F_{1,31} = 0.28$ ,  $p = 0.60$ ; figure 3), and no effects of age ( $F_{6,31} = 0.27$ ,  $p = 0.95$ ) or sex ( $F_{1,31} = 0.53$ ,  $p = 0.47$ ).

## 5. Discussion

We tested the Energetic Expenditure Hypothesis that geolocators attached to aerial insectivores produce a handicap that increases energetic demand. We predicted that if there was a pervasive effect of geolocators it would be reflected in levels of CORT from feathers grown prior to or early-on during post-breeding migration (tree swallows), or at the end of migration (barn swallows). We also expected that our broad geographical and temporal approach of analysing 3 years of CORT<sub>f</sub> data from 12 populations of two species of aerial insectivore on two continents would provide the power to detect an effect of geolocators if one existed. However, our results based on both among-individual (both species) and



**Figure 2.** Levels of corticosterone in feathers (CORT<sub>f</sub>) from barn swallows instrumented with a geolocator compared to controls. All feathers were grown post-breeding (i.e. after deployment of geolocators). Note that some populations contain multiple years of data (table 1).



**Figure 3.** Within-individual change in levels of corticosterone from feathers (CORT<sub>f</sub>) of tree swallows in two consecutive years. For control birds ( $n = 12$ ), this is the change in levels from year 1 to year 2; for geolocator birds ( $n = 28$ ), this reflects the change from pre-deployment (year 1) to post-deployment (year 2) levels.

within-individual (tree swallows only) analyses show that there was no effect of geolocators on levels of CORT<sub>f</sub>. The lack of effect in barn swallows is particularly revealing, considering that they carried the geolocator for considerably longer before moulting than did tree swallows. Thus, to the extent that CORT<sub>f</sub> reflects energetic expenditure, our findings suggest that geolocators apparently did not act as a strong handicap for birds that returned post-deployment. We further speculate that this provides physiological evidence that data about locations and timing of migration obtained from returning geolocator birds (e.g. [11]) may not be biased with regard to levels of stress, although this should be tested directly.

Our findings do not rule out, however, an effect of geolocators on CORT physiology, nor do they necessarily discount CORT as a potential mediator of the effects of geolocators on survival. If non-returning geolocator birds had CORT physiology operating in homeostatic overload (*sensu* [62]) for extended periods, then they could have experienced costs including reduced condition,

increased susceptibility to disease or death (for reviews see [28,38]). Sub-lethal effects of CORT could have reduced the ability of these birds to acquire resources during stopovers or on the wintering grounds, or influenced their decision to not travel as far as controls, resulting in lower return rates to breeding grounds the subsequent year, which have been detected in several of our populations [18,19]. Moreover, factors operating prior to departure from the breeding grounds could have predisposed non-returning geolocator birds to potential negative effects arising from instrumentation. For example, reproductive effort can influence CORT physiology during and at the end of the breeding season [63,64] which, in turn, can have consequences for migration phenology [65,66]. If reproduction was particularly energetically demanding for non-returning geolocator birds (i.e. CORT levels were already near homeostatic overload), then geolocators could have further increased CORT levels and exacerbated costs. Although behavioural data indicate that control and geolocator tree swallows do not appear to differ immediately after instrumentation [18], physiological costs could have carried over into migration which would further increase energetic demands. The duration, speed and distance of the migratory journey, as well as habitat use during stopovers and on wintering grounds, can influence energetics, CORT physiology and return rates of birds [36,37,67–69]. Indeed, migration distance is believed to influence apparent survival rates of geolocator-marked birds [17], and  $CORT_f$  could possibly predict the pace of autumn and spring migration in tree swallows and barn swallows, respectively [65]. Thus, the ecophysiological context before, during and after migration is important for fully understanding how and when geolocators influence survival, the potential fitness consequences to survivors, and the extent to which CORT physiology is involved in these processes.

Regardless of the mechanism, individuals that were better able to manage their CORT physiology may have been better able to avoid costs [62] and thus survive. Measuring CORT from feathers grown post-deployment from non-returning geolocator birds is essential to substantiating this hypothesis but is not possible owing to difficulties recapturing swallows once they leave the breeding grounds. Thus, our ability to identify any obvious physiological differences between returning and non-returning geolocator birds is limited to comparing their pre-deployment  $CORT_f$  levels with controls, yet we found no differences among these three groups. Investigation of plasma CORT at the time of deployment should be a focus of future research. Although we lack evidence of physiological differences between returning and non-returning geolocator birds, it may be the case that only high-quality birds were instrumented to begin with, and this explains why  $CORT_f$  levels of returning geolocator birds did not differ from controls. This is a possibility for tree swallows because geolocators were deployed (albeit randomly) on previously banded birds that had already survived at least two migrations, but we can rule out this hypothesis for barn swallows because deployment of geolocators was completely randomized [19]. Nonetheless, it is important to note that, despite surviving and not having significantly higher levels of  $CORT_f$ , returning geolocator birds may still have incurred a cost of instrumentation. Indeed, initial evidence in European populations of barn swallows suggests that geolocators impair subsequent reproduction [19]. It is unknown what role CORT plays in such effects, so future studies would benefit from determining whether body condition, health, or reproductive variables the spring following instrumentation vary with respect to  $CORT_f$  in returning geolocator birds.

Additional research is clearly needed to identify if physiological costs of instrumentation with geolocators exist and whether these influence survival, and the Energetic Expenditure Hypothesis provides testable predictions of such effects. To the extent that we can use  $CORT_f$  to infer variation in energetic expenditure, our results suggest that geolocators may not have imposed a handicap on returning swallows. Moreover, compared with birds that did not return and breed, returning birds did not have significantly different  $CORT_f$  in the year prior to instrumentation. Whether or not only the best-quality birds survived to be sampled and how CORT physiology may have contributed to this require future research. Longitudinal demographic studies such as ours are particularly informative for addressing how  $CORT_f$  relates to antecedent factors, such as reproductive history, and to downstream fitness costs. Validation studies are needed to determine if the levels of energetic exertion (or physiological stress) necessary to influence  $CORT_f$  are similar among species. As our understanding of migratory movements and stopover areas improve (e.g. [11]), it will become easier to sample geolocator and control birds throughout migration. Comparing physiological profiles of these birds at multiple stages throughout their journey will be essential to substantiating or refuting the Energetic Expenditure Hypothesis.

**Ethics statement.** All feathers were collected in accordance with appropriate ethics permits in North America (University of Saskatchewan 20070041 and 20100084; University of Northern British Columbia ACUC-2011-13; University of Guelph 11R042; Tulane University 0387) and Europe (Office fédéral de l'environnement, Division Espèces, écosystèmes, paysages F044-0799; Regione Lombardia no. 329 and no. 2141; Provincia di Novara no. 905).



Data accessibility. Data for this study have been deposited with Dryad (doi:10.5061/dryad.sq184).

Acknowledgements. We thank S. Gray for the use of her laboratory, kindly appreciate the logistical support of H. de la Giroday and D. Frattinger, and thank S. Cabezas for her help in the laboratory.

Funding statement. This study was funded by Bird Studies Canada (D.W.B.), the Canadian Foundation for Innovation (D.R.N.), Environment Canada (R.G.C., K.A.H.), EU INTERREG program (project ID 157624065), Fondazione Cariplo (grant no. UNIAGI 13357 to N.S.), Milan University (grant no. 2009-ATE-0015 to D.R.), the Natural Sciences and Engineering Research Council of Canada (D.R.N., D.W.B., R.D.D., R.G.C., L.L.B.), the University of Guelph (D.R.N.), the University of Milano-Bicocca (grant no. 2011-ATE-0272 to R.A.), the University of Northern British Columbia (R.D.D., L.L.B.), Tulane University (C.M.T.) and the National Science Foundation (DEB-0933602 to C.M.T.). The Swiss federal office for environment contributed financial support for the development of the data loggers (UTF-Nr. 254, 332, 363, 400).

Author contributions. R.G.C. initially conceived the study, with subsequent contributions from R.D.D., D.R.N., K.A.H., N.S. and C.M.T.; all authors contributed to data collection in the field; G.D.F. and L.L.B. conducted the corticosterone analyses in T.A.M.'s laboratory; G.D.F. analysed the data and wrote the manuscript with all authors providing input; all authors have approved the final version of the manuscript.

Conflict of interests. We have no competing interests.

## References

- Harrison XA, Blount JD, Inger R, Norris DR, Bearhop S. 2011 Carry-over effects as drivers of fitness differences in animals. *J. Anim. Ecol.* **80**, 4–18. (doi:10.1111/j.1365-2656.2010.01740.x)
- Webster MS, Marra PP. 2005 The importance of understanding migratory connectivity and seasonal interactions. In *Birds of two worlds: the ecology and evolution of migration* (eds R Greenberg, PP Marra), pp. 199–209. Baltimore, MD: The Johns Hopkins University Press.
- Norris DR, Marra PP. 2007 Seasonal interactions, habitat quality, and population dynamics in migratory birds. *Condor* **109**, 535–547. (doi:10.1650/8350.1)
- Hobson KA, Wassenaar LI (eds). 2008 *Tracking animal migration with stable isotopes*. New York, NY: Academic Press.
- Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT. 2002 Links between worlds: unraveling migratory connectivity. *Trends Ecol. Evol.* **17**, 76–83. (doi:10.1016/S0169-5347(01)02380-1)
- Robinson WD, Bowlin MS, Bisson I, Shamoun-Baranes J, Thorup K, Diehl RH, Kunz TH, Mabey S, Winkler DW. 2010 Integrating concepts and technologies to advance the study of bird migration. *Front. Environ.* **8**, 354–361. (doi:10.1890/080179)
- Ambrosini R, Møller AP, Saino N. 2009 A quantitative measure of migratory connectivity. *J. Theor. Biol.* **257**, 203–211. (doi:10.1016/j.jtbi.2008.11.019)
- McKinnon EA, Fraser KC, Stutchbury BJ. 2013 New discoveries in landbird migration using geolocators, and a flight plan for the future. *Auk* **130**, 211–222. (doi:10.1525/auk.2013.12226)
- Seavy NE, Humple DL, Cormier RL, Gardali T. 2012 Establishing the breeding provenance of a temperate-wintering North American passerine, the golden-crowned sparrow, using light-level geolocation. *PLoS ONE* **7**, e34886. (doi:10.1371/journal.pone.0034886)
- Stutchbury BJM, Tarof SA, Done T, Gow E, Kramer PM, Tautin J, Fox JW, Afanasyev V. 2009 Tracking long-distance songbird migration by using geolocators. *Science* **323**, 896. (doi:10.1126/science.1166664)
- Laughlin AJ *et al.* 2013 Integrating information from geolocators, weather radar, and citizen science to uncover a key stopover area of an aerial insectivore. *Auk* **130**, 230–239. (doi:10.1525/auk.2013.12229)
- Bairlein F, Norris DR, Nagel R, Bulte M, Voigt CC, Fox JW, Hussell DJ, Schmaljohann H. 2012 Cross-hemisphere migration of a 25 g songbird. *Biol. Lett.* **8**, 505–507. (doi:10.1098/rsbl.2011.1223)
- Fraser KC *et al.* 2012 Continent-wide tracking to determine migratory connectivity and tropical habitat associations of a declining aerial insectivore. *Proc. R. Soc. B* **279**, 4901–4906. (doi:10.1098/rspb.2012.2207)
- Quillfeldt P, Masello JF, Navarro J, Phillips RA. 2013 Year-round distribution suggests spatial segregation of two small petrel species in the South Atlantic. *J. Biogeogr.* **40**, 430–441. (doi:10.1111/jbi.12008)
- Ryder TB, Fox JW, Marra PP. 2011 Estimating migratory connectivity of gray catbirds (*Dumetella carolinensis*) using geolocator and mark-recapture data. *Auk* **128**, 448–453. (doi:10.1525/auk.2011.11091)
- Barron DG, Brawn JD, Weatherhead PJ. 2010 Meta-analysis of transmitter effects on avian behaviour and ecology. *Methods Ecol. Evol.* **1**, 180–187. (doi:10.1111/j.2041-210X.2010.00013.x)
- Costantini D, Møller AP. 2013 A meta-analysis of the effects of geolocator application on birds. *Curr. Zool.* **59**, 697–706.
- Gómez J, Michelson CI, Bradley DW, Norris DR, Berzins LL, Dawson RD, Clark RG. 2014 Effects of geolocators on reproductive performance and annual return rates of a migratory songbird. *J. Ornithol.* **155**, 37–44. (doi:10.1007/s10336-013-0984-x)
- Scandolara C *et al.* 2014 Impact of miniaturized geolocators on barn swallow *Hirundo rustica* fitness traits. *J. Avian Biol.* **45**, 417–423. (doi:10.1111/jav.00412)
- Pennycuik C, Fast PL, Ballerstedt N, Rattenborg N. 2012 The effect of an external transmitter on the drag coefficient of a bird's body, and hence on migration range, and energy reserves after migration. *J. Ornithol.* **153**, 633–644. (doi:10.1007/s10336-011-0781-3)
- Bowlin MS, Henningsson P, Muijres FT, Vleugels RH, Liechti F, Hedenström A. 2010 The effects of geolocator drag and weight on the flight ranges of small migrants. *Methods Ecol. Evol.* **1**, 398–402. (doi:10.1111/j.2041-210X.2010.00043.x)
- Bowlin MS, Cochran WW, Wikelski MC. 2005 Biotelemetry of New World thrushes during migration: physiology, energetics and orientation in the wild. *Integr. Comp. Biol.* **45**, 295–304. (doi:10.1093/icb/45.2.295)
- Quillfeldt P, McGill RA, Furness RW, Möstl E, Ludynia K, Masello JF. 2012 Impact of miniature geolocation loggers on a small petrel, the thin-billed prion *Pachyptila belcheri*. *Mar. Biol.* **159**, 1809–1816. (doi:10.1007/s00227-012-1971-0)
- Wikelski M, Tarlow EM, Raim A, Diehl RH, Larkin RP, Visser GH. 2003 Avian metabolism: costs of migration in free-flying songbirds. *Nature* **423**, 704. (doi:10.1038/423704a)
- Sillett TS, Holmes RT. 2002 Variation in survivorship of a migratory songbird throughout its annual cycle. *J. Anim. Ecol.* **71**, 296–308. (doi:10.1046/j.1365-2656.2002.00599.x)
- Cooke SJ, Hinch SG, Wikelski M, Andrews RD, Kuchel LJ, Wolcott TG, Butler PJ. 2004 Biotelemetry: a mechanistic approach to ecology. *Trends Ecol. Evol.* **19**, 334–343. (doi:10.1016/j.tree.2004.04.003)
- Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, Smith M. 1993 Feast and famine: critical role of glucocorticoids with insulin in daily energy-flow. *Front. Neuroendocrinol.* **14**, 303–347. (doi:10.1006/frne.1993.1010)
- Sapolsky RM, Romero LM, Munck AU. 2000 How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **21**, 55–89. (doi:10.1210/er.21.1.55)
- DuRant SE, Hopkins WA, Hepp GR, Romero LM. 2013 Energetic constraints and parental care: is corticosterone indicative of energetic costs of incubation in a precocial bird? *Horm. Behav.* **63**, 385–391. (doi:10.1016/j.yhbeh.2012.12.001)
- Harvey S, Phillips JG, Rees A, Hall TR. 1984 Stress and adrenal function. *J. Exp. Zool.* **232**, 633–645. (doi:10.1002/jez.1402320332)

31. Dallman MF, Akana SF, Pecoraro NC, Warne JP, la Fleur SE, Foster MT. 2007 Glucocorticoids, the etiology of obesity and the metabolic syndrome. *Curr. Alzheimer Res.* **4**, 199–204. (doi:10.2174/156720507780362236)
32. Landys MM, Ramenofsky M, Guglielmo CG, Wingfield JC. 2004 The low-affinity glucocorticoid receptor regulates feeding and lipid breakdown in the migratory Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*. *J. Exp. Biol.* **207**, 143–154. (doi:10.1242/jeb.00734)
33. Landys MM, Ramenofsky M, Wingfield JC. 2006 Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Endocrinol.* **148**, 132–149. (doi:10.1016/j.ygcen.2006.02.013)
34. Landys-Ciannelli MM, Ramenofsky M, Piersma T, Jukema J, Wingfield JC. 2002 Baseline and stress-induced plasma corticosterone during long-distance migration in the bar-tailed godwit, *Limosa lapponica*. *Physiol. Biochem. Zool.* **75**, 101–110. (doi:10.1086/338285)
35. Holberton RL, Parrish JD, Wingfield JC. 1996 Modulation of the adrenocortical stress response in neotropical migrants during autumn migration. *Auk* **113**, 558–564. (doi:10.2307/4088976)
36. Marra PP, Holberton RL. 1998 Corticosterone levels as indicators of habitat quality: effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia* **116**, 284–292. (doi:10.1007/s004420050590)
37. Holberton RL, Able KP. 2000 Differential migration and an endocrine response to stress in wintering dark-eyed juncos (*Junco hyemalis*). *Proc. R. Soc. Lond. B* **267**, 1889–1896. (doi:10.1098/rspb.2000.1226)
38. Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, Richardson RD. 1998 Ecological bases of hormone-behavior interactions: the 'emergency life history stage'. *Am. Zool.* **38**, 191–206. (doi:10.1093/icb/38.1.191)
39. Suedkamp Wells KM, Washburn BE, Millsbaugh JJ, Ryan MR, Hubbard MW. 2003 Effects of radio-transmitters on fecal glucocorticoid levels in captive dickcissels. *Condor* **105**, 805–810. (doi:10.1650/7174)
40. Pereira RJG, Granzinoli MAM, De Barros FM, Duarte JMB. 2009 Influence of radiotransmitters on fecal glucocorticoid levels of free-ranging male American kestrels. *J. Wildl. Manage.* **73**, 772–778. (doi:10.2193/2008-184)
41. Tremblay Y, Chérel Y, Oremus M, Tveraa T, Chastel O. 2003 Unconventional ventral attachment of time—depth recorders as a new method for investigating time budget and diving behaviour of seabirds. *J. Exp. Biol.* **206**, 1929–1940. (doi:10.1242/jeb.00363)
42. Elliott KH, McFarlane-Tranquilla L, Burke CM, Hedd A, Montevecchi WA, Anderson WG. 2012 Year-long deployments of small geolocators increase corticosterone levels in murrelets. *Mar. Ecol. Prog. Ser.* **466**, 1–7. (doi:10.3354/meps09975)
43. Bortolotti GR, Marchant TA, Blas J, German T. 2008 Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Funct. Ecol.* **22**, 494–500. (doi:10.1111/j.1365-2435.2008.01387.x)
44. Fairhurst GD, Marchant TA, Soos C, Machin KL, Clark RG. 2013 Experimental relationships between plasma- and feather-levels of corticosterone in a free-living bird. *J. Exp. Biol.* **216**, 4071–4081. (doi:10.1242/jeb.091280)
45. Hörak P, Männiste M, Meitern R, Sild E, Saks L, Sepp T. 2013 Dexamethasone inhibits corticosterone deposition in feathers of greenfinches. *Gen. Comp. Endocrinol.* **191**, 210–214. (doi:10.1016/j.ygcen.2013.07.002)
46. Fairhurst GD, Frey MD, Reichert JF, Szelest I, Kelly DM, Bortolotti GR. 2011 Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. *PLoS ONE* **6**, e17663. (doi:10.1371/journal.pone.0017663)
47. Fairhurst GD, Navarro J, González-Solís J, Marchant TA, Bortolotti GR. 2012 Feather corticosterone of a nestling seabird reveals consequences of sex-specific parental investment. *Proc. R. Soc. B* **279**, 177–184. (doi:10.1098/rspb.2011.0884)
48. Meitern R, Sild E, Lind MA, Manniste M, Sepp T, Karu U, Horak P. 2013 Effects of endotoxin and psychological stress on redox physiology, immunity and feather corticosterone in greenfinches. *PLoS ONE* **8**, e67545. (doi:10.1371/journal.pone.0067545)
49. Harriman VB, Dawson RD, Clark RG, Fairhurst GD, Bortolotti GR. 2014 Effects of ectoparasites on seasonal variation in quality of nestling tree swallows (*Tachycineta bicolor*). *Can. J. Zool.* **92**, 87–96. (doi:10.1139/cjz-2013-0209)
50. Will AP, Suzuki Y, Elliott KH, Hatch SA, Watanuki Y, Kitaysky AS. 2014 Feather corticosterone reveals developmental stress in seabirds. *J. Exp. Biol.* **217**, 2371–2376. (doi:10.1242/jeb.098533)
51. Klaassen M. 1995 Molt and basal metabolic costs in males of two subspecies of stonechats: the European *Saxicola torquata rubicula* and the East African *S. t. axillaris*. *Oecologia* **104**, 424–432. (doi:10.1007/BF00341339)
52. Lindström Å, Visser GH, Daan S. 1993 The energetic cost of feather synthesis is proportional to basal metabolic rate. *Physiol. Zool.* **66**, 490–510.
53. Liechti F *et al.* In press. Timing of migration and residence areas during the non-breeding period of barn swallows *Hirundo rustica* in relation to sex and population. *J. Avian Biol.* (doi:10.1111/jav.00485)
54. Stutchbury BJ, Rohwer S. 1990 Molt patterns in the tree swallow (*Tachycineta bicolor*). *Can. J. Zool.* **68**, 1468–1472. (doi:10.1139/z90-217)
55. Rohwer S, Butler LK, Froehlich DR. 2005 Ecology and demography of east-west differences in molt scheduling of neotropical migrant passerines. In *Birds of two worlds: the ecology and evolution of migration* (eds R Greenberg, PP Marra), pp. 87–105. Baltimore, MD: Johns Hopkins University Press.
56. Saino N, Romano M, Caprioli M, Ambrosini R, Rubolini D, Scandola C, Romano A. 2012 A ptilochronological study of carry-over effects of conditions during wintering on breeding performance in the barn swallow *Hirundo rustica*. *J. Avian Biol.* **43**, 513–524. (doi:10.1111/j.1600-048X.2012.05622.x)
57. García-Peréz B, Hobson KA. 2014 A multi-isotope ( $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) approach to establishing migratory connectivity of barn swallow (*Hirundo rustica*). *Ecosphere* **5**, 21. (doi:10.1890/es13-00116.1)
58. Fairhurst GD, Treen GD, Clark RG, Bortolotti GR. 2012 Nestling corticosterone response to microclimate in an altricial bird. *Can. J. Zool.* **90**, 1422–1430. (doi:10.1139/cjz-2012-0096)
59. Blas J, Baos R, Bortolotti GR, Marchant T, Hiraldo F. 2005 A multi-tier approach to identifying environmental stress in altricial nestling birds. *Funct. Ecol.* **19**, 315–322. (doi:10.1111/j.1365-2435.2005.00976.x)
60. Bortolotti GR, Marchant T, Blas J, Cabezas S. 2009 Tracking stress: localisation, deposition and stability of corticosterone in feathers. *J. Exp. Biol.* **212**, 1477–1482. (doi:10.1242/jeb.022152)
61. Bortolotti GR. 2010 Flaws and pitfalls in the chemical analysis of feathers: bad news-good news for avian chemocology and toxicology. *Ecol. Appl.* **20**, 1766–1774. (doi:10.1890/09-1473.1)
62. Romero LM, Dickens MJ, Cyr NE. 2009 The reactive scope model: a new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* **55**, 375–389. (doi:10.1016/j.yhbeh.2008.12.009)
63. Bonier F, Moore IT, Robertson RJ. 2011 The stress of parenthood? Increased glucocorticoids in birds with experimentally enlarged broods. *Biol. Lett.* **7**, 944–966. (doi:10.1098/rsbl.2011.0391)
64. Done T, Gow EA, Stutchbury BJM. 2011 Corticosterone stress response and plasma metabolite levels during breeding and molt in a free-living migratory songbird, the wood thrush (*Hylocichla mustelina*). *Gen. Comp. Endocrinol.* **171**, 176–182. (doi:10.1016/j.ygcen.2011.01.006)
65. Stutchbury BJM, Gow EA, Done T, MacPherson M, Fox JW, Afanasiev V. 2011 Effects of post-breeding molt and energetic condition on timing of songbird migration into the tropics. *Proc. R. Soc. B* **278**, 131–137. (doi:10.1098/rspb.2010.1220)
66. Schultner J, Moe B, Chastel O, Tартu S, Béch C, Kitaysky AS. 2014 Corticosterone mediates carry-over effects between breeding and migration in the kittiwake *Rissa tridactyla*. *Mar. Ecol. Prog. Ser.* **496**, 125–133. (doi:10.3354/meps10603)
67. Klaassen M. 1994 Growth and energetics of tern chicks from temperate and polar environments. *Auk* **111**, 525–544.
68. Marra PP, Holmes RT. 2001 Consequences of dominance-mediated habitat segregation in American redstarts during the nonbreeding season. *Auk* **118**, 92–104. (doi:10.1642/0004-8038(2001)118[0092:codmhs]2.0.co;2)
69. Angelier F, Holberton RL, Marra PP. 2009 Does stress response predict return rate in a migratory bird species? A study of American redstarts and their non-breeding habitat. *Proc. R. Soc. B* **276**, 3545–3551. (doi:10.1098/rspb.2009.0868)