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Regular handling reduces corticosterone stress responses to handling but not condition of semi-precocial mottled petrel (*Pterodroma inexpectata*) chicks

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ABSTRACT

Handling of avian study species is common in ecological research, yet few studies account for the impact of handling in nestlings where exposure to stress may result in negative lifetime fitness consequences. As a result, our understanding of stress reactivity in free-living avian young is limited. In this study we examined the cumulative impact of three levels of research-relevant handling (control, daily and every three days) on the development of the stress response, growth and condition of semi-precocial seabird chicks from near-hatching to near-fledging. By measuring corticosterone concentrations in plasma, we found that mottled petrel (*Pterodroma inexpectata*) chicks were capable of mounting a stress response comparable to adults from near-hatching. There were no differences in plasma corticosterone concentrations in initial samples (< 4 min) between groups at six weeks of age, though by 12 weeks of age plasma corticosterone concentrations in initial samples collected from chicks handled daily were lower than chicks that were handled once every three days, and from control chicks. Corticosterone responses to handling were lower in chicks handled daily at six and 12 weeks of age when compared to other handling groups. Handling chicks daily or every three days had no negative effect on the growth or condition of chicks when compared to control chicks. These findings indicate that daily handling results in chicks became accustomed to handling, with no evidence that regular handling was detrimental to mottled petrel chicks. However, given the unique life-history characteristics of mottled petrels relative to closely related species, we caution that this finding may be species-specific, and wider testing is recommended.

1. Introduction

Studies of wild populations should aim to collect data that are not biased by investigator disturbance, or, at least account for any impact that may bias our understanding of a species' behaviour, physiology or ecology (Carey, 2009; Nisbet, 2000; Serventy and Curry, 1984). Many avian ecological studies require routine and/or repetitive handling of animals for the purposes of obtaining biometrics, attaching logging devices or taking biological samples. Such manipulations are likely to trigger a highly conserved physiological stress response: the activation of the hypothalamic–pituitary-adrenal (HPA) axis and production of glucocorticoid hormones (GCs) such as corticosterone [CORT (Wingfield, 1994; Wingfield and Romero, 2001)]. Short-term elevation of GCs is considered adaptive through the initiation of behavioural and physiological processes that promote survival, such as enhanced cognitive acuity, upregulation of gluconeogenesis and immune function, increased food acquisition behaviours and promotion of escape tactics (Sapolsky et al., 2000; Wingfield and Romero, 2001). However, repeated exposure to a stressor can result in long-term or altered activation of the HPA axis, termed chronic stress, which is negatively associated with condition and survival [reviewed in: (Sapolsky et al., 2000; Wingfield, 1994; Wingfield et al., 1998)].

To date, studies that examine stress in free-living birds typically focus on adult responses (Adams et al., 2005a; Kitaysky et al., 1999b; Wingfield, 1994) with comparatively few investigations of the impact of stress in chicks, despite the increased severity of chronic stress in developing individuals [e.g. (Monaghan, 2008)]. While elevated GC levels in young birds sometimes benefit individuals, such as increased begging and preparation for fledging (Kitaysky et al., 2001; Quillfeldt et al., 2007b)], there can be strong negative effects including

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compromised growth and condition (Kitaysky et al., 1999b), reduced immune function and cognitive abilities, (McEwen et al., 1997; Morici et al., 1997), future fecundity and changes in stress reactivity (Bonier et al., 2009; Cyr and Romero, 2007). Mammalian models show that altered stress reactivity during early life can have long-lasting, negative fitness consequences (Gluckman et al., 2007; Levine, 2002; Meaney et al., 1989; Meaney et al., 1988; Meaney et al., 1991). The phenomenon is less clear in avian taxa, particularly in wild populations, as most research has focused on the impacts of acute stress during development, or the impact of chronically elevated baseline CORT in captive nestlings (Drummond and Ancona, 2015).

When investigating stress in chicks it is important to consider species-specific developmental patterns (Adams et al., 2008). The Developmental Hypothesis, presented by Blas et al. (2006), suggests that non-precocial chicks show a level of hypo-responsiveness to stressors that is relative to their life history strategy on the altricial – precocial continuum. Precocial chicks have been shown to be capable of mounting an adult-level stress response from hatching (Holmes et al., 1990; Marasco et al., 2012), thus allowing them to cope with environmental and external challenges (Blas et al., 2006). Conversely, hypo-responsiveness to stress in non-precocial chicks is considered adaptive, so that valuable resources are not directed away from the growth and tissue development by the initiation of an adult-level stress response to a perturbation from which they are unable to remove themselves (Blas et al., 2006; Quillfeldt et al., 2009; Schwabl, 1999; Wada et al., 2007; Watson et al., 2016). However, evidence from non-precocial/ altricial avian species is sparse and inconclusive, even within family groups (Adams et al., 2008; Quillfeldt et al., 2009), and some groups are not strictly precocial or altricial, making it difficult to predict their stress response.

Petrels, a type of procellariiform seabird, are typically characterised as semi-precocial (Adams et al., 2008; Brooke, 2004; Warham, 1990, 1996). Approximately 50% of this family are listed as both data deficient, and, as having threatened or declining populations (Croxall et al., 2012; IUCN, 2016; Taylor, 2000a; Taylor, 2000b). As a result of these factors, many petrel species are the subject of both intensive research and/or conservation efforts, which entail frequent handling. The impact of handling petrel chicks on the accurate reporting of ecological research, and the successful management of at-risk species, is lacking. To address this, we measured the stress response of mottled petrel (*Pterodroma inexpectata*) chicks. This species is currently subject to intensive conservation management where chicks are being translocated around New Zealand in order to establish new colonies (Sagar et al., 2015). Therefore, we mapped the development of the stress response in this semi-precocial burrow-nesting procellariiform to determine whether chicks exhibit an adult-level stress response throughout development and habituate to research-relevant levels of handling when compared to naïve chicks. More specifically, the aims of the present study were: 1) determine whether mottled petrel chicks exhibit a fully developed, adult-level stress response from hatching and throughout development; 2) compare initial and stress response CORT levels of mottled petrel chicks exposed to three different levels of handling (control, daily and every three days) across the development period (hatch to fledge); and 3) examine whether handling frequency influences chick growth, in particular peak weight, skeletal growth and feather growth.

It was hoped that by acquiring these data we could determine whether or not there were any potentially detrimental effects of regular handling of mottled petrel chicks and thereby promote the successful conservation management of this, and other at-risk avian species.

2. Materials and methods

2.1. Study site and species

This study was undertaken during the mottled petrel chick-rearing period, January – May 2015, on Codfish Island/ Whenua Hou (46° 46′ 0″ S, 167° 38′ 0″ E), a predator-free reserve 3 km west of Stewart Island, New Zealand. Codfish Island is the breeding stronghold of mottled petrels and has an estimated population of 160 000 breeding pairs (Scott et al., 2009). Mottled petrels are synchronous breeders and chicks hatch during late January – early February (mean = 31 Jan ± 5 days; R.L. Sagar unpub. data). The chick-rearing period of mottled petrels is approximately 95 – 105 days and fledging occurs during late April – May (R. L. Sagar, unpub. data).

Typical of procellariiforms, mottled petrels lay a single egg and exhibit bi-parental care and slow chick development (Warham, 1990, 1996; Warham et al., 1977). Chicks are covered in dense down and thermoregulate almost from hatching, and thus are brooded for as little as two hours (Warham et al., 1977). Such traits are consistent with precocial birds, though their reliance on parents to deliver food and inability to locomote far are more consistent with altricial species. Mottled petrel chick growth shows patterns typical of gadfly petrels: weight increases until far exceeding adult weight [up to 220% adult weight (R.L. Sagar unpub. data)], after which chicks rapidly lose weight as they approach fledging. Feather and skeletal growth (often characterised by wing chord length and tarsus length respectively) increases in a parabolic fashion, before reaching an asymptote as chicks approach fledging (Sagar et al., 2015).

2.2. Experimental design

During incubation, 58 nests containing one breeding pair sharing incubation of the single egg were marked with numbered stakes and fitted with wooden lids to allow access to the nest chamber. Nests were monitored daily for hatching, at which time all chicks were weighed and measured (wing chord length, tarsus length) and randomly allocated into the following three handling frequency groups, hereafter referred to as Control Chicks (n = 18), Daily Chicks (n = 19) and Three-day Chicks (n = 21).

Handling frequencies were chosen to mimic studies that seek to describe basic breeding biology, provisioning frequencies and meal sizes and/or chick growth and condition [for example:(Bester et al., 2007; Binder et al., 2013; Cruz-Delgado et al., 2010; Cuthbert, 2006; Miskelly et al., 2009; Sagar et al., 2015; Warham, 1990)]. Each study group were weighed and measured at different frequencies (Table 1).

Daily Chicks were weighed every day, whilst Three-day Chicks were weighed every three days. To reduce error in morphometric measures

Table 1
Handling and measurement regimes of mottled petrel (*Pterodroma inexpectata*) chicks in three groups during the study period. Each time a chick was weighed and/or measured was counted as a handling event. Mean presented ± SEM.

	No. weighing events	No. of wing chord measures	No. of tarsus length measures	Mean no. of handling events per chick
Control chicks *	5	5	5	5.0 (± 0.0)
Daily chicks #	78–88	30–36	5 – 9	82.9 (± 1)
Three-day chicks #	27–34	27–34	5 – 9	30.0 (± 0.4)

*Control chicks that underwent blood sampling were handled a maximum of five times: once at hatching, once at fledging, and during three blood sampling events. To provide a control for blood collection, half of chicks in the group did not undergo blood sampling and were only handled at hatching and fledging.
#Variation in the number of handling events in these groups was due to individual variation in the chick-rearing period (approx. 95–105 days; R.L Sagar, unpub. data), as measurements continued until chicks fledged.

because of slow growth rates, wing-chord length was measured every three days, and both tarsus and wing-chord length were measured every six days in Daily and Three-day groups. Control chicks were handled a total of five times during the study period: once upon hatching and once prior to fledging, and an additional three times for blood sampling, or, to provide comparative measures against blood sampling regimes (see below). Chicks were weighed in cotton bags to the nearest 1 g using a 100 g Pesola scale (Baar, Switzerland; newly hatched chicks only) and to the nearest 5 g using a 600 g Pesola scale. Wing chord lengths (flattened, straightened chord) were measured to the nearest 1 mm using a butted wing rule and tarsus length was measured to the nearest 0.1 mm using vernier callipers. Weighing chicks typically took less than five minutes from extraction from the nest to replacement into the nest. Wing chord measures took an additional one minute and tarsus length measures took an additional two minutes on top of weighing time. Except during bleeding procedures (described below), no chick was handled for a period greater than ten minutes during any single handling event. To account for the unique growth characteristics of procellariiforms, body condition indices (BCIs) were calculated following Quillfeldt et al. (2009), using the observed weight/wing chord length/tarsus length (m) relative to a multi-year mean for each measure (m_{mean}): $BCI = (m/m_{mean}) \times 100$.

2.3. Chick blood sampling procedures

Blood samples were collected from half of chicks in the three study groups, with the remaining chicks in each group acting as a control to compare the effects of the bleeding procedure. To control for any effects of blood sampling procedures, weight and morphometric measures were compared between blood sampled and non-blood sampled individuals within each group. To standardise handling procedures, all study chicks were weighed and had wing chord length and tarsus measured when blood sampling events occurred. Samples were collected from the bled chicks in each group at three intervals across the chick-rearing period: when chicks were approximately one week old (range = 6–9 days old), half-way through development (*circa* six weeks of age; range = 35–49 days old) and close to fledging (*circa* 12 weeks of age; range = 77–91 days old). Blood samples (0.05–0.1 mL) were collected by puncturing the tarsal vein with a 27-gauge needle and collecting blood in heparinised capillary tubes (0.05 mL; Sarstedt, Numbrecht, Germany). To minimise the effects of diel rhythm in CORT secretion, sampling was performed during the day (11:30–16:30 h).

Chicks were removed from the nest by hand, carefully restrained in cotton bags and had initial samples collected within four minutes of the investigator (R.L.S) approaching the burrow. To account for potential disturbance caused by footsteps, time was started when the investigator was 2 m from burrow. Initial sample collection times (mean = 3:40 ± 0:02 min; min. = 2:20 min; max. = 4:00 min) were higher than normal baseline sampling (Romero and Reed, 2005) due to cold conditions, which resulted in lowered blood flow to the chick's extremities, and the need to avoid soiling the chicks, which regurgitate as a defensive mechanism. Accordingly, we have used the term 'initial' as opposed to 'baseline' to reflect this departure from standard sampling methods.

Following blood collection, chicks were weighed and measured following standard procedures described above. The exception was during the sampling of chicks in the youngest age group (post-hatching); chicks were weighed prior to attempting blood sampling to confirm chicks met minimum weight requirements (130 g), so that blood collection did not exceed ethical maximums [deemed as 1% total body weight (Arctander, 1988)]. Following the collection of the initial blood sample and measurement, chicks were left enclosed in individual cotton bags on the ground close to their burrows. At 20 min following capture, chicks had a second 'stress' blood sample collected from the opposite tarsal vein of the initial sample, after which the chick was returned to its nest.

2.4. Adult blood sampling procedure

To determine whether mottled petrel chicks exhibit a fully developed, adult-level stress response from hatching and throughout development, the stress response of non-breeding adults was tested in order to provide a comparative measure. During early February 2015, adults (n = 10) were captured by hand in numbered study burrows that were known to not contain an active breeding attempt (i.e. burrows that did not contain a bird sitting on an egg, or a chick). Birds were sampled twice, in the same manner described for chicks as above (initial sample within four minutes of disturbance, stress sample 20 min following disturbance). Initial sample time mean = 3:25 ± 0:02 min; min. = 2:00 min; max. = 4:00 min). The sampling process was standardised by also weighing and measuring adults following the initial sample collection, and holding birds in dark cotton bags near to their burrows until the second blood sample collected.

2.5. Sample storage and assays

Following collection, blood samples were immediately transferred to 1 mL lidded heparin-lined vials (Sarstedt, Numbrecht, Germany), and were stored at approximately 10 °C until centrifugation. Blood samples were spun within 6 h of collection, after which the plasma was decanted into a fresh vial and stored at -20 °C until hormone assays were carried out, except during overnight cold transport (approx. 4 °C) from the field site to the laboratory at the University of Auckland, New Zealand.

Corticosterone concentrations in plasma diluted in phosphate buffered saline with gelatine (PBSG) were measured by radioimmunoassay following the method of Cockrem et al. (2009) with reagents from MP Biomedicals (USA). Serial dilutions of mottled petrel plasma in PBSG were parallel to the corticosterone standard curve. The quantitative recovery of corticosterone, measured by adding standard corticosterone to mottled petrel plasma, was 92.9 ± 3.5%. The sensitivity of the corticosterone assay, determined as the hormone concentration at the mean minus two standard deviations from the zero hormone point on the standard curves was 0.55 ng steroid mL^{-1} plasma. Solutions of corticosterone in PBSG that gave 20% and 80% binding on the standard curve were used as low and high controls in every assay. The intra-assay coefficients of variation were 8.4 and 7.2%, and inter-assay coefficients of variation were 14.6 and 16.1%.

2.6. Statistical analysis

Statistical tests were performed in JMP 13.0.0 (SAS Institute Inc. 2016).

To evaluate CORT responses between mottled petrel chicks at one week old and non-breeding adults we used a linear mixed model with CORT as the dependant variable, and sample type (initial and stress-induced CORT measures) and group (Chicks, Adults) and the interaction of sample type and group as fixed effects.

To evaluate CORT responses in mottled petrel chicks across the sampling period we used a linear mixed model with CORT as the dependant variable, and sample type (initial and stress-induced CORT measures), handling group (Control, Daily, Three-days), age group (1, 6, 12 weeks old) and the interaction of sample type, handling group and age group as fixed effects. We accounted for repeat measures from individuals by including bird identification as a random factor. For each test, residuals were tested for normality using Shapiro-Wilks tests and initial and stress-response CORT failed to meet the assumptions of normality and were log-transformed (natural log; ln) to meet these assumptions. In all instances, mean CORT values were analysed by ANOVA with False Discovery Rate (FDR) correction and if the p-value was < 0.05, a *post-hoc* Tukey's test was applied. The relationship between significant pair-wise comparisons were determined through back-transformation of least-squares mean values (LSM; LSM^{ln}; see Supplementary Material).

Regression analysis with the fixed effects of handling group, age and blood sampling group (i.e. were, or were not blood sampled) and the interactions between these factors revealed no differences in weight ($t = 1.056$, DF = 2200.583; $p = 0.291$), wing chord length ($t = 1.228$, DF = 1318.992; $p = 0.219$) or tarsus length ($t = 4.591$, DF = 2200.283; $p = 0.098$) between chicks that were bled, or not bled, in all groups, and data for all individuals have been included in the following analyses of chick weight, morphometrics and condition. Weight, wing chord length and tarsus length were examined as a function of chick age [represented by days before fledging, see Sagar et al. (2015)] and residuals failed to meet the assumptions of normality under Shapiro-Wilks tests. As a result, they were examined by regression analysis with the fixed effects of age and handling group, and interaction between age and handling group, with bird identification as a random factor to account for repeated measures. Between-group differences in fledging weight, wing chord length and tarsus length at each age group (1, 6, 12 weeks old) were analysed by one-way ANOVA as residuals met the assumptions of normality. To examine differences in body condition indices (BCIs; weight, wing chord length and tarsus length), we used a linear mixed model, with the fixed effects of handling groups (Daily, Three-day, Control) and the interaction between handling group and age group (1, 6, 12 weeks old) and included bird identification as a random factor. Residuals met the assumptions of normality.

All data are presented as mean ± SEM.

3. Results

3.1. Comparison of chick CORT with adult levels

The model appropriately described the relationships observed (adjusted $R^2 = 0.692$). Age did not affect either initial or stress-induced CORT levels in mottled petrels (Fig. 1; $F_{1,0.035} = 0.431$, FDR-corrected $p = 0.514$): at one week old, mottled petrel chicks (mean = 13.5 ng mL^{-1}; Fig. 1) exhibited initial CORT levels that were similar to adults (mean = 7.6 ng mL^{-1}; Fig. 1). Moreover, one week old mottled petrel chicks were capable producing a similar level of stress-induced CORT response to adults (mean = 77.2 ng mL^{-1} vs. 64.3 ng mL^{-1} respectively; Fig. 1). Stress-induced CORT levels were higher across both age groups than initial CORT levels (Fig. 1; $F_{1,9.153} = 111.117$, FDR-corrected $p < 0.001$).

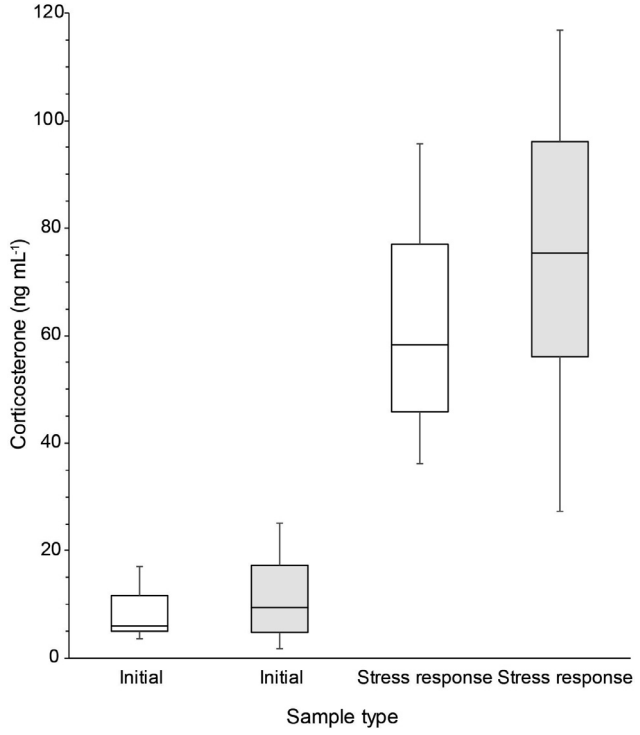

Fig. 1. Comparison of mean (± SEM) plasma initial and stress-induced corticosterone levels (ng mL^{-1}) in young (< 1 week old) chick (white bars) and non-breeding adult (grey bars) mottled petrels (*Pterodroma inexpectata*).

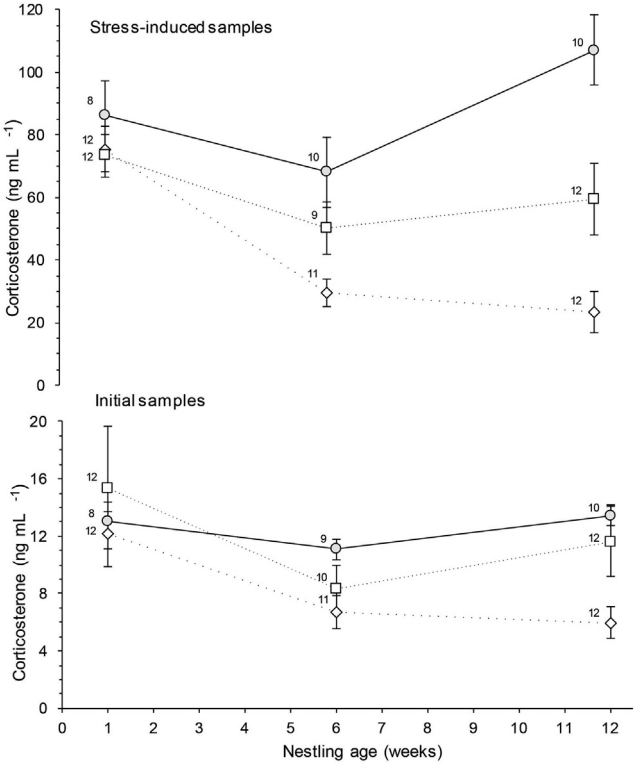

Fig. 2. Mean (± SEM) initial and stress-induced plasma corticosterone levels (ng mL^{-1}) in mottled petrel (*Pterodroma inexpectata*) chicks at three ages exposed to three levels of handling: control (circle), daily (diamond) and three-day (square).

3.2. Impact of handling on the stress response of chicks

The observed relationships were appropriately described by the model (adjusted $R^2 = 0.742$). There was a significant effect of time on mean plasma CORT levels, with stress-induced CORT levels increasing above initial CORT levels (Fig. 2; $F_{1,146.2} = 365.304$, FDR-corrected $p < 0.001$). The interaction between age and handling group significantly affected plasma CORT levels ($F_{4,149.5} = 7.294$, FDR-corrected $p < 0.001$). Pairwise comparisons revealed that compared to all handling groups at 1 week old, and both Control and Three-Day groups at 6 and 12 weeks old, CORT responses in Daily chicks were diminished at six weeks old (approx. 2.3 times less CORT; Fig. 2; see Supplementary Material) and at 12 weeks old had lessened further (approx. 3.3 times less CORT; Fig. 2; see Supplementary Material).

3.3. Impact of handling on the growth and development of chicks

Weight of Daily chicks was higher throughout the development period than for Three-day chicks (Fig. 3A; $F = 4.363$, DF = 1, $p = 0.037$), though there were no differences in weight between all handling groups at fledging (Fig. 3A, Table 3; $F = 0.13$, DF = 2, $p = 0.879$). Wing chord length did not differ between handling groups throughout development (Fig. 3B; $F = 2.528$, DF = 1, $p = 0.112$), or at fledging (Fig. 3B, Table 3; $F = 0.41$, DF = 2, $p = 0.666$). Tarsus length also did not differ between handling groups throughout development (Fig. 3C; $F = 9.210$, DF = 1, $p = 0.214$), or at fledging (Fig. 3B, Table 3;

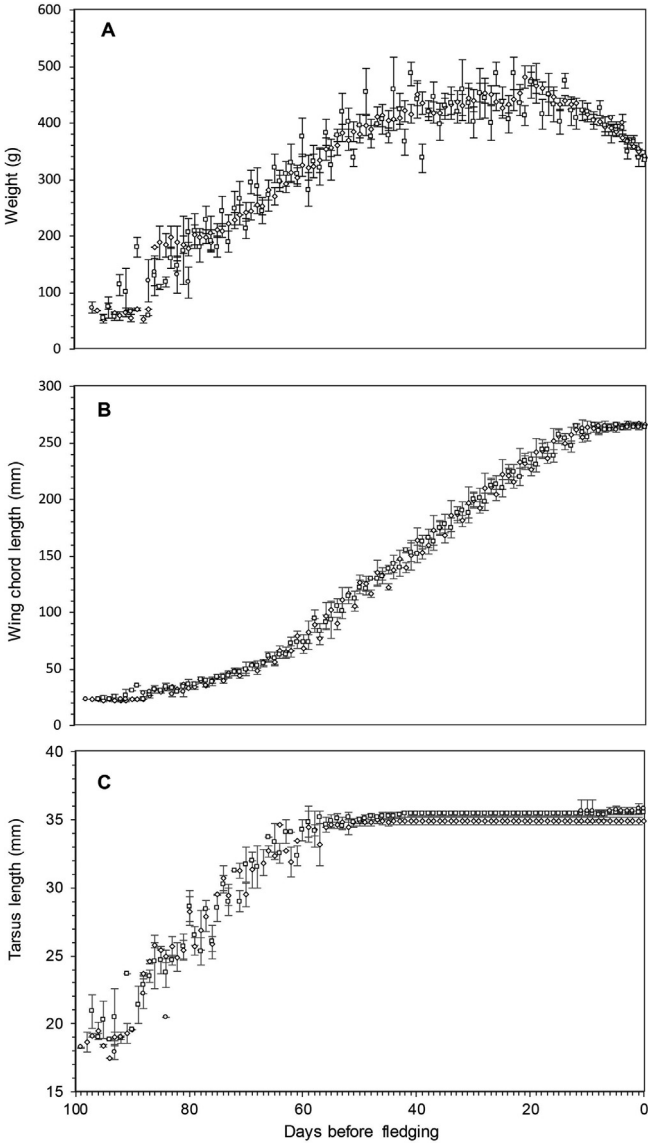

Fig. 3. Mean (± SEM) A. weight (g); B. wing chord length (mm) and C. tarsus length (mm) of mottled petrel (*Pterodroma inexpectata*) chicks exposed to three levels of handling: control (circle), daily (diamond) and three-day (sqaure) as a function of age, expressed as days before fledging.

Table 2
Summary table of mean (± SEM) body condition indices (BCIs) for weights, wing and tarsus lengths across three ages (weeks) for mottled petrel (*Pterodroma inexpectata*) chicks that were handled either daily, every three days, or minimally (control). Within each age group and morphometric, significantly different values are denoted with a different letter.

Nestling age (weeks)	BCI measure	Daily	Three-day	Control
1	Weight	91 (± 5)^{A}	104 (± 16)^{A}	112 (± 17)^{A}
	Wing chord length	94 (± 2)^{B}	96 (± 5)^{B}	97 (± 5)^{B}
	Tarsus length	97 (± 2)^{C}	97 (± 2)^{C}	97 (± 2)^{C}
6	Weight	98 (± 5)^{A}	108 (± 8)^{A}	93 (± 5)^{A}
	Wing chord length	102 (± 3)^{B}	105 (± 6)^{B}	103 (± 6)^{B}
	Tarsus length	101 (± 1)^{C}	102 (± 1)^{C}	103 (± 1)^{C}
12	Weight	102 (± 3)^{A}	112 (± 4)^{A}	103 (± 4)^{A}
	Wing chord length	102 (± 3)^{B}	103 (± 2)^{B}	101 (± 2)^{B}
	Tarsus length	101 (± 1)^{C}	102 (± 1)^{C}	103 (± 1)^{C}

Table 3
Summary table of mean (± SEM) weights, wing and tarsus lengths at hatching and fledging for mottled petrel (*Pterodroma inexpectata*) chicks that were handled either daily, every three days, or minimally (control). For each measure, significant differences are denoted with a different letter.

	Daily	Three-day	Control
Sample size	22	23	23
Number fledged	19*	20*	18*
Weight (g) at hatching	62 (± 2)^{A}	59 (± 2)^{A}	66 (± 3)^{A}
Weight (g) at fledging	339 (± 5)^{B}	335 (± 11)^{B}	342 (± 10)^{B}
Wing length (mm) at hatching	22.7 (± 0.2)^{C}	22.7 (± 0.2)^{C}	23.1 (± 0.2)^{C}
Wing length (mm) at fledging	265.9 (± 1.6)^{D}	264.4 (± 1.3)^{D}	264.3 (± 1.3)^{D}
Tarsus length (mm) at hatching	18.9 (± 0.2)^{E}	18.7 (± 0.1)^{E}	18.6 (± 0.2)^{E}
Tarsus length (mm) at fledging	35.1 (± 0.2)^{F}	35.6 (± 0.2)^{F}	35.8 (± 0.4)^{F}

*Seven chicks died of natural causes during the study period, and a further two chicks had not fledged before the end of the experiment, though both were of adult size and good weight and are predicted to have fledged successfully during the subsequent days.

$F = 2.03$, DF = 2, $p = 0.143$). The model was adequate to test the relationships (adjusted $R^2 = 0.560$) and no differences in any BCI were detected between handling groups at any age (Table 2; $F_{4,66.63} = 1.305$; $p = 0.277$).

4. Discussion

4.1. Stress reactivity in mottled petrel chicks

This study shows that at one week old, mottled chicks are capable of mounting a corticosterone response to handling that is comparable to non-breeding adult mottled petrels. The Developmental Hypothesis (Blas et al., 2006) suggests that chicks show a level of hypo-responsiveness to stress that is relative to their life history strategy on the altricial – precocial continuum. In contrast to this hypothesis, there is now evidence that chicks of two semi-precocial species of Procellariiformes – mottled petrels and grey-faced petrels (*Pterodroma macroptera gouldi*) – exhibit adult-level stress responses from hatching [this study and Adams et al. (2008)]. However, in support of the Developmental Hypothesis within the family Procellariiformes, semi-altricial thin-billed prion [*Pachyptila belcheri* (Quillfeldt et al., 2009)] and Leach's storm petrel [*Oceanodroma leucorhoa* (Fiske et al., 2013)] chicks exhibit a stress response that builds in magnitude with age, in conjunction with their physical and behavioural capacity to respond to the stressor.

The capacity of mottled petrel and grey-faced petrel chicks to initiate a high-level stress response in early life is likely a mechanistic response to deal with extended periods of food deprivation (Adams et al., 2008). Relative to their body size, mottled petrel chicks are fed markedly less frequently than other closely related species [mean = one meal every six days (Sagar et al., 2015)], especially in the period immediately post-hatching (R. L. Sagar, unpub. data). Initiation of an adult-level stress response to food deprivation by mottled petrel chicks at a young age, and throughout development, may facilitate the mobilisation of energy stores (Sapolsky et al., 2000) and increase begging behaviour, resulting in increased food delivery from parents (Kitaysky et al., 2001). These traits could play an important role in carrying these chicks through long periods between meals. Accordingly, we suggest that life-history factors additional to species-specific developmental trajectories on the altricial – precocial continuum should be considered when predicting a species capacity to respond to stress during the nestling period, and we refute the Developmental Hypothesis on this basis.

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4.2. Changes in stress responses in mottled petrel chicks to repeated handling, and the impacts of handling on growth and condition

Corticosterone responses to handling differed between groups as the study progressed: no difference was detected between Daily, Three-day and Control chicks when first sampled at one week old, but at six and 12 weeks of age corticosterone responses to handling of Daily chicks were lower than responses of Three-day and Control chicks. The experimental design of this study allowed us to examine the cumulative impact of research-relevant levels of handling, and to explore whether there is a 'handling threshold' above which corticosterone responses to handling may be altered in semi-precocial mottled petrel chicks. The corticosterone response of Daily chicks was diminished after approximately 35 handling events by six weeks of age, whilst Three-day chicks, exposed to approximately 30 handling events by the final test at 12 weeks, did not show any change in corticosterone responses to handling. This finding suggests that the handling threshold, below which corticosterone-responsiveness is not affected, for mottled petrel chicks is high, around 30–35 handling events.

Few studies have examined the cumulative impact of repeated handling on wild nestlings at progressive stages of development and as result comparisons with other species are limited. Quillfeldt et al. (2009) report that corticosterone responsiveness was not affected in thin-billed prion chicks that were handled daily from hatching, and stress-tested after *circa.* 10, 20 and 40 handling events. Conversely, Lynn et al. (2013) report that corticosterone responses in eastern bluebird (*Sialia sialis*) chicks were reduced by handling after as few as five handling events. However, neither of these studies are directly comparable to the present study, as chicks of these species show a period of hypo-responsiveness to stress when compared to adult levels. Therefore, it is possible that a dampening of the corticosterone response was not observed in thin-billed prion chicks as the HPA axis was not yet sufficiently mature to perceive and mount a full corticosterone response. In keeping with Lynn et al. (2013), our findings suggest that the maturity (and subsequent sensitivity) of the HPA axis affects how and when handling effects are likely to be exhibited in young birds. This is influenced by the life-history strategy of the species, and confirms that investigation of a wider range of species is warranted.

A diminished corticosterone response in Daily chicks by six weeks of age suggests chicks were either experiencing habituation to handling, or physiological desensitization (Cyr and Romero, 2009). Our data support three of the six criteria that Cyr and Romero (2009) use to define hormonal habituation in field studies: 1) stress responsiveness diminished with repeated exposure to the handling stimulus; 2) there was no loss of condition in Daily chicks and 3) all treatment groups were sampled at the comparable age/ life stages. Our data for mottled petrel chicks do not allow consideration of the other three criteria and without testing the chicks' capacity to mount a corticosterone response through measures such as adrenocorticotropic (ACTH) stimulation, we cannot consider whether desensitization might have occurred. With the advent of Multiplex technology that can simultaneously assay ACTH and CORT from the same sample it may be possible to track the development and responsiveness of the HPA axis (i.e. physiological desensitization vs. habituation) among differently handled treatments and fully resolve this.

Despite Three-day and Control chicks continuing to perceive handling as a stressor throughout the study, and wearing the energetic costs associated with regularly mounting a stress response, we measured no differences in growth rates, BCIs or fledging weight, wing chord and tarsus length between all handling groups. Daily chicks were observed to have overall higher weights than Three-day chicks, though there were no differences between all groups at fledging. Mottled petrel chicks may receive up to 140 g in one meal (mean = 67 ± 2 g; R.L. Sagar unpub. data), and may lose up to 50 g in the 24 h following a large meal (mean = 10 ± 2 g; R.L. Sagar unpub. data), resulting in substantial weight flux. This leads us to believe that the discrepancies in weight between handling groups are the result of a higher number of observations for Daily chicks than Three-day chicks, and have little biological significance.

Several other studies have reported that regular handling did not alter growth rates or condition in wild, non-precocial procellariiform chicks (Adams et al., 2005b; Fiske et al., 2013; Quillfeldt et al., 2009; Saffer et al., 2000; Watson et al., 2016). A lowered sensitivity to GC activity on growth and condition may be important for species in this taxon, which may rely on GC-mediated energetic regulation to carry chicks through long periods between meals (see above), and to assist thermoregulation from a young age (Adams et al., 2008; Fiske et al., 2013). Accordingly, we encourage further investigation of a mechanistic link between GC regulation and growth in procellariiform chicks to fully elucidate this relationship.

4.3. Implications and conclusions

Intense handling, as conducted in this study, is commonplace when researching species that lack basic biological information and when monitoring populations undergoing conservation action [e.g. (Binder et al., 2013; Gangloff and Wilson, 2004; Miskelly et al., 2009; Quillfeldt et al., 2007a; Sagar et al., 2015)]. The necessity and intensity of this level of handling begs the question 'how much is too much?' Our work has shown that mottled petrel chicks are resilient to high levels of repeated handling, and investigator disturbance throughout chick development should not bias morphological measurements of chicks. However, we would like to highlight that favourable conditions during the 2015 season resulted in above average breeding success and chick condition (R.L. Sagar, unpub. data). Nutritional stress is known to dramatically alter stress reactivity in seabird chicks, with subsequent negative effects on growth trajectories (Brewer et al., 2008; Kitaysky et al., 1999a; Kitaysky et al., 1999b; Kitaysky et al., 2001). As a result, we caution that the levels of handling undertaken in this study could conceivably result in altered growth and stress reactivity under different environmental conditions, and warrants further investigation.

Our findings provide further evidence that there is no universal rule to explain stress responsiveness in avian young: the development and sensitivity of the stress response has been shown to be species-specific, even between species that exhibit similar life history strategies, and researchers should be aware that no one blanket rule can apply. We suggest that additional species-specific factors beyond developmental trajectories on the altricial – precocial continuum are likely to influence the maturity of the HPA-axis in avian young. Further investigation from a wider range of species with varied early life experiences is required to confirm this.

Finally, we acknowledge that we do not understand how altered stress-reactivity in free-living petrel chicks as a result of handling affects an individual's life-time fitness. In this instance, follow-up studies would be difficult given that mottled petrels are a long-lived species with delayed maturity, and despite their philopatric nature it is very unlikely researchers will encounter study chicks again in a *meta*-colony of more than 160 000 breeding pairs spread over a 10 km^2 island. Where conditions allow, future research should aim to examine the mechanisms of GC regulation in, and, examine the long-term fitness consequences of altered stress reactivity of, a greater range of free-living nestlings.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2018.11.004.

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