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Effects of repeated investigator handling of Leach's Storm-Petrel chicks on growth rates and the acute stress response

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ABSTRACT. Disturbance during development may have lasting effects on the growth rates and stress physiology of birds. Although repeated handling by researchers is often necessary, the possible effects of such handling on the development of semi-altricial young are unclear. We examined the effect of daily handling on growth rates and plasma corticosterone levels of Leach's Storm-Petrel (*Oceanodroma leucorhoa*) chicks on Kent Island in the Bay of Fundy, New Brunswick, Canada, during the 2011 nesting season. From post-hatch day 7 to post-hatch days 14–36, birds in the experimental group were extracted from burrows and measured (wing, tarsus, and mass) for ∼3 min every day, whereas birds in the control group were left undisturbed. After the treatment period, blood was collected from birds in both groups within 3 min of initially reaching into burrows (baseline) and after a 30-min restraint stress test to assess the effect of early life disturbance on programming of the hypothalamic-pituitary-adrenal (HPA) axis. A second acute restraint stress test was conducted three weeks after the end of the treatment period to investigate possible longer term effects of early life disturbance. Growth rates of wings and tarsi were similar for handled chicks ($N = 18$) and non-handled control chicks ($N = 21$), as were baseline and 30-min acute restraint stress-induced corticosterone levels. As also reported in previous studies of other altricial and semi-altricial species, older chicks (42–64 d old) had higher plasma corticosterone levels than younger chicks (21–43 d old) after acute restraint stress tests, reflecting delayed development of the HPA axis. The age-related increase in HPA axis sensitivity observed prior to fledging could facilitate foraging and predator avoidance behaviors while minimizing exposure to high levels of corticosterone earlier in development. Overall, we found no evidence that repeated disturbance influenced either growth rates or HPA axis programming of Leach's Storm-Petrel chicks.

RESUMEN. **Efectos de manipulación repetida por investigadores sobre las tasas de crecimiento y la respuesta a estrés agudo en polluelos de *Oceanodroma leucorhoa***

Perturbaciones durante el desarrollo pueden tener efectos a largo plazo sobre las tasas de crecimiento y el estrés fisiológico de las aves. Aunque la manipulación repetida de las aves con frecuencia es necesaria, los posibles efectos sobre el desarrollo de juveniles semialtriciales son desconocidos. En este estudio, evaluamos el efecto de la manipulación diaria sobre las tasas de crecimiento y los niveles de corticosterona en polluelos de *Oceanodroma leucorhoa* capturados durante la temporada de anidación de 2011 en la isla de Kent, en la bahía de Fundy, New Burnswick, Canadá. Entre los días 7 posteclosión y 14–36 posteclosión, las aves del grupo experimental fueron extraídas de las madrigueras por ∼3 min diarios, tiempo durante el cual tomamos medidas del ala, tarso y masa corporal, mientras que las aves en el grupo control no fueron perturbadas. Después del periodo experimental, colectamos dos muestras de sangre de las aves en cada grupo. La primera, durante los 3 primeros minutos desde nuestra llegada a la madriguera (línea base) y la segunda, después de una prueba de 30 minutos de estrés por retención, con el fin de determinar el efecto de las perturbaciones que ocurren en las primeras etapas de la vida sobre la programación del eje hipotalámico pituitario adrenal (HPA). Realizamos una segunda prueba de estrés agudo tres semanas después del final del periodo de tratamiento, para investigar los posibles efectos a largo plazo de las perturbaciones en las primeras etapas de la vida. Las tasas de crecimiento de las alas y el tarso fueron similares entre los polluelos que fueron manipulados ($N = 18$) y los no manipulados ($N = 21$), así como los niveles de corticosterona en la línea base y después de los 30-min de estrés por retención. Consistente con lo reportado en estudios de otras especies altriciales y semialtriciales, los polluelos mayores (42–64 dias) tuvieron niveles mas altos de corticosterona en el plasma que polluelos mas jóvenes después de las pruebas de estrés agudo por retención, reflejando un desarrollo tardío del eje HPA. La relación entre la edad y el incremento en la sensibilidad del eje HPA previo a la etapa de volantones, puede facilitar el forrajeo y los comportamientos de evasión de depredadores a través de la reducción en los tiempos de exposición a los niveles altos de corticosterona durante el desarrollo temprano. En

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general, no encontramos evidencia que sugiera que las perturbaciones repetidas influencien las tasas del crecimiento o la programación del eje HPA en los polluelos de *Oceanodroma leucorhoa.*

Key words: corticosterone, development, HPA axis, *Oceanodroma leucorhoa*, seabird, stress

Understanding the effects of researcher disturbance on nestling development is crucial for designing appropriate sampling protocols, particularly for species of concern or those known to be sensitive to disturbance. Most studies where the effects of anthropogenic disturbance on birds have been examined have focused directly on adults or indirectly on chicks *vis a vis* the parents (Rodway et al. 1996, Fowler 1999, Blackmer et al. 2004, O'Dwyer et al. 2006, Carey 2011). Only recently have more investigators begun to focus on the effects of disturbance applied directly to chicks (Lord et al. 1997, Müllner et al. 2004, Walker et al. 2005a, Quillfeldt et al. 2009, Sharpe et al. 2009). Previous studies have revealed that effects of anthropogenic disturbance on semi-altricial chicks may include lower body mass, shorter wings, higher mortality rates, and increased secretion of corticosterone (Pierce and Simons 1986, Müllner et al. 2004, Walker et al. 2005a). Investigator disturbance could also have a negative effect on fledging success and post-fledging survival (Müllner et al. 2004). However, not all semi-altricial species are affected by disturbance during early development. For example, investigators found no effect of human disturbance on growth or baseline and stress-induced plasma corticosterone concentrations of Black-legged Kittiwake (*Rissa tridactyla)* chicks (Sandvik and Barrett 2001, Brewer et al. 2008) or Thin-billed Prion (*Pachyptila belcheri*) chicks (Quillfeldt et al. 2009). Importantly, in addition to conflicting effects of repeated disturbance on semi-altricial chicks, investigators in previous studies have not examined the possibility that disturbance during early development may have persistent effects on growth or stress physiology later in development or, in contrast, that chicks recover from acute effects of repeated disturbance. The conflicting results of previous studies highlight the need to study multiple species and breeding systems to better understand the possible effects of common research protocols on avian growth and development. The long semi-altricial period of Leach's Storm-Petrel (*Oceanodroma leucorhoa*) chicks (~70 d) provides a unique opportunity to quantify the effects of repeated handling both early and later in development.

Increases in plasma corticosterone levels in response to handling by researchers have been reported in many species of birds (O'Dwyer et al. 2006, Brewer et al. 2008), and analysis of corticosterone levels can serve as an effective measure of stress responses in birds (Fridinger et al. 2007, Quillfeldt et al. 2007, Brewer et al. 2008). Previous studies have demonstrated that stress early in life can influence development of the hypothalamic-pituitary-adrenal (HPA) axis (Matthews 2002) and lead to maladaptive changes in adult stress physiology (Teicher et al. 2003, Meaney et al. 2007, Lehmann et al. 2011). Chronically elevated corticosterone can lead to immunosuppression, impairment of cognitive abilities, chronic elevation of heart rate and blood pressure, reduced growth rates, lowered body condition, reduced reproductive success, and neuronal cell death (Johnson et al. 1992, Teicher et al. 2003, Kitaysky et al. 2003, McEwen 2008).

Research protocols in studies of semi-altricial species may involve multiple episodes of chick handling, but previous studies examining the effects of this repeated disturbance have yielded conflicting results. For example, some studies have revealed that repeated disturbance of chicks can result in lower body mass, shorter wing length, and higher mortality rates, whereas other studies have revealed no effect of disturbance (Pierce and Simons 1986, Sandvik and Barrett 2001, Müllner et al. 2004, Walker et al. 2005a, Quillfeldt et al. 2009). Our objective was to determine if daily handling of storm-petrel chicks affected growth rates and development of the HPA axis. We also tested the effects of daily handling on growth and stress physiology during two distinct periods of chick development to determine if the effects of disturbance would persist after handling ceased. We predicted that daily handling during post-hatch monitoring would affect the HPA axis such that corticosterone levels at baseline and after acute restraint would be significantly higher in handled chicks than control chicks. We also predicted that growth rates of handled chicks would be significantly lower than those of control chicks. Combining standard ecological monitoring approaches with physiological measures to understand how repeated disturbance early in life may influence individual growth and stress physiology is crucial for developing safe and effective monitoring protocols.

METHODS

Our study was conducted from June to September 2011 at the Bowdoin Scientific Station on Kent Island, New Brunswick, Canada (44°35′N, 66°45′W). We identified 53 burrows containing Leach's Storm-Petrel eggs in June 2011 at two separate locations on the island. Standard burrow-monitoring procedures were used (Huntington et al. 1996). Because prior disturbance may affect the behavior of adults (Brewer et al. 2008), all burrows used in our study had received no prior human disturbance.

The presence of eggs was established by reaching into burrows without removing the adult. Burrows were checked for eggs every other day to determine lay dates ±1 d. Once an egg was found, burrows and incubating adults were left undisturbed. Previous studies have shown that checking burrows a single time after an egg is laid and then leaving them undisturbed until after hatching facilitates high breeding success (73%–93%; Barrett et al. 1987, Harris and Bailey 1992). Beginning 37 d after the established first possible lay date, eggs in burrows were checked for hatching every other day, taking caution to minimize disturbance of incubating adults by reaching in slowly and quietly and being at the burrow for as little time as possible. This allowed us to establish hatching dates within ±1 d.

Experimental design. We randomly assigned 39 petrel chicks to either a non-handled control group ($N = 21$) or a handled experimental treatment group ($N = 18$). Only one chick per burrow was sampled because Leach's Storm-Petrels only have one chick each year. To avoid disturbing brooding adults, chicks in both groups were left undisturbed for 7 d after hatching (Vermeer et al. 1988). After the brooding period, a baseline blood sample was taken from each chick within 3 min of reaching into the burrow to establish pre-treatment baseline plasma corticosterone levels (for experimental timeline, see Fig. 1). The alar vein was punctured with a 27.5-gauge needle and blood was collected into heparinized microhematocrit capillary tubes (~25–100 μl). Blood was kept on ice until returned to the lab (0.5–3 h) and then centrifuged for 10 min at 10,000 rpm to separate the plasma and red blood cells. All plasma samples were stored at −20°C until analysis (Quillfeldt et al. 2007). After the first baseline sample was collected (post-natal day 7), each chick in the experimental group was removed from its burrow and handled for ~3 min daily or left undisturbed for 7 to 29 d depending on hatch date. Time limitations due to field site access and availability of the remote island population resulted in the variation in handling days, with later-hatched chicks being handled fewer times. Handling was conducted by the same researcher removing the chick from the burrow each day and measuring its wing length (to nearest mm), tarsus length (±1 mm), and mass (±0.1 g). Chicks in the control group were left undisturbed in their burrows. This treatment simulated standard research procedures of ornithologists working in seabird colonies (e.g., Huntington et al. 1996).

Acute stress response. At the end of the treatment period, a standard acute restraint stress test was administered, with all chicks restrained for 30 min in a cloth bag. Specifically, baseline blood samples were collected within 3 min of initially reaching into the burrow and again after 30-min acute restraint stress (as in Wingfield et al. 1992, Newman et al. 2008). Wing length, tarsus length, and mass were then measured and chicks returned to their burrows. All birds were then left undisturbed for a further 21 d, and a second acute restraint stress test was then conducted using the same methods described above before the eldest chicks fledged their burrow (Fig. 1).

Corticosterone extractions and analysis. Corticosterone in plasma was quantified using a double-antibody ^{125}I radioimmunoassay (RIA) (Immuchem 07-120103, MPBiomedicals, Orangeburg, NY, USA) that has been modified and optimized for avian plasma (Washburn et al. 2002, Newman et al. 2008). A pilot test of plasma analysis indicated that corticosterone must be pre-extracted from storm-petrel chick plasma. Thus, prior to measurement with RIA, corticosterone was extracted from chick plasma using dichloromethane (DCM) (Wingfield et al. 1992, Newman et al. 2008). To confirm

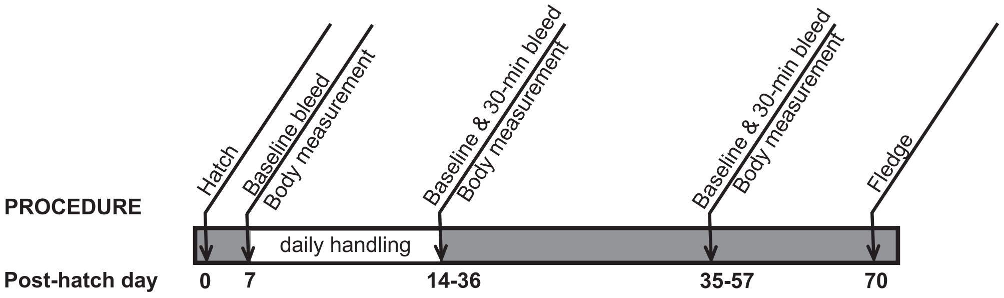

Fig. 1. Experimental timeline to examine the effects of repeated handling during early development on growth and corticosterone levels of Leach's Storm-Petrel chicks. Depending on the date of hatch, one group of chicks was handled daily for 7–29 d after the parental brooding period ended at P7 ($N = 18$). A second group of chicks was left undisturbed for 7–29 d ($N = 21$). All chicks were then left undisturbed for 21 d prior to a second round of blood collection and body measurement.

that DCM extraction was effective, we examined recovery of 24 pg exogenous corticosterone added to a pool of storm-petrel chick plasma before extraction. We also compared serially diluted extracts with the RIA standard curve. Parallelism between the standard curve and serially diluted extracted plasma indicated that DCM extraction effectively removed substances in the plasma that interfere with the assay. For extraction, 4 ml of HPLC grade DCM were added to 8 μL of storm-petrel plasma from an individual chick and vortexed for 60 s. All samples were incubated at room temperature for 120 min. The DCM was then aspirated and transferred to a clean test tube; all tubes were left to dry for ~24 h. Dried samples were then reconstituted in 200 μL buffer, vortexed, and incubated at 4°C overnight. Each plasma sample was analyzed in duplicate as previously described (Newman et al. 2008).

Statistical analysis. We used a mixed-design ANOVA to examine the effects of handling on growth rates and plasma corticosterone levels at baseline and after 30-min acute restraint. We controlled for the number of handling treatment days (7–29) and individual bird ID was included as a random effect. Significant effects were further analyzed using Tukey's honestly significant difference (HSD) post-hoc tests. Values are presented as means ± SE, and results considered significant at $\propto \leq 0.05$. Statistical analyses were conducted using Prism 5 for Mac (GraphPad Software, La Jolla, CA, USA) and PASW Statistics v.18.0 (SPSS, IBM Corporation, Somers, NY).

RESULTS

Comparison of handled and control groups revealed no significant difference ($t = 0.9$, $P = 0.36$) in the mean number of days that chicks were handled daily (14.3 ± 1.0 d, range = 7–26 d) or left undisturbed (15.6 ± 0.9 d, range = 7–29 d).

Growth rate. We found no differences between control and handled groups in growth rates of either wings (control: 2.24 ± 0.08 mm/d; handled: 2.19 ± 0.08 mm/d: ANCOVA, $F_{1, 47} = 0.7$, $P = 0.41$) or tarsi (control: 0.27 ± 0.01 mm/d; handled: 0.26 ± 0.01 mm/d; ANCOVA, $F_{1, 47} = 0.1$, $P = 0.70$).

Effect of repeated handling on corticosterone concentrations. Although there was a range in the number of treatment days, there was no relationship between the number of days handled or left undisturbed (control) for either baseline corticosterone levels (control: $R^2 = 0.01$, $P = 0.72$; handled: $R^2 = 0.02$, $P = 0.57$) or 30-min corticosterone levels (control: $R^2 = 0.09$, $P = 0.19$; handled: $R^2 = 0.002$, $P = 0.88$). A model testing for overall effects of handling on corticosterone levels across chick age classes revealed no significant interaction between treatment and plasma corticosterone levels quantified for during two post-treatment acute restraint stress tests. During both the first and second acute stress tests, chicks in both

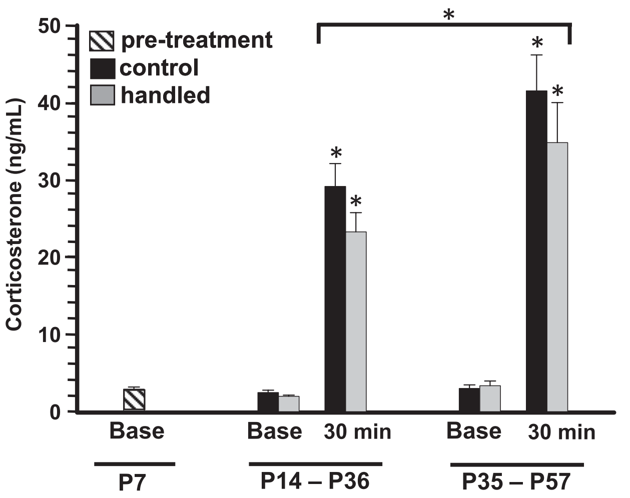

Fig. 2. Effects of repeated handling of Leach's Storm-Petrel chicks from post-hatch day 7 (P7) until P14–P36 on corticosterone levels within 3 min of taking chicks from burrows (baseline) and after a 30-min acute restraint test. All chicks were left undisturbed from P0–P7 and for 21 d prior to P35–P57. Asterisks indicate a significant effect of acute restraint within treatment groups and between 30-min corticosterone levels at P14–P36 and P35–P57.

treatment groups had similar corticosterone levels at baseline and after 30-min acute stress ($F_{4,188} = 1.4$, $P = 0.25$, Fig. 2). Further, although there was no main effect of handling treatment ($F_{1,188} = 1.7$, $P = 0.20$, Fig. 2), we found a significant effect of sample type on corticosterone levels (baseline or stress at three different ages: $F_{4,188} = 294.2$, $P < 0.0001$, Fig. 2). Post-hoc tests revealed that corticosterone levels were significantly higher after 30-min acute restraint stress tests for chicks in both treatment groups. Further analysis showed that the response to 30-min acute stress tests later in development was greater than the response earlier in development (paired *t*-tests; control: $t_{20} = 2.4$, $P = 0.027$; handled: $t_{17} = 2.9$, $P = 0.019$; Fig. 2). Baseline corticosterone levels did not change during development for either the control or handled group (repeated measures ANOVA; control: $F_{2,58} = 2.3$, $P = 0.12$; handled: $F_{2,52} = 2.2$, $P = 0.12$; Fig. 2).

DISCUSSION

We found that daily handling of Leach's Storm-Petrel chicks did not affect baseline or acute stress-induced corticosterone levels prior to fledging, and handling did not influence wing or tarsus growth rates. Despite the propensity of adult Leach's Storm-Petrels to abandon breeding attempts in response to handling by investigators (Blackmer et al. 2004, Carey 2009), developing chicks appear to be robust when exposed to routine handling and measurement. Similar results have been reported for cavity-nesting American Kestrels (*Falco sparverius*) and European Starlings (*Sturnus vulgaris*) (Butler and Dufty 2007), open-cup cliff-nesting Black-legged Kittiwakes (Sandvik and Barrett 2001, Brewer et al. 2008), and burrow-nesting Thin-billed Prions (Quillfeldt et al. 2009). Our results provide further assurance that researcher disturbance has no apparent effect on semi-altricial chicks.

One possible explanation for the absence of an effect of daily handling on Leach's Storm-Petrel chicks is that the duration of daily handling (~3 min) was not sufficient to elicit chronic elevation in baseline and acute stress-induced plasma corticosterone concentrations, as was found in Black-legged Kittiwake chicks (Brewer et al. 2008). Another possibility is that repeated handling was not perceived by the chicks as stressful over the long term. Leach's Storm-Petrel chicks spend most of their 70-d pre-fledge development period alone in burrows, sometimes experiencing long periods (up to 5 d) without food, and are often exposed to poor weather conditions; both lack of food and exposure can potentially result in death. Because Leach's Storm-Petrel chicks have evolved to cope with these experiences, stress resulting from handling may be relatively minor compared to the chronic stress of food restriction and low temperatures experienced in the burrow, as hypothesized by Brewer et al. (2008) in the case of Black-legged Kittiwake chicks. Furthermore, Leach's Storm-Petrels avoid nest predation by nesting on offshore islands devoid of small mammals and other burrow predators. We anticipated that daily removal from the burrow and handling may mimic a repeated acute stressor such as a predation attempt. However, because chicks are typically not exposed to predators, they may not be adapted to respond to acute predation stress at this stage of their life history. These life-history attributes could help explain the lack of chronic stress response elevation as a result of investigator disturbance in other semi-altricial species (Butler and Dufty 2007, Brewer et al. 2008). Finally, we may have detected no effect because corticosterone levels may peak before or after 30 min, a time interval when levels typically peak for passerines (Wingfield et al. 1992, Newman et al. 2008), but possibly not for procellariiforms.

We found that the magnitude of the acute stress response varied with the age of Leach's Storm-Petrel chicks. For both control and handled groups, 30-min corticosterone concentrations were significantly higher after the second stress challenge (35–57 d post-hatching) than after the first stress challenge (14–36 d post-hatching). Previous studies have also revealed an increase in HPA axis responses with increasing age in altricial and semi-altricial chicks of other species (Sims and Holberton 2000, Love et al. 2003, Walker et al. 2005b, Blas et al. 2006, Quillfeldt et al. 2009). The Developmental Hypothesis (Schwabl 1999, Sims and Holberton 2000, Kitaysky et al. 2003) postulates that adrenocortical responses appear at different times during development, depending on species-specific life-history strategies and where a species lies on the precocial–altricial spectrum (Starck and Ricklefs 1998). According to this hypothesis, altricial species will show little or no stress response as nestlings, e.g., Northern Mockingbirds (*Mimus polyglottos*; Sims and Holberton 2000), whereas precocial species such as Mallards will exhibit full adrenocortical responses to stress as hatchlings (Holmes et al. 1990). Many altricial and semi-altricial nestlings lack the behavioral capabilities to respond to and mitigate a stressful situation such as predators or food shortages during the first days of life. For these species, the presence of adult-like adrenocortical responses as nestlings may expose young to chronic corticosterone elevation without providing the benefit of stimulating stress-mitigation behaviors (e.g., Kitaysky et al. 2003). In semi-altricial chicks such as Leach's Storm-Petrels, we might therefore expect to see low adrenocortical responses to stress early in development, with increased responses as chicks approach fledging when they have developed adult-like behavioral and physiological abilities to respond to stress (Blas et al. 2006). This idea is supported by previous studies of the development of the HPA axis in semi-altricial nestlings, including American Kestrels (Love et al. 2003), Magellanic Penguins (*Spheniscus magellanicus*) (Walker et al. 2005b), and Thin-billed Prions (Quillfeldt et al. 2009). Our results provide further support for the Developmental Hypothesis, which predicts that semi-altricial storm-petrel chicks will have a stress hyporesponsive period as hatchlings and will upregulate their adrenocortical stress response later in development. As noted by Sims and Holberton (2000), the lower stress responses of chicks may be an adaptation to prevent exposure to high corticosterone levels during early development to maximize energy allocation to growth and minimize the negative effects of stress hormones. However, the ability of even young storm-petrel nestlings to release corticosterone in response to stress suggests that they are physiologically capable of coping with routine stresses such as food shortages.

There are several additional potential explanations for the upregulation of the HPA axis prior to fledging. Chicks may be increasing their responses to acute stress during the pre-fledging period in preparation for possible predation and foraging stresses after leaving burrows. Short-term increases in corticosterone promote both locomotion and foraging behavior, and could help facilitate successful fledging (Astheimer et al. 1992, Bray 1993). Furthermore, species-specific sensitivities to stress exposure may vary at different stages of development, as was found by Müllner et al. (2004) and Walker et al. (2005a). In our study, chicks were exposed to the handling treatment during early development; exposing petrel chicks to the handling treatment when they were older may have revealed greater sensitivity to investigator disturbance.

Our results suggest that there is no effect of daily researcher handling on growth rates, baseline plasma corticosterone concentrations, or the magnitude of corticosterone stress responses in Leach's Storm-Petrel chicks. In contrast, previous studies have revealed adverse effects of researcher handling on adult Leach's Storm-Petrels (Blackmer et al. 2004). However, the elevation of corticosterone levels that chicks were exposed to in our study as a result of daily handling and acute restraint stress could affect lifetime fitness. The long-term effects of exposure to repeated stress during juvenile development on adult social behavior, susceptibility to disease, reproduction, and responses to stress have been well documented in both humans and model organisms (Teicher et al. 2003, Claessens et al. 2011, Lehmann et al. 2011). For example, Kitaysky et al. (2003) found that Black-legged Kittiwake chicks exposed to experimentally increased levels of corticosterone during weeks 2–4 pos-thatch exhibited cognitive impairment 8 mo later. These effects of increased corticosterone could potentially affect Leach's Storm-Petrels as well, but, because of their extremely low rate of natal philopatry, we were unable to determine if handling during the early stages of development affected adult fitness. However, our results contribute to the understanding of HPA axis development in seabird chicks and have important implications for future work involving handling of semi-altricial chicks by researchers.

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