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Consequences of environmental stress exposure for behaviour and physiology of a cavity-nesting seabird



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the Degree of Doctor of Philosophy

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Abstract

Given the increasing rate of environmental change that free-living animals are exposed to, there is a growing need to understand the causes and consequences of environmentally-induced phenotypic change and to what extent this phenotypic plasticity is adaptive. As well as being of fundamental biological significance, this knowledge is necessary for the development of effective conservation policies. This thesis examines variation in behavioural, morphological and physiological responses to repeated exposure to environmental stressors. In particular, I focus on early-life effects and discuss the potential consequences of developmental plasticity for later-life performance.

Although negative effects of human disturbance on wild animals have been widely reported, few studies have considered the potential for effects on animals occupying burrows or cavities. Procellariiform seabirds are among the most globally-threatened avian taxa and include many species that nest underground. Human disturbance is one of the main threats they face, yet breeding colonies attract increasing numbers of tourists. The European storm petrel *Hydrobates pelagicus* is a small seabird, belonging to the Procellariiformes, that nests in cavities and is strictly nocturnally active at the breeding colony. The UK's largest storm petrel colony at Mousa, Shetland, offers a novel system for investigating the potential impacts of human recreational disturbance on a cavity-dwelling animal and the capacity for environmentally-induced developmental plasticity within the remarkably slow development strategy displayed by nestlings. I show that, despite remaining out of sight, human disturbance above ground can have profound effects on cavity-dwelling animals. Nestling survival was significantly lower in areas exposed to high levels of human recreational activity, compared with those reared in areas subject to very low levels of visitor activity (Chapter 1). Furthermore, disturbed nestlings that survived to fledging displayed depressed growth rates (Chapter 2). Plasticity in developmental pathways, however, enabled nestlings to mitigate for poor growth conditions by prioritising energy allocation to structural components and extending the growth period. While such growth plasticity can be adaptive and avoid short-term costs, there may also be long-term costs associated with this strategy.

Repeated exposure to stressors can give rise to chronic stress and measures of baseline glucocorticoids are widely considered to be useful for detecting stress in natural populations. Nestlings displayed no evidence for chronic stress, as measured by baseline corticosterone (the main glucocorticoid in birds), in response to repeated exposure to human recreational disturbance (Chapter 2) or investigator handling (Chapter 3). Furthermore, baseline corticosterone did not reflect the differences observed in growth trajectories between the two disturbance categories.

Although it is unknown whether the induced changes in growth trajectories of high-disturbance nestlings carry costs, the lack of any effects on corticosterone levels suggests that glucocorticoids are not always an informative measure of individual state. Additional behavioural and physiological data are required to develop an integrated understanding of phenotypic responses to environmental cues. Besides immediate effects on growth and survival, it is well known that conditions experienced during early life can have profound influences on later-life performance. Telomere dynamics potentially provide a mechanistic link between the early environment and fitness outcomes. I show that environmental conditions during growth strongly influence early-life telomere dynamics of entire cohorts, and early-life telomere length is a good predictor of imminent mortality (Chapter 4). Natural variation in the natal environment can lead to marked differences in life-history traits between cohorts and telomere dynamics may be one of the mechanisms underlying cohort effects observed in nature.

Unfavourable natal conditions were also associated with the occurrence of hypothermic responses in nestlings (Chapter 5). Such thermoregulatory responses may be adaptive, by facilitating survival during periods of food shortage or cold, but nonetheless could carry costs for growth and long-term fitness. An enhanced knowledge of the causes and consequences of facultative hypothermic responses can advance our understanding of the effects of environmental change.

Understanding and predicting how populations will respond to environmental change, however, requires accurate estimation of population size. The ecology of Procellariiformes presents a major challenge to achieving this. Using spatially-explicit capture-recapture data from three closely-related species of storm petrel, it was shown that capture probability varies across spatial and temporal scales (Chapter 6). This quantified variation was used to inform optimal sampling strategies. The results demonstrate that spatially-explicit capture-recapture models can be effectively used to monitor vulnerable burrowing seabird populations, but require a species-specific approach.

This thesis addresses key questions concerning the capacity for adaptation and response to environmental stress exposure and the mechanisms underlying individual variation in responses. I demonstrate that multidisciplinary studies of behaviour, morphology and physiology are required to develop an integrated understanding of responses to environmental stressors.

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Author's declaration

I declare that, except where otherwise stated, this thesis is entirely the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution. Authorship is clearly stated and the contributions of each author to each chapter are detailed in the general introduction.

Acknowledgements to others are given, as appropriate, at the end of each chapter.

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General introduction

Stress physiology: linking the environment and life-history evolution

An organism's life history is shaped by the interactions between its intrinsic and extrinsic environment (Stearns 1992). Much of the variation in life histories reflects phenotypic responses to environmental cues and perceived risks (Ricklefs and Wikelski 2002). Phenotypic plasticity is the property of a single genotype to produce different phenotypes under different environmental conditions (West-Eberhard 1989) and reflects the extent to which an organism can change its morphology, physiology or behaviour in response to environmental cues (Dufty Jr et al. 2002). No environment is truly constant and all organisms are exposed to fluctuations in abiotic and biotic conditions throughout their lives. Unpredictable environmental events, such as storms, floods, changes in predator and prey populations, pollution and human disturbance, have the potential to incur stress and disrupt the life cycle. Given current rates of environmental change (both natural and anthropogenic), there is an urgent need to understand how, and to what extent, organisms can respond to altered or new environmental stressors and their capacity to show adaptive phenotypic plasticity. Individual variation in plasticity of life-history traits is likely to be critical in determining the ability of populations to persist in a changed environment (Ghalambor et al. 2007, Lessells 2008). Understanding responses to stressors may provide an 'early warning' of species or populations that are most susceptible to future environmental change (Wingfield and Ramenofsky 1999). As well as having fundamental biological significance, an increased knowledge of how individuals respond to environmental change is necessary for the development of effective conservation policies.

The ability of traits to vary with respect to the environment implies the existence of underlying control mechanisms that link the genome and the environment. An improved knowledge of the mechanisms that regulate expression of life-history traits is essential for our understanding of life-history evolution (Ketterson and Nolan Jr. 1992, Zera and Harshman 2001, Crespi et al. 2013). The endocrine system regulates responses to environmental perturbations, thereby forming a mechanistic link between perception of the environment and phenotypic expression of life-history traits (Lessells 2008, McGlothlin and Ketterson 2008). Hormones often influence multiple traits simultaneously and are thus likely to be important in mediating life-history trade-offs. By mediating suites of correlated traits, hormones may act to facilitate adaptive responses of individuals to their environments; alternatively, the simultaneous influence on multiple traits may act to constrain independent evolution of traits (Ketterson and Nolan Jr. 1992, Wingfield et al. 1998, Ricklefs and Wikelski 2002, McGlothlin and Ketterson 2008).

Hormones thus potentially have a major role to play in advancing our understanding of life-history evolution and the effects of environmental change.

Vertebrates respond to unpredictable environmental stimuli by mounting a 'stress response' - an evolved suite of physiological and behavioural responses that is highly conserved across vertebrate taxa (Romero 2004). The stress response is primarily governed by activation of the hypothalamic-pituitary-adrenal (HPA) axis and the associated production of glucocorticoids (GCs; Wingfield et al. 1998, Wingfield and Kitaysky 2002). A short-term increase in GC secretion can be beneficial by mediating adaptive responses to an acute environmental challenge and promoting survival (Wingfield et al. 1998, Wingfield and Kitaysky 2002, McEwen and Wingfield 2003, Wingfield and Sapolsky 2003). The acute surge in GCs acts to redirect resources away from non-essential activities into those more conducive to maintenance and survival. Short-term effects include the promotion of gluconeogenesis, upregulation of the immune system, increase in foraging behaviour, sharpening of cognition and promotion of escape behaviour (Sapolsky et al. 2000, Wingfield and Sapolsky 2003). These features of the acute adrenocortical response may act to enhance fitness by enabling an individual to 'ride out' a short-lived (i.e. minutes to hours) stressful event in good condition (Wingfield et al. 1998). Repeated exposure to stressors, however, can give rise to chronic stress, typically associated with prolonged (i.e. days to weeks) elevation of baseline levels of circulating GCs (Wingfield and Kitaysky 2002, Romero 2004; though see, Rich and Romero 2005). The apparent benefits of acute activation of the HPA axis stand in stark contrast to the potentially deleterious effects associated with chronic activation. While GCs are necessary for the maintenance of homeostasis (Romero 2004), chronically-elevated baseline levels of GCs result in various detrimental effects including growth suppression, impaired immune function, severe protein loss and compromised cognition (Wingfield et al. 1998, Kitaysky 1999, Sapolsky et al. 2000, Wingfield and Romero 2001, Kitaysky et al. 2003).

Increasingly, measures of GCs are being used as biomarkers to assess the physiological and health status of wild populations and to address conservation issues (Wasser et al. 1997, Romero 2004, Walker et al. 2005a, Wikelski and Cooke 2006, Busch and Hayward 2009, Cooke et al. 2013). GC levels are commonly interpreted as an index of "stress" or allostatic load (McEwen and Wingfield 2003, Romero 2004) and it is assumed that elevated baseline GCs indicate individuals or populations in poorer condition and with lower fitness (Bonier et al. 2009). Elevated baseline GCs have been widely demonstrated under situations of chronic stress in nature associated with predation risk (Boonstra and Hik 1998, Clinchy and Zanette 2004), severe climatic events (Romero and Wikelski 2001), food availability (Kitaysky et al. 1999, 2003, Clinchy and Zanette 2004), pollutants (Hopkins et al. 1997, Wikelski et al. 2002), and human disturbance (Thiel et al. 2011). However, GCs do not always change predictably in response to environmental challenges

(reviewed in Busch and Hayward 2009) and studies fail to find a consistent relationship between baseline GCs and fitness (Breuner et al. 2008, Bonier et al. 2009). Furthermore, within-individual levels of circulating GCs can vary widely with respect to environmental factors, life-history stage, sex, age and social status (Silverin and Wingfield 1998, Goymann and Wingfield 2004, Angelier et al. 2006). Despite this, many studies rely solely on endocrine data to evaluate how ‘stressed’ a population is (though see Boonstra and Hik 1998) and fail to control for relevant covariates that may obscure the interpretation of GCs. Incorporation of demographic, behavioural and/or additional physiological measures into studies will facilitate an enhanced interpretation of GC data and an integrated understanding of responses to environmental stressors (see Busch and Hayward 2009, Breuner et al. 2013).

Early-life conditions

It is well understood that early-life conditions can have profound influences on phenotypic development and long-term fitness. Studies in a range of taxa have demonstrated the important role of developmental conditions in shaping an individual’s phenotype, giving rise to differences in behaviour, morphology or physiology (Mousseau and Fox 1998, Lindström 1999, Metcalfe and Monaghan 2001, Gluckman et al. 2007, Monaghan 2008). As well as reducing immediate growth, survival and immune function (Saino et al. 1997, Birkhead et al. 1999), poor early conditions have been linked with negative effects on a range of life-history traits in adulthood including body size, secondary sexual traits, fecundity, and longevity (Haywood and Perrins 1992, Sedinger et al. 1995, Verhulst et al. 1997, Birkhead et al. 1999, Reid et al. 2003, Lee et al. 2012). The phenotype of the developing organism can be influenced not just by its own environment, but also as a result of current or past environmental conditions experienced by its parents – so-called maternal and paternal effects (Mousseau and Fox 1998). Environmental conditions acting directly on the developing organism have the potential to affect an entire cohort simultaneously, giving rise to substantial differences in life histories between successive cohorts, which can persist throughout the cohort’s lifespan (Albon et al. 1987, Rose et al. 1998, Reid et al. 2003). Cohort effects can subsequently lead to delayed consequences for population dynamics (Lindström and Kokko 2002). Individuals may, to some extent, be able to mitigate negative effects on fitness by trading off costs and benefits between different tissues or life-history stages. For example, allocation of limited resources may be prioritised to certain tissues over others (Nilsson and Svensson 1996). A well-studied example, demonstrating trade-offs between life-history stages, is that of compensatory growth following a period of poor nutrition (Metcalfe and Monaghan 2001, Dmitriew 2011). Individuals may be able to compensate for the poor start and associated slow

growth by extending the growth period or, should conditions improve, accelerating growth rate. While this may be adaptive, enabling an individual to overcome the immediate costs of poor conditions and increase their fitness, compensatory strategies can also carry various long-term costs (Morgan and Metcalfe 2001, Lee et al. 2013).

Telomeres: linking early-life stress with later-life performance

Telomere dynamics link cellular processes with organismal ageing and thus optimisation of telomere length (TL) and attrition may play a major role in life-history evolution (Monaghan and Haussmann 2006). Telomeres comprise highly-conserved, repetitive, non-coding DNA sequences that form protective caps at the ends of eukaryotic chromosomes (Blackburn 2005). The sequence of TTAGGG is consistent across all vertebrates and most other taxa studied to date (Gomes et al. 2010), reflecting the highly-conserved nature of telomeres and their function.

Telomeres play an important role in maintaining genome stability: the terminus of a telomere consists of a single-stranded overhang, which provides a means of differentiating the end of a chromosome from double-stranded breaks in DNA (reviewed in Verdun and Karlseder 2007). In the absence of the enzyme telomerase, telomeres shorten with each round of somatic cell division due to the “end-replication problem”, which results in incomplete DNA replication at the 3' end of the template (Watson 1972). The end-replication problem, however, may only account for a small proportion of telomere loss and there is evidence that attrition is accelerated by other factors, such as oxidative stress (von Zglinicki 2002). When a critical length is reached, telomeres become dysfunctional and cells enter a state of replicative senescence (Blackburn 2005, Verdun and Karlseder 2007).

Subsequently, senescent cells either die or adopt an altered secretory profile, resulting in secretion of inflammatory cytokines, growth factors and degradative enzymes that contribute to age-related declines in tissue and organ function (Campisi 2005). The accumulation of senescent cells thus appears to be important to the ageing phenotype, thereby influencing organismal lifespan (Campisi 2005).

Longitudinal studies have shown that TL declines progressively with age in humans, other mammals and birds (Zeichner et al. 1999, Brümmendorf and Mak 2002, Salomons et al. 2009, Aviv et al. 2009, Bize et al. 2009, Heidinger et al. 2012, Barrett et al. 2013), though short-term lengthening of telomeres can occur (Mizutani et al. 2013). It was once thought that, within a species, TL varied little between individuals of the same age and telomere shortening occurred at a constant rate; therefore, telomeres were thought to represent a mitotic clock and be of use as a tool for ageing organisms (Haussmann and Vleck 2002, Vleck et al. 2003). However, we now know that substantial within-species variation exists in both TL and the rate of telomere shortening among individuals of the same age (Hall et al.

2004, Aviv et al. 2009, Bize et al. 2009). Accumulating evidence links TL and dynamics with survival, demonstrating that individuals with the shortest telomeres or the highest loss rate have the poorest survival prospects (Cawthon et al. 2003, Haussmann et al. 2005, Salomons et al. 2009, Bize et al. 2009, Olsson et al. 2011, Heidinger et al. 2012, Barrett et al. 2013). While studies in humans have concerned later-life disease and mortality (Cawthon et al. 2003), avian studies suggest that telomere dynamics are also associated with survival during early life (Haussmann et al. 2005, Heidinger et al. 2012).

Initial TL is partly determined by inheritance (Njajou et al. 2007, Olsson et al. 2011), though much of the inter-individual variation in TL may relate to environmental factors. The idea that physiological stress accelerates organismal ageing has long been held, but only recently has mechanistic evidence of links between chronic stress and telomere loss been revealed. Exposure to stressful conditions including psychological stress (Epel et al. 2004, Kotrschal et al. 2007), large-scale climatic processes (Mizutani et al. 2013), low habitat quality (Angelier and Vleck 2013) and reproduction (Kotrschal et al. 2007, Heidinger et al. 2012) have all been associated with short telomeres and/or accelerated telomere attrition. Few studies have measured both GCs and TL, but elevated GCs have been directly linked with short telomeres and oxidative stress (Epel et al. 2006, Haussmann et al. 2012, Herborn et al. 2014). The effects of the environment on telomere loss may be primarily mediated by increased oxidative damage (von Zglinicki 2002) and TL may be regarded as an integrative measure of both oxidative stress and telomere dynamics (Haussmann and Marchetto 2010). Telomere dynamics and oxidative stress may thus mechanistically link chronic stress with survival. In this way, exposure to environmental stressors may act to enhance senescence rates and decrease longevity.

Telomere dynamics are a strong candidate for mechanistically linking developmental conditions with later-life performance and senescence (Monaghan 2010). Poor early nutrition and catch-up growth have been shown to result in accelerated telomere loss in both laboratory rats (Jennings et al. 1999, Tarry-Adkins et al. 2009) and wild birds (Hall et al. 2004, Geiger et al. 2012), and early-life TL was found to be a better predictor of longevity than TL in adulthood in captive zebra finches (Heidinger et al. 2012). Recent links between GCs and TL indicate there may be long-term survival costs associated with early exposure to elevated GCs, as a consequence of increased telomere attrition during development (Haussmann et al. 2012, Herborn et al. 2014). Despite the potential significance of early-life telomere dynamics in influencing life-history traits, very little is known about how variation in early-life conditions influences telomere loss during development in natural environments.

Sources of environmental stress

i. Human disturbance

There are few places on earth left untouched by the activities of humans. An increasing frequency of unpredictable perturbations associated with anthropogenic activities is set against a backdrop of a changing climate, which may also lead to organisms facing dramatic changes to predictable aspects of their environment and annual cycle. The effects of human activities on free-living animals have been widely studied and it is well-understood that human disturbance can have negative effects on wildlife (reviewed in Boyle and Samson 1985, Carney et al. 1999, Nisbet 2000). Historically, studies have primarily focused on behavioural consequences of anthropogenic disturbances (Lord et al. 1997, Duchesne et al. 2000, Gill et al. 2001a, Stankowich 2008). Reduced reproductive success, as a consequence of changes in reproductive behaviour, has been widely demonstrated in response to human disturbance (particularly in birds); reduced parental care (Fernández and Azkona 1993, Verhulst et al. 2001) or abandonment of the breeding attempt (Safina and Burger 1983, Bolduc and Guillemette 2003) have been frequently revealed. However, behavioural change may not necessarily be an appropriate index of disturbance effects. If the strength of an animal's response to a disturbance event is positively correlated with the animal's condition, the relative balance of costs and benefits associated with changing behaviour will vary with individual state; individuals that don't respond may be those in poorer condition for whom the costs of a change in behaviour may be too great (Beale and Monaghan 2004a). Increasing attention is now being paid to investigating physiological responses to disturbance events. Some studies have detected no behavioural changes in response to human disturbance, but have revealed altered physiology, such as increased heart rate (MacArthur et al. 1982, Weimerskirch and Shaffer 2002), body temperature (Regel and Pütz 1997) and glucocorticoid levels (Creel et al. 2002, Müllner et al. 2004, Thiel et al. 2011). While physiological responses may not necessarily affect the outcome of the current breeding attempt, they may carry long-term costs that may be traded off against future reproduction or survival (Kitaysky et al. 2003, 2006).

Research examining the consequences of human disturbance for free-living animals has also tended to focus on the effects on adults with less attention on the consequences for developing young. However, the effects may be more severe in early life. While adult Magellanic penguins appear to habituate to human disturbance (Fowler 1999), chicks show a heightened adrenocortical response (Walker et al. 2005b). Repeated exposure to human disturbance has been linked to reduced fledging mass (Albores-Barajas et al. 2009) increased body temperature (Regel and Pütz 1997), and elevated stress-induced levels of GCs (Walker et al. 2005b) in developing young. As public access to wildlife is increasingly

encouraged, it is of growing importance to understand the consequences of recreational activities for all life-history stages. Besides the potential for immediate effects on survival and growth, changes in phenotypic development of growing individuals may carry significant costs, which could persist well into adulthood (as discussed earlier). Furthermore, monitoring both physiological and behavioural responses to anthropogenic disturbances is critical to gain an integrated understanding of all the possible outcomes.

ii. Natural environmental variation

To understand responses to anthropogenic stressors, it is necessary to understand how individuals and populations respond to natural variation in environmental conditions. Long-lived iteroparous organisms experience variation in environmental conditions over their lifetime; temperature, rainfall, frequency of extreme weather events, predator abundance and prey availability all vary from one year to another. A growing need to understand the effects of natural environmental variation is being driven by the increasing intensity and frequency of unpredictable environmental events associated with global climate change. The combination of climate change and increasing human impact is expected to increase long-term stress exposure among many organisms and may yield devastating consequences for many populations and species over the coming decades (Wingfield 2008). The monitoring of populations over successive breeding seasons allows the measurement of traits over a range of environmental conditions. The quality of a breeding season typically varies between years and inter-annual variation in vertebrate breeding success has been widely associated with variation in foraging conditions, influenced by both food availability and weather conditions (Bergallo and Magnusson 1999, Oro et al. 1999, Inchausti et al. 2003, Reid et al. 2003). Besides immediate effects on survival and fecundity, environmental factors that affect the whole cohort simultaneously can give rise to large and persistent effects on the life histories of entire cohorts (Albon et al. 1987, Rose et al. 1998, Reid et al. 2003). It is not always clear what environmental correlates are relevant, but quality of the natal environment can be measured by the overall breeding performance of a population (Reid et al. 2003).

The study system

Seabirds are among the most globally-threatened groups of birds (Croxall et al. 2012). It is estimated that by the year 2100, at least 15% of the world's seabird species will be extinct (Şekercioğlu et al. 2004). The life histories of seabirds are characterised by slow development, low fecundity, delayed sexual maturity and high longevity. The limited variation in seabird life histories is surprising, given the diversity of physical conditions, diets, foraging strategies, development patterns,

nest sites and phylogenies represented (Ricklefs 1990). Low variation implies the existence of constraints on life-history evolution, which in turn suggests constraints in the underlying control mechanisms (Wingfield and Silverin 1986). According to life-history theory, animals are expected to adjust current reproductive investment relative to the costs of future survival and reproduction (Stearns, 1992). Long-lived animals are therefore expected to favour self-maintenance over the current reproductive attempt and behave as “prudent parents” (Drent and Daan 1980, Bókony et al. 2009). Breeding seabirds may thus be particularly responsive to unpredictable environmental stressors and more likely to reduce parental effort compared to shorter-lived animals.

Human disturbance has been identified as one of the main threats seabirds face (Croxall et al. 2012), yet breeding colonies attract increasing numbers of tourists throughout the world. Increasing recreational demands at breeding colonies have led to studies quantifying the impacts on breeding seabirds, but these have mostly focused on diurnally-active, surface-nesting species (e.g. Beale and Monaghan 2004b). Burrowing seabirds belonging to the Procellariiformes (shearwaters and petrels) and the Alcidae (auks) are already facing extensive threats at breeding sites from loss and degradation of habitat as a result of human interference and introduction of mammalian predators, as well as impacts at sea, such as mortality in long-line fisheries (Croxall et al. 2012). It is generally assumed that cavity- and burrow-nesting animals are less vulnerable to the effects of human activities above ground (Burger 1981, Lunn et al. 2004). However, even when humans do not pose a direct mortality risk, animals may still perceive them as a predation risk and respond accordingly (Frid and Dill 2002, Beale and Monaghan 2004b). While some authors have quantified the effects of investigator disturbance (Pierce and Simons 1986, Blackmer et al. 2004, O’Dwyer et al. 2006, Carey 2009), the effects of recreational disturbance may be far greater than those of scientific investigators (Giese 1996). I am only aware of one study that has considered the potential for effects of human recreational disturbance on burrowing seabirds, in which human approach by tourists was found to elicit an increase in heart rate in Humboldt penguins secreted in their burrows (Ellenberg et al. 2006).

The island of Mousa, located in the Shetland archipelago (60°0’N, 1°10’W), supports the UK’s largest colony of the European storm petrel *Hydrobates pelagicus* (hereafter, storm petrel). The storm petrel is a small seabird belonging to the Procellariiformes that breeds in the north-east Atlantic and winters in the southern hemisphere. Storm petrels nest out of sight in cavities and are strictly nocturnally-active at the breeding colony. The storm petrel population of Mousa was estimated at 11,781 apparently-occupied sites (AOSs) in 2008 (Bolton et al. 2010). Although Mousa has remained uninhabited since 1853, it is serviced by a daily ferry service during the summer months (April-September) bringing a total of 4000-5000 visitors, over the course of the breeding season, attracted by wildlife and

archaeological interests. The site is designated under the EU Birds Directive as a Special Protection Area, for which the storm petrel is a qualifying feature and is managed as a nature reserve by the Royal Society for the Protection of Birds (RSPB). The island covers 2 km² and comprises flat coastal grassland, with cliffs and boulder beaches along the shoreline. On Mousa, storm petrels nest in crevices in dry stone walls, boulder beaches, loose rock scree, and abandoned buildings including a 2000-year-old Iron Age broch (dry stone tower). Dogs are not allowed on Mousa and the island remains free of introduced predators, such as rodents and cats.

Like other procellariiform seabirds, the storm petrel has a very long breeding cycle. The storm petrel lays a single egg that is incubated by both parents for c. 40 d, during which they alternate 2-3 d incubation shifts with foraging trips out to sea (Davis 1957, Scott 1970). After hatching, the nestling is brooded for about a week, during which time one adult remains with the chick while the other is foraging (Mínguez and Oro 2003). During the remainder of the chick-rearing period, the nestling remains alone in the nest, while both parents are foraging, returning most nights to feed the nestling. Storm petrel nestlings are semi-precocial (Starck and Ricklefs 1998): they hatch with a thick layer of down and become thermally independent at an early age, but they do not leave the nest until fledging at 60-70 d (Davis 1957) and are completely dependent on the parents for food. Following fledging, young storm petrels depart the colony and embark on their first migration, heading south to the southern oceans.

The storm petrel colony at Mousa offers a novel system for addressing questions regarding the effects of human activities on both breeding adults and developing young of a cavity-dwelling animal. Due to the long breeding cycle and slow pace of development, both incubating adults and chicks are potentially vulnerable to recreational disturbance associated with tourism for extended periods. They remain within the confines of the underground nest cavity and are unable to relocate to avoid a disturbance event. Very little is understood about the capacity for environmentally-induced developmental plasticity within such a remarkably slow growth strategy. While much of the literature on early-life effects concerns altricial mammals and birds, there is much to learn about the potential effects of exposure to stressors during early life in a semi-precocial bird and within such a slow development strategy. Furthermore, since only one offspring is raised per breeding attempt, the effects of potentially confounding factors concerning within-nest environmental conditions, such as sibling competition (Love et al. 2003b), on growth and physiological development can be eliminated.

Aims of the thesis

The overall aim of the thesis is to examine the behavioural, morphological and physiological effects of repeated exposure to environmental stressors in the storm petrel. In particular, the thesis focuses on the effects of exposure to stressors during early life and the potential consequences for growth and physiological development. The thesis comprises six manuscripts that are either published, in review or in preparation for submission for publication. **Chapter 1** examines how repeated exposure to human recreational disturbance influences reproductive success. The consequences of disturbance at both the individual and population level are quantified, enabling recommendations for conservation actions to be derived. In **Chapter 2**, the significance of repeated exposure to human disturbance during early life is investigated. Specifically, the paper examines the effects of disturbance on circulating GCs and the scope for developmental plasticity in postnatal growth trajectories in response to exposure to repeated disturbance. An experimental manipulation of investigator handling in **Chapter 3** examines the effects of neonatal handling on pre-fledging physiology and body condition. **Chapter 4** examines the effects of variation in environmental conditions during growth on TL and dynamics, which are subsequently discussed in terms of the potential consequences for life-history variation between successive cohorts. The potential for early-life TL to predict fledging success is quantified and parent-offspring relationships in TL are investigated. **Chapter 5** describes the occurrence and nature of hypothermic responses observed in storm petrels in relation to inter-annual variation in environmental conditions. **Chapter 6** represents the output of collaborative work led by the RSPB to quantify variation in capture probability for three species of storm petrel and inform on optimal sampling designs for spatially-explicit capture-recapture population estimates. This work also sought to achieve a greater understanding of the frequency and extent of movement of adult European storm petrels within the colony, building our knowledge of their behavioural ecology. Chapters 1-4 were conceived and designed by myself and my supervisors, Pat Monaghan and Mark Bolton. I collected all the data, with support from M.B. and a number of field assistants. I conducted all lab analyses, with the exception of some molecular sexing and initial qPCR validation (for Chapter 4); I performed all data analyses and wrote the manuscripts. Chapter 5 was entirely my own work. My contribution to Chapter 6 was in the design and undertaking of the radio-tracking fieldwork, in conjunction with M.B.; I also provided input on the manuscript.

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1. Out of sight but not out of harm's way: human disturbance reduces reproductive success of a cavity-nesting seabird

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Abstract

While negative effects of human disturbance on animals living above the ground have been widely reported, few studies have considered effects on animals occupying cavities or burrows underground. It is generally assumed that, in the absence of direct visual contact, such species are less vulnerable to disturbance. Seabird colonies can support large populations of burrow- and cavity-nesting species and attract increasing numbers of tourists. We investigated the potential effects of recreational disturbance on the reproductive behaviour of the European storm petrel *Hydrobates pelagicus*, a nocturnally-active cavity-nesting seabird. Reproductive phenology and outcome of nests subject to high and low levels of visitor pressure were recorded in two consecutive years. Hatching success did not differ between disturbance levels, but overall nestling mortality was significantly higher in areas exposed to high visitor pressure. Although visitor numbers were consistent throughout the season, the magnitude and rate of a seasonal decline in productivity were significantly greater in nests subject to high disturbance. This study presents good evidence that, even when humans do not pose a direct mortality risk, animals may perceive them as a predation risk. This has implications for the conservation and management of a diverse range of burrow- and cavity-dwelling animals. Despite this reduction in individual fitness, overall colony productivity was reduced by 61.6% compared with that expected in the absence of visitors. While the colony-level consequences at the site in question may be considered minor, conservation managers must evaluate the trade-off between potential costs and benefits of public access on a site- and species-specific basis.

Introduction

Public access to wildlife is vital for generating support for biodiversity conservation, yet it is widely recognised that human disturbance can have negative effects on wildlife (reviewed by Boyle and Samson 1985, Carney et al. 1999). Reported effects of human disturbance are varied and include changes in demography (e.g. Bolduc and Guillemette 2003), behaviour (e.g. Duchesne et al. 2000), physiology (e.g. MacArthur et al. 1982) and distribution (e.g. Gill et al. 2001a). Most studies have focused on diurnal animals that primarily live above ground and out in the open. While some authors have considered the potential effects of human disturbance on animals concealed in refuges such as burrows, dens or cavities (Blackmer et al. 2004, Linnell et al. 2000, Magle et al., 2005, Steiner and Leatherman 1981, Tempel and Gutiérrez 2003), it is generally assumed that, by remaining out of sight, these animals are less vulnerable to the presence of human activities above ground (Burger 1981, Lunn et al. 2004, though see Ellenberg et al. 2006). However, even when humans do not pose a direct mortality risk, animals may still perceive them as a predation risk and respond accordingly (Frid and Dill

2002). While some animals may be able to relocate to an undisturbed location, in response to human disturbance, others may be forced to remain despite the disturbance (Gill et al. 2001b). The risks associated with leaving a refuge may be higher than staying and enduring a disturbance event. Furthermore, relocation may not be possible if breeding has already commenced or may be limited by energetic constraints or availability of alternative refuges. Even if breeding individuals can relocate, they may still suffer a reduction in reproductive output (Swenson et al. 1997).

Colonies of breeding seabirds can support a large number of burrow- and cavity-nesting species and are attracting increasing numbers of visitors. Seabirds are among the most globally-threatened groups of birds and human disturbance has been identified as one of the main threats they are facing (Croxall et al. 2012). Increasing recreational demands at breeding colonies have led to studies quantifying the impacts on breeding seabirds, but these have mostly focused on surface-nesting species (e.g. Beale and Monaghan 2004). A few studies have examined the effects of investigator disturbance on burrowing seabirds and produced varied results: a reduction in hatching success was reported in response to nest-monitoring (e.g. puffins, Pierce and Simons 1986) and handling of adults (e.g. storm petrels, Blackmer et al. 2004, e.g. shearwaters, Carey 2011), while other studies found no effect of handling on breeding success (e.g. shearwaters, O'Dwyer et al. 2006). One study showed that merely the daily presence of a single researcher within a colony resulted in reduced fledging mass of young Cassin's auklets (Albores-Barajas et al. 2009). The effects of recreational disturbance, however, may be far greater than those of scientific investigators (Giese 1996). While typically less invasive than scientific research, recreational visits are usually longer, less closely regulated and involve more people. A handful of studies have examined the effects of recreational activities on burrowing penguins. Reported effects include elevated corticosterone stress responses (Walker et al. 2005) and increased heart rate (Ellenberg et al. 2006), while another study showed no effects of tourism on breeding success (Yorio and Boersma 1992). Rapid habituation to recreational disturbance was demonstrated in adults of the burrowing Magellanic penguin (Walker et al. 2006), but not in chicks (Walker et al. 2005). Unlike many other burrowing seabirds, however, penguins are active at the colony during the day and may come into visual contact with humans. There is clearly a need for conservation managers to quantify the effects of recreational activities and weigh up the costs and benefits of human access, but the information required to assess these impacts is rarely available (Gill 2007).

In the present study, we investigated the effects of diurnal human disturbance associated with tourism on the reproductive performance of the European storm petrel *Hydrobates pelagicus*, a small seabird that nests in cavities and is strictly nocturnally-active at breeding colonies. Due to protracted development periods,

incubating adults and chicks are present within the confines of the underground nest – and potentially vulnerable to disturbance - for extended periods. Although storm petrels remain out of visual contact with humans, they are exposed to odours, noise and vibrations associated with human activity close to or directly above their nests. We examined whether pairs nesting in areas exposed to high recreational disturbance differed in reproductive timing or outcome from those nesting in areas with little visitor activity. Reductions in breeding success have been widely reported in response to human disturbance. Birds may spend less time incubating their eggs, in response to disturbance (Verhulst et al. 2001, Wiegmann and Baylis 1995), which may result in an extension to the incubation period and subsequent reduction in the likelihood of hatching (Chaurand and Weimerskirch 1994). Reduced time spent brooding (Burger, 1981) or provisioning (Fernández and Azkona 1993, Verhulst et al. 2001) young may increase offspring mortality. Alternatively, the breeding attempt may be abandoned altogether (Bolduc and Guillemette 2003). We quantified the effects of disturbance on laying date, incubation period, hatching success, fledging success and overall productivity. While negative consequences for individual fitness are clearly important, we also quantified the magnitude of the observed effects at the colony level. Colony-scale effects will depend on both colony size and the proportion of breeding pairs affected by disturbance.

Methods

Study site and species

Mousa is a small (2 km²) island located in the Shetland archipelago, UK (60°0'N, 1°10'W). The site is managed as a nature reserve by the Royal Society for the Protection of Birds and is designated under the EU Birds Directive as a Special Protection Area, for which the storm petrel is a qualifying feature. The island comprises flat coastal grassland, surrounded by cliffs and boulder beaches. During the breeding season, Mousa receives 4000–5000 visitors during daylight hours, attracted by wildlife and archaeological interests. The island has remained uninhabited since 1853 and is not exposed to any regular sources of human disturbance outside of tourism during the summer months. The average number of diurnal visitors per day is consistent throughout the main part of the breeding season (June: 27.8 ± 3.1; July: 26.3 ± 3.7; August: 27.9 ± 3.1). Although access to the island is unrestricted, visitor management ensures that the majority of activity is restricted in space and time. A single daily ferry sailing provides access to the island and limits the duration of visits (usually <3 h). The provision of maps and information signs acts to concentrate activity within a 3.2 km circular route in the central portion of the island (Fig. 1.1); furthermore, the key points of interest are all situated along this trail, therefore there is little incentive to stray far from the path. While there is some tourism at night, comprising guided walks to observe storm

petrels as they enter and leave the colony just after darkness, this is strictly controlled (no torches; duration c. 1.5 h), and restricted to a small area of the island and the early part of the breeding season.

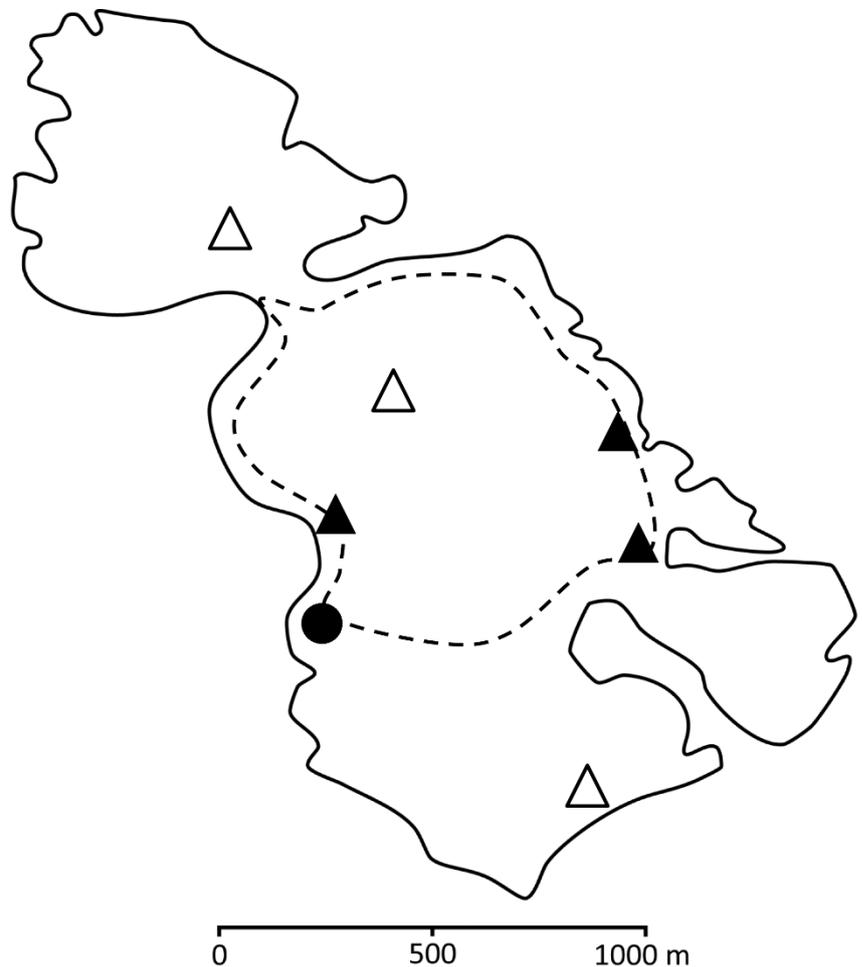


Figure 1.1. Map of study site illustrating the visitor path (dashed line), location of main archaeological interests including the Iron Age broch (closed circle), and location of study plots supporting storm petrel nests in areas subject to high (closed triangles) and low (open triangles) levels of recreational disturbance.

On Mousa, storm petrels nest in crevices in dry stone walls, boulder beaches, loose rock scree, and abandoned buildings including a 2000-year-old Iron Age broch (dry stone tower). Like many other burrow- and cavity-nesting seabirds, storm petrels are only active within the colony at night. During the day, birds either remain in the underground nest, out of sight, or are foraging at sea. As a consequence of their nocturnal habits and the inconspicuousness of nests, diurnal visitors are generally unaware of their proximity to breeding storm petrels. Storm petrels prefer dark chambers and nests are usually at least 30 cm below ground and without a direct view to the exterior; therefore, there is no visual contact between diurnal visitors and storm petrels. Storm petrels do not use earth burrows at this site and therefore there are no risks concerning erosion or burrow collapse due to the

presence of recreational activity. The storm petrel lays a single egg that is incubated by both parents for c. 40 d (Davis 1957). The nestling is brooded for about a week, during which time one adult remains with the chick while the other is foraging (Mínguez and Oro 2003). During the remainder of chick-rearing, the nestling remains alone in the underground nest, while both parents are foraging, returning most nights to feed the nestling; the chick fledges at 60–70 d (Davis 1957). The island remains free from mice, rats and cats; the only mammalian predator present is the Eurasian otter *Lutra lutra*, which rarely predaes on storm petrel nests.

Based on observations of visitor behaviour, we concluded that recreational activity predominates within a belt of 10 m either side of the marked path. For this reason, we considered nests located within 10 m of the path to be subject to ‘high’ levels of visitor pressure. Visitors rarely explore beyond this area and we adopted a conservative threshold distance of 150 m from the path, beyond which we considered nests to be subject to ‘low’ levels of visitor pressure. A total of 95 study nests were distributed among six ‘plots’, geographically spread across the island (Fig. 1.1). Three plots, encompassing 43 nests, were in areas subject to high disturbance (i.e. ≤ 10 m of the path), while the other three plots, supporting 52 study nests, were in areas subject to low disturbance (i.e. >150 m from the path). Study nests were located in either walls or rock scree and both of these nesting habitats were equally represented within the high and low disturbance categories. Nesting densities were similar between high- and low-disturbance plots. There were no other differences in surrounding habitat or environmental conditions between plots. Only one of the high-disturbance plots was exposed to any nocturnal human disturbance (see above).

Effects on individual reproductive performance

In the consecutive breeding seasons of 2010 and 2011, 75 and 82 of the study nests, respectively, were occupied by a breeding pair of storm petrels. Nests were monitored to determine laying date, hatching date and breeding outcome. Nests were visually inspected most days from early June, allowing the determination of laying date with a maximum error of 3 d for at least 85% of breeding records. To minimise disturbance, nests were inspected briefly with the aid of a torch. Storm petrels are highly sensitive to disturbance induced by handling during incubation, so neither the egg nor incubating adults were removed from nests. In 2010, nests continued to be inspected regularly after laying to record nest attendance (not reported here). In 2011, once laying was confirmed, inspections ceased until 38 d after the earliest possible laying date - close to expected hatching. The average level of investigator disturbance experienced by nests was thus constant between disturbance levels within years, but higher in 2010 than in 2011. Limited visibility at c. 15% nest sites prevented hatching dates from being accurately determined from visual checks. At these sites, the age of nestlings (and subsequently hatching

date) was estimated from tarsus length. Up to c. 30 d the tarsus grows at a constant rate (Bolton, unpubl.) allowing age to be estimated from the linear regression of age on tarsus length of known-age chicks (2010: $r^2 = 0.94$; 2011: $r^2 = 0.96$). This enabled estimation of age to within 3 d of actual age in 94% of nestlings. Due to increasingly adverse weather conditions restricting access to the island late in the season, most nests were not followed through to fledging. The majority of chicks were between 50 and 60 d at the final check and all were >30 d, beyond which failure is unlikely and successful fledging can be confidently assumed (Davis 1957). Of 116 chicks hatched over two years, just one died beyond 18 d. Nests were inspected at the beginning of the following season to provide additional confirmation of whether the chick fledged successfully or not. Nest predation is rare and obvious, since the only predator present capable of removing a chick from a nest is the otter, which always leaves clear signs of excavation.

Population-level effects

To quantify the effect of recreational disturbance at the population level, we first needed to quantify the proportion of the colony that is subject to high levels of visitor disturbance. Although the colony had recently been censused in 2008 (Bolton et al. 2010), this did not give separate estimates for each of the areas subject to high and low disturbance. Between 21 and 27 July 2012, we conducted a census of all potentially-suitable nesting habitat within 10 m of the visitor path. We employed the playback methodology, developed by Ratcliffe et al. (1998) and used in two previous whole-island surveys (Bolton et al. 2010, Ratcliffe 1997), in which a male purr call is played at 1 m intervals along transects and the number of individual responses recorded. In addition to censusing wall, boulder and rock scree habitats (as per Ratcliffe 1997), we also surveyed the Mousa broch, an Iron Age stone tower, which was not included in previous island surveys. The broch's structure essentially consists of two 13 m-high concentric walls. For the purposes of the survey, each of the two walls were considered to consist of 13 individual metre-high "walls" situated one on top of another. The lowest two "walls" (i.e. from 0–1 m and 1–2 m above ground) and the uppermost "wall" (12–13 m) of both the external and internal walls of the broch were surveyed following the methodology described for wall habitats (see Ratcliffe 1997). Since not all birds will respond to playback, it was necessary to calibrate the number of responses recorded (as per Ratcliffe et al. 1998). Playback was conducted at 55 sites known to be occupied by a breeding pair. The response rate was calculated as the proportion of occupied sites from which a response was elicited on a single day. The response density for each surveyed area was subsequently adjusted for the response rate and multiplied by the total length (walls) or total area (rocks or boulders) to give an estimate of apparently occupied sites (AOSs). Summing the estimated number of AOSs for all surveyed areas gave us the total number of AOSs subject to high levels of visitor disturbance.

Subtracting the number of high-disturbance AOSs from the most recent colony estimate of 11781 AOSs (Bolton et al. 2010) provided us with an estimated number of AOSs subject to low visitor disturbance. Subsequently, using productivity rates from high- and low-disturbance areas determined from the present study, we predicted the number of offspring fledged from the colony in the presence and absence of recreational disturbance in 2012. We express the colony-level consequences associated with recreational disturbance in terms of the predicted annual reduction in colony productivity due to tourism.

Statistical analysis

All statistical analyses were performed in R 3.0.0 (R Core Team 2013). Linear mixed models with a normal error structure were employed to examine variation in laying date in relation to disturbance using the lme4 package (Bates et al. 2013). We considered the random effects of nest identity and nest identity nested within plot. First, the optimal random effects structure was determined by performing likelihood ratio tests (LRTs) on nested models fitted by restricted maximum likelihood (REML), with a saturated fixed component and different random effects structures. The variance associated with the random effect of plot in all models was close to zero and did not significantly improve the model fit, so only nest identity was retained as a random effect. The saturated model included the fixed effects of disturbance and year and the interaction between disturbance and year. The optimal fixed effects structure was obtained by stepwise deletion, sequentially removing non-significant parameters ($P > 0.05$) from models fitted by maximum likelihood (ML) estimation. The significance of model parameters was estimated by comparison to a probability distribution obtained from 10,000 Markov Chain Monte Carlo simulations using `pvals.fnc()` in languageR, which does not require the estimation of degrees of freedom. Results are presented from the minimum adequate model fitted by REML. Differences in incubation period (the number of days from laying to successful hatching) between nests subject to high and low visitor pressure were examined using Wilcoxon rank-sum tests for each year separately. Unless stated otherwise, all results are presented as means \pm SE.

Since complete nest histories were available for all nests, reproductive success was analysed using a binary (0/1) response variable. Generalised linear mixed models (GLMMs) with a binomial error structure and logit link function were fitted to data on (i) overall productivity, (ii) hatching success and (iii) fledging success. Again, the variance associated with the random effect of plot was close to zero and only the random effect of nest identity was retained in models. Saturated models included the fixed effects of disturbance (high or low), laying date (covariate), year (2010 or 2011) and nest type (wall or scree). We also considered relevant two-way interactions to control for seasonal (laying date x disturbance) and inter-annual (year x disturbance) variation in the effect of disturbance on the

response variable and inter-annual variation in the effect of laying date on the response variable (year x laying date). The optimal fixed effects structure was attained by a stepwise deletion process, in which each term was removed separately and LRTs were performed between each of the reduced models and the fuller model. The criteria for removal of a term was a log-likelihood ratio P -value >0.05 . MCMC sampling is not yet implemented for generalised linear mixed models in lme4; therefore, the significance of parameter estimates from minimum adequate models was estimated using Wald z -tests. The predictive accuracy of models was assessed using Receiver Operating Characteristic plots fitted using the ROCR package (Sing et al. 2005). The resulting area under the curve (AUC) offers a measure of predictive performance; a value of 1.0 indicates a perfect model, while a value of 0.5 indicates that a model performs no better than random. AUC values are reported for final models.

Results

Visitor pressure and reproductive performance

A summary of reproductive performance of storm petrels breeding in areas subject to high and low levels of recreational disturbance in the two study years is presented in Table 1.1. Egg-laying began in early June and continued through to early August in both years. There was no effect of visitor pressure ($t = 1.00$, $P = 0.264$) or year ($t = -2.24$, $P = 0.113$) on laying date (Fig. 1.2) and the effect of disturbance did not vary between years (disturbance (low) x year: $t = 0.85$, $P = 0.452$). The length of the incubation period was unaffected by visitor pressure in either 2010 (median and range: high: 40 d, 39–43 d; low: 40 d, 39–42 d; $W = 397$, $P = 0.565$, $n = 55$) or 2011 (median and range: high: 42 d, 39–66 d; low: 42 d, 39–69 d; $W = 451$, $P = 0.697$, $n = 62$). Failure during the nestling stage was recorded in 12 and 25 nests in 2010 and 2011 respectively. Excluding one chick that died at 56 d, the majority of mortalities (78%) occurred at 68 d (median: 6 d; range: 1–18 d; $n = 36$).

The likelihood of reproductive success was significantly lower in nests exposed to high levels of visitor disturbance compared with low-disturbance nests (Tables 1.1 and 1.2; $P = 0.021$). However, a significant interaction between laying date and disturbance (Table 1.2; $P = 0.016$) confirmed different patterns of seasonal decline between high- and low-disturbance nests. The rate and magnitude of seasonal decline in probability of successful breeding was greater in areas subject to high levels of visitor pressure (Table 1.2, Fig. 1.3A). While the likelihood of successful hatching decreased significantly with increasing laying date (Table 1.2; $P = 0.017$), there was no effect of disturbance ($z = -0.29$, $P = 0.771$) or year ($z = -0.06$, $P = 0.949$) on hatching success (Fig. 1.3B). In contrast, a significant interaction between laying date and disturbance (Table 1.2, Fig. 1.3C; $P = 0.027$) revealed a marked seasonal decline in probability of fledging success in high-

disturbance nests, while there was very little seasonal change in fledging success in nests subject to low levels of recreational pressure. Both the likelihoods of reproductive success (Table 1.2, Fig. 1.3A; $P = 0.020$) and fledging success (Table 1.2, Fig. 1.3C; $P = 0.011$) were significantly lower in 2011 compared with 2010 across all nests, yet the effect of disturbance persisted. There was no significant difference in productivity ($z = -1.63$, $P = 0.102$), hatching success ($z = -0.72$, $P = 0.473$) or fledging success ($z = -1.73$, $P = 0.084$) between wall and scree nesting habitats.

Table 1.1. Summary of reproductive performance of storm petrels nesting in areas subject to high and low recreational disturbance in 2010 and 2011 at Mousa, Shetland.

	n	Productivity	Hatching success	Fledging success
2010				
High disturbance	34	0.44	0.71	0.63
Low disturbance	41	0.68	0.76	0.90
2011				
High disturbance	37	0.38	0.78	0.48
Low disturbance	45	0.51	0.73	0.70

Colony-level effects

The playback survey elicited a total of 66 responses to playback from 1076 survey points. Of 55 calibration sites known to be occupied by a breeding pair, a response to playback was elicited from 12 sites, giving a response rate of 0.218. This is comparable to response rates determined in previous censuses (Ratcliffe 1997, Ratcliffe et al. 1998, Bolton et al. 2010). The mean response densities, adjusted for the response rate, were 0.41 AOSs m² for walls (including the broch) and 0.18 AOSs m² in natural habitats. When multiplied by the total area of suitable habitat, this yielded estimates of 330 AOSs and 197 AOSs in walls and natural habitats respectively, giving an estimated total of 527 AOSs located within 10 m of the visitor path and subject to high visitor pressure in 2012. Based on a predicted population size of 11,781 breeding pairs (see Bolton et al. 2010), this equates to 4.5% of the colony being subject to high levels of recreational disturbance in 2012 and, therefore, reduced annual productivity, compared with the remainder of the population, which is subject to low levels of disturbance. Using productivity estimates from both 2010 and 2011, between 70 and 128 fewer chicks were estimated to have fledged from the colony in 2012 compared with a hypothetical

scenario where the whole population is subject to low disturbance (Table 1.3). This represents a reduction in overall colony productivity ranging from 1.2–1.6%.

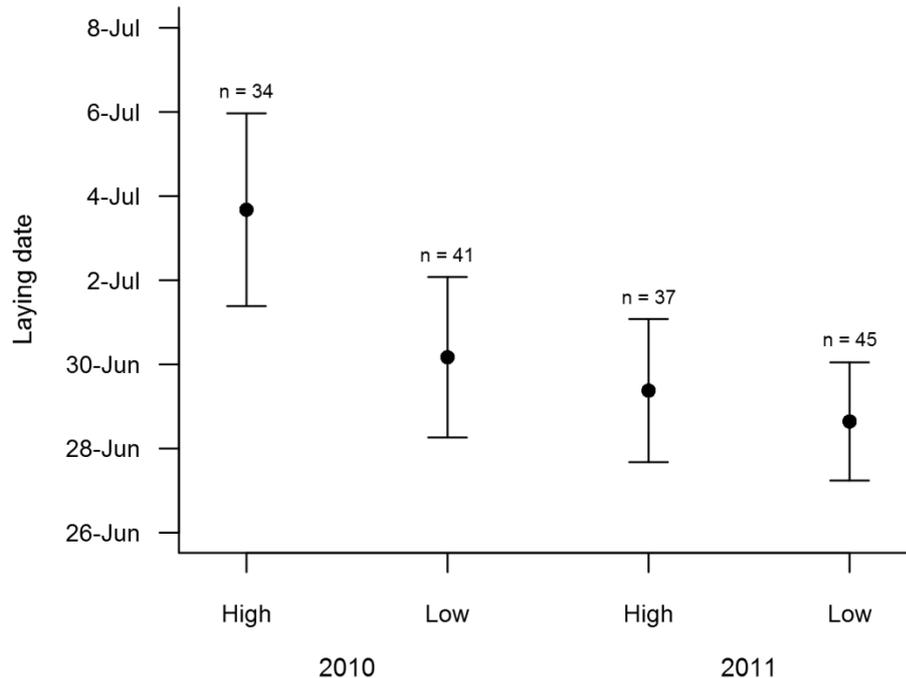


Figure 1.2. Laying date of storm petrels nesting in areas subject to high and low recreational disturbance in 2010 and 2011 at Mousa, Shetland (all $P > 0.1$). Means \pm SE are presented with corresponding sample sizes.

Discussion

Despite nesting underground and out of sight, reduced reproductive success of a cavity-nesting nocturnal bird was associated with diurnal human disturbance above ground due to tourism. There were no differences in incubation period or hatching success between high- and low-disturbance nests, suggesting birds exposed to repeatedly high levels of visitor disturbance can maintain incubation effort, but early nestling mortality was significantly higher. We showed that the relationship was spatially and temporally stable, as demonstrated by repeatable results between the different plots within each disturbance treatment and between two consecutive seasons, respectively. The relationship between disturbance and breeding success persisted in both a ‘good’ and a ‘poor’ year for overall colony productivity and therefore presumably under very different environmental conditions. The colony has been exposed to tourism at this level for a number of years, suggesting that, if any habituation to human presence has occurred, it is not sufficient to offset negative impacts. There was no evidence to support alternative explanations for the observed reduction in breeding success. Availability of nesting habitat is not limited at the study site and different nesting habitat types (wall and rock scree) were equally represented within the two disturbance levels. There were no site-specific

differences between high- and low-disturbance plots besides the level of visitor activity. Investigator pressure was the same at high- and low-disturbance nests within years and, in fact, the probability of reproductive higher. Furthermore, breeding success was comparable to that recorded by low-intensity annual monitoring in the same years in low-disturbance areas (0.50 and 0.56, respectively, Okill, unpubl.), suggesting that investigator disturbance did not significantly influence breeding success.

Table 1.2. Summaries of minimum adequate GLMMs fitted to data on productivity (n = 157), hatching success (n = 157) and fledging success (n = 117) of storm petrels nesting in areas subject to high and low levels of recreational disturbance in 2010 and 2011^{a,b}.

Dependent variable	Fixed effect	Estimate ± SE	z-value	P
Productivity	Laying date	-0.117 ± 0.03	-3.65	<0.001
	Disturbance (low)	-16.38 ± 7.15	-2.29	0.021
	Year (2011)	-0.885 ± 0.38	-2.33	0.020
	Laying date x Disturbance (low)	0.095 ± 0.04	2.40	0.016
Hatching success	Laying date	-0.042 ± 0.02	-2.43	0.017
Fledging success	Laying date	-0.137 ± 0.04	-3.16	0.002
	Disturbance (low)	-22.45 ± 10.8	-2.09	0.037
	Year (2011)	-1.569 ± 0.62	-2.53	0.011
	Laying date x Disturbance (low)	0.132 ± 0.06	2.21	0.027

^aAll models included the random effect of nest identity

^bThe predictive performance of models was assessed using Receiver Operating Characteristic plots, which yielded values of 0.88, 0.81 and 0.94 for the area under the curve of each respective model

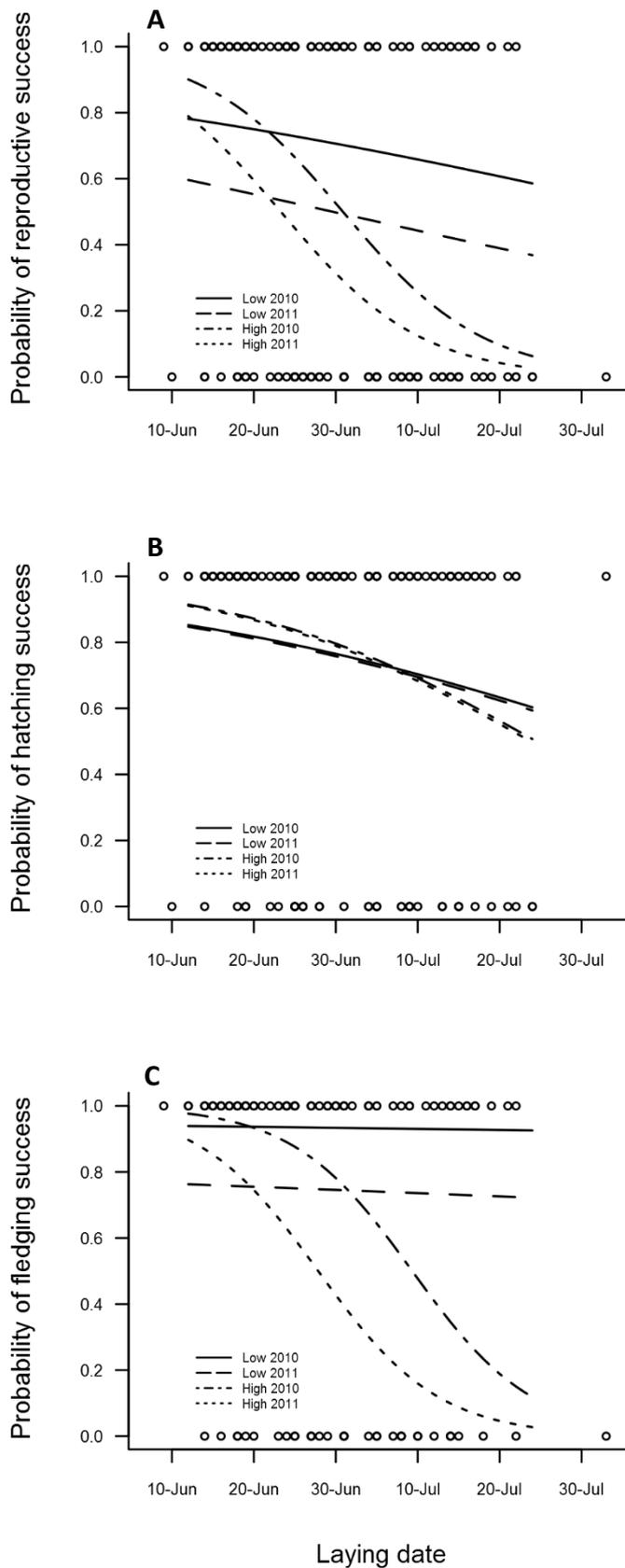


Figure 1.3. Seasonal trend in the probability of (A) reproductive success (n = 157), (B) hatching success (n = 157), and (C) fledging success (n = 117) of storm petrels nesting in areas subject to high and low recreational disturbance in a ‘good’ (2010) and a ‘poor’ (2011) year for overall colony productivity. Lines represent predictions from GLMMs fitted within the range of observed values (open circles). See Table 1.2.

An alternative explanation for the differences in reproductive success observed between disturbed and undisturbed areas could be variation in individual quality which could potentially arise through perceived differences in habitat quality according to human disturbance pressures. In the absence of an experimental manipulation of visitor activity (which was not possible at this site), we cannot wholly discount this possibility. However, if disturbed areas did support birds of lower quality, it would be expected that birds in high-disturbance areas would breed later in the season (Verhulst et al. 1995). In fact, we found no difference in the timing of breeding between disturbance levels, which suggests that the differences observed in reproductive success between high- and low-disturbance areas were not linked to age, experience or condition of adults (Mills 1973). Prior to laying, storm petrels spend little time, if any, at the colony during daylight and are therefore limited in their ability to assess cues related to diurnal human disturbance. We thus expect individuals to be randomly distributed, with respect to individual quality, between disturbed and undisturbed areas.

The cause of reproductive failure in response to disturbance is unknown, though there was no evidence for predation or damage to nests. Breeding failure due to human disturbance is often attributed to birds fleeing the nest, leaving the contents vulnerable to predation or at the mercy of the weather (e.g. Bolduc and Guillemette 2003). Storm petrels were, however, never observed to flee from the nest during the day; the risks associated with leaving the nest are likely to be far greater than staying and enduring a disturbance event. The majority of nestling mortality occurred shortly after hatching; the determination of cause of death would have required a high level of investigator disturbance at the nest. It is therefore not possible to determine whether mortality was driven by abandonment, poor parental care or due to an intrinsic characteristic of the chick itself. There are thus several potential routes by which disturbance could influence nestling survival, either via direct effects on the chick itself, for example via increased physiological stress or elevated energetic demands, or via indirect effects on parental care during incubation and/or the early chick-rearing period. According to life-history theory, animals are expected to adjust current reproductive investment relative to the costs of future survival and reproduction (Stearns 1992). The trade-off between current reproduction and survival is likely to be more marked in long-lived animals, which are expected to favour the maintenance of their own body condition over that of their young (Drent and Daan 1980). Breeding seabirds may thus be particularly responsive to disturbance by humans and more likely to reduce parental effort compared with shorter-lived animals. Although there were no differences in the length of the incubation period or hatching success between high- and low-disturbance nests, it has previously been shown that the prenatal environment can exert constraints on postnatal development even in the absence of effects on incubation period or hatching success (Nilsson et al. 2008).

Previous studies have assumed that visual contact is necessary to invoke a disturbance event (Lunn et al. 2004, Yorio and Boersma 1992) and, therefore, refuge-dwelling animals are less vulnerable to the effects of human disturbance. However, it was shown that a human passing a nest-burrow, out of visual contact, was sufficient to elicit an elevation in heart rate in incubating Humboldt penguins (Ellenberg et al. 2006). The present study supports the work of Ellenberg et al. (2006), demonstrating that negative effects of human activities can occur even when animals do not see the intruder and appear to be protected from impact. This study presents good evidence that, even when humans do not pose a direct mortality risk, they may still be perceived as a predation risk. The suggestion that disturbance can affect animals in refuges via routes other than visual contact, due to noise, vibrations or even odours, has implications for the conservation and management of a wide range of species that utilise refuges.

Most studies of the impacts of recreational disturbance have focused on individual responses, with little consideration for consequences at the population level (though see Mallord et al. 2007, Patthey et al. 2008). While the fitness consequences of human disturbance are clearly important at the individual level, whether these effects result in population-level impacts will depend on the scale at which the disturbance occurs (Gill 2007). By quantifying the effects at the colony level as well as the individual level, we were able to demonstrate that, at the current level of provision of visitor access at the study site, less than 5% of the population are vulnerable to the effects of recreational disturbance. The storm petrel colony at Mousa has more than doubled in size since 1996 (Bolton et al. 2010), in the presence of tourism, and therefore, under the present visitor management scenario, it is not expected that visitor activity will prevent further growth of this colony. However, even a small reduction in population growth rate could have significant implications for seabirds, which are characterised by low fecundity and delayed maturation. Population-level consequences may be more dramatic in other situations, particularly if the population is under additional pressure, for example due to non-native predators or being located at the edge of the species' range.

Conservation management always requires an evaluation of the trade-off between the costs and benefits of public access; in the present case, a small reduction in colony productivity may be accepted in favour of the potential benefits of tourism. This study demonstrates that the potential impacts of visitor activity can be minimised through a combination of a number of management measures including the safeguarding of core areas as refuges, the use of suitably-located and well-marked trails and viewing points, and limited times of access. Recreational users may be unaware of the presence of animals in cavities or burrows and, in contrast to investigators, are less likely to adjust their behaviour accordingly to minimise impacts. It is therefore essential to provide clear guidance to visitors to facilitate low-impact recreational access. Small changes in visitor management,

however, have the potential to significantly alter the scale and magnitude of effects. This highlights the importance for conservation managers to have access to sufficient information to enable them to quantify potential impacts at both the individual and population level and use this to effectively inform management practices. Managers should explore both traditional and novel opportunities for offsetting restrictions on access by offering visitors an enhanced experience of their visit; to name but a few suggestions, this could include provision of high-quality interpretation boards, remote viewing facilities and interactive web-based resources that visitors can access following their visit.

Conclusions

Increasing interest in nature-based tourism is expected to lead to increased pressures on coastal and marine habitats and the seabird colonies they support. Burrowing seabirds belonging to the Procellariiformes (shearwaters and petrels), Alcidae (auks) and Sphenisciformes (penguins) are already facing extensive threats at breeding sites from loss and degradation of habitat as a result of human interference and introduction of mammalian predators, as well as impacts at sea, such as mortality in long-line fisheries

(Croxall et al. 2012). The effects of tourism could exacerbate the population declines that are widely reported amongst seabirds. Although further research is required to determine the proximate cause of failure associated with disturbance, the study presents good evidence that, even in the absence of visual contact, the presence of human activity above ground can have negative effects on animals remaining out of sight in refuges. Humans may be perceived as a predation risk and stimulate appropriate anti-predator responses, even in the absence of a direct mortality risk. Negative consequences for individual fitness, however, do not necessarily translate to population-level effects and thus it is critical that conservation managers have the necessary information to evaluate impacts at both the individual and population level. The relative costs and benefits of public access should be evaluated on a site- and species-specific basis. While the results have most immediate relevance to the conservation and management of seabird colonies across the world, they also highlight the potential for impacts of recreational disturbance on a wider range of species utilising refuges. Conservation managers should consider the potential for impacts on less obvious animals and it should not be assumed that animals remaining out of sight are out of harm's way.

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2. Effects of repeated exposure to human disturbance on avian postnatal growth and stress physiology

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Abstract

Given the rapid rate of anthropogenic-induced environmental change, it is of increasing importance to understand the consequences of human activities on all life-history stages in free-living animals. Unfavourable environmental conditions during early life can not only impair immediate growth and phenotypic development, but can also translate into long-term fitness consequences. Measures of adrenocortical function are increasingly used to assess the physiological status of free-living animals, but studies have failed to show any consistent relationship between glucocorticoid levels and fitness. In breeding storm petrels, nestling mortality is higher in nests subject to repeated exposure to human recreational disturbance. Here, we examine whether surviving nestlings reared in disturbed environments are also affected and how this might translate into changes in corticosterone secretion and growth trajectories. Nestlings reared in high-disturbance areas displayed lower rates of both structural and mass growth, compared with nestlings reared in undisturbed areas. However, neither baseline corticosterone nor fledging body size or mass were negatively affected by human disturbance. While developmental plasticity enabled high-disturbance nestlings to achieve a similar body size and larger mass at fledging, an extended rearing period and probable delay in fledging may carry hidden long-term costs for parents and offspring. The results emphasise the importance of studying individual responses throughout the entire development period to fully understand environmentally-induced plasticity. We recommend an integrated approach incorporating robust behavioural, morphological and physiological monitoring to fully understand the consequences of repeated exposure to human disturbance during early life.

Introduction

Organisms are currently being exposed to rapid rates of anthropogenic-induced environmental change, yet we know relatively little about how exposure to human disturbance during early life may affect aspects of growth and physiological development in wild populations. Environmental influences during development play an important role in shaping an individual's phenotype, giving rise to differences in behaviour, morphology and physiology (Lindström 1999, Monaghan 2008). Stressful conditions in early life can not only reduce immediate growth and survival, but can have long-term negative consequences for fitness, extending far beyond the timescale of the initial stressor (Haywood and Perrins 1992, Birkhead et al. 1999, Lee et al. 2013). As public access to wildlife is increasingly encouraged, it is of growing importance to understand the consequences of recreational activities for individual development, which may ultimately affect population viability. As well as having fundamental biological significance, an increased knowledge of how free-living organisms respond to human disturbance can facilitate the achievement

of conservation goals and maintenance of ecological and economic sustainability of nature-based tourism.

Developmental growth rates can be highly plastic and have been widely documented to change in response to environmental cues (reviewed in Arendt 1997). In birds, studies investigating the costs associated with exposure to human disturbance during early life typically measure effects at a single time-point - usually at the end of the nestling stage - and have produced mixed outcomes. Human disturbance has been linked with reduced body mass at fledging in birds (yellow-eyed penguin *Megadyptes antipodes* McClung et al. 2004, Cassin's auklet *Ptycoramphus aleuticus* Albores-Barajas et al. 2009), while other studies have found no difference in body mass between nestlings reared in tourist-exposed sites and undisturbed sites (Magellanic penguin *Spheniscus magellanicus* Yorio and Boersma 1992, gentoo penguin *Pygoscelis papua* Cobley and Shears 1999, hoatzin *Opisthocomus hoazin* Müllner et al. 2004). However, the degree of human disturbance will likely differ between study sites. Changes in offspring growth rates in response to human disturbance have been assumed, for example when provisioning rates are reduced (European oystercatcher *Haematopus ostralegus* Verhulst et al. 2001), though data on actual growth rates are rarely collected. While the environment may constrain growth below an optimum rate, individuals may be able to compensate for slow growth by subsequently accelerating growth, should conditions become more favourable, or extending the period of time to reach a given developmental stage (reviewed in Dmitriew 2011). By allowing adjustment of growth trajectories, such developmental plasticity may enable individuals to overcome the short-term costs of slow growth. However, even if an organism is able to recover from a poor start in life and attain the usual body size and mass, there may be long-term detrimental effects on fitness of the adult phenotype and even fitness of their offspring (Metcalf and Monaghan 2001, 2003).

The endocrine system plays a major role in mediating the effects of exposure to environmental stressors on phenotypic development via the hypothalamic-pituitary-adrenal (HPA) axis and the production of glucocorticoids (GCs; Wingfield and Kitaysky 2002). Although short-term secretion of GCs can be beneficial, by mediating adaptive responses to an acute environmental challenge (Wingfield and Kitaysky 2002), chronically-elevated levels of circulating GCs can have detrimental effects for growth, immune function, cognition, and survival (Davison et al. 1983, Sapolsky et al. 2000, Kitaysky et al. 2003, Blas and Bortolotti 2007). Increasingly, measures of GC levels are being used as biomarkers to assess the physiological status of populations and address conservation issues (Wasser et al. 1997, Romero 2004, Walker et al. 2005b, Wikelski and Cooke 2006, Busch and Hayward 2009). GC levels are interpreted as an index of "stress" or allostatic load (Romero 2004) and it is assumed that elevated baseline GCs indicate individuals or populations in poorer condition and with lower fitness (Bonier et al. 2009). However, GCs do not

always change predictably in response to environmental challenges (reviewed in Busch and Hayward 2009) and studies fail to find a consistent relationship between baseline GCs and fitness (Bonier et al. 2009). Most studies of human disturbance focus on the effects on adults, while relatively few authors have considered the potential consequences for developing young. Repeated exposure to human disturbance has been linked to elevated baseline and stress-induced levels of GCs in developing avian young (Magellanic penguin Walker et al. 2005a). Conversely, repeated investigator handling was shown to reduce baseline and/or stress-induced GC levels in nestlings of the American kestrel *Falco sparverius* (Love et al. 2003a, Whitman et al. 2011), although another study demonstrated no relationship between human disturbance and GCs in American kestrel chicks (Strasser and Heath 2011).

Seabirds are among the most globally-threatened groups of birds (Croxall et al. 2012). Human disturbance has been identified as one of the main threats that they face (Croxall et al. 2012), yet breeding colonies attract increasing numbers of tourists. Daily human recreational disturbance is associated with increased nestling mortality in the European storm petrel *Hydrobates pelagicus* (hereafter, storm petrel), a cavity-nesting nocturnal seabird (Chapter 1, Watson et al. 2014). In the present study, we examined whether surviving young reared in areas exposed to high tourist activity are also affected through changes in their stress physiology and/or growth trajectories, compared with nestlings reared in areas subject to low visitor pressure. Due to their slow development, incubating adults and nestlings can be exposed to repeated disturbance at the underground nest over a period in excess of 6 wk; they remain within the confines of the nest cavity and are unable to relocate to avoid a disturbance event. Developing young could thus be affected by direct exposure to disturbance and/or indirectly via influences on adults. By taking regular measurements of body size and mass throughout postnatal development, we were able to study different components of growth in nestlings reared in disturbed and undisturbed areas. We examined whether baseline plasma corticosterone (CORT – the primary GC in birds) in nestlings changes in response to repeated exposure to disturbance and is related to growth trajectories.

Methods

Study site and species

The study was conducted at the island of Mousa, located in the Shetland archipelago, UK (60°0'N, 1°10'W). The island is designated under the EU Birds Directive as a Special Protection Area, for which the storm petrel is a qualifying feature. On Mousa, storm petrels nest in crevices in dry stone walls, boulder beaches and derelict buildings. The storm petrel lays a single egg, which is incubated for c. 40 d; the chick is brooded for c. 7 d and fledges at 65-70 d (Davis 1957). Storm petrel nestlings are semi-precocial: they hatch with a thick layer of

down and become thermally independent at an early age, but they do not leave the nest until fledging and are completely dependent on the parents for food. By studying a species that produces only one offspring per breeding attempt, we were able to eliminate the confounding factor of sibling competition on growth and physiological development (Love et al. 2003b).

Mousa receives 4000-5000 visitors during the breeding season due to its wildlife and archaeological interests. The average number of visitors per day was consistent throughout the breeding seasons of 2010 and 2011 (mean \pm SE: June: 27.8 ± 3.1 ; July: 26.3 ± 3.7 ; August: 27.9 ± 3.1). The island has remained uninhabited since 1853 and is not exposed to any regular human disturbance outside of tourism during the summer months. Visitor management ensures that the majority of activity is concentrated within a 3.2 km circular route. Based on observations of visitor behaviour, human activity is largely confined to a belt of 10 m either side of the marked path. For this reason, we considered nests located within 10 m of the path to be subject to 'high' levels of visitor pressure. Visitors rarely explore beyond this area and we adopted a conservative threshold distance of 150 m from the path, beyond which we considered nests to be subject to 'low' levels of visitor pressure. Study nests were distributed among six 'plots', geographically spread across the island. Three plots were subject to high levels of visitor pressure, while the other three plots were subject to low levels of visitor pressure. Study nests were located in either walls or rock scree and both of these nesting habitats were equally represented within the high- and low-disturbance categories. There were no other differences in surrounding habitat or environmental conditions between plots.

Biometrics and blood sampling

Data were collected from 55 nestlings that survived through to fledging in the breeding seasons of 2010 (28 nestlings) and 2011 (27 nestlings). Of these, 24 nestlings were reared in nests subject to high levels of recreational disturbance and 31 were from nests subject to low disturbance. Hatching dates spanned a range of 41 d. Blood was collected from all but one nestlings ($n = 54$) on two occasions (where possible), in the first half (median: 12 d; range: 8–33 d; $n = 47$) and second half (median: 43 d; range: 32–57 d; $n = 47$) of postnatal development. Repeated samples were obtained from 41 nestlings and the median number of days elapsed between subsequent measurements from the same individual was 31 d (range: 22–46; $n = 41$). Whole blood was obtained by venepuncture of the brachial vein and collected in heparinised capillary tubes under licence from the UK Home Office. All blood samples were collected within 3 min of the onset of disturbance (determined as when the investigator approached to within 1 m of nest), which is considered representative of baseline CORT (Romero 2004) and there was no effect of blood sample time on CORT levels (LMM: $t = 1.24$, $P = 0.218$). Blood samples

were stored at $<5^{\circ}\text{C}$ for up to 8 hr prior to being separated by centrifugation. Plasma and red blood were stored at $<5^{\circ}\text{C}$ in the field for a maximum of 3 d before being transferred to -20°C for up to 3 months; following this, samples were stored at -80°C until laboratory analysis was performed. Following blood sampling, tarsus length, body mass and wing length (where relevant) were measured, as described below.

Tarsus length and mass were regularly measured throughout the nestling period between 8 ± 1.0 and 51 ± 1.3 d (means \pm SE; $n = 55$). The median number of days on which individuals were measured was 7 d in 2010 and 16 d in 2011. While investigator disturbance thus differed between years, it did not differ between treatments within years. The length of the tarsus was measured to the nearest 0.1 mm using callipers and mass was recorded to the nearest 0.25 g using a spring scale. Wing length (maximum flattened chord) was measured to the nearest 1 mm using a stopped rule, once the outermost primary feather emerged from the quill sheath (c. 30 d). Nestling mass varies greatly according to feeding history and the time elapsed since last feeding; chicks lose weight at a constant rate throughout the day and can lose up to 7 g d^{-1} (Bolton 1995). To account for differences in timings of weighings, mass was corrected to a standardised time (18:00 h) according to the age-related rate of proportional weight loss (see Bolton 1995).

Determination of baseline plasma corticosterone

Plasma CORT levels were determined using corticosterone enzyme immunoassay kits (Enzo Life Sciences Inc.). CORT was first extracted from plasma with 2 ml diethyl ether in a dry ice-methanol bath, evaporated under nitrogen gas and re-suspended in 300 μl of the assay buffer. Prior to extraction, plasma was spiked with [^3H]-CORT to calculate recovery rate. Average recovery of samples was 84.5%. Samples were run in duplicate at a 1:20 dilution and repeated samples were run on the same plate. The standard curve was measured in triplicate with six standards ranging from 20,000 to 15.63 pg ml^{-1} . After adding stop solution, absorbance was measured immediately using a Thermo Multiskan EX plate photometer at 405 nm and corrected for 570 nm. Values were corrected for initial plasma volume and individual recovery. Mean inter-assay and intra-assay coefficients of variation were 6.4% and 9.3%, respectively. The detection limit of the assay was 0.46 ng ml^{-1} ; this was calculated by taking two standard deviations away from the mean of the total-binding wells. One sample fell below the detection limit and was assigned the detection limit. Sex was determined following the molecular method described by Griffiths et al. (1998).

Statistical analyses

All statistical analyses were performed in R 3.0.0 (R Core Team 2013). Full details are provided in the Supplementary material, Appendix 1. Linear mixed models

(LMMs) with a normal error structure and including a random effect of nestling identity were fitted to data on nestling CORT (log-transformed) and wing length. To investigate whether baseline CORT or the change with age varied between disturbance levels, a saturated model included the fixed effects of disturbance (high or low), age (covariate) and their two-way interaction. To control for seasonal and inter-annual variation, we included the fixed effects of hatching date (covariate) and year (2010 or 2011) and their interactions with age. The inclusion of the two-way interactions of hatching date x disturbance and year x disturbance controlled for seasonal and inter-annual variation, respectively, in the effect of disturbance on baseline CORT. We also considered a number of other candidate explanatory variables that may influence baseline CORT including sex, body condition index (residuals from regression of mass on age; covariate), and time of day (covariate). As with analysis of CORT data, we wanted to know if wing length varied between disturbance levels and whether the rate of growth (i.e. change in length with age) varied between disturbance levels, as represented by the interaction between age and disturbance. Again, a saturated model included the fixed effects of hatching date and year, as well as their two-way interactions with age and disturbance. We also considered that length and growth rate might differ between the sexes by including sex as a fixed factor and the interaction between sex and age, respectively.

Nonlinear mixed-effects models using the Gompertz function were fitted to data on tarsus length and body mass to test for variation in nestling growth trajectories in relation to human disturbance. Non-linear models were fitted using the FlexParamCurve package in R (Oswald et al. 2012). The Gompertz function describes the relationship between tarsus length (mm)/mass (g) and age (d) as a function of A the asymptotic length (mm)/mass (g), k the growth rate constant (rate of change of slope with age), and i the inflection point (age at maximum growth rate). The asymptote and inflection point were allowed to vary randomly with respect to nestling identity. Hatching date and year were included as fixed effects to control for seasonal (for k and i only) and inter-annual (A , k , and i) variation, respectively. Since there were fewer observations around the asymptote for later-hatching nestlings, hatching date was not allowed to affect the asymptote. Seasonal and inter-annual variation in the effect of disturbance were considered by including the interactions of hatching date x disturbance (again for k and i only) and year x disturbance (A , k , and i). Sex was allowed to affect all three parameters. All results are presented from minimum adequate models attained by stepwise deletion (refer to Supplementary material, Appendix 1). Results are presented as means \pm SE, unless otherwise stated.

Results

Variation in baseline CORT with disturbance

There were no differences in the concentration of baseline CORT between nestlings reared in low-disturbance sites and high-disturbance sites (Fig. 2.1; disturbance(low): $\beta = -0.07 \pm 0.15$, $t = -0.46$, $P = 0.654$). Baseline CORT significantly decreased with increasing age during postnatal development in all nestlings (Fig. 2.1; $\beta = -0.026 \pm 0.004$, $t = -6.03$, $P < 0.001$), but this change with age did not differ between disturbance levels (age x disturbance(low): $\beta = -2.06e-3 \pm 9.1e-3$, $t = -0.23$, $P = 0.826$). There was no effect of sex, hatching date, year, body condition or time of day on baseline CORT (all $P > 0.3$). The variance (mean \pm SD) associated with the random effect of nestling identity was $4.1e-18 \pm 2.0e-09$.

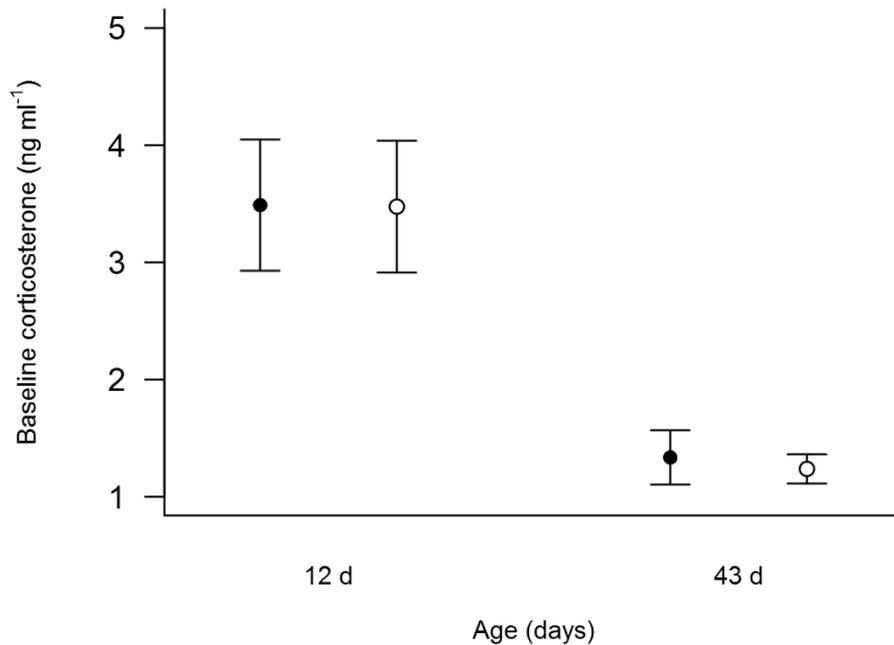


Figure 2.1. Mean \pm SE baseline corticosterone during early and late postnatal development of storm petrel nestlings reared in nests subject to high (closed circles) and low (open circles) levels of human recreational disturbance. $n = 100$; number of nestlings = 54.

Variation in nestling growth with disturbance

Wing

There were no differences in length (disturbance(low): $\beta = 1.33 \pm 1.82$, $t = 0.73$, $P = 0.415$) or growth rate (age x disturbance(low): $\beta = -0.017 \pm 0.06$, $t = -0.30$, $P = 0.408$) of the wing between nestlings reared in high- and low-disturbance areas (Fig. 2.2). Neither wing length (sex(male): $\beta = 0.05 \pm 1.80$, $t = 0.03$, $P = 0.442$) nor growth rate of the wing (age x sex(male): $\beta = -0.09 \pm 0.05$, $t = -1.62$, $P = 0.642$)

differed between the sexes. There was no significant effect of hatching date or year on wing length or growth rate (all $P > 0.05$). The wing grew at an average rate of 2.5 mm day^{-1} . The variance (mean \pm SD) associated with the random effect of nestling was 42.36 ± 6.5 .

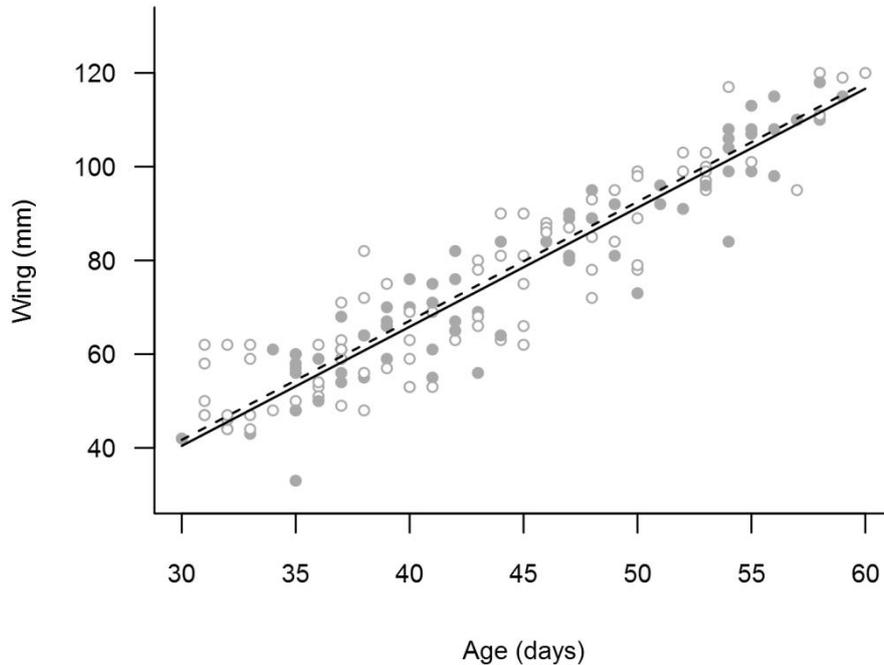


Figure 2.2. Wing growth of storm petrel nestlings reared in nests subject to high (closed circles; solid line) and low (open circles; dashed line) levels of human recreational disturbance. Lines represent predictions from the minimum adequate LMM. $n = 159$; number of nestlings = 55.

Tarsus

The growth rate constant (k) for tarsus growth of nestlings reared in high-disturbance nests was significantly lower than that of nestlings from low-disturbance sites (Fig. 2.3A; disturbance(low): $\beta = 0.006 \pm 0.002$, $t_{398} = 2.46$, $P = 0.014$). The k value for disturbed nestlings was 0.95 times that of undisturbed nestlings. There was no seasonal (hatching date \times disturbance(low): $\beta = 0.0001 \pm 0.0003$, $t_{389} = 0.51$, $P = 0.610$) or inter-annual (year(2011) \times disturbance(low): $\beta = 0.010 \pm 0.006$, $t_{396} = 1.86$; $P = 0.064$) variation in the effect of disturbance on k . Neither the asymptote (disturbance(low): $\beta = 0.17 \pm 0.23$, $t_{394} = 0.72$, $P = 0.470$) nor inflection point (disturbance(low): $\beta = -0.58 \pm 0.58$, $t_{392} = -1.01$, $P = 0.315$) varied between disturbance levels (Fig. 2.3B). The inflection point occurred at a later age in later-hatching chicks ($\beta = 0.06 \pm 0.03$, $t_{398} = 2.29$, $P = 0.022$) but there was no effect of hatching date on k ($\beta = 0.0002 \pm 0.0001$, $t_{395} = 1.43$, $P = 0.154$). There were no inter-annual effects on either A , k or i . For the average nestling (based on an average hatching date of 6 August), the time taken to reach 90% of the asymptote was 35 d and 36 d in nestlings reared in low-disturbance sites and high-

disturbance sites, respectively. During this period of growth, the average daily increase in tarsus length was 0.33 mm and 0.31 mm in low- and high-disturbance nestlings, respectively. There were no differences in tarsus growth between male and female nestlings (all $P > 0.4$). The estimated variances (mean \pm SD) associated with the random effect of nestling were 0.46 ± 0.70 (A) and 2.71 ± 1.65 (i), with a correlation of 0.04.

Mass

All three parameters describing the rate of mass gain in nestlings were significantly affected by disturbance (Fig. 2.3B). Nestlings reared in low-disturbance nests exhibited a lower asymptote ($\beta = -2.98 \pm 1.35$, $t_{521} = -2.21$, $P = 0.028$), higher growth rate constant ($\beta = 0.017 \pm 0.006$, $t_{521} = 2.67$, $P = 0.008$) and earlier inflection point ($\beta = -2.22 \pm 0.81$, $t_{521} = -2.71$, $P = 0.007$). The average nestling from a low-disturbance nest exhibited a k value that was 0.85 times that of an average nestling from a high-disturbance nest. There were no significant interactions between disturbance and hatching date and disturbance and year on any parameter, demonstrating there was no seasonal or inter-annual variation in the effect of disturbance on mass growth (all $P > 0.3$). For the average nestling, the time taken to reach 90% of the asymptotic mass was 32 d in nestlings from low-disturbance sites and 38 d in nestlings reared in high-disturbance sites. From the inflection point to 90% of the asymptote, the average daily increase in mass was 0.88 g and 0.74 g in low- and high-disturbance nestlings respectively. The asymptote ($\beta = 3.31 \pm 1.23$, $t_{521} = 2.70$, $P = 0.007$) and inflection point ($\beta = 4.49 \pm 0.80$, $t_{521} = 5.62$, $P < 0.001$) were significantly higher and later, respectively, in 2011, compared with 2010, while there was no inter-annual variation in k ($\beta = -0.01 \pm 0.01$, $t_{519} = -1.30$, $P = 0.195$). Nestlings that hatched later in the season exhibited a significantly lower growth rate constant ($\beta = -0.001 \pm 0.00$, $t_{521} = -3.10$, $P = 0.002$). While the best-fit model retained a fixed effect of hatching date on i , the effect was not quite significant ($\beta = 0.06 \pm 0.03$, $t_{521} = 3.47$, $P = 0.05$). There were no differences in mass gain between male and female nestlings (all $P > 0.4$). The estimated variances (mean \pm SD) associated with the random effect of nestling were 15.85 ± 4.0 (A) and 4.61 ± 2.1 (i), with a correlation of 0.67.

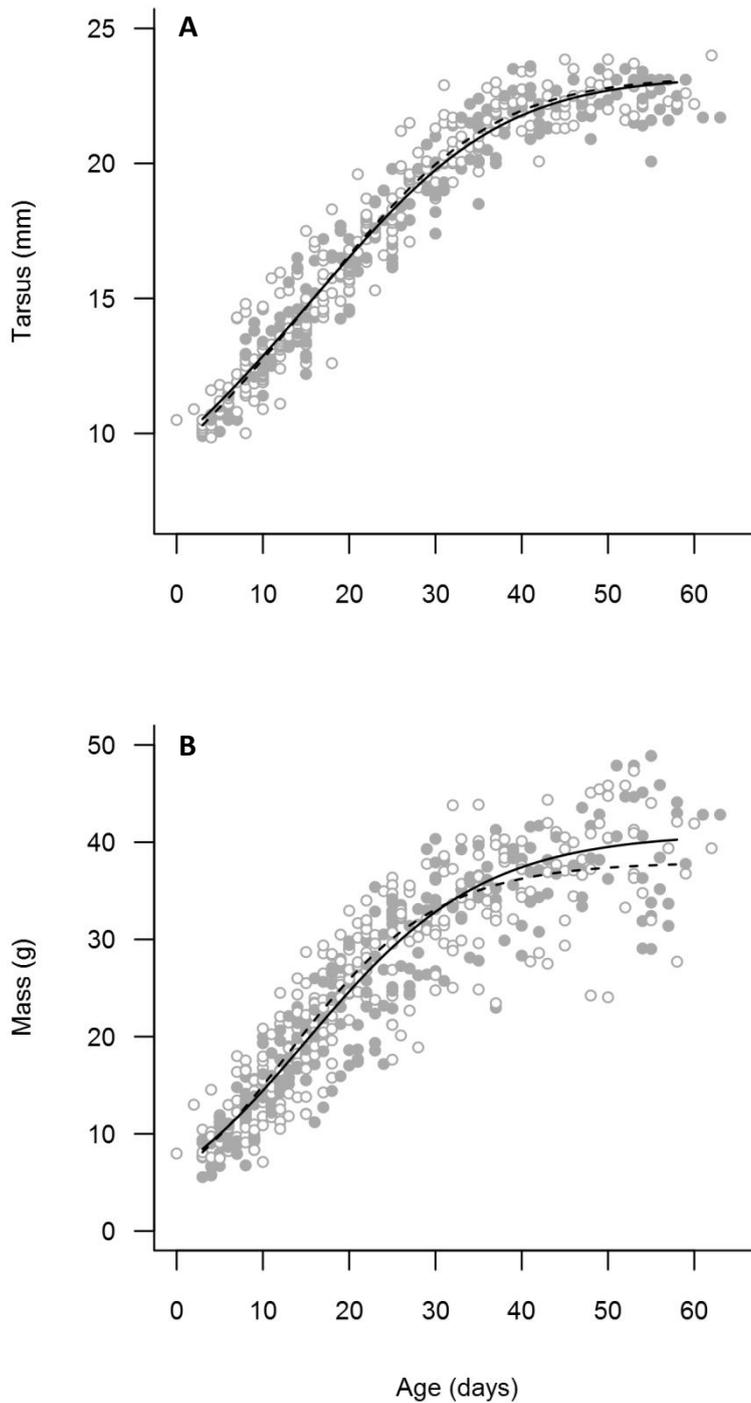


Figure 2.3. Growth curves for (A) tarsus ($n = 459$) and (B) mass ($n = 585$) of storm petrel nestlings ($n = 55$) reared in nests subject to high (closed circles; solid line) and low (open circles; dashed line) levels of recreational disturbance. Lines represent predicted growth curves for the average nestling generated from non-linear mixed models fitted by the Gompertz function and within the range of observed values. Although the magnitude of the effect of disturbance on tarsus growth is small, the growth rate constant of high-disturbance nestlings was significantly lower, compared with low-disturbance nestlings.

Discussion

Despite exhibiting overall slower growth in respect of both structural development (tarsus, but not wing) and mass gain and delayed maximal growth (mass only), nestlings reared in an environment subject to high levels of daily recreational disturbance caught up with nestlings from low-disturbance nests by extending the growth period. This enabled them to achieve a similar body size and, on average, higher mass. Despite the differences in asymptotic mass, it is not known if there were differences in the extent of mass regression between high- and low-disturbance nestlings and whether differences in mass persisted at the time of fledging. There was no evidence for high-disturbance nestlings being chronically stressed, as suggested by the absence of any differences in baseline CORT. However, since we did not measure stress-induced levels, we cannot exclude the possibility that repeated stress exposure may have modified responsiveness of the HPA axis to stressors. Repeated exposure to a stressor during early life has been shown to lead to both hyper- (Spencer et al. 2009) and hypo-responsiveness (Love et al. 2003a) of the HPA axis. While surviving nestlings were able to compensate for sub-optimal development conditions, almost half of nestlings reared in high-disturbance nests did not survive to fledging (Chapter 1, Watson et al. 2014). By studying only the surviving young, we were most likely looking at the least affected individuals. At the current level of disturbance, the effect sizes are small, though could be exacerbated under scenarios of elevated recreational pressure.

The depressed growth rates and extended growth period displayed by high-disturbance nestlings may carry costs for both parents and offspring. Slow growth and a subsequent extension of the growth period have been widely associated with delayed fledging (Bize et al. 2003, Searcy et al. 2004). Storm petrels experience very low nest predation rates and demonstrate highly asynchronous fledging; therefore, a small delay in fledging is unlikely to pose an appreciable survival cost for nestlings hatching early in the season. Any costs associated with a delay in fledging, however, may be far more significant for late-hatching young, which grow more slowly and consequently have an even longer growth period. Slow growth was previously found to be associated with a delay in fledging of up to 12 d in storm petrel nestlings (Scott 1970). Parents that fed their chicks at a reduced rate in early development were able to compensate for this by continuing to feed nestlings for considerably longer than the normal nestling period (i.e. of those that were well fed throughout development). The increased demands of an extended rearing period may thus reduce subsequent parental reproductive success or survival, since life-history theory predicts that increased current reproductive effort should reduce reproductive success or survival in future years (Stearns 1992). In this way, an environmental stressor, such as human disturbance, may alter the trade-off between

current and future reproduction which could have profound effects on individual life histories.

Despite the potential costs, growth plasticity can be adaptive, since the immediate survival benefit of attaining a normal size probably has a greater impact on fitness than costs incurred later in life. The ability to reach a large body mass is advantageous, since body mass at fledging is generally positively correlated with survival and recruitment in birds (Krementz et al. 1989, McClung et al. 2004). High-disturbance nestlings appeared to prioritise structural growth over mass accumulation. The growth rate of the wing was no different to that of low-disturbance nestlings and the tarsus grew at only 95% of the rate of undisturbed nestlings. While it only took one extra day for the tarsus of high-disturbance nestlings to achieve 90% of the asymptotic size, the same development period was extended by an extra six days in respect of mass gain. The differential patterns of growth observed suggest the existence of trade-offs in energy allocation to different structural components and energy storage, in which high-disturbance nestlings invested more resources in skeletal and plumage development than mass gain. Similar patterns of differential growth allocation have been demonstrated in a number of avian species (e.g. Nilsson and Svensson 1996). While a growth strategy in which allocation to wing growth is prioritised is often explained by selection for synchronised fledging in altricial species (Nilsson and Svensson 1996), in aerially-active birds, such as storm petrels, the selective pressure may be the optimisation of wing load at fledging. The ability to display plasticity in the developmental strategy enabled nestlings to overcome the poor early growth environment and avoid the potential short-term costs of fledging at a smaller size.

Slow postnatal growth can arise via intrinsic or extrinsic energetic constraints on the developing individual. In other words, growth rate can be limited by either physiological constraints of the chick or the rate of provisioning by parents. The low magnitude of effects on developmental growth and absence of evidence for chronic stress suggest that any direct effects of disturbance on nestlings were small. The small differences in mass and structural growth could be mediated by changes in energetic demands (see Regel and Pütz 1997), which could arise if, for example, disturbance were to result in increased activity of chicks within nests. Alternatively, constraints imposed on prenatal development, as a result of exposure of adults to disturbance during incubation, may persist into postnatal development. Although there were no differences in the length of the incubation period or hatching success between high- and low-disturbance nests (Chapter 1, Watson et al. 2014), it has previously been shown that the prenatal environment can exert constraints on postnatal growth even in the absence of effects on incubation period or hatching success (Nilsson et al. 2008). Another potential route for indirect effects of disturbance on nestling growth is via effects on postnatal parental care and provisioning rates. Storm petrels forage far out to sea during the day, only

returning to the colony to provision the chick at night. Due to this spatial and temporal dissociation between human activity and foraging sites and chick feeding, respectively, postnatal parental care should not be affected by direct human interference. Parental care during the chick-rearing phase, however, could be influenced via carry-over effects as a consequence of exposure of adults to disturbance during incubation. Provisioning rates are likely to be dependent on adult condition (e.g. Lorentsen 1996); if adults breeding in disturbed areas perceive disturbance as a stressor, they may respond with physiological adjustments that have a negative impact on body condition and subsequently reduce their ability to provision chicks at an optimal rate. None of these mechanisms discussed are mutually exclusive and they may be acting in tandem to influence postnatal growth rates. Further studies are required to determine both the proximate and ultimate mechanisms underlying the variation in nestling growth rates, but here we present good evidence for the existence of plasticity in developmental growth in response to recreational disturbance.

Despite exhibiting growth-rate depression and an extended growth period, neither baseline CORT nor pre-fledging body size or mass were negatively affected by human disturbance. Further research is required to fully understand whether this environmentally-induced plasticity is adaptive and the relative costs and benefits of different development strategies. The results emphasise the importance of studying individual responses throughout the entire development period to fully understand the consequences of exposure to repeated stressors. There is no doubt that the use of physiological tools are valuable for developing an integrated understanding of individual or population responses to environmental perturbations, but the present study emphasises the need for caution in simplistic interpretations of GC measures alone, without additional physiological or behavioural data. Future studies should consider the measurement of additional physiological metrics, such as oxidative stress, immune function and telomere dynamics (see Breuner et al. 2013), to develop a more integrated understanding of the impacts of repeated exposure to environmental stressors on individual life histories. Given the increasing pressures associated with nature-based tourism and the potential short- and long-term costs associated with sub-optimal growth, it is critical that scientists and practitioners afford more attention to the potential effects of repeated exposure to human disturbance during early life and the consequences for individual fitness and population viability.

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Supplementary material

Appendix 1

Statistical analyses

Linear analysis of baseline CORT and wing length

All statistical analyses were performed in R 3.0.0 (R Core Team 2013). General linear mixed models were fitted to log-transformed baseline corticosterone and wing length in the lme4 package (Bates et al. 2013). First, the optimal random effects structure was found by comparing nested models, fitted by restricted maximum likelihood (REML) estimation, using likelihood ratio tests (LRTs). Nested models had a saturated fixed component, but different random effects structures. We considered the inclusion of a 3-tiered nested random effect of nestling identity, nest identity and plot (listed inner to outer). The random effects of nestling identity and nest identity accounted for the lack of independence between repeated measures from individuals (within years) and nests (between years), respectively. Although we do not know the identity of adults, it is possible that nests were occupied by the same breeding pairs in both years. The random effect of plot accounted for potential spatial autocorrelation in nest or parental quality within different plots. The estimated variances (mean \pm SD) associated with nest identity and plot were close to zero and did not significantly improve the model fit. Only the random effect of nestling identity was retained in models.

Starting with a saturated model, the optimal fixed effects structure was obtained using a stepwise deletion approach, removing terms that did not significantly differ from zero ($P > 0.05$). The significance of model parameters was estimated by comparison to a probability distribution obtained from 10,000 Markov Chain Monte Carlo (MCMC) simulations using `pvals.fnc()` in language, which does not require estimating degrees of freedom. Results are presented from models fitted by REML.

Non-linear analysis of tarsus length and mass

Nonlinear mixed-effects models were fitted to data on tarsus length and mass to test for variation in nestling growth trajectories in relation to human disturbance. This approach offers many advantages over traditional methods of analysing growth data (see Sofaer and Chapman 2013). Using the FlexParamCurve package (Oswald et al.

2012) in R, we first identified the nonlinear functions that best fitted data on the change in tarsus length and mass with age. Both a four-parameter function and a three-parameter Gompertz function were identified as strong candidates for fitting tarsus and mass data. While four-parameter models offer more flexibility in terms of describing the shape of a curve, they are more demanding with respect to data quality and may result in overfitting of a model (Zach 1988). Although storm petrels are known to undergo a period of mass recession prior to fledging, best described by a four-parameter curve, such mass loss was not detected for most individuals, either because it occurred beyond the end of data collection or did not occur at all (e.g. Barrett and Rikardsen 1992). In respect of both tarsus and mass data, the three-parameter Gompertz functions fitted more individuals and we thus opted for the more parsimonious models to avoid overparameterisation.

Individual curves were first fitted to each nestling using `nlsList()` to derive suitable initial parameter estimates to be carried forward to nonlinear mixed models fitted in the `nlme` package (Pinheiro et al. 2013). Since `nlme()` can only fit `nlsList` objects with a single grouping variable and earlier analyses found the estimated variances associated with the random effects of nest and plot were close to zero and did not significantly improve model fits, only the random effect of nestling identity was included. To find the optimal random effects structure, we used an information theoretic approach based on the Akaike Information Criterion (AIC). Different combinations of the three curve parameters (A , k , and i) were allowed to vary randomly with nestling identity and the AIC values of models were compared (see Sofaer et al. 2013). If a model failed to converge, it was considered to be overparameterised and not considered further in the model selection process. The random effect of nestling identity was retained in nonlinear mixed models fitted to tarsus and mass data in respect of two parameters - the asymptote (A) and inflection point (i). The relationship between the inflection point and the asymptote was not consistent across individuals and so there was strong support for a model that allowed both parameters to vary randomly. For mass data, this model had strongest statistical support (i.e. lowest AIC). For tarsus data, allowing k (growth rate constant) to vary randomly with respect to nestling identity improved the model fit, but the estimated variance was close to zero (mean \pm SD: $1.19e^{-04} \pm 0.01$) and there was reasonable correlation between k and i (0.48). For these reasons, only A and i were allowed to vary randomly in respect of nestling identity.

We tested for differences in the asymptote, growth rate constant and inflection point for tarsus and mass growth between nestlings reared in high- and low-disturbance nests, while the shape parameter m was fixed at the mean across datasets: 3.5 for tarsus and 0.8 for mass. The optimal fixed effects structure was obtained by stepwise deletion performing LRTs on nested models fitted by maximum likelihood. Since MCMC sampling is not implemented for nonlinear mixed effects modelling in R, the significance of parameter estimates was estimated

using conditional *t*-tests, which requires estimating degrees of freedom. Refer to Pinheiro and Bates (2000) for the method of estimation of degrees of freedom in nlme().

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3. Repeated handling in early life does not affect baseline corticosterone or telomere length in semi-precocial nestlings

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Abstract

Nestling birds are regularly handled for ecological research, but few studies consider the potential for stress induced by repeated handling to influence hormonally-mediated phenotypic development or bias interpretations of physiological measurements. We experimentally manipulated the handling experience of European storm petrel *Hydrobates pelagicus* nestlings and examined the effects on pre-fledging baseline corticosterone (CORT), telomere length (TL) and body condition. Following initial handling to estimate age and record biometrics, nestlings were either left undisturbed or exposed to regular handling. The cumulative number of handling episodes ranged from 1-7 over the course of 14-39 d during postnatal development. Neither baseline CORT, TL nor body condition varied with the number of handling episodes. We find no evidence that handling induced chronic stress, typically associated with elevated baseline CORT, in nestlings. Few studies have simultaneously examined GCs and TL, yet GCs have been shown to mediate damaging effects of stress on TL. Repeated elicitation of an adrenocortical response can lead to acclimation, without modification of baseline CORT levels, but might be expected to result in accelerated telomere attrition and short telomeres. The absence of effects on TL therefore suggests that habituation did not occur, though in the absence of stress-induced CORT measures, we cannot exclude this possibility. It is possible that the level of handling applied was not sufficient to induce detectable effects or nestlings did not perceive handling to be stressful. Eliciting a response to a stressor may be maladaptive for young that are unable to escape or defend themselves in a predation attack. In conclusion, within the limits of handling applied in a typical field study, the results suggest that storm petrel nestlings appear robust to investigator handling.

Introduction

It is well understood that early-life experiences can have profound and long-lasting effects on phenotypic development (Lindström 1999, Monaghan 2008). Unfavourable conditions during early life can influence immediate growth, survival, and immune function, as well as affect later-life performance (Haywood and Perrins 1992, Sedinger et al. 1995, Verhulst et al. 1997, Birkhead et al. 1999, Reid et al. 2003, Lee et al. 2012). The hypothalamic-pituitary-adrenal (HPA) axis regulates responses to environmental cues and thereby forms a mechanistic link between perception of the environment and phenotypic expression (Lessells 2008, McGlothlin and Ketterson 2008). Vertebrates respond to an acute stressor by activation of the HPA axis and the production of glucocorticoids (GCs; Wingfield et al. 1998, Wingfield and Kitaysky 2002). A short-term increase in GC secretion can be beneficial by mediating adaptive responses to an acute environmental challenge and promoting survival (Wingfield and Kitaysky 2002). Repeated exposure to a

stressor, however, can give rise to chronic stress, typically associated with prolonged elevation of baseline circulating GC levels (Wingfield and Kitaysky 2002, Romero 2004; though see, Rich and Romero 2005). Chronically-elevated GCs can have various deleterious consequences, including growth suppression, impaired immune function, severe protein loss and compromised cognition (reviewed in Wingfield et al. 1998, Sapolsky et al. 2000).

In ecological research, animals are routinely and repeatedly handled for various purposes including the collection of biometrics, attachment of tracking devices, blood sampling, and mark-recapture survival analysis. There is a sizeable literature on the effects of marks and devices on behaviour, survival and reproduction (e.g. Sharpe et al. 2009, Dennis and Shah 2012). However, few studies consider the potential for handling to affect hormonally-mediated phenotypic development (Dufty Jr et al. 2002) or bias subsequent interpretations of physiological or demographic data (though see Haydon et al. 1999, Clinchy et al. 2001). Measures of GC levels are widely used as indices of physiological condition (Romero 2004, Walker et al. 2005, Busch and Hayward 2009); therefore, it is fundamental that we understand how GC measures and their interpretation may be influenced by repeated investigator handling. Several examples of neonatal handling clearly demonstrate how early-life experiences can modify development of the HPA axis; regular handling of captive and domesticated mammals and birds during postnatal development has been shown to result in a reduction of baseline GCs and/or the adrenocortical stress response (Meaney et al. 1988, 1991, Hemsworth et al. 1994, Collette et al. 2000, Weaver et al. 2000, Adams et al. 2005). Regular and repeated handling of nestling birds for research and conservation purposes is common, yet we know relatively little about the effects of repeated handling for development of endocrine function in free-living animals and the few studies carried out have produced mixed results. While regular handling induced a dampening of the stress response in altricial nestlings of American kestrels (daily handling, Whitman et al. 2011) and eastern bluebirds (handling every other day, Lynn et al. 2013), neither baseline nor stress-induced levels were affected in semi-precocial nestlings of the thin-billed prion (daily, Quillfeldt et al. 2009), black-legged kittiwake (every 4 d, Brewer et al. 2008) or Leach's storm petrel (daily, Fiske et al. 2013).

Chronic stress (Epel et al. 2004, 2006) and elevated GCs (Hausmann et al. 2012) are also associated with increased oxidative stress, short telomeres and low telomerase activity. Telomeres comprise highly-conserved non-coding DNA sequences at the ends of eukaryotic chromosomes (Blackburn 2005). In the absence of the enzyme telomerase, telomeres shorten with each round of somatic cell division and, upon reaching a critical length, trigger cellular senescence (Blackburn 2005). The accumulation of senescent cells contributes to tissue and organ dysfunction and age-related pathologies (Campisi 2005). TL declines progressively

with age (Brümmendorf and Mak 2002, Aviv et al. 2009, Bize et al. 2009, Heidinger et al. 2012) and individuals with the shortest telomeres or the highest loss rate have the poorest survival prospects (Hausmann et al. 2005, Salomons et al. 2009, Bize et al. 2009, Heidinger et al. 2012). Stressful experiences including poor growth conditions (Hall et al. 2004, Tarry-Adkins et al. 2009, Geiger et al. 2012), psychological stress (Epel et al. 2004, Kotrschal et al. 2007) and reproduction (Bauch et al. 2012) have all been associated with reduced TL. Telomere loss is thought to be greatest during early life, presumably as a result of the rapid growth and cell division that occurs at this time (Zeichner et al. 1999, Salomons et al. 2009, Heidinger et al. 2012). In one study, early-life TL was shown to be a stronger predictor of lifespan than TL in adulthood (Heidinger et al. 2012). Environmentally-induced variation in early-life TL may therefore be particularly important in shaping individual life-history development.

The objective of this study was to examine the effects of repeated handling during postnatal development on pre-fledging baseline corticosterone (CORT – the main GC in birds), TL and body condition in the European storm petrel *Hydrobates pelagicus* (hereafter, storm petrel). The storm petrel is a nocturnal cavity-nesting colonial seabird. The young are semi-precocial (Starck and Ricklefs 1998); although nestlings hatch with a layer of down and can thermoregulate within a few days of hatching, they do not leave the confines of the underground nest until fledging at 65-70 d (Davis 1957). Nestlings were handled on between 1 and 7 d over the course of postnatal development, facilitating identification of a possible threshold of cumulative handling events above which physiological responses are induced. Most studies have failed to consider that the effects of handling may be threshold-dependent (though see Lynn et al. 2013). Few studies have explored the links between GCs and TL and there are currently no published studies examining TL as a measure of physiological costs associated with neonatal handling.

Methods

Study site and species

The study was conducted at the island of Mousa, located in the Shetland archipelago, UK (60°00'N, 01°10'W). The single storm petrel nestling is brooded for c. 7 d, following which the chick remains alone in the nest during daylight hours. Parents return to provision the nestling only at night, so diurnal handling following cessation of brooding will not impede parents tending nests. The single-egg clutch of this species enables the isolation of experimental effects from potentially confounding effects of the within-nest environment (e.g. sibling competition, Love et al. 2003b).

Experimental manipulation of handling

In mid-late August 2011, 26 nests were identified that were occupied by an unbrooded chick. Human recreational disturbance has been shown to result in increased nestling mortality at the study site; however, all nests were located >150 m from areas of human recreational activity and therefore outside of the zone of effect (Chapter 1, Watson et al. 2014). Nests were not exposed to investigator disturbance prior to the cessation of brooding. All nestlings were handled at this first encounter to enable the estimation of age from tarsus length (M. Bolton, unpubl. data). Due to the high asynchrony in timing of breeding, nestlings spanned a range of ages at the start of the experiment (median age: 16 d, range: 3-30 d). After the initial handling, nestlings were subsequently handled between 0 and 6 times over the course of the experiment until late postnatal development (median age: 45 d, range: 41-55 d). The treatment period (number of days elapsed between initial handling and end of experiment) therefore varied depending on the age of chicks at the beginning of the experiment. Eight nestlings were left undisturbed until the end of the experiment in late postnatal development (handling episodes = 1), while four were handled on one further occasion at 22 d after initial handling (handling episodes = 2). The remaining nestlings (n = 14) were all handled with the same frequency -approximately every 5 d - until the end of the treatment period; the cumulative number of handling episodes thus ranged between 3 and 7 times (including initial identification and handling), depending on hatching date and age at the start of the treatment period. Handling was standardised across the experiment and designed to simulate that employed in a typical field study. Each handling event involved the removal of the nestling from the nest while biometrics were recorded. From the initial onset of disturbance (e.g. moving rocks to access nest), handling lasted 3-4 min. Tarsus length was measured to the nearest 0.1 mm using callipers; body mass was recorded to the nearest 0.1 g using a spring scale and wing length (maximum flattened chord) was measured to the nearest 1 mm using a stopped rule once the outermost primary feather emerged from the quill sheath (c. 30 d). We did not expect handling to influence parental care, since nestlings were only handled during the day and once brooding had been terminated, so there was no separation between parents and offspring.

Nestlings were exposed to their assigned treatment until late postnatal development (median age: 45 d, range: 41-55 d), when blood was collected and biometrics were recorded. A minimum of 5 d elapsed between final handling and blood sampling. Whole blood was obtained by venepuncture of the brachial vein and collected in heparinised capillary tubes under licence from the UK Home Office. All blood samples were collected within 3 min of the onset of disturbance (determined as when the investigator approached to within 1 m of nest), so can be considered representative of baseline (Romero 2004). CORT measures were not

TACCCT-3'). The single-copy control gene used was ornithine decarboxylase (OCD), isolated from the European storm petrel, which was amplified using the primers: OCD Fwd1 (5'-GACCTTGCCATCATTGGAGTTAG-3') and OCD Rev1 (5'-AAGGCA TCCCTATTGTTAGGTAGA-3') sourced from Integrated DNA Technologies (Leuven, Belgium). qPCR was performed using 10 ng of DNA per reaction. The concentrations of primers used were 500 nM for telomere and 70 nM for OCD reactions. Telomere qPCR conditions started with 15 min at 95°C, followed by 27 cycles of 15 s at 95°C, 30 s annealing at 58°C and 30 s extending at 72°C. OCD reactions started with 15 min at 95°C, followed by 40 cycles of 30 s at 95°C and 30 s at 60°C. For both telomere and OCD reactions, the number of PCR cycles required for accumulation of sufficient products to exceed a threshold of fluorescent signal (C_t) was determined. A standard curve, run on each plate, consisted of a serial dilution of a reference sample ranging from 40 ng to 2.5 ng. The C_t threshold for each reaction was determined from the reference sample. All samples, including the standard curve, were run in triplicate; mean values were used to calculate the T/S ratio for each sample relative to the reference sample according to the formula $2^{\Delta\Delta C_t}$, where:

$$\Delta\Delta C_t = (C_t^{\text{telomere}} - C_t^{\text{OCD}})_{\text{reference}} - (C_t^{\text{telomere}} - C_t^{\text{OCD}})_{\text{sample}}$$

Inter- and intra-assay variations were 3.6% and 1.4% respectively for telomere reactions and 0.82% and 0.33% respectively for OCD reactions. Amplification efficiencies were within an acceptable range (Mean \pm SD: Telomere: $106.6 \pm 3.4\%$; OCD: $114.5 \pm 3.9\%$) and all samples fell within the bounds of the standard curve.

Statistical analyses

All analyses were performed in R 3.0.0 (R Core Team 2013). General linear models with a normal error structure were fitted to data on (i) baseline CORT (ii) telomere length and (iii) body condition to examine variation in the response variables in relation to the number of handling events (covariate). We considered the need to control for age (covariate), total number of treatment days (covariate), sex (female or male), body mass (covariate), body size (wing length; covariate) and time of day (CORT model only). We also tested for a two-way interaction between number of handling events and days of treatment. The length of the treatment period was highly correlated with age at first handling (0.79), so only the former was considered for inclusion. Dependent variables were transformed, where necessary, to achieve normality. Starting with a null model, a conservative approach to model fitting was adopted in which fixed effects were added sequentially in a forward stepwise regression to reach a minimum adequate model. The criterion for inclusion of a variable was a maximum likelihood ratio P -value of ≤ 0.05 . Each time a new variable was added to the model, the significance of existing variables was re-

examined. All results are presented as means \pm SE, unless otherwise stated. We performed post-hoc power analyses using the package *pwr*, based on Cohen (1988), to determine the minimum detectable effect sizes given a power of 0.8 and α of 0.05.

Results

Experimental manipulation of handling

Neither baseline CORT (Fig. 3.1A; $\beta = 0.063 \pm 0.03$, $t_{20} = 1.92$, $P = 0.070$), TL (Fig. 3.1B; $\beta = -0.018 \pm 0.02$, $t_{24} = -0.88$, $P = 0.385$) nor body condition (Fig. 3.1C; $\beta = -0.338 \pm 0.23$, $t_{21} = -1.50$, $P = 0.149$) varied with handling experience, ranging from 1 to 7 cumulative handling episodes. Although there was a tendency for baseline CORT to increase with the number of days of handling, the effect was not significant and inclusion of the variable did not improve the model fit. Baseline CORT significantly increased with nestling age ($\beta = 3.73 \pm 0.90$, $t_{21} = 4.14$, $P < 0.001$), but none of the other explanatory variables explained a significant amount of the variation in TL or body condition. Although the length of the treatment period varied among individuals (median: 27 d, range: 14-39 d), it did not affect variation in CORT ($\beta = 0.002 \pm 0.005$, $t_{18} = 0.42$, $P = 0.681$) or TL ($\beta = 0.001 \pm 0.002$, $t_{22} = 0.42$, $P = 0.682$) in response to handling, as tested for by the interaction between number of handling days and number of treatment days. Although the interactive effect of handling and treatment period on body condition produced a parameter estimate that is significant at the 0.05 level ($\beta = 0.059 \pm 0.02$, $t_{19} = 2.50$, $P = 0.020$), inclusion of the main effects and interaction did not significantly improve the model fit compared with a null model, when added in a forward stepwise regression. Power analyses revealed that, for a power of 0.8 and α of 0.05, the minimum detectable effect sizes were 0.49, 0.33 and 0.38, respectively, for the dependent variables of CORT, TL and body condition.

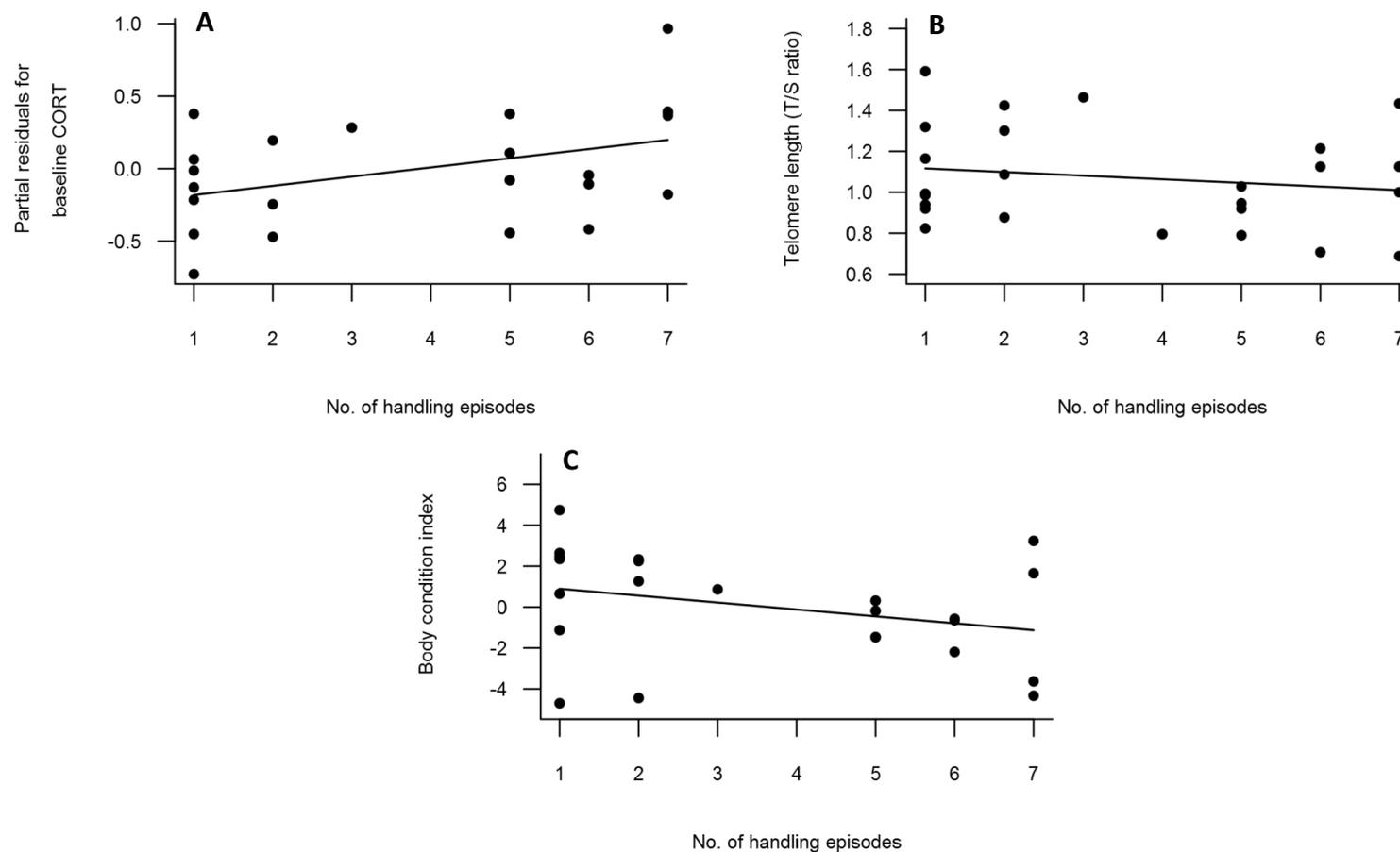


Figure 3.1 Effects of repeated handling during early life on (A) baseline plasma corticosterone (CORT; $t_{20} = 1.92$, $P = 0.070$); (B) telomere length ($t_{24} = -0.88$, $P = 0.385$); and, (C) body condition ($t_{21} = -1.50$, $P = 0.149$) in late postnatal development (median age: 45 d). Values plotted in (A) are partial residuals, having accounted for the effect of age on baseline corticosterone.

Discussion

The effects of neonatal handling on the HPA axis have been well-studied in captive and domesticated animals, but we know little about the physiological effects of handling in free-living animals. Experimental manipulation of handling experience during early life did not affect pre-fledging baseline CORT, TL or body condition in storm petrel nestlings. Power analyses suggest the sample size was sufficient to only detect relatively large effects, though previous studies have shown differences in GCs in excess of 50% between unhandled and handled individuals (e.g. Meaney et al. 1988, Adams et al. 2005, Lynn et al. 2013). It appears that nestlings are robust to handling as frequently as every 5 d and up to 7 times during development. The effects of repeated handling might only be manifest beyond a threshold of cumulative number of handling episodes, yet most studies fail to consider this (though see Lynn et al. 2013). The experimental approach was designed to facilitate detection of a possible threshold, by examining effects along a covariate of handling experience, within the range of a typical field study. It is possible that the frequency of handling or maximum number of handling episodes was not sufficient to induce detectable effects in storm petrel nestlings. However, the frequency and extent of handling applied reflects that employed in a typical field study suggesting that, within these limits, physiological measurements will not be biased by investigator handling.

One possible explanation for the absence of any effects of repeated handling is that nestlings did not perceive handling to be stressful. Nestlings face a trade-off between the costs and benefits of eliciting an adrenocortical response and strict regulation of the HPA axis during development may be necessary to avoid the potentially deleterious costs associated with exposure to chronically-elevated GCs. It may be maladaptive for cavity-dwelling chicks that are at low risk of predation and unable to escape or defend themselves in the event of a predation attempt to elicit an adrenocortical response to an acute stressor. The ecology and life-history of the storm petrel may therefore favour a dissociation of the HPA axis from stressful stimuli in early life. While young mammals and altricial nestlings typically demonstrate a hyporesponsive period to stressors in early life (Sapolsky and Meaney 1986, Wada et al. 2007, Wada 2008), precocial and semi-precocial chicks appear to demonstrate a robust stress response soon after hatching (Adams et al. 2008, Chin et al. 2013). However, the response was found to be reduced in early development, compared with late postnatal development, in thin-billed prions (Quillfeldt et al. 2009) and Leach's storm petrels (Fiske et al. 2013). Despite being able to elicit an adrenocortical response to a standardised stressor, a recent study demonstrated that daily handling has no effect on either baseline or stress-induced CORT levels, as well as growth rate, in the Leach's storm petrel *Oceanodroma leucorhoa* (Fiske et al. 2013). A suppression of the HPA axis may be adaptive,

reducing overall exposure to CORT during the vulnerable period of development. Handling in the latter study was restricted to the first half of postnatal development; the present study extends this research by demonstrating that handling in late postnatal development also has no effect on baseline CORT in storm petrel nestlings.

Repeated exposure to stressors can modify GC secretion by inducing chronic stress or through the process of acclimation or habituation. While chronic stress is generally considered to be consistent with elevated baseline GCs and reduced body condition (Wingfield et al. 1998, Sapolsky et al. 2000, Romero 2004; though see Rich and Romero 2005), acclimation is characterised by a reduced adrenocortical response to the stressor (Meaney et al. 1988, Romero 2004, Whitman et al. 2011). A dampening of the stress response following exposure to repeated neonatal handling of captive and domesticated animals has commonly been attributed to acclimation (Meaney et al. 1988, Collette et al. 2000). Since we did not measure stress-induced CORT levels, we cannot exclude the possibility that handling may have affected the sensitivity of the HPA axis, which could permanently modify an individual's responsiveness to stress. While modification of the functioning of the HPA axis may be important for long-term fitness, chronic elevation of baseline GCs is associated with various immediate and deleterious effects, the consequences of which may be particularly severe in developing young (Sapolsky et al. 2000). An advantage of baseline measures is that they are quicker to collect and more practically integrated into field studies, compared with the collection of stress-induced measures, which involves restraining animals for up to 30 min or longer, presumably incurring greater stress to individuals. Furthermore, the optimum time for detecting the peak of the response is unknown and can vary greatly both between species and within-species depending on age (Wada et al. 2007, Quillfeldt et al. 2009) or body condition (Heath and Dufty 1998).

Although our understanding of the links between GCs and TL is limited, stress hormones have been shown to mediate the damaging effects of stress on telomere length and maintenance (Epel et al. 2004, 2006, Hausmann et al. 2012). Repeated acute elevation of circulating CORT could contribute to elevated telomere attrition and short telomeres via increased oxidative stress and cell division (Epel et al. 2004, 2006). Besides the detrimental effects associated with modifications to levels of circulating GCs, individuals exposed to a repeated stressor may also experience fitness costs via the reduced survival prospects associated with accelerated telomere loss and short telomeres (Cawthon et al. 2003, Hausmann et al. 2005, Heidinger et al. 2012). While acclimation to handling may not be evident in a measure of baseline CORT, it might be expected that regularly-handled young still have shorter telomeres compared with those that have not been handled so frequently. The absence of any differences in TL further supports the idea that storm petrel nestlings do not perceive handling to be stressful and do not elicit a

robust adrenocortical response. Although initial TL and rate of telomere loss during development are unknown, nestlings represent a random sample from the population and, therefore, are not expected to display consistent differences in initial TL. It would therefore be expected that any effect of handling on rate of telomere attrition would be evident in a single measure of TL in late development.

The absence of any differences in CORT or TL could also potentially be explained if handling on a single occasion were sufficient to induce habituation, with the consequence that individuals no longer respond in the same way. While the experiment enabled the examination of the effects of handling in isolation from prior investigator disturbance and other sources of human disturbance, a consequence of this was that all nestlings were handled on at least one occasion. It was previously shown that a single exposure to an acute stressor is enough to induce habituation in adult eastern bluebirds (Lynn et al. 2010), but not in nestlings (Lynn et al. 2013). The ability for young to habituate so rapidly seems unlikely, but understanding intra- and inter-specific variation in the capacity for, and rate of, acclimation to stressors is an important area for further research.

The present study suggests that storm petrel nestlings are robust to handling as frequently as every 5 d and as many as 7 times during development. Considering handling experience as a covariate enabled us to confirm that, at least within the range of handling intensity typically applied in field studies, there are no large effects of investigator handling and handling is unlikely to bias physiological measurements. In the absence of any evidence for chronic stress or acclimation, it seems that nestlings are able to avoid the deleterious effects associated with elevated baseline CORT, accelerated telomere attrition and short telomeres during early life. Smaller effects, however, which could still have biological relevance, may not have been detected within the limits of the study design. Despite the routine nature of handling nestling birds, few studies consider the potential for repeated handling to affect hormonally-mediated phenotypic expression or bias interpretations of focal measures (though see Haydon et al. 1999, Clinchy et al. 2001). Despite the absence of any measureable effects of repeated handling in this study, effects may vary between populations or depending on exposure to additional stressors. With the increasing use of physiological markers for assessing population status and addressing conservation issues, it is imperative that more studies consider the potential for repeated handling to influence focal measures. Future research should also address the potential long-term consequences of repeated handling during early life.

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4. Variation in early-life telomere dynamics in a long-lived bird: links to environmental conditions and survival

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Abstract

Conditions experienced in early life can have profound consequences for both short and long-term fitness. Variation in the natal environment has been shown to influence survival and reproductive performance of entire cohorts in wild vertebrate populations. Telomere dynamics potentially provide a mechanistic link between the early environment and fitness outcomes, yet we know little about how the environment can influence telomere dynamics in early life. We found that environmental conditions during growth play an important role in early-life telomere length (TL) and attrition in nestlings of a long-lived bird, the European storm petrel *Hydrobates pelagicus*. Nestlings reared under unfavourable environmental conditions experienced significantly greater telomere loss during postnatal development and had shorter telomeres at fledging, compared with nestlings reared under more favourable natal conditions, which displayed a negligible change in TL. The results of this study suggest that early-life telomere dynamics may contribute to the marked differences in life-history traits that can arise among cohorts reared under different environmental conditions. Early-life TL was also found to be a significant predictor of survival during the nestling phase, providing further evidence for a link between variation in TL and individual fitness. To what extent the relationship between early-life TL and mortality during the nestling phase is a consequence of genetic, parental and environmental factors is currently unknown, but an interesting area for future research. Accelerated telomere attrition under unfavourable conditions, as observed in this study, might play a role in mediating the effects of the early-life environment on later-life performance.

Introduction

One of the principal aims of evolutionary ecology is to understand the mechanisms underlying individual variation in longevity and fecundity. Telomere dynamics link cellular processes with organismal ageing and thus optimisation of telomere length (TL) and attrition may play a major role in life-history evolution (Monaghan and Haussmann 2006). Telomeres comprise highly-conserved non-coding DNA sequences that form protective caps at the ends of eukaryotic chromosomes (Blackburn 2005). By protecting coding sequences from attrition, telomeres play an important role in maintaining genome stability (reviewed in Verdun and Karlseder 2007). In the absence of the enzyme telomerase, telomeres shorten with each round of somatic cell division; when a critical length is reached, telomeres become dysfunctional and cells enter a state of replicative senescence (Blackburn 2005, Verdun and Karlseder 2007). Senescent cells subsequently die or adopt an altered secretory profile, resulting in secretion of inflammatory cytokines, growth factors and degradative enzymes that contribute to age-related declines in tissue and organ

function (Campisi 2005). The accumulation of senescent cells thus appears to be important to the ageing phenotype, thereby influencing lifespan (Campisi 2005).

Longitudinal studies have shown that TL declines progressively with age in humans, other mammals and birds (Zeichner et al. 1999, Brümmendorf and Mak 2002, Salomons et al. 2009, Aviv et al. 2009, Bize et al. 2009, Heidinger et al. 2012, Barrett et al. 2013). Large within-species variability in TL and the rate of telomere shortening is reported among individuals of the same age (Hall et al. 2004, Aviv et al. 2009, Bize et al. 2009). Inter-individual variation in TL has been found to predict fitness components in natural populations; individuals with the shortest telomeres or the highest loss rate have the poorest survival prospects (Cawthon et al. 2003, Haussmann et al. 2005, Bize et al. 2009, Olsson et al. 2011, Heidinger et al. 2012) and TL was found to be positively correlated with lifetime reproductive success (Pauliny et al. 2006). While early-life TL is partly determined by genetic factors (Njajou et al. 2007, Olsson et al. 2011), much of the inter-individual variation in TL may relate to environmental influences. Exposure to repeated stress (Epel et al. 2004, Kotrschal et al. 2007, Herborn et al. 2014), large-scale climatic processes (Mizutani et al. 2013), low habitat quality (Angelier and Vleck 2013) and reproduction (Kotrschal et al. 2007, Heidinger et al. 2012) have all been associated with accelerated telomere attrition, possibly mediated by increased oxidative damage (von Zglinicki 2002). TL therefore potentially reflects variation in individual state and past experiences.

It is well known that early-life conditions can have profound influences on phenotypic development and long-term fitness consequences (Lindström 1999, Metcalfe and Monaghan 2001). Variation in the natal environment has been shown to influence survival and reproductive performance in a number of vertebrate species (Albon et al. 1987, Haywood and Perrins 1992, Sedinger et al. 1995, Rose et al. 1998, Reid et al. 2003). Environmental conditions during early life can affect an entire cohort simultaneously, giving rise to substantial differences in life histories between successive cohorts, which can persist throughout the cohort's lifespan (Albon et al. 1987, Rose et al. 1998, Reid et al. 2003). Several studies on mammalian and avian species have shown that telomere loss is greatest during early life, presumably as a result of the rapid growth and cell division that occurs during this period (Zeichner et al. 1999, Baerlocher et al. 2007, Salomons et al. 2009). Poor early nutrition and catch-up growth have been shown to result in accelerated telomere loss in laboratory rats (*Rattus norvegicus* Jennings et al. 1999, Tarry-Adkins et al. 2009) and wild birds (European shag *Phalacrocorax aristotelis* Hall et al. 2004, king penguin *Aptenodytes patagonicus* Geiger et al. 2012) and increased exposure to glucocorticoids led to increased telomere attrition in nestlings of a wild bird (European shag Herborn et al. 2014). Early-life TL was found to be a better predictor of longevity than TL in adulthood in captive zebra finches *Taeniopygia guttata* (Heidinger et al. 2012). Effects of early-life conditions on TL may therefore

mechanistically link developmental conditions with later-life senescence (Monaghan 2010). Despite the potential significance of early-life telomere dynamics in influencing life-history traits, we still know relatively little about how variation in early-life conditions influences telomere loss during development in natural environments.

In this study, we examined the effects of inter-annual variation in the natal environment on nestling TL and telomere dynamics in the European storm petrel *Hydrobates pelagicus*. The storm petrel is a long-lived seabird belonging to the Procellariiformes, displaying low annual fecundity (obligate clutch of one) and low adult mortality. We compared cohorts from two consecutive breeding seasons of differing quality, as indicated by overall reproductive performance of the colony. Productivity was significantly lower in 2011, compared with 2010. This enabled us to examine the relationship between environmental conditions, telomere dynamics and survival during early life, and identify potential cohort effects. We also investigated whether exposure to an additional source of stress, arising from human recreational disturbance, had consequences for early-life telomere dynamics. Parent-offspring relationships in TL were investigated within a single cohort.

Materials and methods

The study was conducted at the island of Mousa, located in the Shetland archipelago, UK (60°0'N, 1°10'W). Storm petrel nestlings are brooded for c. 7 d and do not leave the underground nest cavity until fledging at c. 65-70 d (Davis 1957). Fledging success (of eggs hatched) was significantly lower in 2011 (0.78), compared with 2010 (0.60) (see Chapter 1, Watson et al. 2014). This was probably partly linked to an increased frequency of extreme weather events in 2011 (Watson 2013). Blood was collected from 32 nestlings in 2010 and 29 nestlings in 2011 and from both parents at 22 nests (n = 44) in 2011 only. Whole blood was obtained by venepuncture of the brachial vein under licence from the UK Home Office.

Nestlings were sampled on two occasions (where possible) in the first (median age: 11 d, range: 4-33 d, n = 59) and second (median: 45 d, range: 34-63 d, n = 52) half of postnatal development. The median number of days elapsed between repeated measurements was 31 d (range: 22-47 d). Fifty nestlings were sampled twice. An initial sample was available for a further 11 nestlings; however, follow-up samples were not available either because nestlings died (n = 6), were not accessible in the nest or samples were accidentally destroyed. The overall proportion of chicks that died was greatly underrepresented in the data and the difference in mortality between the two cohorts was not reflected; this is because the majority of chick mortality occurred within a few days of hatching and before initial blood sampling was carried out. Tarsus length (nestlings only), mass and wing length (maximum flattened chord; adults only) were recorded. Nestling mass was corrected to a

standardised time (18:00 h) to account for the age-related rate of proportional weight loss that chicks undergo during diurnal fasting (Bolton 1995).

Determination of TL

Blood samples were stored at $<5^{\circ}\text{C}$ for up to 8 hr prior to being separated by centrifugation. Plasma and red blood cells were stored at $<5^{\circ}\text{C}$ in the field for a maximum of 3 d before being transferred to -20°C for up to 3 months; following this, samples were stored at -80°C until laboratory analyses were performed. DNA was extracted from red blood cells using Machery-Nagel NucleoSpin Blood kits following the manufacturer's protocol. Sex was determined following the molecular method described by Griffiths et al. (1998). TL was measured by quantitative PCR (qPCR) as described by Cawthon (2002) and adapted by Criscuolo et al. (2009) for birds. The method gives a relative value for TL by determining the ratio of number of telomere repeats to that of a single-copy control gene, relative to a reference sample. Consequently, the measure will include any interstitial repeats of the telomeric sequence (Foote et al. 2013). However, good correlations have been found between TL measured including and excluding the interstitial repeats using telomere restriction fragment (TRF) analysis (Foote et al. 2013) and between TRF and qPCR analyses (Criscuolo et al. 2009, Aviv et al. 2011). Since the extent of interstitial repeats is not expected to change with age, qPCR is well suited to detecting within-individual changes.

Amplification of telomere sequences was achieved using the forward and reverse primers: Tel1b (5'-CGGTTTGGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3') and Tel2b (5'-GGCTTGCCCTTACCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'). The single-copy control gene used was ornithine decarboxylase (OCD), isolated from the European storm petrel (GenBank: DQ881744.1), which was amplified using the primers: OCD Fwd1 (5'-GACCTTGCCATCATTGGAGTTAG-3') and OCD Rev1 (5'-AAGGCATCCCTATTGTTAGGTAGA-3') sourced from Integrated DNA Technologies (Leuven, Belgium). qPCR was performed using 10 ng of DNA per reaction. The concentrations of primers used were 500 nM for telomere and 70 nM for OCD reactions. Telomere qPCR conditions started with 15 min at 95°C , followed by 27 cycles of 15 s at 95°C , 30 s annealing at 58°C and 30 s extending at 72°C . OCD reactions started with 15 min at 95°C , followed by 40 cycles of 30 s at 95°C and 30 s at 60°C . For both telomere and OCD reactions, the number of PCR cycles required for accumulation of sufficient products to exceed a threshold of fluorescent signal (C_t) was determined. A standard curve, run on each plate, consisted of a serial dilution of a reference sample ranging from 40 ng to 2.5 ng. The C_t threshold for each reaction was determined from the reference sample. All samples, including the standard curve, were run in triplicate; mean values were used to calculate the T/S ratio for each sample relative to the reference sample according to the formula $2^{\Delta\Delta C_t}$, where:

$$\Delta\Delta Ct = (C_t^{\text{telomere}} - C_t^{\text{OCD}})_{\text{reference}} - (C_t^{\text{telomere}} - C_t^{\text{OCD}})_{\text{sample}}$$

Inter- and intra-assay variations were 3.6% and 1.4% respectively for telomere reactions and 0.82% and 0.33% respectively for OCD reactions. Amplification efficiencies were within an acceptable range (Mean \pm SD: Telomere: $106.6 \pm 3.4\%$; OCD: $114.5 \pm 3.9\%$) and all samples fell within the bounds of the standard curve.

Statistical analysis

All statistical analyses were performed in R 3.0.0 (R Core Team 2013). Data were transformed where necessary to achieve normality. Linear mixed models (LMMs) with a normal error structure were fitted to data on nestling telomere length to examine the within-individual change in TL. LMMs were fitted in the lme4 package (Bates et al. 2013). First, the optimal random effects structure was found by comparing nested models, fitted by restricted maximum likelihood (REML), using likelihood ratio tests (LRTs). We considered the inclusion of the random effects of nest identity and nestling identity (nested within nest). A random effect of nest identity was to account for the fact that nests may be occupied by the same breeding pair in subsequent years and 30% nests were sampled in both years. A random effect of nestling identity accounted for non-independence of repeated measures from the same individuals. The variances associated with nest identity were estimated to be zero and inclusion of the random effect did not significantly improve model fits. Only the random effect of nestling identity was retained in models. The estimated variances (mean \pm SD) associated with the random effect of nestling identity in final models were 0.01 ± 0.1 and 0.00 ± 0.00 , respectively.

Since we were primarily interested in the within-individual change in nestling TL, we employed within-subject centring (van de Pol and Verhulst 2006) to separate between-individual (cross-sectional) and within-individual (longitudinal) effects. The age variable was split into two variables: age at first sampling (between-individual effects) and delta age (the change in age since first sampling; within-individual effects). With nestling TL as the dependent variable, candidate explanatory variables included cohort (year: 2010 or 2011), sex and disturbance level (high or low, defined as ≤ 10 m or > 150 m from the visitor path, respectively) as two-level fixed factors and age, hatching date, body size (tarsus length), and body mass as covariates. As well as testing for the effects of candidate explanatory variables on absolute TL, we tested for effects on within-individual change in TL by considering all respective interactions with delta age. We also tested for a potential interaction between cohort and disturbance. Due to relatively small sample sizes and a large number of variables to potentially control for, a conservative approach to model fitting was adopted; starting from a null model, fixed effects that significantly differed from zero ($P < 0.05$) were added sequentially in a forward

stepwise regression. Each time a new variable was added to the model, the significance of existing variables was re-examined. The significance of parameter estimates was estimated by comparison to a probability distribution obtained from 10,000 Markov Chain Monte Carlo simulations using `pvals.fnc()` in language R, which does not require the estimation of degrees of freedom. Parameter estimates are quoted from models fitted by REML.

Linear models (LMs) with a normal error structure were fitted to data on initial and follow-up nestling TL. Candidate explanatory variables included cohort, nestling sex and human disturbance as fixed factors and age, hatching date, body size (tarsus length), and body mass as covariates. We considered the need to fit a mixed effects model and include a random effect of nest identity. The estimated variances associated with nest identity were zero and its inclusion did not significantly improve model fits. Parent-offspring relationships in the 2011 cohort (including only nestlings that survived to fledging) were also examined in a linear model analysis with initial nestling TL as the dependent variable. Candidate fixed effects included the covariates of maternal TL, paternal TL and nestling age and nestling sex as a fixed factor. We also tested for potential effects of nestling age and sex on parent-offspring relationships with the respective two-way interactions between parental TL and nestling sex and age. Having shown, in the previous analysis, that neither hatching date, body size, body mass nor visitor disturbance affected initial nestling TL, these variables were not included in the parent-offspring analysis.

Finally, generalised linear models (GLMs) with a binomial error structure and logit link function were fitted to data on early TL (≤ 16 d, median: 11 d, $n = 41$) to examine whether early TL was a good predictor of survival of the nestling phase. The threshold of 16 d was adopted since 95% of the first measurements fell below this upper bound. These data did not include any repeated measures from the same individuals or nests. In addition to the fixed effect of nestling TL at ≤ 16 d, candidate explanatory variables included year, sex and visitor disturbance as fixed factors and nestling age, body size, body mass, hatching date, growth rate (average daily incremental growth in tarsus and mass) as covariates. We also tested for two-way interactions between TL and nestling age, and mass and tarsus growth rates. The minimum adequate model was evaluated using Receiver Operating Characteristic plots (Sing et al. 2005). The resulting area under the curve (AUC) offers a measure of predictive performance for a binomial model; a value of 1.0 indicates a perfect model, while a value of 0.5 indicates that a model performs no better than random. Again, fixed effects were added sequentially in a forward stepwise regression; for LMs and GLMs, nested models were compared using LRTs. The criterion for entry of a variable was a log-likelihood ratio P -value of <0.05 . Each time a new variable was added to the model, the significance of existing variables was re-examined. All means are presented with standard errors, unless otherwise stated.

Results

Initial nestling TL was significantly longer in individuals from the 2011 cohort, compared with the 2010 cohort (Fig. 4.1A; year(2011): $\beta = 0.093 \pm 0.042$, $t_{51} = 2.21$, $P = 0.031$). The follow-up TL measurement in late postnatal development was shorter in 2011, though the estimated effect was borderline significant (Fig. 4.1A; year(2011): $\beta = -0.099 \pm 0.051$, $t_{49} = -1.97$, $P = 0.054$). The within-individual change in nestling TL was significantly different between the two cohorts: in 2010, there was little change in individual TL during the nestling period, whereas TL declined significantly with age in nestlings reared in 2011 (Fig. 4.1B; delta age x year(2011): $\beta = -0.007 \pm 0.002$, $t = -3.09$, $P = 0.007$). Neither nestling TL nor the within-individual change in TL (tested by the respective interactions with delta age) were affected by any of the other variables considered, including sex, hatching date, body size, body mass, or visitor disturbance (all $P > 0.2$). The inter-annual variation in TL did not differ between disturbance levels ($\beta = -0.019 \pm 0.098$, $t = 0.20$, $P = 0.843$). Between-individual differences in TL were not significant (age at first sampling: $\beta = -0.005 \pm 0.003$, $t = -1.61$, $P = 0.111$).

Of the 61 nestlings sampled, 6 died prior to fledging (3 individuals from each cohort). When examining whether early TL was a good predictor of survival of the nestling phase, we found that the probability of surviving to fledging increased significantly with TL (Fig. 4.2A; $\beta = 0.99$, 95%CI: 0.79-1.0, $z = 2.23$, $P = 0.026$). On average, mean TL of chicks that did not survive to fledging was 22% shorter than that of chicks that successfully fledged (Fig. 4.2B). The probability of fledging also increased with increasing rate of mass gain ($\beta = 0.93$, 95%CI: 0.37-1.0, $z = 1.62$, $P = 0.105$); although the effect was not significant, inclusion of the variable significantly improved the model fit. One of the chicks that did not fledge had very short telomeres (T/S ratio = 0.69) and so the analysis was re-run excluding this nestling. The outlier had no influence on the relationship, having no effect on the magnitude or significance of the effect of TL ($\beta = 0.99$, 95%CI: 0.76-1.0, $z = 2.19$, $P = 0.029$). The probability of fledging was not affected by sex, hatching date, year, or visitor disturbance (all $P > 0.1$). The relationship between nestling TL and probability of fledging was independent of age and rates of structural and mass growth, as demonstrated by the respective interactions with TL (all $P > 0.1$). The AUC of the minimum adequate model was 0.91, indicating the model performed very well in terms of accuracy.

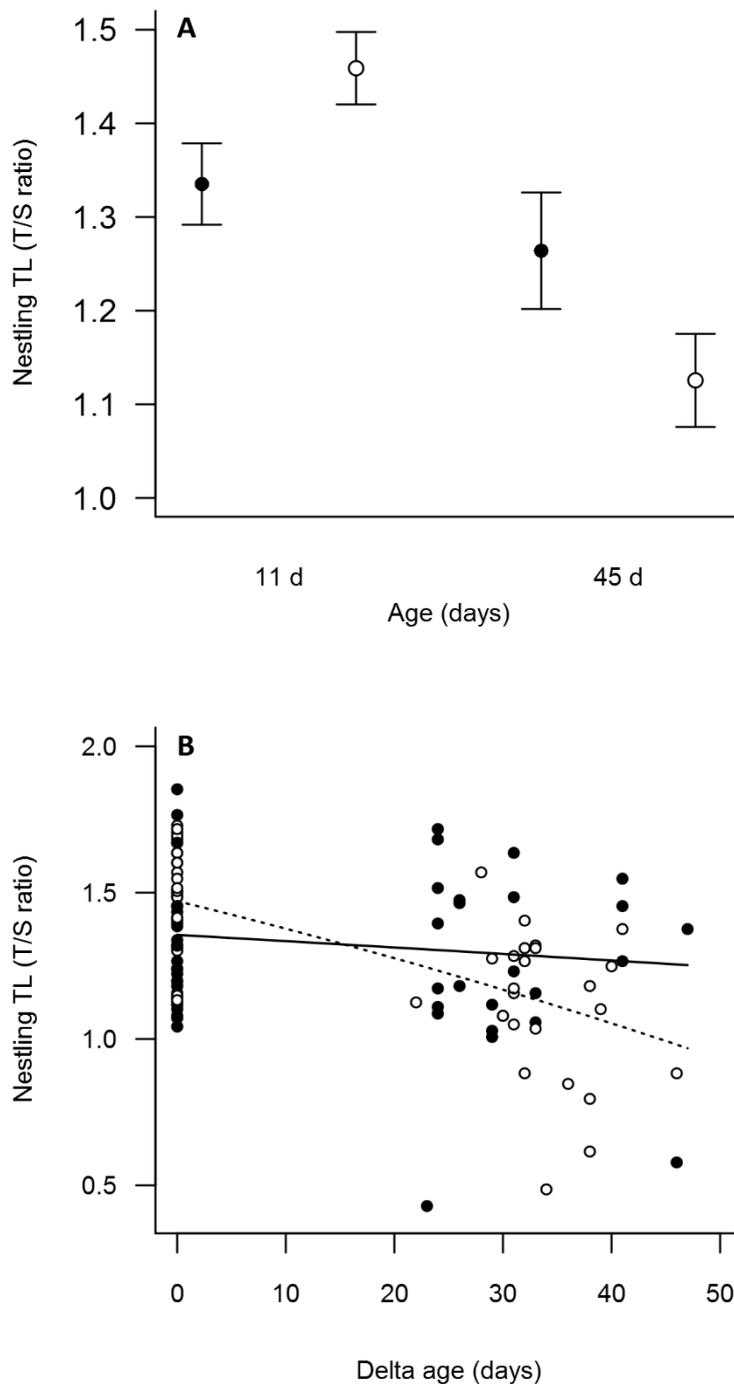


Figure 4.1. Telomere length (TL) and change in TL during growth in storm petrel nestlings ($n = 55$) from two consecutive cohorts reared in different natal conditions: (A) Mean \pm SE initial (LM: $t_{51} = 2.21$, $P = 0.031$) and pre-fledging (LM: $t_{49} = 1.97$, $P = 0.054$) TL in 2010 (closed circles) and 2011 (open circles). (B) within-individual change in TL in 2010 (closed circles; solid line) and 2011 (open circles; dashed line). The x-axis represents the change in age since initial sampling (day 0). Median age at initial sampling was 11 d. Fitted lines represent model predictions from the LMM (delta age x cohort: $t = -3.09$, $P = 0.007$).

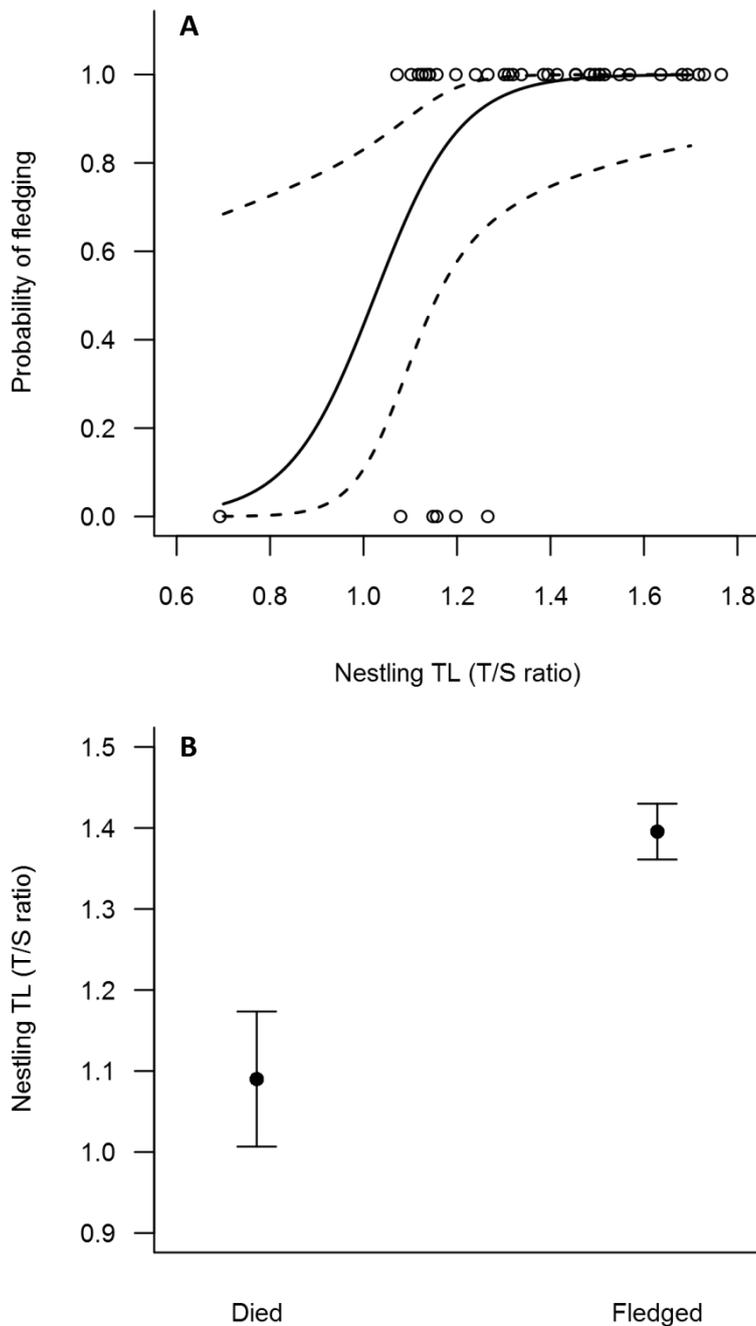


Figure 4.2. Relationship between nestling telomere length (TL) and survival during postnatal development: (A) predicted probability of fledging (solid line; GLM: $z_{38} = 2.23$, $P = 0.026$) in relation to early TL (≤ 16 d) with 95% confidence intervals (dashed lines) and fitted within the range of observed values (open circles; $n = 41$). Removal of the outlier at the lower end of the TL scale did not affect model predictions. (B) Mean \pm SE TL of nestlings that died ($n = 6$) during the nestling phase and those that survived to fledging ($n = 35$).

Analysis of parent-offspring relationships in the 2011 cohort revealed that nestling TL was not significantly correlated with either maternal TL (Fig. 4.3A; $\beta = 0.077 \pm 0.10$, $t = 0.80$, $P = 0.437$) or paternal TL (Fig. 4.3B; $\beta = 0.034 \pm 0.09$, $t =$

0.39, $P = 0.703$). Parent-offspring TL relationships were unaffected by nestling age or sex, as shown by testing the respective two-way interactions (all $P > 0.2$).

Discussion

Although it has been widely suggested that the most rapid telomere loss occurs early in life in a range of vertebrates (Zeichner et al. 1999, Baerlocher et al. 2007, Salomons et al. 2009), only a few studies have investigated telomere dynamics within the timeframe of postnatal development itself (see Foote et al. 2011, Geiger et al. 2012, Herborn et al. 2014). We found that TL and dynamics in growing nestlings were strongly influenced by the quality of the natal environment. Favourable environmental conditions during development, as reflected by moderate colony productivity in 2010, were associated with a negligible change in TL during early life, whereas conditions resulting in low chick survival among the 2011 cohort were associated with significant telomere attrition and low pre-fledging TL. Initial TL was, however, higher in the 2011 cohort. This could potentially be explained if the high early mortality observed within the 2011 cohort removed individuals with short telomeres from the population or poor-quality and/or older birds did not breed. Alternatively, nestlings could have hatched with longer telomeres as a consequence of more favourable conditions within the prenatal environment. While human recreational disturbance was previously shown to reduce survival probabilities (Chapter 1, Watson et al. 2014) and affect growth patterns (Chapter 2) in nestling storm petrels, there was no effect of visitor activity on telomere dynamics.

It is well known that cohort effects can arise in response to natal conditions. Survival and recruitment among cohorts have previously been shown to be positively correlated with the natal environment in mammals (Albon et al. 1987, Rose et al. 1998) and birds (Sedinger et al. 1995, Reid et al. 2003). An increasing body of evidence links short telomeres and accelerated rate of telomere loss to a shorter lifespan (Cawthon et al. 2003, Bize et al. 2009, Olsson et al. 2011, Heidinger et al. 2012) and lower reproductive success (Pauliny et al. 2006). The results of this study suggest that early-life telomere dynamics may contribute to the marked differences in life-history traits that can arise among cohorts reared under different environmental conditions. Indeed, TL in early life has been shown to be a strong predictor of lifespan and a better predictor than TL in adulthood (Heidinger et al. 2012). In addition to the increase in chick mortality observed in this study in response to unfavourable natal conditions, the population-level effects might be exacerbated by a reduction in the fitness of entire cohorts compared with those fledging under more favourable conditions. Such cohort-wide effects can subsequently destabilise population dynamics (Lindström 1999). Although our understanding will benefit from examination of longer-term data of multiple

cohorts, this study supports the idea that telomere dynamics may be a mechanism linking early-life conditions with later-life performance.

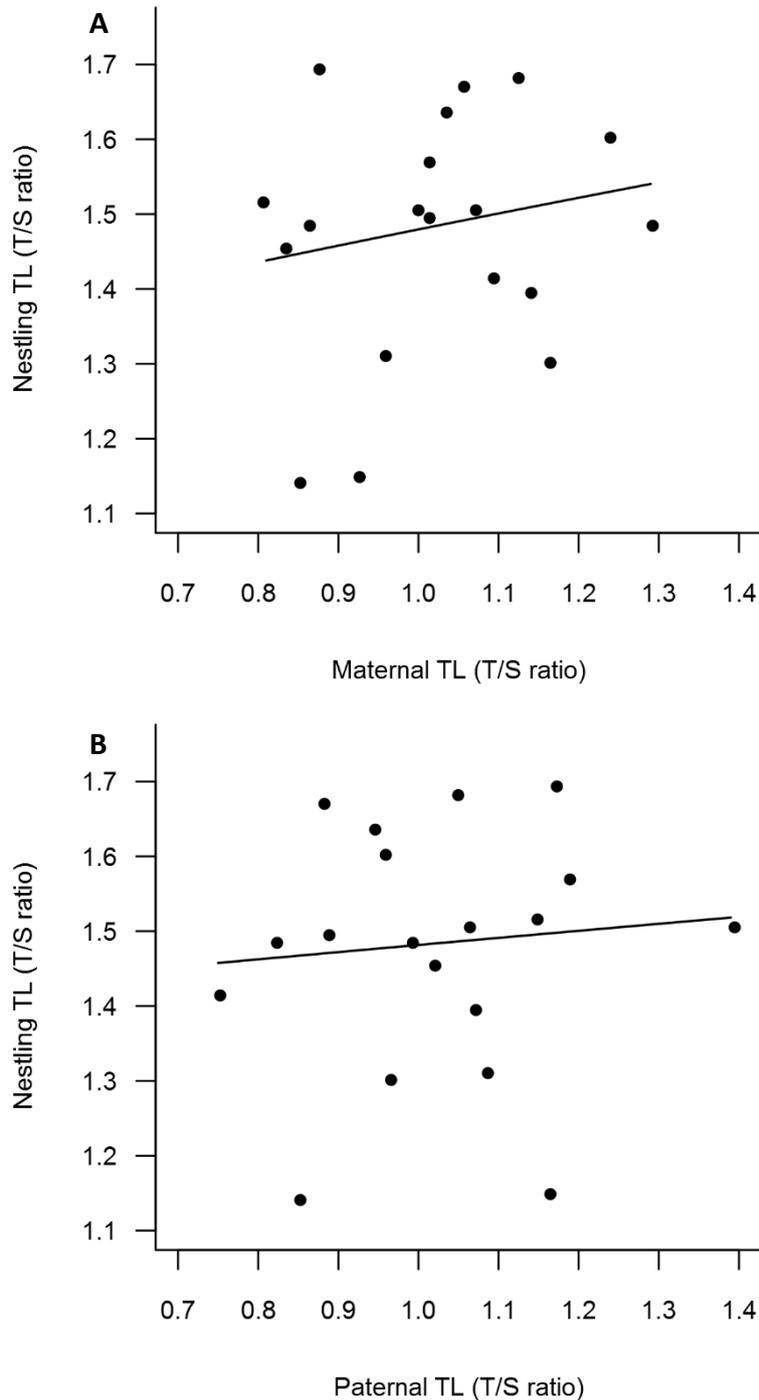


Figure 4.3. Relationship between initial nestling telomere length (TL) and (A) maternal TL (LM: $t = 0.80$, $P = 0.437$) and (B) paternal TL (LM: $t = 0.39$, $P = 0.703$) in the 2011 cohort ($n = 19$). Fitted lines represent model predictions from the respective linear models.

The maintenance of TL during postnatal development under favourable natal conditions in storm petrels contrasts with studies in other birds suggesting that rapid telomere loss occurs early in life (Hall et al. 2004, Baerlocher et al. 2007, Salomons

et al. 2009, Heidinger et al. 2012). A negligible change in early-life TL was also demonstrated in chicks of the long-lived king penguin experiencing favourable growth conditions (Geiger et al. 2012). A slow rate of telomere shortening could be causally linked to a higher resistance to oxidative stress (Ogburn et al. 2001) and/or elevated telomerase activity (Verdun and Karlseder 2007, Haussmann et al. 2007). Stressful conditions, however, have been shown to lead to accelerated telomere loss via downregulation of antioxidants in birds (Stier et al. 2009) and telomerase in telomerase (Epel et al. 2004). When faced with a costly reproductive event, adult Adélie penguins *Pygoscelis adeliae* are able to increase antioxidant defences and avoid accelerated telomere attrition (Beaulieu et al. 2011). The results of this study suggest that storm petrel nestlings, when faced with unfavourable natal conditions, are unable to modulate or activate such regulatory mechanisms. If poor early conditions were to lead to irreversible downregulation of antioxidants or telomerase, accelerated telomere loss may even persist beyond the nestling period, exacerbating the effects of early conditions on later-life senescence. An area for future research is to understand the links between telomere loss, oxidative stress and telomerase activity during early life.

While recent studies have investigated the relationship between TL in early life and survival in captive (Heidinger et al. 2012) and wild (Caprioli et al. 2013) birds, we are aware of only one study that has directly examined the association between early-life TL and mortality within the development phase itself. Just as king penguin chicks that died prior to fledging displayed shorter telomeres (Geiger et al. 2012), we also found that post-hatching TL of storm petrel nestlings is directly related to imminent mortality. Despite a small sample size, the model performed well (as measured by AUC) and the results were not influenced by an outlying observation. Although confidence intervals are wide at low TLs, they do not overlap with confidence intervals when the probability of fledging is equal to one. TL was a much better predictor of fledging success than the rate of mass gain. While food load and rate of mass gain are likely to be of importance in determining survival within the first few days of hatching, storm petrel nestlings are well-buffered against periodic food shortages and mortality beyond the brooding stage is unlikely to be strongly influenced by environmental factors affecting rate of provisioning and subsequent mass gain. Additionally, since they nest in cavities, the risk of nest predation is very low. Consequently, it seems most likely that mortality was linked to intrinsic factors. While telomere shortening is unlikely to be the direct cause of nestling mortality, it may be indicative of poor pre-natal conditions or high exposure to oxidative stress. Early TL is partly determined by genetic factors (Njajou et al. 2007), but the rate of attrition is also known to be affected by environmental influences (Epel et al. 2004, Tarry-Adkins et al. 2009, Geiger et al. 2012). The results demonstrate that early-life TL is a good predictor of imminent mortality.

While a strong paternally-inherited component to offspring TL has been demonstrated in humans (Njajou et al. 2007, Nordfjäll et al. 2010) and reptiles (Olsson et al. 2011), recent evidence suggests that TL in birds is maternally inherited (Horn et al. 2011). Parent-offspring relationships in this study may have been obscured by parental age, which was not known; although telomere length was unrelated to age in two other long-lived avian species (Hall et al. 2004). Since we only have data on parent-offspring relationships for the cohort reared under unfavourable conditions, it is also possible that environmental effects may have outweighed genetic and maternal effects on nestling TL. Future research should seek to disentangle the relative contributions from genetic, parental and environmental effects on TL and dynamics. This will help to develop our understanding of the role of telomere dynamics in driving the evolution of life histories.

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5. The occurrence of hypothermia in nestlings of the European storm petrel *Hydrobates pelagicus*

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Although procellariiform nestlings develop independent endothermic thermoregulation at an early age (Wheelwright and Boersma 1979, Ricklefs et al. 1980), they may not be able to meet the high energetic costs of maintaining a constant high body temperature during prolonged periods of fasting or cold exposure (Geiser 2008). The ability to reduce body temperature and enter torpor could therefore represent an important survival strategy in the Procellariiformes. Torpor constitutes a facultative hypothermic response, characterised by a regulated reduction in metabolic rate and body temperature, which reduces energy expenditure and potentially facilitates survival under energetically-challenging conditions (McKechnie and Lovegrove 2002, Geiser 2008). This is distinct from an unregulated drop in body temperature that may be the result of an unavoidable pathological response. It is widely acknowledged that procellariiform chicks have the ability to reduce their energy requirements and enter a state of torpor, yet there are few (mainly brief) documented observations of hypothermia in procellariiform young (Davis 1957, Scott 1970, Wheelwright and Boersma 1979, Pettit et al. 1982, Lockley 1983, Simons and Whittow 1984, Boersma 1986), while some authors have found little or no evidence for hypothermic responses (Bech et al. 1991, Gębczyński 1995, Weathers et al. 2000).

Here, I describe incidental observations of hypothermia in nestlings of the European storm petrel *Hydrobates pelagicus* (hereafter, storm petrel), recorded while undertaking research at the UK's largest colony at Mousa, Shetland (60°00'N 01°11'W). Hypothermia in the storm petrel has previously been reported in young unattended chicks and in older chicks during periods of food deprivation; although Davis (1957), during three years of study, only observed a single 'torpid' chick, Scott (1970) provided greater detail, including measurements of body temperature, of five 'semi-torpid' chicks. Although I did not measure body temperature, the behavioural observations described here provide further evidence of the occurrence and nature of hypothermia in developing procellariiform chicks.

In the breeding seasons of 2010 and 2011, 75 and 82 occupied storm petrel nests, respectively, were monitored from laying through to late postnatal development. In 2011, eight nestlings were found exhibiting behaviours consistent with hypothermia, whereas no such observations had been made in the previous year. In each instance, an unbrooded chick was observed in the nest cavity, displaying no obvious signs of life. On removal from the nest, the only initial indication that a nestling might still be alive was that the downy plumage was dry; otherwise, chicks appeared limp and lifeless with the eyes closed. Following about a minute of being held in the palm of the hand, perceptible signs of life began to appear. Minor movements were initiated, usually starting with the legs, then the head and gaping of the mouth, before a definite heartbeat became detectable, both visually and to the touch. None of the nestlings started calling or regained activity levels typical of healthy chicks, though they may have done so if they had been

handled, and thus warmed, for longer. The time during which nestlings were handled and kept outside of the nest was kept to a minimum to minimise investigator effects.

Table 5.1 Details of eight European storm petrel *Hydrobates pelagicus* nestlings (denoted by the letters A-H) observed exhibiting hypothermic responses. Data presented include age at which first unattended, age when first observed in hypothermic state, days elapsed between first record of hypothermia (H) and recovery (R) or death (D), mass at hypothermia (H) and recovery (R), and fledging success. The table is divided into those nestlings presumed to be incompetent and competent of endothermic regulation at the onset of hypothermia and thus incapable and capable of displaying torpor, respectively.

Nestling ID	Age unattended (d)	Age (d)	Days elapsed (H - R or H - D)	Mass _H (g)	Mass _R (g)	Fledged
Presumed incompetent of endothermic regulation						
A	2	2	1D	4.9	NA	No
B	3	3	1D	5.3	NA	No
C	3	4	1R, 3D	5.4	8.1	No
D	5	5	1D	5.9	NA	No
Presumed competent of endothermic regulation						
E	6	6	1D	9.0	NA	No
F	6	10	1R	7.1	11.7	Yes
G	5	11	1D	10.4	NA	No
H	7	20	4R	17.4	25.1	Yes

Presumably these nestlings were experiencing large reductions in body temperature, resulting in behaviours characteristic of hypothermia. Drops in body temperature of more than 10°C below the mean were reported, in association with similar behaviours to those described here, in chicks of the European storm petrel (Scott 1970) and fork-tailed storm petrel *Oceanodroma furcata* (Boersma 1986). Four of the storm petrel nestlings in this study (denoted A - D; see Table 5.1) were aged between 2 d and 5 d when found in a hypothermic state and were unlikely to be capable of independent thermoregulation. Being a facultative hypothermic response, the ability to display torpor is concomitant with endothermic thermoregulation. In 2010, nestlings were brooded for around 7 d (median; range: 4-11 d) after hatching; this is similar to that reported in other studies (Davis 1957, Mínguez and Oro 2003) and probably coincident with complete development of endothermy. The brooding period was significantly shorter in 2011 (median: 6 d, range: 3-8 d; Wilcoxon rank-sum: $W = 730$, $P < 0.001$) and it was not uncommon to observe chicks alone in the nest within the first few days after hatching. Hypothermia exhibited by these very young chicks was likely the result of an

unavoidable pathological response, as opposed to regulated torpor, associated with the premature cessation of brooding. Indeed, all of these chicks died within three days of entering hypothermia.

The other four hypothermic nestlings (denoted E - H; see Table 5.1) ranged in age from six to 20 d and presumably were all competent of endothermic thermoregulation. Only two of these nestlings (F & H) showed signs of recovery from their hypothermic state, presumably associated with a rise in body temperature back towards normothermia, while the other two chicks (E & G) were found dead the following day. I refer to recovery as a gain in mass and regain of normal lively behaviour, irrespective of subsequent successful fledging. The two former nestlings were found to have regained a normal lively state, following receipt of food, as evidenced by increases in mass of 4.6 g and 7.7 g within 24 h and 96 h respectively (all mass measurements were adjusted to a standardised time to account for mass loss during the day between feeds, according to Bolton (1995)). Parental brooding of these chicks had ceased and they had already been unattended for 4 d and 13 d, respectively, prior to becoming hypothermic. These two instances of hypothermia may represent examples of genuine torpor - an adaptive and facultative response to an energetic challenge, presumably due to exposure to a long period of cold or starvation. While a third nestling (C) did show signs of recovery within 24 h, it was found dead a further two days later; at just 4 d, the nestling was unlikely to have fully developed endothermy and thus the hypothermic response observed may not have represented actual torpor. This chick was in fact accompanied in the nest by an adult, when observed in a hypothermic state, but was not being actively brooded.

The onset of torpor in birds has been linked to a shortage of food (Koskimies 1948, Prinzinger and Siedle 1988) and body temperature was found to be strongly correlated with food load in the fork-tailed storm petrel (Boersma 1986). Disruptions in provisioning causing prolonged starvation could thus trigger entry into a facultative hypothermic state. Scott (1970) noted a marked reduction in feeding frequency by European storm petrels when the wind was Beaufort Force 7 ($>14 \text{ m s}^{-1}$) or greater, while Davis (1957) found evidence for a reduction in meal size under such rough conditions. However, neither author linked weather conditions with observations of hypothermia. In the first 16 days of August 2011, during which six instances of hypothermia were recorded, the average daily wind speed at Mousa (recorded from the nearest station located at Lerwick, c. 33 km away) was significantly higher (median: 6.2 m s^{-1} , range: $3.1\text{--}10.8 \text{ m s}^{-1}$) than in 2010 (median: 4.6 m s^{-1} , range: $2.1\text{--}6.2 \text{ m s}^{-1}$; Wilcoxon rank-sum: $W = 46.5$, $P = 0.006$), when no hypothermic chicks were observed. On seven out of those 16 days, the average wind speed was equal to or greater than 7.2 m s^{-1} (max.: 10.8 m s^{-1}) in 2011, while it never exceeded 6.2 m s^{-1} during the same period in the previous season. While these daily mean wind speeds do not approach the speeds referred to by Scott (1970) and Davis (1957), the differences observed may still be sufficient to

affect provisioning rates; data on maximum wind speed are not known. Mean ambient temperatures were not significantly different during the same period in 2011 (median: 12.5°C, range: 10.3–15°C), compared with 2010 (median: 12.7°C, range: 11.8–14.5°C; Wilcoxon rank-sum: $W = 145$, $P = 0.184$), suggesting that the occurrence of hypothermia in 2011 was not driven by prolonged exposure to low temperatures.

Although I did not observe provisioning behaviour, it seems highly likely that the occurrence of temporary hypothermia was the result of indirect effects of strong winds resulting in reduced feeding frequency and/or food load. Nestling survival was significantly lower in 2011 (45.1%) compared with 2010 (57.3%, GLMM: $z = -2.53$, $P = 0.011$), a further indication of less favourable conditions for successful chick-rearing. Regardless of whether the hypothermic responses described are facultative (i.e. genuine torpor) or not, the observations indicate that the body temperature of developing storm petrels can be highly plastic, even once endothermic thermoregulation has fully developed. The ability to survive periods of hypothermia may be adaptive, facilitating survival under less favourable conditions, though it may also constrain growth (Boersma 1986) and delay fledging date (Davis 1957). The observations documented here offer further insight into the nature and occurrence of torpor in Procellariiformes, but more detailed studies, particularly the recording of body temperature over time, are required to improve our understanding of the ability to display a regulated hypothermic response and the consequences for individual growth and development.

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NB The figure for breeding success in 2011 was quoted incorrectly in the published version of this article; this has been corrected in this version and a request has been submitted to the publisher to publish an erratum in the next issue.

6. Optimisation of survey effort for spatially-explicit capture-recapture population estimates

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Abstract

Mark-recapture techniques provide long-established and well-developed methods to estimate population size across a vast range of taxa. Such techniques rely on often untested assumptions that all individuals have equal probability of capture, or that variation in individual capture probability is adequately accounted for in the modelling procedure. Capture probability is likely to vary spatially, according to the distance from trapping sites to individuals' activity centres (e.g. breeding location) and trapping devices, and temporally, according to individuals' activity cycles. Whilst spatial heterogeneity can be accounted for in spatially-explicit capture-recapture (SECR) models, there is little guidance about the duration of survey effort. We obtained spatially-explicit mark-recapture data for three species of nocturnal burrow-nesting seabirds which alternate extended periods at the nest with foraging bouts at sea. In one species, studied by radio telemetry, we found substantial variation in capture probability with the nest attendance cycle, distance from the nest site and between coastal and inland nests. We calculated the optimal spacing of capture sites and duration of capture episodes to minimise inter-individual variation in capture probability. A SECR model successfully estimated population size of two further species captured in spatially-referenced mist-nets. We found large differences in home range size and capture probability between these two species, which breed in different seasons on the same island. Whilst the methodological and analytical approaches adopted here are applicable to a wide range of other taxa, the considerable differences we found among the three closely related species highlight the need for an informed species-specific survey design.

Introduction

Capture-recapture methods provide widely used techniques for estimating population size in ecological and conservation studies. The technique has a long history (Petersen 1894) and initially relied upon assumptions of random mixing of marked individuals within a closed population (an absence of births, deaths, immigration or emigration) between capture and recapture episodes. Subsequent developments in both the methods of data collection (involving repeated recapture episodes) and analysis permitted the estimation of several demographic parameters (Sekar and Deming 1949, Seber 1986). More recently, spatially-explicit capture-recapture (SECR) methods have been developed, which incorporate spatial information to estimate the approximate location of each individual's home-range centre and improve the reliability of population size estimation (see Borchers 2012 for an overview). Such models emphasise the need to consider the spatial characteristics of any sampling procedure to estimate population size, since most capture-recapture exercises are inherently spatial in nature. SECR methods allow post-hoc calculation of the effective sampling area of the capture method.

Information on patterns of behaviour and movement of the study species is required for efficient sampling design (Sollmann et al. 2011, Chambert et al. 2012, Noss et al. 2012). Even when spatial information is incorporated into the survey design and analysis, mark-recapture techniques rely fundamentally on the assumption that capture probabilities are equal for all individuals (Williams et al. 2002), or that variation in capture probability can be effectively modelled (Lebreton et al. 1992). The assumption of equal capture probability is rarely likely to be valid (Begon 1983) due to spatio-temporal heterogeneity in individual capture probability (Lebreton et al. 1992) resulting from intrinsic factors such as age, gender, life-history stage and activity patterns, or extrinsic factors (Crespin et al. 2008, Cubaynes et al. 2010) such as distance of breeding site or home range centres from capture locations. The design of any mark-recapture study must therefore employ a spatial and temporal sampling regime that minimises variation in capture probability among individuals, or collects additional data that allow such variation to be reliably modelled.

Procellariiform seabirds are among the most threatened avian taxa globally (Croxall et al. 2012) and include many species that nest underground on remote islands, remain concealed in the nest by day and are active in the colony only during the hours of darkness. These characteristics present severe challenges to estimation of population size, which hampers assessment of conservation status and ecological studies (Sanz-Aguilar et al. 2010). Current methods for population estimation rely principally on diurnal response to playback of recordings of nest song (Ratcliffe et al. 1998, Mitchell et al. 2004), which requires access to all potential nest entrances which may be impractical in some colonies. In addition, the typically low response rate necessitates the application of a large correction factor, with large associated confidence limits (e.g. Ratcliffe et al. 1998, Bolton et al. 2010). Such imprecision is exacerbated for colonies that are too large for a complete census and a sampling approach is employed. Capture-recapture methods may provide an alternative approach to population estimation for nocturnal burrow-nesting seabirds. Attempts have been made to use recapture data obtained by mist-netting birds in the colony at night to estimate population size for some colonies (Insley et al. 2002), but uncertainties remain concerning the extent of the area that is effectively sampled by mist-netting birds in flight, given the localised nature of the capture area within the colonies and lack of information about the extent of movement of breeding birds within colonies (Sanz-Aguilar et al. 2010). In addition, Procellariiformes undertake long incubation bouts, lasting up to a week or more whilst the off-duty partner forages at sea (Warham 1990). It is not known whether incubating birds leave the nest burrow at night, making them available for capture, or whether they are active in flight within the colony only on nights when they resume or cease incubation shifts. These issues affect the spatio-temporal mixing of marked birds available for

capture in mist-nets in the colony and therefore affect estimation of population size with capture-recapture models.

Here we adopt two approaches to quantify the extent of spatio-temporal variation in individual capture probability. Firstly, we use radio-tracking to examine intra-colony movements in relation to breeding status, distance from the nest site, and nest attendance routines for European storm petrels *Hydrobates pelagicus* nesting on a 200 ha island in Scotland, UK. Radio-tracking allows the collection of detailed information since birds' nest locations and nest attendance patterns are known. Secondly, for two further species of storm petrel (Madeiran *Oceanodroma castro* and Monteiro's *O. monteiroi*) nesting on a very small (7.5 ha) islet in the Azores, North Atlantic, we used an intensive programme of mist-netting at multiple sites, conducted over a 13-month period, to estimate population size using SECR methods. Mist-netting allowed collection of recapture data from much larger numbers of birds than permitted by radio-tracking, which was further facilitated by the presence of two storm petrel species nesting on the island. We simulated the estimation of density from varying numbers and spatial configurations of mist-nets to assess minimum sampling effort, and use this simulation in combination with effective sampling radius of each mist-net to recommend an optimal sampling design for future surveys. This analysis provides a robust assessment of the spatial and temporal sampling effort required for estimating the abundance of mobile species using a SECR approach.

Materials and methods

Study species

Storm petrels are the smallest of all seabirds (25 g to 45 g) and typically undertake incubation shifts of 2-5 d duration (Davis 1957, Harris 1969, Bolton 1996). Being vulnerable to predators, at most breeding locations storm petrels are active in flight around the colony only during the hours of darkness and remain concealed within their nest crevices or forage at sea during daylight.

Radio-tracking of European storm petrels

Radio-tracking was conducted on the island of Mousa (60°00'N 01°11'W) in the Shetland archipelago, UK, between May and August 2012. The island holds the largest breeding colony of European storm petrels in the UK (Mitchell et al. 2004), most recently estimated by playback census at 11,781 pairs in 2008 (Bolton et al. 2010). Whilst the colony extends over most of the island, nest-sites are restricted to crevices among stones and boulders and approximately half the population nest in discrete areas of boulder beach and coastal boulder talus, with the remaining birds nesting in dry stone walls and abandoned buildings, mainly located away from the coast (M. Bolton, unpublished data). Intra-colony movements of storm petrels were

tracked by fitting low-power coded radio tags (0.54 g Micro PIP, Biotrack UK) to the four central tail feathers using a small strip of adhesive tape. The tag antennae were trimmed to a length of 6 cm and field trials indicated that tags could generally be detected no further than 40 m from tracking stations. Nine birds were fitted with tags at a sub-colony on the East coast and eleven birds were tagged at a sub-colony in the interior of the island (Fig. 6.1A). All tagged individuals were known to be breeding at the time of tag deployment and their nest locations were known. Only one member of each pair was tagged. An automated tracking station (“DataSika” Data Logging Receiver, Biotrack UK) with an omni-directional antenna was located at each sub-colony, operated by a timer to scan for tagged birds every 2.5 s from 30 min before local dusk (when the first birds return from foraging at sea) until 30 min after local dawn (after the departure of any incubating birds). Any birds detected after dawn or before dusk were assumed to be present at their nests all day. Two further automated tracking stations were deployed for periods of 2-7 d at distances ranging from 50 m to 1250 m from the study sub-colonies, in coastal and inland (>100 m from Mean High Water) locations (see Fig. 6.1A). The two mobile tracking stations were always located in areas known to be occupied by breeding storm petrels.

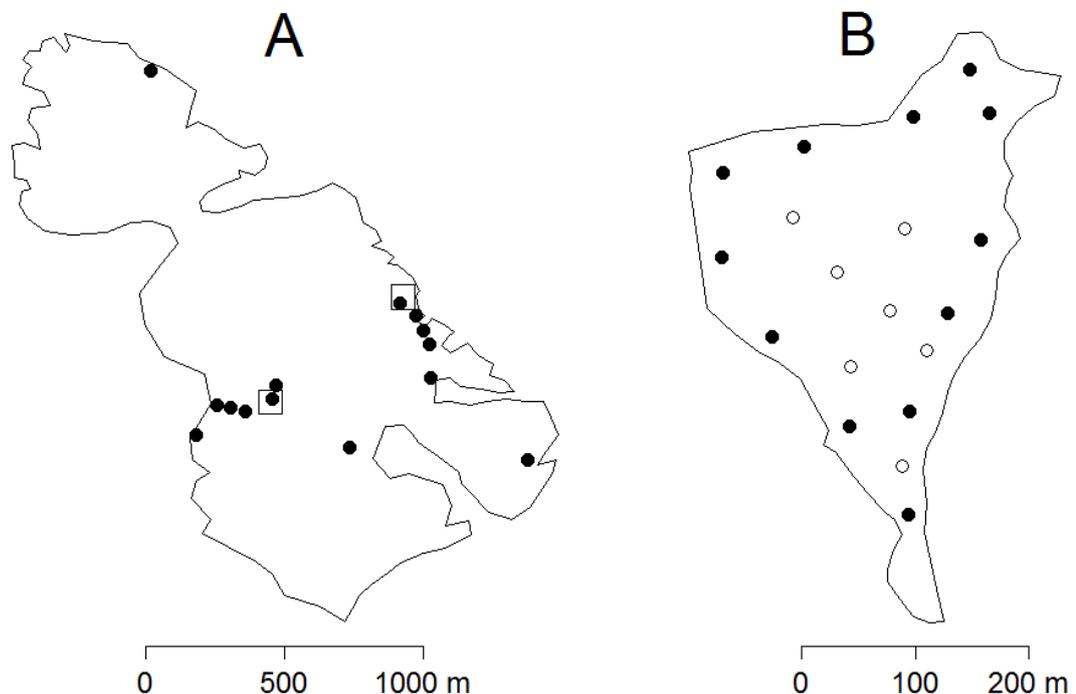


Figure 6.1. (A) Mousa Island, Shetland, UK: location of tracking stations (closed circles) and nesting colonies (open squares) of radio-tagged European storm petrels; (B) Praia Islet: location of mist-nets for capture-recapture study of Madeiran (all circles) and Monteiro's storm petrels (closed circles only).

Tagged birds were marked with a small spot of non-toxic acrylic paint to enable individual recognition and all nests attended by tagged birds were

subsequently monitored daily by visual inspection, to verify the correct detection of tagged birds by the local tracking stations, to detect if tags were shed or had failed, and to determine the outcome of breeding attempts. A sample of further nests where no adult was tagged was similarly monitored to verify that radio tagging had no effect on the incidence of temporary egg neglect or nest failure rates of nests where one member was tagged (percent of days of egg neglect: $8.3 \pm 2.5\%$ ($n = 20$) and $11.6 \pm 3.6\%$ ($n = 13$), respectively; Kruskal Wallis: $X^2_1 = 0.258$, $P = 0.61$; failure rates 15% ($n = 20$) and 25% ($n = 16$), Fisher Exact Test: $P = 0.67$).

We classified an individual's nest attendance routine for each night of tracking on the basis of attendance at the nest on the preceding and following days into six categories (see Supplementary material, Appendix 1 for further details):

Returning: not present at the nest on the previous day, present on the following day

Departing: present on the previous day, not present on the following day

Attending: present on both the previous and following day, incubating the egg or brooding the chick

Off duty: not present on either the previous or following day, when the nest contained an egg

Provisioning: not present on either the previous or following day when the nest contained a chick capable of thermoregulation and did not require day-time attendance

Failed: nest that contained an egg but was no longer attended by either parent by day

Statistical analysis of radio-tracking data

Radio tag detection data were analysed using a Generalised Linear Mixed Model (GLMM), implemented in SAS[®] v.9.2, using a binomial error structure and logit link function. Since the dataset comprised repeated detections of the same 20 tagged individuals, we used individual identity as a random term. We examined nightly detection probability (1 = detected, 0 = not detected) at each tracking location as a function of: distance from the nest (in metres), nest attendance routine (a six-level factor as defined above), nest location (coastal/inland) and detection location (coastal: $\leq 100\text{m}$ from Mean High Water; or inland). We examined the interaction between attendance routine and distance, to determine whether nest attendance affected birds' movements around the colony. We also included the interaction between nest location and detection location to determine whether there were differences in detection probabilities of coastal and inland nesting birds according to detection location. Where the model indicated significant effects of factors or their interactions, Bonferroni-corrected post-hoc pairwise comparisons were conducted to determine statistical significance among factor levels.

Simulations of the effect of trap effort on variance in individual capture probability

We used the empirical relationships derived from the model above to simulate how variation in trap effort (number of traps), and trap placement (coastal or inland) would affect variation in colony-wide detection probabilities of individuals. We simulated a circular 200 ha colony (radius 800 m), assuming that nests were uniformly distributed, one at each intersection of a 10 m grid. We considered three scenarios:

- i. Locating increasing numbers of traps (2-12) at equally spaced locations around the coastline.
- ii. Locating one trap in the centre of the island in addition to an increasing number (2-12) of coastal nets, as above.
- iii. Locating traps without a coastal bias, in the manner that minimised the mean distance from each nest to the nearest trap.

We calculated the probability of detection of birds from all nests at all trap sites and then calculated the overall colony-wide detection probability, P , of birds from every nest from the following equation:

$$P = 1 - \prod_{i=1}^n (1 - P_i)$$

where P_i = probability of detection by trap i . Outputs from all models are shown in Supplementary material Appendix 2, Fig. A2.1.

Whilst the aim of the capture protocol is to minimise variance in individual capture probability (which will tend to decrease as capture probability itself decreases), the trapping regime must also generate sufficiently high capture probabilities to generate acceptably small confidence limits. Therefore we also examined how variation in trap effort and placement affected mean individual detection probability and the mean:variance ratio.

Mist-netting of Madeiran and Monteiro's storm petrels

The SECR study of Madeiran and Monteiro's storm petrels was conducted on the islet of Praia, in the Azores archipelago (39°03'N, 27°57'W), where the two species nest sympatrically, but with temporal segregation of breeding seasons (Monteiro and Furness 1998, Bolton et al. 2008). Both species nest in rock crevices and in burrows dug into the topsoil. The population sizes were estimated at 200 and 100 breeding pairs respectively (Monteiro et al. 1996) on the basis of the extent of nocturnal vocal activity, the numbers of nests found and the apparent availability of suitable nesting habitat. The relatively small size and accessible terrain of Praia Islet facilitated an intensive and regular deployment of mist-nets, which were placed at 19 locations spaced on average 60 m apart across the entire island (Fig. 6.1B). Although Monteiro's storm petrels were believed to nest in the interior of the island,

it was not possible to erect mist-nests there during their breeding season due to the presence of nesting terns.

From October 2000 – August 2001 mist-nets were operated on 20 nights during the breeding season of Madeiran storm petrels (15th October 2000 – 14th April 2001) and 19 nights during the breeding season of Monteiro's storm petrel (14th April – 1st August 2001). On each night, one to four mist-nets were operated at a subset of the 19 capture localities and at each locality we operated mist-nets for 1–11 nights. In total, we operated 47 net-nights during the breeding season of Madeiran storm petrel and 37 net-nights during the breeding season of Monteiro's storm petrel.

Spatially-explicit capture-recapture models to estimate storm petrel density

Spatially-explicit capture-recapture (SECR) models use the individual identification of animals at different locations within the study area to estimate the approximate location of each individual's home range centre (Efford et al. 2009, Royle et al. 2009, Borchers 2012). These methods utilise additional spatial information compared to standard capture-recapture analyses to estimate abundance, and require no *a priori* definition of the effective sampling area (Borchers 2012). For nesting storm petrels, the 'home-range centre' can be conceptually defined as the location of the nesting burrow and capture probabilities in mist-nets near nesting burrows would be expected to be higher than in mist-nets that are farther away from nesting burrows. We considered each night during which mist-nets were operated as a discrete encounter occasion. For analysis, we only used encounter occasions for each species that fell into the time range when the species was actually breeding (and thus present) on the island. One of the major assumptions of mark-recapture models is that the population is closed during the study period. The long study duration rendered it likely that prospecting birds may have visited the island, or that failed breeders may have departed from the island. We used a closure test to evaluate whether our data met the assumption of population closure (Stanley and Burnham 1999). If the closure assumption was violated, we reduced all occasions to a single occasion and simply used the spatial encounter history of individuals to estimate density (Borchers and Efford 2008).

We used a half-normal detection function and assumed a Poisson point process for the distribution of animal home range centres (Borchers and Efford 2008, Noss et al. 2012). For numerical integration, the likelihood function was evaluated at points spaced 10 m apart and distributed evenly throughout the island area of Praia. All SECR models were fit in R 2.13.0 (R Development Core Team 2010) using the library 'secr' (Efford 2011). We estimated the total island population size for each species by multiplying the estimated density by the area of Praia Islet (7.5 ha). To better compare the estimated shape parameter of the detection function (σ) with the radio-tracking data, we assumed a bivariate

normal distribution for individual home ranges and converted the sigma estimate to the mean radius of a home range using the formula provided by Noss et al. (2012). We present all model estimates as means \pm SE.

To assess the effect of sampling effort on density estimates derived from SECR models, we estimated densities for each species on Praia using subsets of data and the model described above. To create subsets, we removed the net with the lowest number of captures and we repeated this step until only data from three nets remained. We defined the minimum adequate sampling effort as the minimum number of nets that resulted in a mean density estimate that was within the 95% confidence intervals of the estimated density using the full data set.

Temporal variation in capture probability in relation to nest attendance cycles

Birds returning to or departing from nests may have higher probability of capture than those engaged in ongoing incubation (which may remain in their nests all night), or those which are off-duty, presumably foraging at sea. If such variation in capture probability exists, it will be important to conduct sampling over a sufficiently long period to equalise probability of capture among individuals. We therefore determined: (i) the duration of the nest attendance cycle; (ii) how attendance-related variation in nightly capture probability would affect mark-recapture population estimates as the duration of the sampling period increased. The nest attendance cycle was determined for the three study species by daily visual inspection of nests where both adults were individually marked (see Davis 1957). The effect of increasing sampling duration on population estimates was conducted for European storm petrels using the observed frequencies of each nocturnal attendance behaviour (returning, departing, attending, off-duty) and the relationship between each of these behaviours and detection probability (determined by radio telemetry) as input parameters into a deterministic simulation model. We modelled the nightly detection probability of 100 individuals that followed the observed nest attendance pattern and simulated the capture history of each over the course of 10 consecutive nights on the basis of the attendance-specific detection probabilities. We calculated the cumulative proportion of the population detected, the number of recapture events, and mark-recapture estimate of population size as nightly sampling effort increased. For further details see Supplementary material Appendix 3.

Results

Radio-tracking of European storm petrels

Effect of nest attendance routine and distance from the nest on detection probability

For European storm petrels tracked with radio-telemetry, there were large differences in nightly detection probability according to nest attendance pattern (Fig. 6.2, $F_{5,904.8} = 22.62$, $P < 0.001$) and there was considerable heterogeneity in the effect of distance from the nest on detection probability according to attendance routine (Fig. 6.3, interaction effect $F_{5,1203} = 9.83$, $P < 0.001$). The modelled relationships indicated that detection probabilities were higher for birds commencing or finishing a bout of nest-attendance (incubating or brooding), than for off-duty birds (most of which are assumed to be foraging at sea) and for birds currently engaged in an ongoing bout of incubation or brooding small chicks. The detection probability of birds which had failed in their breeding attempts (two individuals) was intermediate between these levels. Detection rates of the five individuals which, by the end of the study were only returning at night to provision thermally-independent chicks, were highly variable (Fig. 6.2).

Whilst detection probability was highest for birds returning to and departing from nests, detection rates for these birds declined sharply with increasing distance from the nest and mean values were similar to those of other attendance routine categories beyond 400 m (Fig. 6.3). In contrast, storm petrels attending nests where chicks no longer required brooding ('provisioning'), showed little reduction in detection rates with increasing distance from the nest. Tracking data from the two individuals whose nests failed during the course of the study revealed that these birds also roamed widely around the colony after nest failure (Fig. 6.3). Detection probability was lowest for birds attending the nest overnight (incubating or brooding small chicks). Such individuals rarely left their nests overnight and, if so, they remained within the vicinity of the nest site (Fig. 6.3).

Effect of nest and trap locations on detection probability: coastal versus inland sites

Having accounted for the influence of distance from the nest and attendance routines described above, tagged birds were more likely to be detected at coastal than inland tracking stations ($F_{1,1203} = 9.45$, $P = 0.002$), and there was a significant interaction between the effect of nest site location and tracking station location, on detection probability (Fig. 6.4, $F_{1,1203} = 7.65$, $P = 0.006$). At coastal tracking sites, there were no differences in the detection rates of both inland- and coastally-nesting storm petrels. Similarly, inland-nesting birds were equally likely to be detected at coastal sites as inland. However, coastal-nesting storm petrels had significantly lower detection rates at inland sites compared with coastal ones.

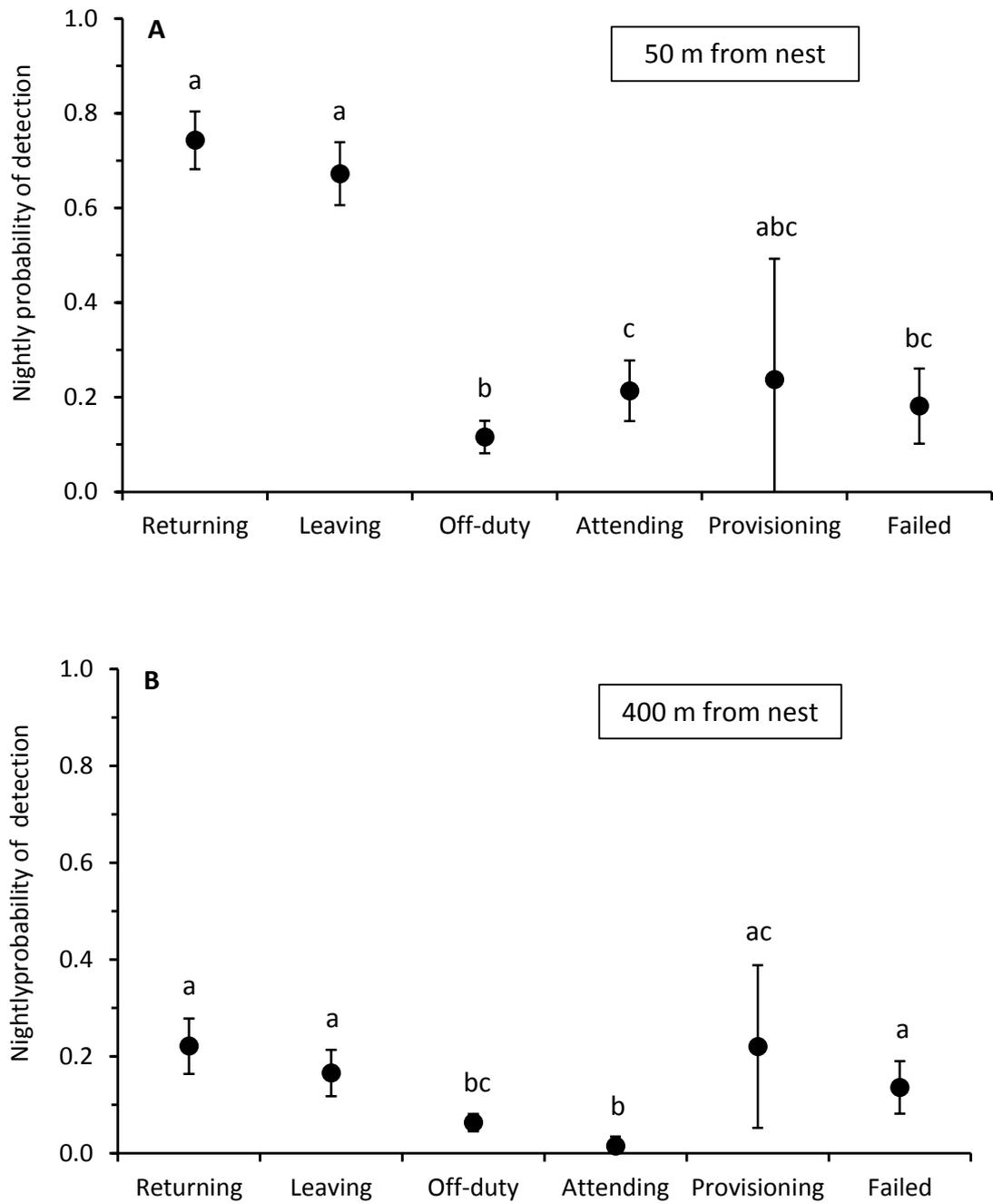


Figure 6.2. Detection probabilities (\pm SE) of radio-tagged European storm petrels in relation to nest attendance routine at (A) 50 m and (B) 400 m from nest location. Common superscripts indicate groups that did not differ at $P < 0.05$.

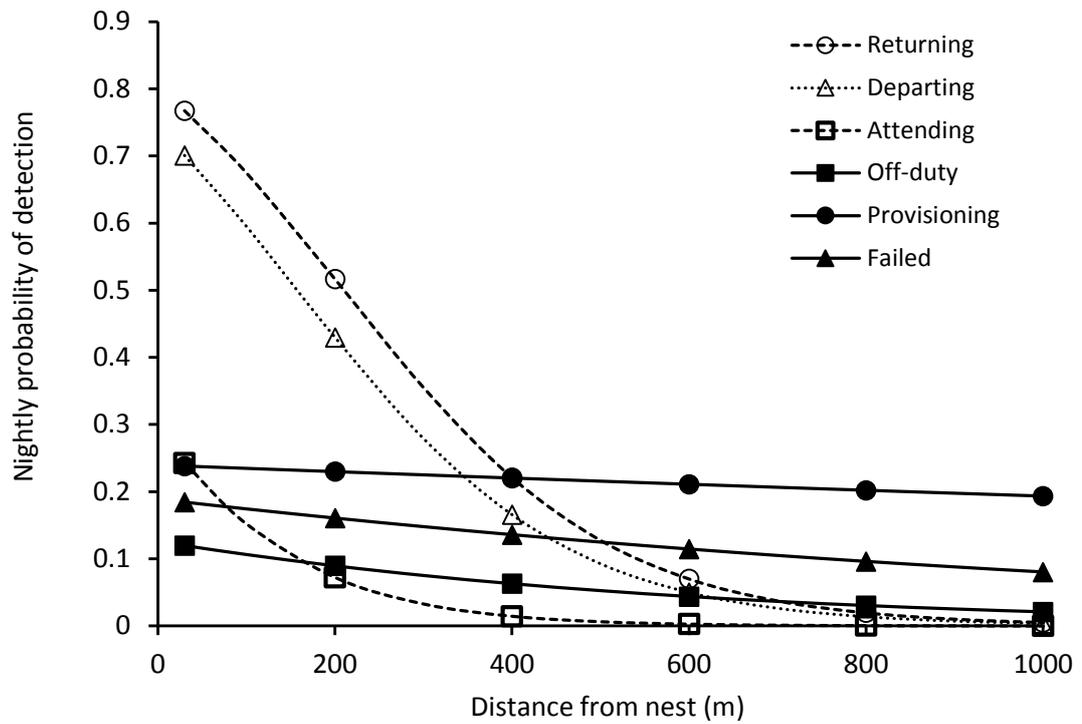


Figure 6.3. Change in probability of nightly detection of radio-tagged European storm petrels with increasing distance from the nest, according to nest attendance routine.

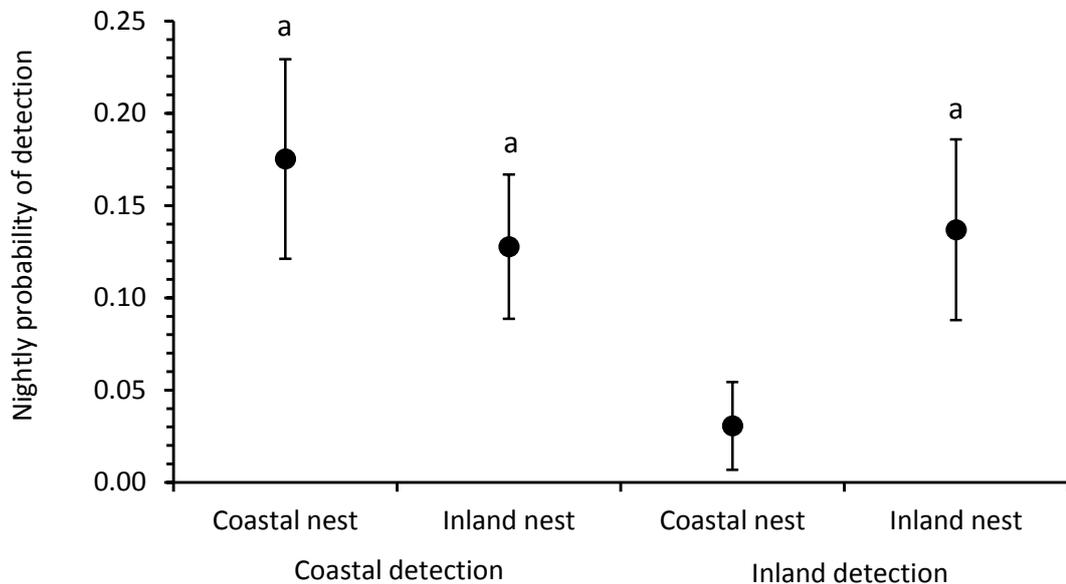


Figure 6.4. Influence of location of nest sites and tracking stations on detection probability (mean \pm SE) of radio-tagged European storm petrels. Groups that do not differ at $P < 0.05$ are indicated by a common superscript.

Effect of trap effort and trap placement on capture probability

In the simulation of differing trap numbers and placement, increasing the number of coastal trap stations from two to 12 resulted in an increase in mean individual capture probability from 0.10 to 0.49 (Fig. 6.5A). Variance in capture probability decreased from 0.0085 to 0.0066 as the number of traps increased from two to five, but increased as trap effort increased further as the centre of the colony became poorly sampled relative to the periphery. The addition of a single, centrally placed inland trap dramatically reduced variance as trap effort increased to nine traps (i.e. eight coastal and one central), but increased thereafter. Placement of traps in the manner that minimised the mean nest-trap distance across the entire colony resulted in higher variance in capture probability (Fig. 6.5B). The optimal sampling strategy, which maximised the mean:variance ratio, comprised a single centrally-located trap in conjunction with coastal traps, and peaked at nine traps (Fig. 6.5C; Supplementary material Appendix 2, Fig. A2.1), where the eight coastal traps were 612 m apart, and each was 800 m from the central trap.

SECR model of storm petrel population size on Praia islet, Azores

We captured 511 individual Madeiran storm petrels over the course of 20 nights of mist-netting during winter 2000/2001 and 144 individual Monteiro's storm petrels over 19 nights in summer 2001. The closure tests indicated that neither of the populations were closed during their respective breeding seasons (Madeiran storm petrel: $z = -2.26$, $P = 0.01$; Monteiro's storm petrel: $z = -2.96$, $P = 0.002$), hence we reduced the encounter history of all mist-nets to a single occasion. Madeiran storm petrels had a lower baseline capture probability than Monteiro's storm petrels (capture probabilities if the individual's activity centre was located at the capture site: 0.02 ± 0.00 and 0.37 ± 0.11 respectively) and the estimated density of Madeiran storm petrels was 259 ± 26 birds ha^{-1} compared with 126 ± 23 birds ha^{-1} for Monteiro's storm petrel. The 'effective search area' of each mist-net, reflecting the area over which birds captured in a given mist-net may nest, was estimated at 1.97 ha and 1.1 ha for Madeiran and Monteiro's storm petrels respectively, and estimated home-range radii (reflecting the distance at which the capture probability fell to zero) were 566 ± 203 m and 46 ± 5 m respectively. The estimated total population size for Monteiro's storm petrel on Praia Islet was 942 (95% CI: 654–1357) birds, and 1942 (95% CI: 1590–2373) birds for Madeiran storm petrel.

Removing mist-nets (and thus reducing sampling effort) predictably resulted in less precise and more biased density estimates for both species. The effect of low sampling effort was both more severe and in an opposite direction for the species with the smaller estimated home-range radius (Monteiro's storm petrel, Fig. 6.6). The difference was due to the lower detection probability for this species when there are few nets which inflated the population estimates, but the opposite occurred for the species with the larger home range. We estimated that a minimum of five

mist-nets would be required to obtain unbiased density estimates of Madeiran storm petrel, and a minimum of nine mist-nets for Monteiro's storm petrel (Fig. 6.6).

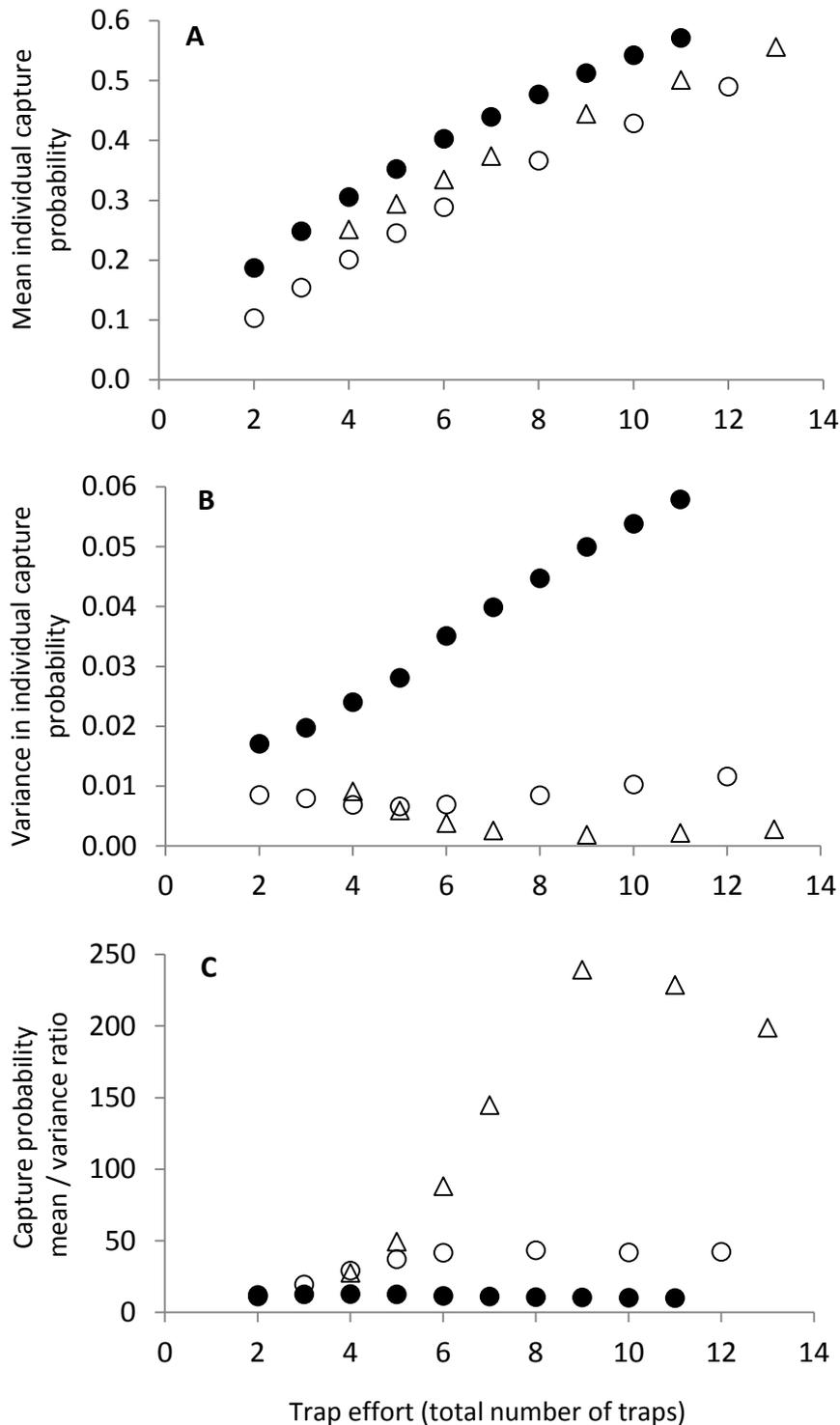


Figure 6.5. Influence of trap effort (number of traps deployed) on (A) mean, (B) variance and (C) mean:variance ratio of individual capture probability of radio-tagged European storm petrels under three different scenarios of trap locations: coastal traps only (open circles), coastal traps plus a single centrally-located trap (open triangles) and minimising mean distance between each nest and traps (closed circles). See Supplementary material Appendix 2, Fig. A2.1 for more details.

Temporal variation in capture probability in relation to nest attendance cycles

The nest attendance cycle had a mean duration of 5 d for European storm petrels, 7 d for Monteiro's and 10 d for Madeiran storm petrels (Table 6.1). The simulation model of capture probability over a range of trapping durations showed that an extended trapping period would not be necessary to generate reliable population estimates. Despite large differences in nightly capture probability associated with nest attendance behaviour indicated by radio-tracking, using these values as model inputs indicated that during three nights of trapping more than half the population would be sampled and the resulting estimate of population size would be within 14% of the true value (Fig. 6.7). Detection probabilities were highest when birds departed and returned to the nest, which occurred on average every 2.5 nights (Table 6.1). On the third night of trapping, birds caught on the first night as they departed from, or returned to, their nests, would have high recapture probabilities as they departed from, or returned to, their nests respectively. Doubling the survey effort to six nights resulted in no improvement in survey precision, although estimates based on 10 nights of trapping were within 5% of the true value.

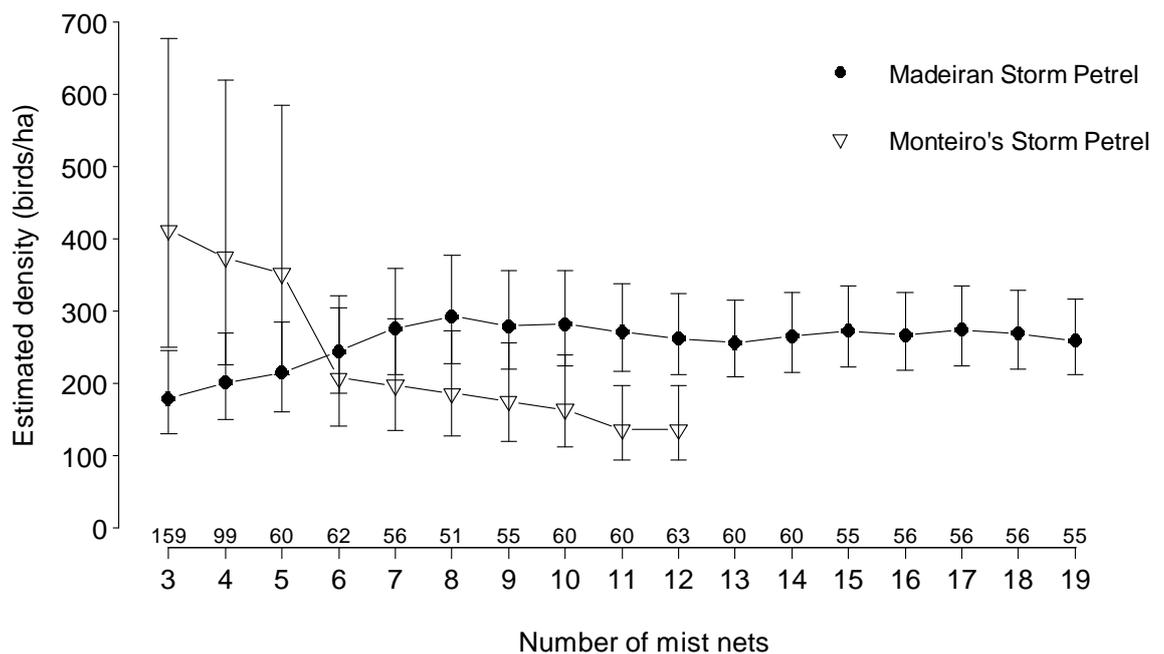


Figure 6.6. Estimated density (\pm 95% confidence intervals) of Madeiran (closed circles) and Monteiro's (open triangles) storm petrels on Praia Islet generated by spatially-explicit capture-recapture models based on a varying number of mist-nets. Numbers above the x-axis reflect the average nearest-neighbour distance (m) between mist-nets in the data set used to estimate density.

Table 6.1. Duration (d) of components of the nest attendance cycle of European storm petrels (Mouša, Shetland) and Madeiran and Monteiro's storm petrels (Praia Islet, Azores). Mean of individuals' mean values \pm SE are given with the number of individuals in brackets.

Species	Incubation shift	At-sea foraging	Total cycle
European storm petrel	2.30 \pm 0.06 (68)	2.78 \pm 0.13 (68)	5.08 \pm 0.13 (68)
Madeiran storm petrel	4.89 \pm 0.18 (60)	5.21 \pm 0.18 (60)	10.10 \pm 0.30 (60)
Monteiro's storm petrel	3.49 \pm 0.11 (82)	3.92 \pm 0.17 (82)	7.40 \pm 0.21 (82)

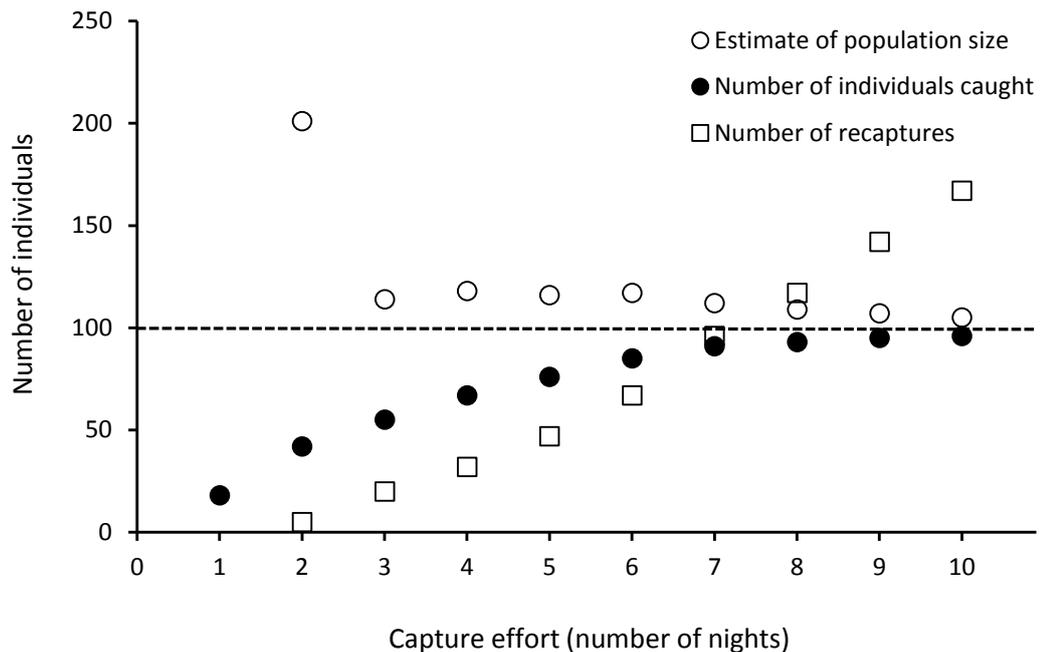


Figure 6.7. Deterministic simulation model outputs of the effect of increasing the capture effort on the number of individuals captured (closed circles), number of individuals recaptured (open squares) and estimate of population size (open circles). The virtual population comprised 100 individuals (dashed line).

Discussion

The use of mist-netting and spatially-explicit capture-recapture models provides a viable and robust method to estimate the density and abundance of nocturnal burrow-nesting seabird species on small islands. Our analyses from radio-tracking and mist-netting showed that a period of 3-10 d of mist-netting at localities 60-600 m apart would likely be sufficient to estimate the population size of storm petrel species that are not possible to count visually. The detection probabilities of radio-tagged birds reported here are likely to be higher than capture rates of storm petrels using mist-nets. The radio-tracking stations were likely to detect the presence of all birds that remained within c. 40 m of the receiving antenna long enough to register four radio pulses, which were emitted every 1.9–2.1 s (i.e. minimum period of 5.7–

6.3 s). Storm petrels may evade capture in mist-nets if they fly parallel to or above a net, and a proportion of those individuals which strike the net subsequently escape, particularly in windy conditions. However, the likely lower capture rate of mist-nets does not affect our conclusions: reducing the trap-wise capture rate by 50% yielded the same pattern of relationships between trap effort and variance in colony-wide capture probability and mean:variance ratios shown in Fig. 6.5.

Nightly detection probabilities of tagged birds away from the nest varied according to nest attendance routines. We found that the probability of detection of individuals engaged in ongoing nest attendance duties (incubation or brooding), and those of off-duty and failed breeders, was not zero. Davis (1957) found that nests of European Storm petrels were visited on nights in which changeovers did not occur and although the identity of overnight visitors was unknown, he considered that the visits were most probably made by the off-duty bird since changeovers occurred at irregular intervals yet gaps in incubation were rare. He showed that nests were visited on 63 - 83% of nights whereas changeovers occurred on just 35-44% of nights. Whilst individuals engaged in all stages of the nest attendance cycle, plus failed breeders, may be detected in the colony at night, the substantially higher detection probability of individuals commencing or ceasing a nest-attendance shift provides a source of variation in individual capture probability that must be accounted for either in the survey design or in the analysis if adequate covariates of such variation are available. There is currently no method to unambiguously determine the nightly nest-attendance status of mist-netted individuals, although there may be scope to develop such methods in the future based on weight loss associated with fasting during incubation (Bolton 1996). Failed breeders and those with hatched young may be identifiable from the re-feathering of a non-vascularised brood patch. Further work should be undertaken to assess the reliability of these criteria to assign activity status to individuals. Until such information is available, a capture episode should be conducted for a sufficient period to enable all individuals to have a similar probability of capture. A simulation model suggested that for European storm petrels a sampling period of three days, representing half the entire nest-attendance cycle, produced population estimates within 14% of the true value, and to reduce this substantially further, survey effort needed to exceed seven days. In situations where mist-netting may be constrained by inclement weather, extended sampling periods may not be practical, or necessary. However, conventional playback surveys typically require a period of one to two weeks to complete, and even small colonies require a minimum of one week due to the need to calibrate the response rate for each colony (Ratcliffe et al. 1998). Hence, for small colonies of European storm petrels a mist-netting capture-recapture approach may represent the more time-efficient survey method.

Since failed breeders and those provisioning thermally independent chicks have lower detection probabilities, which are not expected to vary in a cyclical way,

such individuals will be under-represented in the sample, leading to under-estimation of population size. We recommend that mark-recapture surveys be conducted during the peak of incubation, before substantial numbers of chicks hatch or nests fail, to minimise the numbers of individuals falling in these categories. Conducting surveys early in the season will also have the additional benefit of excluding prospecting immature birds which tend to roam widely among the colonies later in the year (Scott 1970, Fowler and Hounscome 1998).

Detection at coastal stations of birds from inland nests was not significantly lower than that of birds nesting coastally, presumably since all birds will need to cross the coastal margins of the colony on return from the sea. However, the reverse was not the case, showing that coastal-nesting birds are less likely to venture inland than those attending inland nests. An important consequence of this pattern for the design of a SECR study is that a network of coastal capture stations should sample both coastal- and inland-nesting birds at similar rates, at least for a colony of similar dimensions to Mousa. Further work in colonies that cover larger areas would be necessary to validate the generality of this finding. Simulation models showed that for a colony the size of Mousa, where nests are uniformly distributed or nest distribution is unknown, the most efficient sampling design which minimised variance in colony-wide capture probability and maintained high mean capture rates would be to deploy eight traps equally spaced around the coast (c. 600m apart) and a single net in the centre of the island.

Empirical assessment of the effect of the number of mist-nets on reliability of survey estimates in Azores showed that population size estimates stabilised at net spacing of about c.60 m for both Madeiran and Monteiro's storm petrels, which compares to estimated home range radii of 566 m and 46 m respectively. The design for SECR mist-netting studies can thus follow the same guidelines as for camera trapping studies, which suggest that the spacing of detectors (mist-nets or camera traps) should be small enough to facilitate the capture of individuals at more than one location (Efford et al. 2009, Noss et al. 2012). Sollmann et al. (2011) suggest that the distance between traps could be as large as twice the extent of individual movement. Simulated reductions in trap density showed that even when most animals were captured at only one trap, population estimates remained relatively accurate and the SECR model was only unable to estimate population size reliably when very few animals were captured at more than one trap.

Our population estimates derived from the SECR approach were considerably higher than previous estimates of population size on this island (Monteiro et al. 1996). However, earlier estimates were more subjective, based principally on the extent of nocturnal vocal activity, the number of known nest sites, the perceived nest density and the extent of breeding habitat available. The capture of 511 individual Madeiran storm petrels over the course of 20 nights suggests that the population was considerably larger than the estimate of 200 pairs in 1996

(Monteiro et al. 1996). A further cause of discrepancy is the inclusion of an unknown number of non-breeding individuals in the estimates presented here. In common with the earlier estimates, we found that the population of Madeiran storm petrels on Praia Islet was approximately twice as large as that of Monteiro's storm petrels. Intriguingly, Madeiran storm petrels were found to have a much larger home-range radius, reflected in a wider pattern of recaptures among mist-nets and therefore a larger effective search area of each net. Whilst some of this larger range radius is probably explained by the larger number of mist-nets, facilitating both coastal as well as inland captures for Madeiran storm petrels, a re-analysis using only coastal mist-nets still found a much larger (167 m, 95% CI: 145-192) home-range radius for Madeiran than for Monteiro's storm petrels. This difference may be related to their larger population size and competition for nest sites (Ramos et al. 1997, Bolton et al. 2004) leading to a higher incidence of non-breeding individuals among birds of breeding age. Such individuals are likely to roam more widely within the colony and to have a lower overall capture probability as they attend the colony less regularly. In contrast, Monteiro's storm petrels were much more localised in their spatial pattern of recapture, indicating close association to a breeding site for most individuals.

Conclusions

We found considerable variation in the capture probabilities of individuals in relation to the distances between activity centres (nest sites) and trap locations, and nest-attendance behaviour. This variation can be minimised by ensuring an adequate spacing and placement of traps, and the use of spatially-explicit mark-recapture analysis methods. We found considerable differences in the capture probabilities and spatial pattern of recaptures among the closely-related study species, which highlights the need for a species-specific approach to optimise design of mark-recapture surveys. Nonetheless, a carefully designed spatial arrangement of traps, coupled with a sufficiently long capture effort, will facilitate the robust estimation of population size of nocturnal burrow-nesting seabirds – a feat that was until recently considered almost impossible (Sanz-Aguilar et al. 2010). In addition, the adoption of a SECR-based approach for annual monitoring would also facilitate the estimation of vital demographic parameters such as adult survival and thus provide an efficient approach for the monitoring of vulnerable seabird populations.

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Supplementary material

Appendix 1

Processing of radio-tracking data

Data were screened to remove erroneous tag detections. Occasionally, tag codes were incorrectly read by the tracking stations, evidenced by the registration of tag codes not deployed in the study (1,909 of 543,962 detections (0.4%) related to such invalid codes, which likely arise when pulses from multiple tags coincide temporally). At times, certain incorrect codes were repeatedly registered by the same tracking station over short periods of time (up to 5 min), which led to concern that incorrect reading of tag codes could lead to false detections of deployed tag codes. We therefore examined the frequency of registrations of incorrect codes. While unused codes were recorded up to three times in a minute (10 occurrences), they were never recorded four times per minute, so this value was selected as the threshold for regarding the detection of a deployed code as valid (see Table A1.1). The use of this criterion led to the exclusion of 7,185 (1.3%) apparent tag detections.

Two individuals (one from each study sub-colony) were censored 2 d post-deployment since tags were shed. Both birds continued to attend their nests. Equipment malfunction led to interruptions in recording of tracking data at the inland colony on the nights of 28-30th July, so data were censored for this tracking station for these nights.

We assumed birds would be available for capture using conventional sampling methods (i.e. capture in mist-nets) on nights they returned to, or departed from, a nest containing either an egg or chick, and when detected at tracking locations remote from the nest site. For ‘attending’ birds (i.e. individuals engaged in incubation or brooding on both the preceding and following day), we assessed whether an individual left the nest temporarily and was available for capture, by examining the data from all tracking stations. Since local tracking stations were able to continuously detect the presence of tagged birds in their nests, an interruption in the detection of a bird at the local tracking station and/or detection at remote tracking stations indicated that an attending bird had left the nest. Tracking confirmed that, on occasion, incubating birds left the nest and flew around the colony on nights that a change-over did not occur; following this, they resumed incubation duties at the nest, as confirmed by nest inspections on the following day. Inspection of the local detection history (i.e. detected by the tracking station situated within the sub-colony) of birds incubating or brooding small chicks overnight revealed that on 16 of 48 such bird-nights, storm petrels left the vicinity of the nest for periods of between one and 79 min. On one such occasion, a bird left the nest twice overnight (for periods of 60 and 79 min) and was detected at three separate times at both mobile stations, each over 100 m away. We assumed that if the local

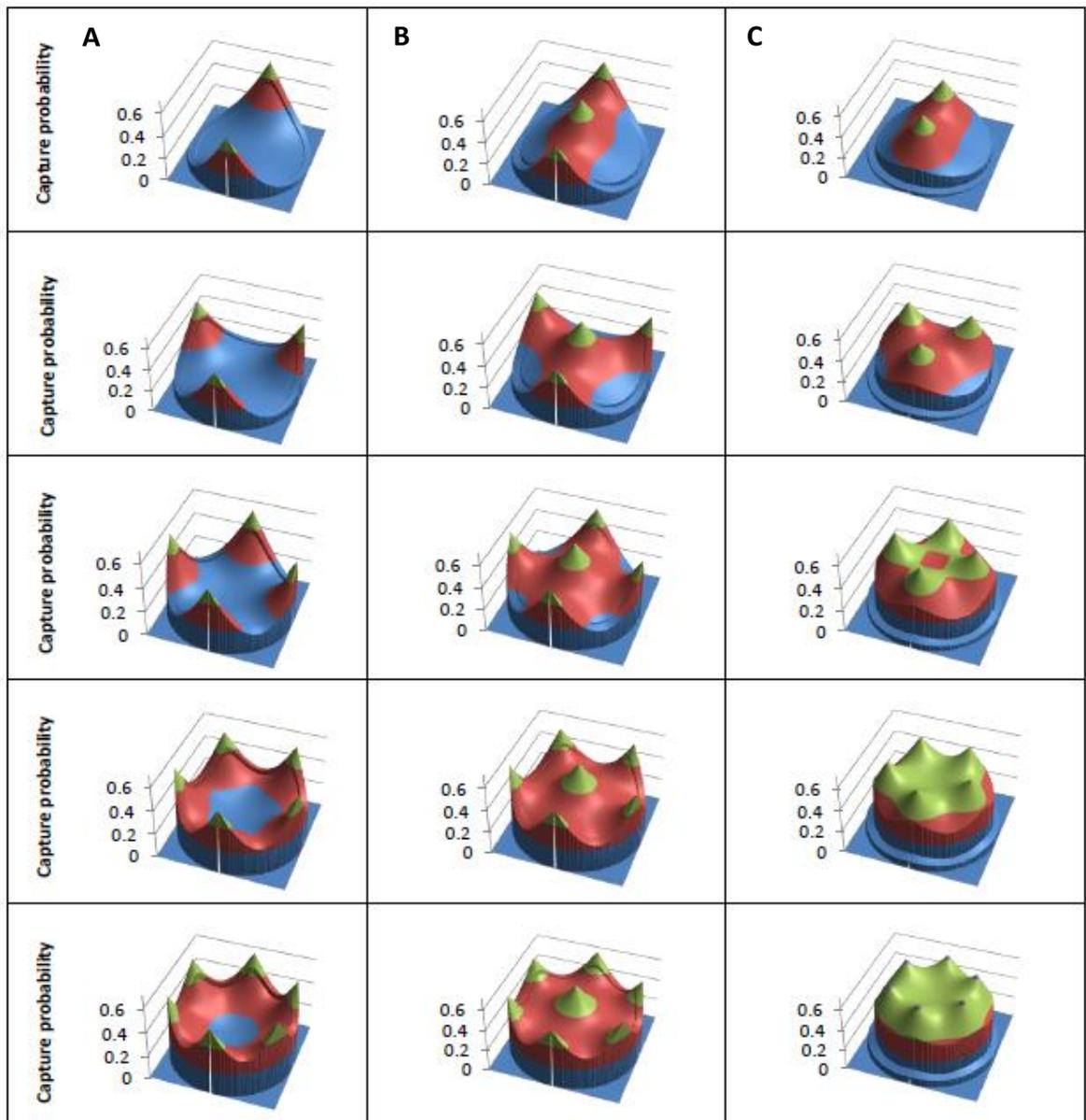
detection of a bird incubating or brooding at a nest overnight was interrupted for a period exceeding one minute, the bird had left the nest and would have been available for capture by a mist-net.

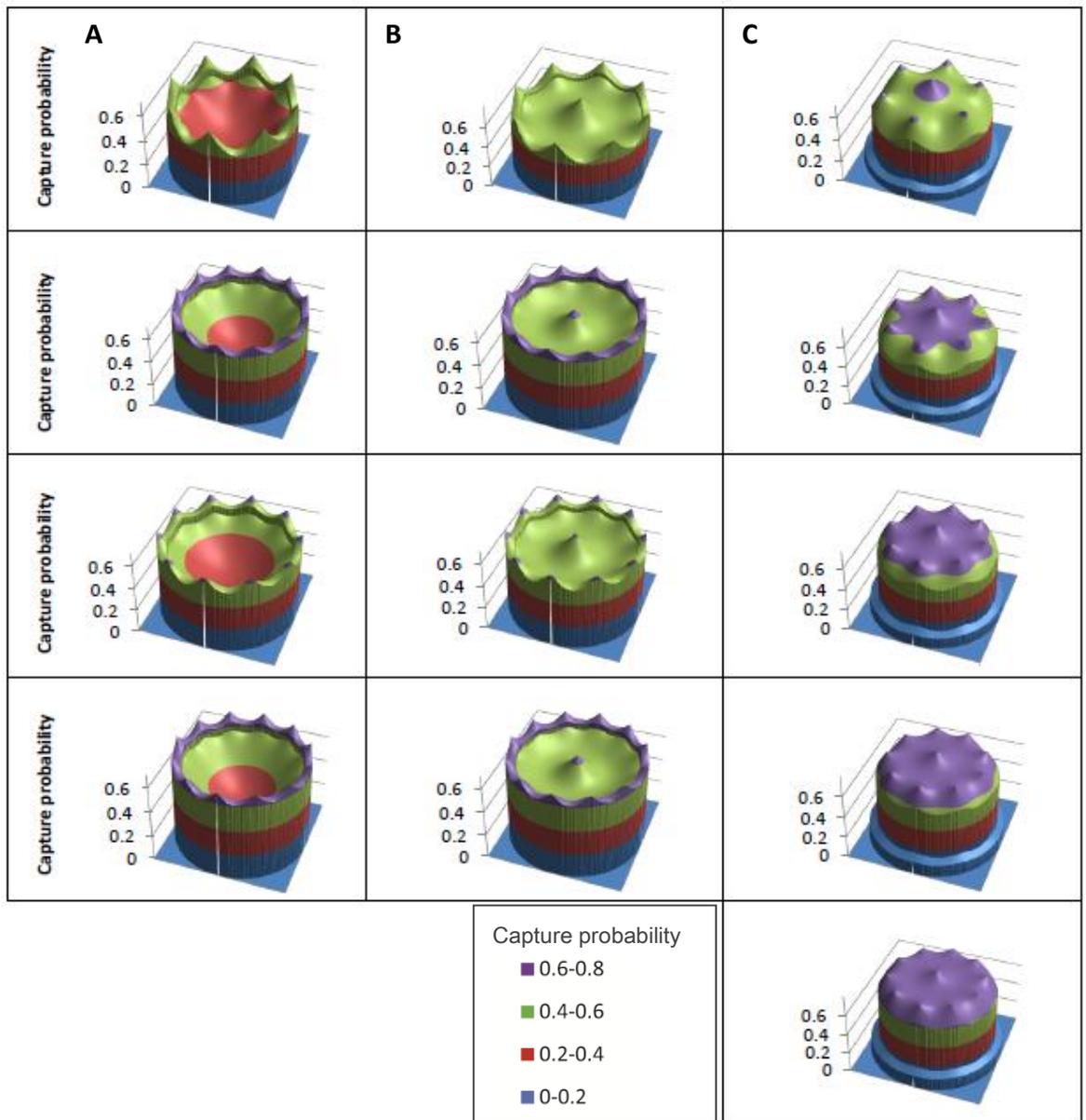
Table A1.1. The frequency of occurrence of registrations of incorrect tag codes at various time intervals.

Number of registrations	Time interval						
	15 s	30 s	1 min	2 min	3 min	4 min	5 min
2	43	59	168	216	272	303	334
3	0	2	10	34	57	75	95
4	0	0	0	5	10	20	28

Appendix 2

Figure A2.1. Variation in colony-wide capture probabilities in relation to nest location with increasing trap effort and trap location. The colony was simulated as a circle (radius 800 m, area 200 ha) with nests uniformly distributed at the intersections of a 10 m x 10 m grid. Simulations were based on three different spatial distributions of traps: (A) traps equally spaced around the coast; (B) one centrally-located trap in addition to equidistant coastal traps; and, (C) traps spaced to minimise mean distance to each nest. Peaks in the detection probability contour represent locations of traps.





Appendix 3

Specification of deterministic model of sampling duration on estimation of population size

Capture probabilities were estimated from a binomial errors GLMM of radio-telemetry detections of individuals attending nests during the egg stage, solved for a distance between the nest and trap of 249 m (the mean distance associated with the optimal trap spacing and placement from the simulation model described in the methods). The probabilities of nightly capture associated with each of the four nest attendance behaviours are given in Table A3.1.

Table A3.1. Nightly capture probabilities in relation to nest attendance behaviour.

Nightly nest attendance behaviour	Capture probability
Returning	0.545
Departing	0.437
Attending	0.061
Off-duty	0.117

General discussion

Although the effects of human disturbance on free-living animals have been widely studied (reviewed in Boyle and Samson 1985, Carney et al. 1999, Nisbet 2000), few authors have considered the potential for effects on animals occupying burrows or cavities (though see Steiner and Leatherman 1981, Blackmer et al. 2004, Magle et al. 2005). The research presented within this thesis demonstrates sound evidence that, despite remaining out of sight, animals residing in cavities or burrows can be negatively affected by human activities above ground. Human recreational disturbance was shown to negatively affect nestling survival (Chapter 1, Watson et al. 2014) and growth trajectories of surviving nestlings (Chapter 2) in a cavity-nesting nocturnally-active seabird, the European storm petrel *Hydrobates pelagicus*. Human disturbance has a consistent effect even in the presence of natural environmental variation in these traits. Although of most immediate relevance to burrowing seabirds, the outcome of Chapters 1 and 2 highlights the potential for impacts of anthropogenic activities on a wider range of species utilising refuges. Against a backdrop of changing climatic conditions, it is especially important that increasing anthropogenic pressures are managed to minimise additive effects on the population declines that are widely reported in seabirds (Croxall et al. 2012). The guidance for conservation action arising from this research is fairly intuitive and encapsulates aspects that should already be a core part of site-based visitor management. However, if prevailing attitudes consider that burrow- and cavity-dwelling animals are less vulnerable to human disturbance (Burger 1981, Lunn et al. 2004), appropriate management is unlikely to be in place. Furthermore, we should be advocating the need for conservation policy to be based on sound scientific evidence. It is only when impacts are quantified at both the individual and population level that robust actions can be effectively implemented with strong legislative and policy support (Gill et al. 2001).

Further studies are required to determine both the proximate and ultimate mechanisms underlying reproductive failure and slow nestling growth in response to disturbance. Impacts could arise as a result of direct effects on the chick itself or via indirect effects, as a consequence of exposure of adults to disturbance during the long incubation period. The route for indirect effects could either be via influences on parental care during incubation itself, therefore influencing the prenatal environment of the developing egg, or via carry-over effects influencing parental care in the chick-rearing stage. The majority of reproductive failure, in response to disturbance, occurred in the very early chick-rearing stage and before data could feasibly be collected from either adults or nestlings. Consequently, it remains unclear whether the cause of elevated mortality in high-disturbance nesting areas is a consequence of intrinsic properties of the chick or parental behaviour. While

nestlings appear robust to investigator disturbance at the nest, the sensitivity of adults (Davis 1957) presents challenges to the collection of physiological and morphometric data. A cross-fostering experiment would enable the separation of disturbance effects acting during incubation and the chick-rearing period, by a reciprocal translocation of either eggs (close to hatching) or nestlings (shortly after hatching) between high- and low-disturbance nests. This would also isolate potential effects on nestlings from effects on adults. It is, however, not known if cross-fostering can be performed successfully in storm petrels. An experimental manipulation of visitor pressure at the study site is desirable to confirm causal relationships between human disturbance and observed responses, although was not achievable within the context and timescale of this study. Experimental manipulations within wild populations often face challenges associated with the involvement of numerous stakeholders and the need to comply with site and species protection legislation. That is not to say these challenges cannot be overcome, but can be difficult to achieve within a limited timeframe.

Nestlings reared in nests exposed to high levels of human disturbance were able to overcome their poor start in life - characterised by slow growth - by an extension of the growth period (Chapter 2). That is, phenotypic changes were induced to mitigate for the negative effects of slow growth. While reduced growth rates have been assumed in response to human disturbance (e.g. Verhulst et al. 2001), I am not aware of other studies that have investigated the potential for variation in growth trajectories as a consequence of disturbance. The existence of developmental plasticity in growth has been widely demonstrated amongst the young of avian species (Schew and Ricklefs 1998) and may not be surprising for a species, such as the storm petrel, that is presumably well-adapted to cope with unpredictable periods of food shortage (Ricklefs 1968). However, relatively little is understood about the capacity for growth plasticity within the slow development strategy of procellariiform seabirds, and even less so in relation to stressors distinct from food supply and foraging conditions (Pettit et al. 1984, Boersma 1986, Weidinger 1997). It is expected that developmental plasticity will facilitate phenotypic adjustment of individuals in response to unpredictable environmental perturbations, which will favour persistence of populations (Ghalambor et al. 2007, Lessells 2008). However, while some storm petrel nestlings are able to demonstrate plasticity in response to repeated exposure to human disturbance, many die shortly after hatching. Whether those nestlings that fail to fledge are unable to display an adequate phenotypic modification or are not given the opportunity as a consequence of extrinsic constraints imposed by parental care is unclear (see above).

The developmental plasticity displayed by storm petrel nestlings exposed to high disturbance may be adaptive if fitness is higher than in its absence. Nonetheless, there may also be costs associated with an extended growth period. Presumably, the extension to the growth period results in a delay in fledging, which

is widely considered to be costly in other species (Bize et al. 2003, Searcy et al. 2004). Determination of exact fledging dates would shed more light on the magnitude of an effect on timing of fledging. However, although earlier fledging is presumed to be beneficial, enabling fledglings to get a head start on their long migration before weather conditions become less favourable, it is not known if there are major fitness consequences of late fledging for storm petrels. The chances of recapturing individuals beyond fledging are slim, presenting a major challenge to linking growth conditions with post-fledging survival and performance in storm petrels. Although the consequences of human disturbance at the population level are predicted to be small (Chapter 1), taken altogether, the effects may be exacerbated in years when breeding is delayed. The effect of disturbance on nestling survival increases in magnitude with increasing laying date and late-hatching nestlings display an even lower growth rate, which presumably leads to a larger extension to the growth period and subsequent delay in fledging. Against a backdrop of increasingly unpredictable weather conditions and food availability – associated with poor productivity of many seabirds in the north Atlantic (Mitchell et al. 2004) – the effects of human disturbance may become exacerbated with time, even in the absence of changes to visitor pressure.

Repeated exposure to a stressor can give rise to chronic stress and measures of baseline GCs are widely considered to be useful for detecting stress in natural populations (Wasser et al. 1997, Romero 2004, Walker et al. 2005, Wikelski and Cooke 2006). However, studies fail to find a consistent relationship between baseline GCs and fitness (Breuner et al. 2008, Bonier et al. 2009) or predictable changes in GCs in response to environmental cues (reviewed by Busch and Hayward 2009). There is no evidence that repeated exposure to stress associated with either high levels of human disturbance (Chapter 2) or investigator handling (Chapter 3) caused nestling storm petrels to be chronically stressed or to experience greater overall exposure to CORT. There are a number of possible explanations for an absence of changes in baseline GCs. Human disturbance and handling may not have been sufficient to cause chronic stress. Alternatively, storm petrels may be unable to exhibit an adrenocortical response in early life, as found in many mammals and altricial nestlings (Wada et al. 2007, Wada 2008). While some precocial and semi-precocial nestlings have been shown to demonstrate a robust stress response (Adams et al. 2008, Quillfeldt et al. 2009, Fiske et al. 2013), some species still display a hypo-responsive period during early development (Quillfeldt et al. 2009, Fiske et al. 2013). A period of reduced sensitivity to stressors could be adaptive, limiting overall exposure to CORT during the vulnerable period of development. Since stress-induced levels of CORT were not measured, one cannot rule out the possibility that storm petrels habituated to stressors, which is typically associated with a reduced sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis.

If one were to consider only pre-fledging CORT, body size and body mass – a reflection of an individual's status at a single point in time – the conclusion would be drawn that there are no negative effects of exposure to human disturbance on developing storm petrel nestlings (Chapter 2). However, these measures do not reflect significant differences in the developmental routes that high- and low-disturbance nestlings followed to reach the same phenotype. Although it is not known if there are long-term costs associated with the developmental strategy displayed by high-disturbance nestlings, the results indicate a need for caution in making simplistic interpretations of GC measurements. Taken altogether, the findings of this research suggest that GCs are not always an informative measure for identifying responses to environmental stressors and of individual state. Although previously highlighted by other authors, many studies continue to present only endocrine data, in the absence of additional behavioural, physiological or demographic data (reviewed in Breuner et al. 2008, Busch and Hayward 2009). Although GCs are the primary regulators of responses to environmental cues, energetic challenges can lead to changes in metabolic rate, immune function, oxidative stress and telomere dynamics. Furthermore, the results demonstrate that caution should be exercised in drawing conclusions about environmental effects based on data collected at only a single time point within the course of a long and slow postnatal development. A thorough understanding of environmentally-induced developmental plasticity may require the collection of repeated measurements throughout the period of growth to fully understand developmental pathways and potential costs associated with different strategies.

Exposure to environmental stressors during early life can have profound consequences for later-life fitness (Lindström 1999, Monaghan 2008). Variation in the natal environment has been shown to be a strong force driving differences in longevity and fecundity between cohorts (Albon et al. 1987, Rose et al. 1998, Reid et al. 2003). Telomere length and dynamics influence the rate of accumulation of senescent cells, which contributes to tissue and organ dysfunction (Campisi 2005), and thus may mechanistically link early-life stress with later-life survival and performance. In this thesis, it has been demonstrated that environmental conditions during development not only induce changes in growth trajectories, but also strongly influence early-life TL and rate of telomere loss in storm petrel nestlings (Chapter 4). Early-life TL was also found to be a good predictor of imminent mortality during development. Given the increasing body of evidence linking telomere length and loss to survival and reproductive performance, the results support the emerging idea that early-life telomere loss may be a driver of reduced fecundity or longevity following unfavourable growth conditions. Despite the potential significance of early-life telomere loss being highlighted by a number of authors, few studies have investigated variation in telomere loss within the timeframe of development (Foote et al. 2011, Geiger et al. 2012, Herborn et al.

2014). This study therefore adds crucial evidence to our understanding of how the early environment influences telomere dynamics. There is a need for future research to disentangle the relative importance of genetic, parental and environmental effects on early-life TL.

In the face of an escalating frequency of unpredictable environmental events, such as storms or changes in prey availability, it is of increasing importance to understand the ability for individuals to be able to exhibit facultative thermoregulatory responses to energetically-challenging conditions. Although descriptive in nature, observations on the occurrence and nature of hypothermia in storm petrel nestlings (Chapter 5, Watson 2013) add to a limited body of literature on this topic. Hypothermic responses were recorded only in individuals reared in a poor natal environment, as measured by overall reproductive success of the colony. It is unclear exactly which environmental correlate(s) are driving the inter-annual variation in traits, as reported throughout this thesis, though the increased frequency of strong winds likely contributed via a reduction in parental care. While a hypothermic response to an energetic challenge may be adaptive and maximise fitness under the current circumstances, there may also be long-term costs. Future studies should seek to investigate the potential costs of facultative hypothermic responses for growth rate and later-life survival and performance. An increased understanding of the causes and consequences of facultative thermal responses will enhance our understanding of the effects of changing environmental conditions. Understanding and predicting how populations may respond to future environmental change also requires accurate estimates of population sizes. The ecology of storm petrels and other procellariiform seabirds presents major challenges to the estimation of population size, since many species nest underground on remote islands, remain concealed in the nest during the day and are only active within the colony at night. Procellariiform seabirds are among the most globally-threatened avian taxa (Croxall et al. 2012) and the absence of reliable population estimates subsequently hampers assessment of conservation status. Although capture-recapture techniques are well-established for estimating population size, they often largely ignore the potential for spatial and temporal variation in individual probability of capture (Williams 2002). Using spatially-explicit capture-recapture data from three closely-related species of storm petrel at two different study sites and using two different methods, Chapter 6 shows that capture probability varies both within and between species across both spatial and temporal scales. Using simulations of variable capture effort, the optimal spacing and location of traps to minimise individual variation in capture probability was estimated for the different species and sites. While the methodological and analytical approaches employed have wide applications, they also indicate that an optimal survey design requires a species-specific approach. This paper demonstrates that spatially-explicit capture-recapture models provide a robust approach for

estimating population size and their adoption will enhance rigorous and effective monitoring of vulnerable seabird populations.

Summary

In the face of increasing rates of anthropogenic environmental change, the research contained within this thesis investigates key questions concerning the capacity for adaptive responses to repeated environmental stressors. The research has focused on the causes and consequences of phenotypic plasticity during development and the mechanisms underlying individual variation in responses. It has been demonstrated that, despite remaining out of sight, human disturbance can negatively affect animals dwelling in underground refuges. Developmental plasticity enabled developing young to overcome the negative effects of unfavourable early conditions, though further research is required to fully understand whether this is adaptive and how costs and benefits vary between different development strategies. The research highlights that GCs are not always an informative measure for identifying responses to environmental stressors and additional behavioural and physiological metrics should be collected alongside GC measurements to fully understand the effects of repeated exposure to stressors during development. The results add to increasing evidence of the important influence of the early-life environment on telomere dynamics, as well as highlighting the potential for early-life TL to predict imminent mortality.

Table i. Summary of the effects of the environmental stressors of human disturbance - recreational and investigator (i.e. handling) - and inter-annual environmental variation on various traits measured in the European storm petrel. With the exception of breeding success, all traits refer to nestlings. The direction of significant effects is shown as an increase (>) or decrease (<) in trait value. - indicates relationships that were not investigated.

	Breeding success	Growth ^a			CORT	Telomeres		Body condition
		Wing	Tarsus	Mass		Length ^b	Rate of loss	
Recreational disturbance	*	NS	*	**	NS	NS	NS	-
High	>		< (k)	> (A, i)				
				< (k)				
Low	<		> (k)	< (A, i)				
				> (k)				
Investigator disturbance	-	-	-	-	NS	NS	-	NS
Inter-annual variation	*	NS	NS	**	NS	*	**	-
Good natal environment	>			> (A, i)		< (1 st)	<	
						> (2 nd)		
Poor natal environment	<			> (A, i)		> (1 st)	>	
						< (2 nd)		

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NS = not significant.

^aGrowth parameters: A = asymptote, k = growth rate, i = inflection point. ^bTelomere length measurements: 1st = initial and 2nd = follow-up.

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Appendices

Appendix 1

Quantifying human recreational disturbance

Few studies of the effects of human disturbance on free-living animals quantify the level of disturbance (though see Beale and Monaghan 2004, Strasser and Heath 2013). From the outset, a key aim for this thesis was to quantify the level of human disturbance within each of the six study plots at the study site of Mousa (see Chapter 1, Fig. 1.1). Three study plots were located <10 m from the visitor trail and considered to be subject to high levels of human recreational disturbance, while three were located >150 m from the trail and considered to be subject to low levels of disturbance. Refer to Chapter 1, Watson et al. 2014 for more details of the study site and study design. The main access point for the island is a pier in the West Ham on the island's west coast. The majority of visitors arrive into the pier via the scheduled ferry service, which usually offers a single sailing each day; it is therefore feasible to count the number of visitors each day. Access to the island is unrestricted, yet the majority of visitors follow the marked circular trail, thereby passing through the three high-disturbance study plots located alongside the visitor path. However, the exact choice of route and length of time people spend in different areas will vary depending on their interests and motivations, physical capabilities, and the weather, among other factors. Thus, while a count of daily visitors tells us the number of people present on the island, this figure does not convey anything about the relative levels of visitor pressure experienced in each of the six study plots.

The aim, therefore, was to quantify daily visitor pressure in each of the study areas throughout the breeding season in the consecutive years of study in 2010 and 2011. Two systems were employed to achieve this objective: beam counters and automated camera systems. However, a number of limitations were encountered with both methods and, consequently, a quantification of visitor disturbance was not incorporated into the studies presented in Chapters 1 and 2. The design, application and limitations of the two methods are discussed below.

Beam counters

Beam counters were designed and built by MVLS Bioelectronics, University of Glasgow. I was heavily involved in the design process, to ensure the end product achieved the objectives effectively in the field. There were many considerations to take into account to ensure smooth operation in a remote field environment including protection from weather, ease of collecting data, changing and charging batteries, and detection range. The system consists of a passive infrared (PIR) sensor connected to a recording unit and powered by a single 12V lead acid battery.

The sensors use narrow infrared single-beam technology to count and record traffic each time the beam is broken. The system will not necessarily record the exact number of people passing, since if several people pass and break the beam simultaneously, the unit records only one passage. The data collected by the system can therefore be considered to reflect a measure of visitor pressure, rather than absolute numbers.

A single counter was located within each of the six study plots before onset of the breeding season in 2010 (May) and left in operation through 'til the end of the visitor season in late chick-rearing (mid-September). Sensors were either secreted within dry-stone walls or mounted on posts, with the associated recording unit located under a cairn nearby. Due to the presence of sheep on the island, sensors were all mounted c. 1.3 m above the ground to reduce the likelihood of sensors being triggered by passing sheep. Initial tests in the field revealed that the detection range achieved was up to 10 m. The systems had no built-in memory, so, in order to record a daily count, the systems had to be manually inspected and re-set to zero either at the beginning or end of each day. Following deployment of the systems, quantitative validation of beam counters located in high-disturbance areas was carried out. Observations of visitor activity were recorded over two separate 30-minute periods and compared with the corresponding count from the beam counter at two of the high-disturbance plots. This demonstrated that recorded counts from beam counters fell within the range of 75% and 150% of the total number of visitors passing. This quantitative validation, combined with the continuous monitoring of the total number of visitors to the island each day, meant that it was possible to detect any spurious counts generated by the beam counters. It became apparent early on in the season that spurious data were regularly being generated by counters. On some days when there had been no visitors to the island (e.g. when the ferry was cancelled), daily counts would be phenomenally high – sometimes as much as 500% of the total number of visitors – even in areas of known low visitor activity. Equally, on some days when there had been large numbers of visitors present on the island, daily counts in areas of high visitor activity would fall well below 50% of the known visitor numbers. The occurrence of anomalous counts was not consistent between counters and there were no obvious reasons for their occurrence. Further tests of the beam counters revealed that the detection range of some beam counters had diminished, in some cases only extending to c. 2.5 m; while this may in part explain unrealistically low counts, this could not, however, explain unrealistically high counts. Only one system worked smoothly and reliably throughout the season, while, at the other counters, the frequency of erroneous counts ranged from 9.5-47% of the cumulative number of days of counts. In addition to these problems, one counter stopped working mid-way through the season and was out of action for 22 days while a new electronic chip was manufactured.

Modifications were performed to the systems prior to the second field season in 2011 by MVLS Bioelectronics to attempt to address the issues experienced in the first season. Software modifications were also performed so that the system would record and save to memory the total number of passages within a 24-hour period, before re-setting to zero for the next 24-hour period. The memory stores up to five daily counts, thereby decreasing the labour required to collect data, since daily counts could now be collected without the need for checking and resetting the systems every day. Unfortunately the modifications did not adequately address the problems encountered and spurious data continued to be generated by counters. In conclusion, given the unreliability of the data collected by the beam counters, it was considered to not be sufficiently accurate to be able to generate robust quantitative measures of human recreational disturbance.

Automated camera systems

Due to the problems encountered with the beam counters, trail cameras, more typically used for game scouting, were employed from July 2010 onwards as an alternative means of collecting quantifiable data on human disturbance. Three different models of camera were used - Wildview X2, Wildview X5 and Bushnell Trophy Cam – though all operated on the same principles and had identical programming features. Similar to the beam counters, the cameras are triggered to capture a photographic image or video sequence when the beam produced by a PIR sensor is broken by passing traffic. Cameras offered many advantages over the beam counters: cameras have a greater sensitivity than beam counters, with a detection range of c. 15 m and, by employing a domed PIR sensor, they offer a wide field of view. Cameras are powered by 4 x 'C'-cell batteries and images are stored on SD cards; their relatively low power consumption and ability to program collection of low-resolution images means that cameras require much less effort to operate and maintain in the field, only necessitating batteries and SD cards to be replaced every few weeks. Since the camera records data in the form of an image with a time- and date-stamp, they can collect data at a much higher resolution than beam counters, providing data not just on the number of people passing through the area, but also the timing of passages, and activity of visitors. Most importantly, however, given the issues encountered with beam counters, inspection of the images captured enables the distinction between triggers from sources besides humans. As with beam counters, there were numerous captures that were not triggered by humans. In some instances, the cause could be attributed to sheep or a bird, for example, but often, there was no apparent cause. However, these unknown triggers could be quickly filtered out, leaving only photos which could be attributed to visitor disturbance. The major disadvantage to the use of automated cameras to collect data on human disturbance is the laborious nature of processing and analysing of data.

Despite the many advantages and potential for cameras to collect high-quality data on human disturbance, a number of limitations were encountered in their application within this study. Unfortunately, cameras were installed too late in 2010 to collect adequate data from the incubation period. However, trials suggested cameras could achieve the objective and it was hoped that robust data could be collected in 2011. Cameras did not work entirely flawlessly; occasional malfunction resulted in up to 10% of days of no data collection at some sites. While this was an acceptable margin of error, unfortunately problems were repeatedly encountered with one of the cameras in the second half of the breeding season in 2011. These problems seemed to be related to age of the systems, as two brand-new cameras worked effectively throughout the entirety of the season, but insufficient research funds meant that it was not possible to source more new cameras. With high-quality data for only five of the six study areas and in only one year of the study, it was concluded that, within the scope of the study, it was not possible to achieve a robust analysis of variation in behaviour and physiology in relation to a quantitative measure of human disturbance.

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