

We may like them, but how do they feel  
about us?

Little Penguins (*Eudyptula minor*) and  
Human Disturbance

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## Abstract

Penguins are a much-loved taxon, and are frequently the subject of both scientific research and tourist visitation. Many species of penguin do not show a behavioural response to human presence, so it is often assumed that the penguins are not negatively affected. However, in the absence of a visible behavioural response, physiological changes such as increases in heart rate may use up vital energy resources and lead to population-level consequences. The personality of individual penguins may also affect how they react to human disturbance.

There are an increasing number of commercial operations taking advantage of the apparent indifference of Little Penguins (*Eudyptula minor*) to human presence, such as the Oamaru Blue Penguin Colony at Oamaru, New Zealand. My study aimed to quantify the effects of human disturbance on Little Penguins. I recorded the heart rate (HR) of Little Penguins at Oamaru using artificial eggs, to measure responses to typical researcher and visitor interactions with penguins. Researcher interactions were: human speech, band checking, and weighing, and a penguin call playback was used as a control. I calculated the amount of energy expended by a Little Penguin in response to an invasive researcher interaction, i.e. weighing. HR responses to researcher interactions were compared with corticosterone responses obtained from the same individual penguins. I used chick mass data to compare chick growth and fledging weights between a colony visited by tourists and a control colony.

Little Penguins at Oamaru had stronger HR responses to being weighed than to hearing penguin calls or human speech. However, some individual penguins reacted as strongly or more strongly to having their band checked than to being weighed. There was some correlation between HR responses and corticosterone responses, suggesting that individual penguins respond consistently on a shy-bold personality continuum. The HR of incubating penguins averaged over four-hour periods did not differ significantly between a colony visited by tourists and a control colony. However, female penguins in the ‘medium disturbance’ zone of the visited colony had significantly higher resting heart rate (RHR) than their male counterparts. The amount of energy used by a Little Penguin in response to being weighed was found to be negligible. Chick fledging weights at the visited colony were significantly lower than those at the control colony, which may affect their first-year survival.

A balance must be reached between humans having close contact with wildlife, for purposes of research and education, and leaving the wildlife to itself. At Oamaru, current management practices are successful in facilitating the penguin colony’s growth while also permitting thousands of visitors to view the penguins every year. Future research focusing on individual

penguins and the responses of particular personality types to environmental changes, including human disturbances, will aid the ongoing success of this colony.

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# Table of Contents

Abstract.....	ii
Acknowledgements.....	iv
List of Tables .....	vii
List of Figures.....	viii
Introduction .....	1
1.1 Human Disturbance – Visitors or Predators? .....	1
1.1.1 Tourism and Human Disturbance .....	2
1.1.2 Seabird Research and Human Disturbance.....	3
1.2 Human-Penguin Interactions .....	4
1.2.1 Penguin Research.....	5
1.2.2 Differences in Disturbance Response .....	5
1.3 Penguin Personalities.....	7
1.4 Little Penguins and Human Disturbance .....	8
1.4.1 Oamaru Little Penguin Colony .....	9
1.5 Measuring Physiological Response .....	9
1.5.1 Corticosterone Sampling.....	10
1.5.2 Heart Rate Telemetry.....	10
1.6 Research Aims.....	12
Methods .....	13
2.1 Study Species.....	13
2.2 Study Site.....	15
2.3 Egg Dummy Construction .....	17
2.4 Data Collection .....	18
2.4.1 Pilot study .....	19
2.4.2 Measuring Researcher Disturbance .....	20
2.4.3 Measuring Tourist Disturbance .....	21
2.5 Data extraction.....	22
2.5.1 Manual Analysis using Audacity .....	22
2.5.2 Matlab Analysis .....	24
2.6 Further Analyses.....	25
2.6.1 Researcher Disturbance .....	25
2.6.2 Relationship between Heart Rate and Corticosterone Response .....	26
2.6.3 Tourist Disturbance .....	28

2.6.4	Chick Growth Modelling .....	28
Results	.....	30
3.1	Researcher Disturbance .....	30
3.1.1	Energy Expenditure .....	34
3.2	Relationship Between Heart Rate and Corticosterone Response.....	35
3.3	Tourist Disturbance .....	36
3.4	Chick Growth Modelling .....	39
Discussion	.....	41
4.1	Researcher Disturbance .....	41
4.1.1	Little Penguin Energy Budget.....	42
4.1.2	Response and Personality .....	43
4.2	Corticosterone and Heart Rate Responses .....	43
4.2.1	Comparing Heart Rate and Corticosterone .....	44
4.2.2	Persistence of Personality .....	45
4.3	Tourist Disturbance .....	45
4.4	Modelling Chick Growth.....	47
4.5	Conclusions and Recommendations .....	48
References	.....	51
Appendix A	.....	63
Appendix B	.....	65
7.1	Energy Consumption Calculations .....	65
7.2	Specific Example: Penguin #45167.....	66
7.3	Hypothetical Significant Response.....	67

## List of Tables

Table 2.1	Comparison of outputs from manual and Matlab analyses.....	24
Table 2.2	Stimuli for which response data were obtained from individual penguins.....	26
Table 2.3	Mean resting heart rate percentage increase in response to each of four stimuli....	27
Table 2.4	Hypothetical mean resting heart rate percentage increase values for individual penguins.....	27
Table 3.1	Mean and standard deviation values for Little Penguin heart rate increase as a percentage of resting heart rate and time taken for heart rate to return to baseline following a stimulus, in response to each of four stimuli.....	31
Table 3.2	Output from linear models evaluating the magnitude of Little Penguin heart rate increase in relation to pairs of four stimuli.....	32
Table 3.3	Output from linear models evaluating the length of stimuli response time in relation to pairs of four stimuli.....	33
Table 3.4	Input variables in relation to Little Penguin resting heart rate.....	36
Table 3.5	Candidate set of models explaining variation in Little Penguin resting heart rate in relation to Sex, Zone, and the interaction between these factors.....	37
Table 3.6	Output from linear models comparing resting heart rate of male to female penguins within zones of human disturbance.....	38

## List of Figures

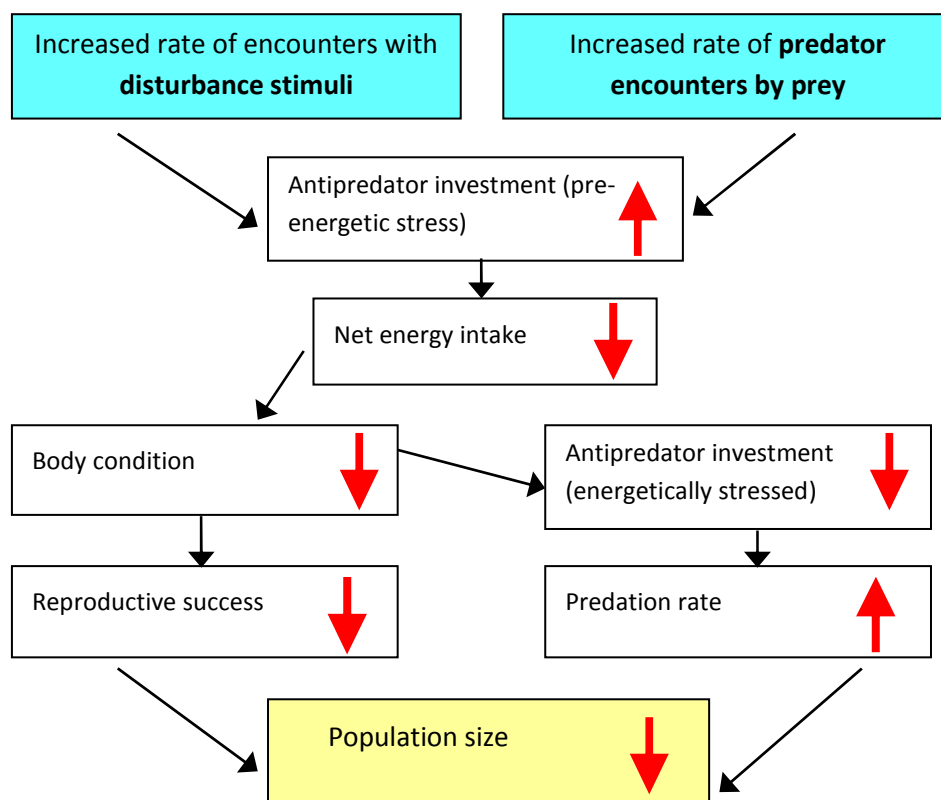
Figure 1.1	Behavioural mechanisms contributing to population decline.....	1
Figure 2.1	External and internal view of Egg Dummy (ED).....	18
Figure 2.2	Comparison of clear and poor Heart Rate signal with Audacity.....	23
Figure 3.1	Little Penguin heart rate in response to stimuli.....	30
Figure 3.2	Little Penguin heart rate response as a percentage of baseline heart rate .....	31
Figure 3.3	Time taken for Little Penguin heart rate to return to baseline after stimuli.....	32
Figure 3.4	Heart Rate response of individual Little Penguins to stimuli.....	33
Figure 3.5	Little Penguin energy expenditure in response to weighing as a fraction of daily energy budget.....	34
Figure 3.6	Little Penguin rankings of magnitude of response to blood sampling and human intervention.....	35
Figure 3.7	Mean Little Penguin resting heart rate in relation to sex of penguin.....	37
Figure 3.8	Mean Little Penguin resting heart rate in relation to human disturbance level.....	37
Figure 3.9	Mean Little Penguin resting heart rate in relation to human disturbance level and sex of penguin.....	38
Figure 3.10	Individual Little Penguin chick weights in relation to age.....	39
Figure 3.11	Little Penguin fledging weight in relation to colony location.....	40
Figure 3.12	Little Penguin chick weight in relation to age and colony location.....	40



# Introduction

## 1.1 Human Disturbance – Visitors or Predators?

Human disturbance can be defined as any human activity that changes the contemporaneous behaviour and/or physiology of one or more individuals (Nisbet, 2000). The risk-disturbance hypothesis predicts that an animal's response to disturbance should follow the same economic principles used by prey encountering predators; hence perceived predation risk and disturbance stimuli are considered analogous (Frid & Dill, 2002). Predation *risk* differs from predation itself in that predation always ends in a complete reduction in the individual's fitness, whereas predation *risk* necessitates the prey to decide whether or not to compromise the rate of resource acquisition or other activities to reduce the probability of death (Frid & Dill, 2002). Therefore it is irrelevant that disturbance stimuli are non-lethal (Beale & Monaghan, 2004b). Figure 1.1 illustrates this analogy, and demonstrates how human disturbance can ultimately lead to decreased population size.



**Figure 1.1:** Conceptual model outlining the behavioural mechanisms by which increased rates of human disturbance or of predator encounters by prey could cause population size to decline. Downward-facing arrows inside boxes indicate a negative response and upward-facing arrows indicate a positive response. Redrawn from Frid & Dill (2002).

### 1.1.1 Tourism and Human Disturbance

As humankind becomes ever more removed from wildlife, and biodiversity is continually diminished, human preference will impact, if not dictate, which species endure (Stokes, 2007). An important way of increasing the value of wildlife to the public, and hence increasing the impetus to conserve it, is to allow the public access through close encounters (Gill, 2007). However, the desire of people to have close encounters with wildlife in a 'natural' setting needs to be carefully balanced against the level of disturbance that the wildlife can tolerate (Taylor, 2000a). Consequently, wildlife tourism faces a conundrum: human presence generates awareness and financial support, but has the potential to be ecologically unsustainable (Ellenberg *et al.*, 2006; Stokes, 2007).

It is crucial to be able to identify when human presence constitutes disturbance, and to quantify the extent of this threat (Gill, 2007). Many studies assessing human disturbance monitor animal behaviour, and use behavioural changes as an indication of disturbance. For example, the vigilance behaviour of male polar bears (*Ursus maritimus*) was found to increase in the presence of a single vehicle (Dyck & Baydack, 2004), and bottlenose dolphins (*Tursiops truncatus*) were found to engage in more milling behaviour in the presence of tourist boats (Constantine *et al.*, 2004).

However, the effects of disturbance are not always visible. Without showing behavioural changes, animals may undergo changes in physiological function which alter the energy fraction available for vital processes (Weimerskirch *et al.*, 2002). Sub-lethal physiological effects such as increased heart rate and decreased body weight could reduce individual fitness and may ultimately have population-level consequences (Ellenberg *et al.*, 2006; McClung *et al.*, 2004).

Disturbance does not affect all individuals equally. The responses of wildlife to human disturbance may be influenced by a range of factors, including species, age, sex, individual personality, reproductive condition, nutritional condition, prior experience, and available habitat (Bejder *et al.*, 2009; Ellenberg *et al.*, 2009; Holmes *et al.*, 2005).

It is essential that wildlife managers consider the potential impacts of human disturbance on the animals being visited, and plan human-wildlife interactions in a manner which minimizes disturbance effects. This will ensure that tourism does not contribute to the decline of wild populations.

### 1.1.2 Seabird Research and Human Disturbance

Another source of disturbance is from researchers and those who monitor population health. When studying animal populations ‘in the wild’ researchers aspire to collect data on animals in their ‘natural’, or undisturbed, state (Carey, 2011). However, the process of collecting data may alter this state, and thus bias conclusions (Carey, 2009; Viblanc, *et al.*, 2012). For example, researcher disturbance could reduce offspring survival, which would interfere with the accurate assessment of population parameters and thus exacerbate population decline (Carey, 2011).

Seabirds are studied around the world for a vast number of reasons. As almost half of all seabird species are now known or suspected to be in decline (Croxall *et al.*, 2012), a great deal of recent seabird research has focused on links between changes in seabird population size and larger-scale ecosystem or climate changes, (e.g. Mallory, *et al.*, 2010; Sydeman, *et al.*, 2012; Wolf *et al.*, 2010).

Researcher interactions with seabirds are usually more intrusive than tourist visitation, a fact which is often overlooked (Ibáñez-Álamo & Soler, 2010). Typical seabird research may involve checking nests, banding birds, or weighing chicks; but the effects of these activities on the bird or on the population are rarely measured (Vertigan *et al.*, 2012). A review of literature that had measured the effects of basic researcher activity on seabirds found that in over half (7 out of 13) of studies of surface-nesting seabirds, researcher disturbance significantly affected survival and/or growth (Vertigan *et al.*, 2012). Burrow-nesting seabirds seemed to be less susceptible to disturbance, but not exempt: Cassin’s Auklet (*Ptycormaphus aleuticus*) chicks subjected to visitors frequently walking past their nests fledged at lower weights than their undisturbed conspecifics (Albores-Barajas *et al.*, 2009).

Many seabird populations now have a continuous human presence as part of long-term management strategies. Seabird colonies which are exploited for tourism are likely to also be subject to intensive research and monitoring, due to accessible locations and the need to inform management decisions. An awareness of the possibility that researcher presence affects animal behaviour and physiology is essential for changing how we think about scientific experiments in the wild, and the inferences we gain from those experiments (Viblanc *et al.*, 2012). Ideally all wildlife research should include a component to assess the impact of the research itself (Carey, 2009).

## 1.2 Human-Penguin Interactions

Penguins are a captivating and much-loved taxon. The upright walking stance of penguins endears them to the general public, as this predisposes the penguins to anthropomorphism (Stokes, 2007). The charisma and aesthetic appeal of penguins makes penguin colonies an ideal ecotourism destination (Curtin, 2010; Ellenberg *et al.*, 2009). This presents an enigma: penguin colonies are a tourist drawcard, offering opportunities for education and the generation of revenue to promote conservation; however the presence of humans may reduce the fitness of these colonies.

Ecotourism ventures are increasingly aware of the stress human presence may put on animals, but conversely are pressured to offer the best ‘quality’ wildlife experiences by getting people as close to the animals as possible (Higham & Shelton, 2011; Martinez-Abraín *et al.*, 2008). An understanding of the impact human presence is having on a group of animals, such as a penguin colony, is crucial both for the success of the ecotourism venture, and the ongoing survival of the group in question.

Although penguin colonies endure close to urban environments in countries including South Africa, Argentina, Australia and New Zealand, most localities were occupied by penguins long before people (Gilling *et al.*, 2008). Of 18 extant species of penguin, 15 are now rated as ‘near threatened’ or worse on the IUCN redlist (IUCN, 2014). Many species of penguin show no immediate behavioural response to the presence of humans at their nesting sites, which may lead to the conclusion that they are not affected, or disturbed, by this human presence (Carney & Sydeman, 1999). This misconception could lead to false and potentially damaging conclusions, such as ‘Antarctic and sub-Antarctic seabirds, particularly penguins and albatrosses... are tolerant of regular weighing and general human activity around the nest and/or moult site’ (Portugal & Guillemette, 2011).

The lack of external response shown by these animals makes sense in the light of evolutionary history: without land-based predators there was no reason for penguins to evolve a behavioural response to being approached on land (Ellenberg *et al.*, 2009). However, an absence of alarm behaviours does not equate to an absence of stress. Physiological changes indicative of a stress response often occur with no visible signs (Ellenberg *et al.*, 2006). Of course, the occurrence of a stress response in itself is not a detrimental thing – this is a response which gears the animal to evade predators by activating muscle groups for rapid movement. However, repeated activation of this response may be detrimental, as the energy used in mounting the response is no longer available for other essential tasks (Ellenberg, *et al.*, 2013).

### 1.2.1 Penguin Research

Scientists may choose to study penguins for many reasons. Penguins are a top predator and serve as a sentinel for ecosystems both at sea and on land. Penguins are a model study species for a vast range of research topics, from basic questions concerning the biology of a particular species to large-scale studies of climatic change and food systems (Forcada *et al.*, 2006; Reilly & Cullen, 1981; Saraux *et al.*, 2011). Typical seabird characteristics, such as monogamy while breeding, longevity, and extensive parental care, make penguins ideal subjects of investigation, as their behaviour is consistent and can be tracked over many years (Chappell *et al.*, 1993). Furthermore, coloniality and flightlessness render penguins relatively easy to locate (Chappell *et al.*, 1993).

A number of studies have focused on human disturbance in penguins (Ellenberg *et al.*, 2006; Holmes, 2007; Viblanc *et al.*, 2012). The range of different responses to human disturbance found among even closely related penguin species clearly indicates that no one rule or management practice can be put in place regarding human interaction with penguins (Ellenberg *et al.*, 2006). The absence of overt behavioural responses to human presence of many penguin species means that detailed investigations of physiological responses are crucial to accurately quantify the impact of human disturbance (Giese, 1998).

### 1.2.2 Differences in Disturbance Response

Within the penguin taxon there are striking interspecific differences in reactions to human disturbance (Ellenberg *et al.*, 2006). The breeding success of Humboldt (*Spheniscus humboldti*), Yellow-eyed (*Megadyptes antipodes*), and Adélie (*Pygoscelis adeliae*) Penguins decreased in relation to frequency of human visitation; whereas Magellanic Penguins (*Spheniscus magellanicus*) did not exhibit this trend (Ellenberg *et al.*, 2006; Giese, 1998; McClung *et al.*, 2004). As Adélie Penguins do not always display overt behavioural responses associated with distress when faced with humans, they have previously been regarded as relatively immune to human disturbance (Giese, 1996, 1998). However, a growing body of evidence implies that this is far from being the case (Bricher *et al.*, 2008; Culik & Wilson, 1991). Proximity to human activities plays a highly significant role in directing long-term trends in Adélie Penguin demographics (Bricher *et al.*, 2008).

Yellow-eyed Penguins are among the most vulnerable to the negative impacts of human disturbance of all penguin species studied thus far. The mere presence of people on beaches can disrupt the post-forage landing of Yellow-eyed Penguins, potentially causing a reduced amount of food to be delivered to chicks (McClung *et al.*, 2004). This may have long-reaching

repercussions, such as slower chick growth, lower fledging mass, and ultimately decreased survival rates (Ellenberg *et al.*, 2007; McClung *et al.*, 2004).

Many studies examining the effects of human presence on various penguin species targeted changes in large-scale parameters such as distribution, behaviour, demography, and population size (Gill, 2007), breeding success or recruitment (Anderson & Keith, 1980), juvenile survival (McClung *et al.*, 2004), or visible changes such as behaviour (Holmes, 2007; Holmes, *et al.*, 2005; Yorio & Boersma, 1992). These approaches are critical in giving an indication of long-term trends or extreme responses, but they do not provide insights into the mechanisms which cause demographic change, such as increases in heart rate or stress hormone levels; or focus on individuals within the group (Ellenberg *et al.*, 2007). Behavioural responses may differ in relation to a range of factors such as body condition, sex, and time of year, so basing conclusions on behaviour alone may be misleading (Beale & Monaghan, 2004a). The response of penguins to human presence is not just be species-specific (Ellenberg *et al.*, 2006), and may vary across different stages of the life cycle and even among individuals (Cockrem, 2007; Ellenberg *et al.*, 2009; Gill, Norris, & Sutherland, 2001).

#### 1.2.2.1 Physiological Response

Penguin responses to the approach of a person on land may range from obvious behavioural changes, such as fleeing, hunching, hissing, or lunging at the intruder, to no visible response (Martinez-Abraín *et al.*, 2008; Waas, 1990). However, physiological changes to prepare the bird for action, such as increased heart rate and increased production of ‘stress hormones’ that go on to power muscles, may be taking place (Cockrem *et al.*, 2009; Walker *et al.*, 2006). Such changes may use a significant portion of the bird’s energy budget (Ellenberg *et al.*, 2013; Groscolas *et al.*, 2010). Prolonged exposure to stress can be physiologically damaging to individuals, potentially resulting in higher susceptibility to disease, reduced fertility and lower life expectancy (Sapolsky *et al.*, 2000; Bókonyi *et al.*, 2009).

For example, Adélie Penguins approached to 15m had significantly elevated heart rate (HR) responses although there were no behavioural indications of this response (Giese, 1998). Here behaviour was not a reliable indicator of disturbance. Gentoo Penguins (*Pygoscelis papua*) were more affected by the specific behaviour rather than the presence of a human, with abrupt movements of nearby people causing HR to spike (Nimon *et al.*, 1996). Royal Penguins (*Eudyptes schlegeli*) showed increased HR in response to a single pedestrian visit, however visits appeared unlikely to elicit responses considered greater than minor or transitory (Holmes *et al.*, 2005). Penguin resting heart rates varied within and between individuals based on factors such as sex and body condition (Holmes *et al.*, 2005).

### 1.2.2.2 *Habituation to Disturbance*

Penguins may alter their response to human disturbance with repeated exposure over time, with some species showing evidence of habituation. Habituation is defined as ‘a reduced response to repeated stimulation not attributable to fatigue or sensory adaptation’ (Domjan, 2003). It leads to the absence of a ‘fear’ response, so that energy is not wasted on this response (Higham & Shelton, 2011).

For example, King Penguins (*Aptenodytes patagonicus*) became habituated to common forms of disturbance such as footsteps approaching; but not to more intensive disturbance such as handling (Viblanco *et al.*, 2012). King Penguins in areas where they were frequently disturbed had lower HR responses than those in undisturbed areas, indicating habituation. Magellanic Penguins in areas visited by tourists had lower corticosterone levels and exhibited fewer defensive behaviours, which also indicates habituation (Walker *et al.*, 2006).

### 1.2.2.3 *Sensitization to Disturbance*

Conversely, other penguin species seem to become sensitized to human disturbance. When disturbed, sensitized individuals show a significantly increased stress response compared to naïve conspecifics (Ellenberg *et al.*, 2007). Humboldt penguins were extremely sensitive to human disturbance, unlike the closely related Magellanic Penguins (Ellenberg *et al.*, 2006; Yorio & Boersma, 1992). Yellow-eyed Penguins frequently visited by tourists had a significantly greater hormonal stress response than those only visited for research purposes, implying that the frequently visited birds had become sensitized to human presence (Ellenberg *et al.*, 2007).

## 1.3 Penguin Personalities

Personality can be defined as a coherent set of behavioural and physiological stress responses which is consistent over time and which is characteristic of a certain set of individuals (Ellenberg *et al.*, 2009). If survival and reproduction of penguins is selective, based on individual personality traits, human disturbance may be shaping the evolution of a particular colony (Reale *et al.*, 2007). Within any species, individuals vary on a bold-shy continuum, with bolder animals being more aggressive, and shyer animals more passive (Coppens *et al.*, 2010). Shy animals may be less able to cope with disturbance, causing them to fail to breed or leave the area (Viblanco *et al.*, 2012). If individuals that are more tolerant of human disturbance are selected for, this could mean that certain genes become eliminated from the population. This outcome is concerning, as a population with greater genetic similarity is less resistant to environmental change or disease (Reale *et al.*, 2007; Viblanco *et al.*, 2012).

## 1.4 Little Penguins and Human Disturbance

Many colonies of Little Penguin (*Eudyptula minor*) persist remarkably close to areas of human habitation, implying that these penguins can co-exist to a degree with humans and all their associated disturbances. Several significant Little Penguin colonies in New Zealand and Australia are managed as ecotourism ventures (e.g. Phillip Island, Victoria; Penguin Island, Perth; Oamaru Blue Penguin Colony, NZ). A new venture has been developed at Pilot's Beach, Dunedin in 2013. As more and more people seek to view these penguins, a better understanding of how human-driven environmental change is impacting the penguins' lives is urgently needed. This begins with quantifying the ways in which interacting with humans on land affects this species.

Remarkably, Little Penguin colonies are located within the confines of each of Australia's two largest cities, Melbourne and Sydney. In Melbourne, Little Penguins reside on a breakwater at St Kilda beach. This anthropogenic structure was first colonised by penguins in 1974, and the colony has doubled in size over the last 18 years (Giling *et al.*, 2008; Priddel *et al.*, 2008). A fence divides the breakwater, separating nesting areas into regions accessible and inaccessible to the public. Penguins nest in higher density out of public reach, implying that human presence has a negative effect on breeding success (Giling *et al.*, 2008). Some of the colony remains accessible to the public because the benefits of increasing public awareness of the species is thought to outweigh the negative consequences of decreased breeding success (Giling *et al.*, 2008). Conversely, penguins in the publicly-visited part of the Phillip Island colony in Victoria, Australia, are thought to have become accustomed to the presence of humans and bright lighting, as they have similar breeding success to penguins in regions without public access (Giling *et al.*, 2008).

In some places, human disturbance is contributing to reduced success in Little Penguin colonies. Human disturbance has been shown to significantly reduce burrow occupancy and hatching success of Little Penguins on Penguin Island, Perth (Klomp *et al.*, 1991), and is given as a factor contributing to the decline of the white-flipped subspecies of Little Penguins on Banks Peninsula, NZ (Allen *et al.*, 2011). A recent study found that the behaviour of Little Penguins in captivity at Melbourne Zoo, Australia, differed significantly with the presence and absence of visitors (Sherwen *et al.*, 2015).

The stresses of human disturbance potentially have a greater impact on smaller bird species, because the safety margin of energy stores decreases with decreasing body size (Weimerskirch *et al.*, 2002). This means that it is vital to determine the conditions under which Little Penguins are vulnerable to human disturbance, and to quantify the extent of the effects. As of yet, few



studies have specifically addressed the impacts of human disturbance on the Little Penguin. Vertigan *et al.* (2012) investigated the effects of researcher disturbance on egg-laying, chick survival and fledging mass in relation to nest checking and chick handling, with indeterminate results. Hence, further research in this area is essential.

### 1.4.1 Oamaru Little Penguin Colony

Little Penguins nest on the coast around the township of Oamaru, New Zealand. Two main populations exist: the Creek colony which is not accessible to the public; and the Quarry colony, which is open to paying visitors. The latter site was an operational quarry from 1865 until the mid-1980s. It was first colonised by Little Penguins in the 1970s, at which time the birds were considered a pest (Higham & Lück, 2002). This site opened as an ecotourism operation in 1993, and is particularly significant to Oamaru's economy as the nightly 'penguin parade' prompts many tourists to select this town as an overnight destination (Higham & Lück, 2002). On average 80,000 people per year visit the penguins, more than six times the town's population (Agnew, 2007).

Comparisons have been made between the two Oamaru colonies to evaluate the effects of tourism, with the Creek colony acting as a control. Penguins are monitored regularly at both colonies. Although demographics have largely been consistent and both colonies are increasing in size, in the 2006 breeding season it was found that the mean fledging weights of chicks were significantly higher in the Creek colony than in the Quarry colony (Agnew, 2007). Fledging weight is thought to be a predictor of first year survival for many species of seabird (Priddel *et al.*, 2008; Reilly & Cullen, 1982). Hence, ongoing monitoring is essential to determine the extent of the effects induced by human disturbance (Taylor, 2000b). The ongoing success of these two colonies is used as an example of how properly-managed tourism can benefit the local economy, as well as fulfilling the central goal of benefitting the wildlife (Agnew & Houston, 2008).

## 1.5 Measuring Physiological Response

Without showing behavioural changes, penguins may undergo changes in physiological function which alter the energy fraction available for vital processes, similar to that found by Weimerskirch *et al.* (2002) in albatrosses. Measurements of physiological parameters such as heart rate, body temperature, rate of oxygen consumption, or corticosteroid levels enable the impacts of disturbance to be quantified. These methods hold more credibility than basing conclusions solely on behavioural observations, as behaviour may be dependent on a range of factors other than disturbance (Regel & Pütz, 1997). Subtle changes in physiological

parameters may reduce individual fitness, and have the potential to cause declines at the population level, or even species level (Ellenberg *et al.*, 2006; McClung *et al.*, 2004).

### 1.5.1 Corticosterone Sampling

When an animal is stressed, the hypothalamo-pituitary-adrenal (HPA) axis is activated and glucocorticoid hormones, corticosterone (CORT) in birds, are released from the adrenal gland (Cockrem, 2007; Fowler, 1999). The release of these hormones helps the animal to cope with or escape from stressors (Walker *et al.*, 2006). To assess the magnitude of a stress response in penguins, blood samples can be taken at timed intervals: initially to gauge baseline CORT levels, and subsequently the increase in CORT which occurs as a part of the stress response (Romero & Reed, 2005).

An important factor to be mindful of when assessing the impact of human disturbance on animals is the stress caused by the measuring technique itself (Carey, 2009). CORT sampling is a useful way of comparing the degree of environmental stress experienced by populations with differing levels of disturbance, or the stress caused by capture and restraint (Ellenberg *et al.*, 2007; Walker *et al.*, 2006). However, because this sampling method itself induces a stress response, it is not suited to monitor the magnitude of a response to a different stressor over time.

### 1.5.2 Heart Rate Telemetry

Measuring heart rate (HR) is a practical way of assessing the impacts of environmental factors on the metabolism of penguins. HR increase occurs in response to stimuli that are novel, challenging or threatening (Nimon *et al.*, 1996). The magnitude and duration of a HR response can be used to quantify a penguin's perception of a disturbance event when no behavioural reaction is apparent (Ellenberg *et al.*, 2013; Nimon *et al.*, 1996). HR has been linearly correlated with the rate of oxygen consumption, and thus metabolic rate, in several penguin species, and this relationship can be applied to all penguin species (Green *et al.*, 2005). This relationship has recently been calibrated for Little Penguins (Green *et al.*, 2008). Converting HR into metabolic rate allows the energetic cost of a stress response to be estimated, so that the biological significance of the response can be determined (Green *et al.*, 2008).

Using an artificial egg or egg dummy (ED) to record HR allows changes in HR to be monitored with minimal disturbance to the animal inflicted by the sampling procedure. This sampling procedure reduces any confounding effects of researcher disturbance (Giese *et al.*, 1999). However, EDs require contact with the birds' brood patch to record heart rate, so this method is only effective during the incubation period. Birds may stand up in response to disturbance

events, which breaks the signal with the ED. This may cause the peak HR response to be underestimated, which is a potential source of bias (Giese *et al.*, 1999). Despite these limitations, the animal being sampled is thought to be unaware of the sampling apparatus (Nimon *et al.*, 1996) so any HR changes can be related to disturbance stimuli rather than the sampling procedure.

An early limitation of this technique was that the ED needed to be attached to a wooden board in order for the sensor to remain upright, so parents would spend an extended period of time trying to rotate the ED before returning to incubation (Nimon *et al.*, 1996). Giese *et al.* (1999) eliminated this limitation by weighting the eggs with lead so that they would return to the desired orientation if rotated. Penguins were highly protective of these eggs when researchers attempted to retrieve them, indicating that the EDs were successfully adopted (Giese *et al.*, 1999).

## 1.6 Research Aims

My study aims to quantify the physiological effects of human disturbance on Little Penguins.

To achieve this, my investigation will:

1. Quantify Little Penguin heart rates responses to realistic researcher disturbance events.
2. Quantify Little Penguin heart rates responses to realistic tourism disturbance events.
3. Compare growth rates and fledging weight of Little Penguin chicks from the publicly accessible Oamaru Blue Penguin Colony and the inaccessible Oamaru Creek Penguin Refuge.
4. Determine whether inter-individual differences in stress responses (measured using corticosteroid response) reflecting personality differences are related to heart rate responses.
5. Calculate the energetic costs of typical researcher interactions occurring at the Oamaru Blue Penguin Colony.

## Methods

### 2.1 Study Species

For an endotherm to survive in seawater while retaining enough heat for metabolism, a minimum mass of 6.8kg is predicted (Thomas & Fordyce, 2008). This expectation is especially defied by the existence of the Little Penguin (*Eudyptula minor*, ‘good little diver’), otherwise known as the Blue Penguin, Little Blue Penguin, Fairy Penguin; or Korora. This species, the smallest of the penguins, is highly adapted for an aquatic lifestyle, and averages a mass of only 1.1kg and height of 30cm (Gaskin & Peat, 1991; Stahel & Gales, 1987). The Little Penguin occupies its own genus and is highly plesiomorphic, exhibiting characteristics which arose early in the evolution of the penguin taxon (Nakagawa *et al.*, 2001). The total population of Little Penguins is estimated at between 350,000 and 600,000 breeding pairs (Vertigan *et al.*, 2012). Six sub-species of Little Penguin have been identified. One of these inhabits the southern coast of Australia and the Otago coast, and the other five are found around New Zealand’s coastal regions and offshore islands (Gales, 1987).

Little Penguins are nocturnal on land, leaving and returning to their burrows only under cover of darkness (Stahel & Gales, 1987). In the evenings, they tend to assemble offshore until light levels are low enough to deter aerial predators, permitting a return to their nests (Stahel & Gales, 1987). This facilitates a synchronised ‘parade’ of penguins across the foreshore to their nesting area (Overeem *et al.*, 2008).

Breeding begins at two or three years of age, and Little Penguins may stay with the same partner for many years (Reilly & Cullen, 1981). The breeding success of Little Penguins is highly dependent on local conditions, especially food supply, and may fluctuate considerably from one season to the next (Agnew *et al.*, 2014; Heber *et al.*, 2008; Perriman *et al.*, 2000; Reilly & Cullen, 1981). The timing of egg laying varies with latitude and food availability, but typically begins in July or August and continues until November (Vertigan *et al.*, 2012). The survival of fledglings during their first year at sea is partially dependent on weight at fledging, with chances of survival generally improving with increased mass (Priddel *et al.*, 2008).

More experienced penguins are more likely to successfully raise chicks to fledging (Agnew *et al.*, 2014; Reilly & Cullen, 1981). Clutches typically contain two eggs, laid 2-4 days apart (Kemp & Dann, 2001). Little Penguins are unique among penguin species in that they may fledge two clutches in one season (Gales, 1985). Little Penguins that lay a second clutch are known as ‘double brooders’. Laying of the second clutch typically begins in October or November, once chicks from the first clutch have fledged and left the nest (Johannesen *et al.*,

2003). In the Otago region of southern New Zealand, egg laying typically occurs in early spring (August or September), and eggs are incubated for 36 days before the chicks hatch (Perriman *et al.*, 2000). During this incubation period, one parent remains on the nest during the day while the other parent leaves to forage at sea. The two parents typically swap roles every 2-3 days, but one parent may spend as long as 10 days away fishing before returning to swap with its partner (Numata *et al.*, 2000).

Some individual penguins are more adept at breeding and raising young than their conspecifics; these individuals are deemed of higher 'quality' (Johannesen *et al.*, 2003). 'Quality' birds are those which lay two clutches in one season, giving them a greater probability of laying two clutches in the subsequent season, and of ongoing survival (Johannesen *et al.*, 2003).

Little Penguins are visual hunters that forage during the day, so the duration of their foraging period is dependent on daylength (Saraux *et al.*, 2011). They are regarded as significant predators within their marine communities, with each consuming an estimated 37kg of food annually (Gales & Green, 1990). Their diet is thought to consist mainly of pilchard, sprats, squid, and other cephalopods, but more research is required to determine variations in food preferences amongst sub-species (Allen *et al.*, 2011). Winter is thought to be the most challenging season for Little Penguins as daylength is reduced and there is less food available, so foraging is both more difficult and under stricter time constraints (Johannesen *et al.*, 2002). Energy expenditure exceeds the amount of energy acquired from feeding during this period. This net energy loss causes the penguins to lose body mass (Gales & Green, 1990). Little Penguins have one of the shortest foraging ranges among seabirds, making them particularly vulnerable to environmental changes such as altered prey densities (Saraux *et al.*, 2011).

Little Penguins have successfully overcome these seasonal challenges for thousands of years, but some colonies have found the added challenges related to human presence insurmountable. Most mainland colonies of Little Penguin have suffered declines in population size over recent decades, with several going extinct (Heber *et al.*, 2008). This abatement is largely attributed to predation by introduced terrestrial mammals, enabled by a lack of proper management (Allen *et al.*, 2011). Offshore islands on which predation is minimized through intensive monitoring remain population strongholds (Taylor, 2000b).

The southern sub-species of Little Penguin, *Eudyptula minor minor*, inhabits the Otago region and numbers between 5,000 and 10,000 breeding pairs (Taylor, 2000b). Population analyses within this region suggest that most mainland colonies are in decline (Taylor, 2000b). Little Penguins no longer nest at seven previous nesting sites around the Otago region (Dann, 1994). Despite this, colonies of southern Little Penguins around Oamaru are breeding successfully, with numbers growing steadily over the last decade (Agnew, 2007). Furthermore, the

population of White-Flipped Little Penguins (*Eudyptula minor albosignata*), found only on Banks Peninsula, is increasing (Allen *et al.*, 2011). These latter findings are heartening, as they indicate that Little Penguin populations can recover quickly when aided by appropriate management.

Little Penguins may nest in rock crevices, under trees or buildings, or dig burrows into sand or soil. Many large Little Penguin colonies are provided with artificial nesting boxes, which the penguins readily inhabit. These boxes are less prone to collapsing or flooding than regular nests, and may allow a greater density of penguins to occupy a suitable site (Houston, 1999; Perriman & Steen, 2000). Nest boxes also allow researchers to gain access to the penguins much more easily than to regular nests.

## 2.2 Study Site

Perhaps because penguins are so easily anthropomorphized, people enjoy viewing them going about their lives. Hence, penguin colonies can be used as a source of education and revenue. Little Penguins are especially suited to such enterprise as they appear unperturbed by human presence: they parade up the shore each evening at sunset as they travel home to their nests, and their colonies are often located near areas of human habitation (Overeem *et al.*, 2008). Several Little Penguin colonies have been developed as tourist destinations.

The Oamaru Blue Penguin Colony (OBPC) is one such colony. The success of the OBPC in terms of both growing revenues from tourists and a growing penguin population, provides an example of how properly managed tourism can benefit the local economy as well as the wildlife itself (Agnew, 2007).

The OBPC (45°07'S, 170°58'E), is situated less than two kilometres from the centre of the town of Oamaru. The site became designated breeding habitat for the penguins in 1992, and has since been operated as a tourism venture (Johannesen *et al.*, 2003). The colony features approximately 300 wooden nest boxes in earth mounds, as well as natural burrows, on one hectare of land (Houston, 1999). Penguins have a choice of boxes to occupy, and, once settled on a box, typically return to this same box throughout the breeding season and even in subsequent years.

Visitors to the OBPC face several options as to how they encounter the penguins, depending on the amount of time they have available and how much money they want to spend. Tours operate year-round, so visitors may be in the colony while penguins are breeding. During the day, visitors can embark upon a 'Self-Guided Day Tour' in which they walk along boardwalks and pathways amongst the nesting boxes, and learn about the penguins by means of informative

signage. This type of tour costs \$10 for an adult, and is estimated to take 10-20 minutes. Visitors are unlikely to see much penguin activity in the nesting boxes strewn around the pathways, as during daylight hours the penguins on land are typically resting or asleep. If any penguins are to be spotted, this will only be as a pair of eyes peering out of an entrance tunnel, or perhaps a glimpse of tail feathers as a well-aimed poo is shot out of an entranceway.

The OBPC features a free-standing room known as the 'Blue Wing', especially designed so that visitors may view Little Penguins in their burrows during the day. This room has ten nesting boxes built into its walls, each with a transparent glass lid and viewing tunnel. Penguins choose to nest in these boxes, and the same individuals have returned here for more than one season. Visitors can peer down the tunnels and observe the penguins during daylight hours. Penguins will typically be huddled in a corner of their nest boxes, resting or sleeping.

During the day, visitors may also opt for the 'Guided Day Tour'. This is much the same as the 'Self-Guided Day Tour', the main differences being that the guided tour includes the eponymous guide, and a larger area of the colony is able to be accessed. The guide begins the tour with a 20 minute commentary, providing information about the penguins. This tour option increases the visitors' chances of spotting penguins peering out from the entrance of their nest boxes. The Guided Day Tour costs \$16 for an adult, and typically lasts 30-40 minutes.

As it gets dark, 'Evening Viewing' is available to visitors. Attendants watch as the Little Penguins return from the ocean to their nests in substantial numbers. Visitors are seated in stands on either side of the main pathway that the penguins take from the rocky shoreline into the penguin colony. A tour guide provides commentary about the penguins. This costs \$28-\$40 for adults, depending on the chosen seating option.

The OBPC has been monitoring its resident penguins since 1993 (Numata *et al.*, 2000). Penguins living in the colony are fitted with flipper bands for individual identification. Many penguins are also marked with passive inductive transponder (PIT) tags, which allow their movements into and out of the colony to be recorded electronically. Colony staff routinely record the weights of adult penguins, and chicks are weighed every few days from hatching to fledging.

A separate colony of Little Penguins is located near the outflow of a creek into Oamaru Bay, approximately 980m from the quarry site. This site is known as the Oamaru Creek Penguin Refuge (OCPR). Public access to this area is prevented by means of a fence and signage. Penguins living in the OCPR are monitored by OBPC staff once a week. Staff compare the welfare of penguins at each of the colonies to assess the impact of visitors at the former quarry



site. Data show no difference in reproductive success between the two colonies (Johannesen *et al.*, 2003).

The OBPC can be divided into three areas: (1) low tourist presence, where nests are monitored regularly by staff but there is no visitor access; (2) medium tourist presence, where visitors are allowed to walk among the nest boxes; and (3) high tourist presence, i.e. the nest boxes that can be viewed from the Blue Wing. Ten nest boxes are on view in the Blue Wing, but in a typical season only six or seven will be occupied.

## 2.3 Egg Dummy Construction

Egg dummies (EDs) were constructed to resemble Little Penguin eggs. These artificial eggs contained microphones to record the penguins' heart beats (Fig. 2.1).

A Little Penguin egg, provided by Philippa Agnew (resident scientist at OBPC) was used to construct a two-part silicone (Pinkysil) mold. Each half of the mold was coated with epoxy resin into which fibreglass strands were arranged so as to take on the shape of the mold. Once dry, the fibreglass half eggs were sanded so that the edges fitted together without gaps. A hole was drilled into the dome of one half to accommodate a microphone with radius 10mm. The base of each half was drilled to accommodate the microphone cable, radius 8mm.

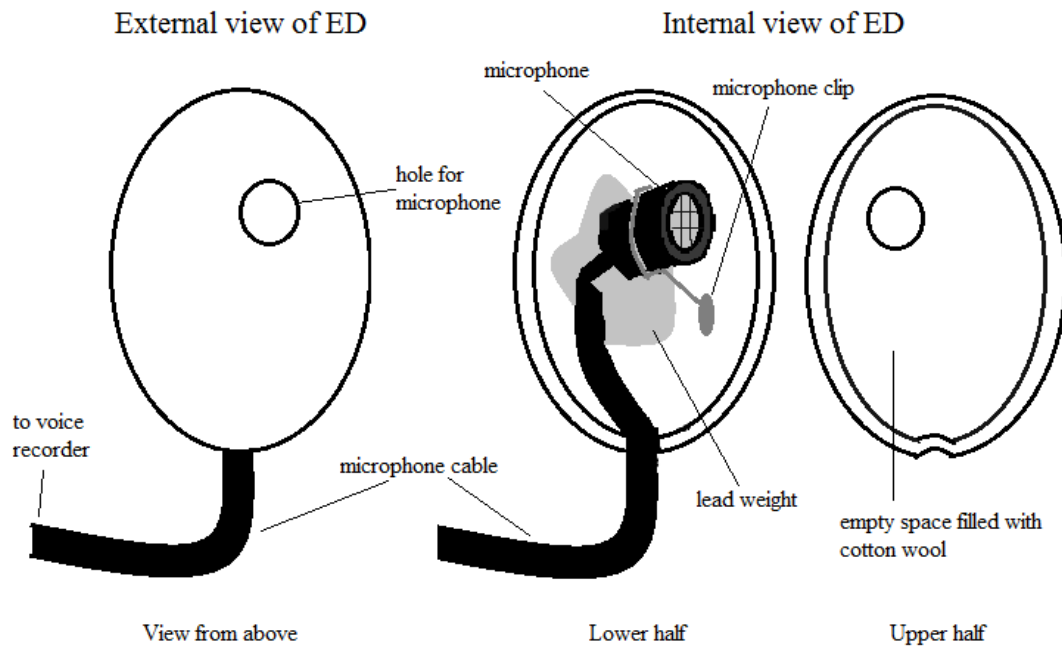
A Shure 183 omnidirectional lavalier condenser microphone was positioned in the egg half with the hole drilled in the dome, so that the upper surface of the microphone was flush with the surface of the egg. A lead weight was glued with resin into the other half of the egg, countering the microphone. This helped the microphone to maintain the correct orientation to facilitate optimum sound recordings. The lead weight brought the total weight of the ED up to approximately 54g, this being approximate to the mean weight of a Little Penguin egg (53.67g) (Kemp & Dann, 2001).

The remaining space within the egg shells was filled with cotton wool for soundproofing, and the two halves were fixed together with Tesa tape. The microphone was attached to a 2m length of cable, reinforced so that penguins could not peck through it. The cable was plugged into a Panasonic RR-US300 Voice Recorder powered by one AAA battery.

The microphone could record penguin heart beats only when in contact with a bird's brood patch, a highly vascularised area of skin on the bird's abdomen. However, Little Penguins periodically turn their eggs during incubation so that eggs are maintained at a constant temperature (P Agnew, pers. comm.). This problem was overcome by taping cable and egg together at the point where the cable entered the base of the egg, so that the microphone pointed

upwards when the cable took on its natural coil. This caused the egg to return to the correct orientation when moved by the penguin.

Three EDs were constructed in this manner.



**Figure 2.1:** External and internal view of Egg Dummy (ED).

## 2.4 Data Collection

The data collected for this project were approved by the Department of Conservation (DoC).

University of Otago Animal Ethics Approval has been granted for this project (permit number 34/12).

The Ngāi Tahu Research Consultation Committee support this project.

### 2.4.1 Pilot study

A pilot study was conducted from 17-23 October 2012 to determine the amount of time taken from ED deployment (i.e. replacing a real egg with the ED) for penguin heart rates to return to basal levels, with the aim of gaining practical experience with equipment and the experimental protocol. I conducted manipulations with the aid of Philippa Agnew.

A Little Penguin egg was removed from under an incubating adult in its nest box, and the ED positioned in its place, via the following procedure.

The front section of the nest box lid was used to shield my hand from the penguin's beak. While holding this lid section in place with one hand, the other hand was used to remove an egg from underneath the penguin, place this on the ground nearby, and then place the ED in position through the entrance tunnel of the box. The ED was arranged underneath the penguin with the microphone pointing upwards. The lid was then replaced, and the voice recorder was set to record.

The egg was then carried by hand from the nesting box to an incubator situated within the research facility at the colony. The egg was in transit for no longer than five minutes. The incubator was heated to 36°C, this being the incubation temperature within a Little Penguin nest (P. Agnew, pers. comm.). If more than one egg was in the incubator at one time, eggs were labelled with a vivid marker with the number of the nest box they came from to ensure that they were returned to the correct nests. After a recording period of up to three hours, the egg was replaced and the ED removed in a reversal of this procedure. All eggs hatched successfully.

The recorded sound file was used to calculate the penguin's heart rate in beats per minute, and determine changes in heart rate in relation to ED deployment. From this, knowledge was gained of resting heart rate (RHR), and the time taken to resume RHR after disturbance.

This procedure was repeated with eight penguins until consistent recordings of reasonable quality were obtained. Some penguins were also observed during the recording period via an infra-red camera with live feed to a monitor situated in the colony building. This enabled activities such as egg-turning and preening to be matched with the resulting sound in the recording, so that I became familiar with the causes of such sounds.

The resulting recordings from this pilot study showed that Little Penguin heart rate typically slowed to 120-130 beats per minute (bpm) well within half an hour of egg deployment. This was the slowest heart rate recorded in three-hour long recordings, so was understood to be the typical RHR range for the penguins. Previous research has recorded Little Penguin resting heart rate at 112-117 bpm (Green *et al.*, 2008).

## 2.4.2 Measuring Researcher Disturbance

My study required penguins that were incubating eggs. Data collection for the main part of the study began late in the first round of egg laying for the season (late October 2012), so that many penguins nests already contained chicks and were therefore excluded. Penguins with eggs that were due to hatch within five days of the sampling date were excluded. Twenty nest boxes within the OBPC were selected based upon box location, stage of breeding, and the previous experience of adult penguins with researchers. If penguins had been fitted with data loggers in the past, they were excluded from the study on the basis that they may have an altered response to handling; although birds fitted with loggers were found to have no detectable differences in breeding parameters (Agnew *et al.*, 2013). This component of my study required nesting boxes that were located more than two metres from visitor pathways, and out of direct line-of-sight from these pathways, so that passing tourists would have minimal impact on the penguins' heart rates during recordings.

### 2.4.2.1 Disturbance Stimuli

Four stimuli were presented to each penguin. Three of these stimuli were designed to mimic typical researcher interactions with incubating penguins, and the fourth served as a control. These stimuli were:

- 1) 'Human speech playback': a playback of a 30s recording of human speech, (Newstalk ZB, Murray Lindsay) from an HP Pavilion laptop at full volume, one metre from the nest box entrance. Care was taken while approaching the nest to not be visible through the entrance tunnel, so that the sound, rather than visible approach, was the stimulus.
- 2) 'Lifting nest box lid': removal of the lid of the nest box to check the penguin's flipper band. Eye contact was made with the penguin for 10 seconds before replacing the lid and walking away.
- 3) 'Weighing penguin': weighing the adult penguin. A cloth bag was presented to the bird for it to bite, so that it could be grabbed without the researcher's hand being bitten. The penguin was then lifted out of the nest box, placed into the bag, and weighed. Weights were measured using a 2500g Pesola scale. Once weighing was complete, the lid was replaced onto the box, then the penguin was released into the entrance tunnel.
- 4) 'Penguin call playback' (control): a playback of a 30s recording of a Little Penguin braying call (courtesy of Abbie Mason) again at full volume from an HP Pavilion laptop at a one metre distance from the nest box entrance.

#### 2.4.2.2 *Recording Protocol*

The ED was swapped with a penguin egg following the procedure outlined in section 2.4.1. After a 30 minute adjustment period, as determined in the pilot study, the four stimuli were enacted in a randomized order with 30 minute intervals between each one. The ED was left in place for 30 minutes after the last stimulus, meaning that it remained in the nest for a total of two hours 30 minutes. After this period the egg was returned to the nest and the ED removed. Data were then downloaded from the voice recorder to my laptop. This procedure was repeated for the inhabitants of 20 nest boxes, totalling 40 adult penguins in the incubation stage of chick rearing.

Twenty penguins were sampled from 23-29 October 2012. A further twenty penguins were sampled between 26 November and 4 December 2012, resulting in a total sample size of 40 penguins.

#### 2.4.3 Measuring Tourist Disturbance

Nest boxes within the OBPC were classified into one of three ‘tourist presence’ zones in relation to their degree of public accessibility. The ‘low’ zone was comprised of nests that were more than two metres from the pathways accessible to tourists during the day, and not oriented towards these pathways. Nests in the ‘medium’ zone were within two metres of a pathway, with the entrance tunnel facing the pathway. Nests in the ‘high’ zone were within the ‘Blue Wing’. The Oamaru Creek Penguin Refuge (OCPR) or ‘creek’, which is inaccessible to the public, was used as a control site.

Data were collected from both penguins in ten nesting boxes at each of the ‘low’, ‘medium’ and ‘creek’ sites, resulting in twenty samples per site. Data were collected from only seven birds in the Blue Wing, as only four nests were occupied. A total of 67 penguins were sampled for their response to tourist presence. EDs were deployed in the manner described in section 2.4.1 and left in nests for a total of four hours, before penguin eggs were replaced. ED deployment occurred between 9am and approximately 2.30pm, so that the four hour recording period would occur during normal visiting hours to the colony.

So as to determine whether HR was influenced by a range of factors other than proximity to visiting tourists, data on relevant variables were included in my analyses. For each penguin sampled, data on the number of years of previous breeding experience, the number of chicks fledged in the previous breeding season, and sex (male/female) were obtained from resident scientist Philippa Agnew. HR is known to differ between sexes in sexually dimorphic seabirds (Green, Butler, Woakes, Boyd, & Holder, 2001; Weimerskirch *et al.*, 2002). The variables

‘number of chicks fledged in the previous season’ and ‘years of breeding experience’ were included as means of determining whether HR could be related to breeding success, to investigate whether the more successful breeders are the individuals less affected by disturbance. Human disturbance is known to cause decreased breeding success in many species of seabird (Anderson & Keith, 1980).

The behavioural responses of each penguin during ED deployment were recorded, to determine if there was a relationship between physiological and behavioural response. The absence of a behavioural response to disturbance may indicate habituation (Viblanco *et al.*, 2012). Each penguin was assigned one of the following ‘aggression’ ratings:

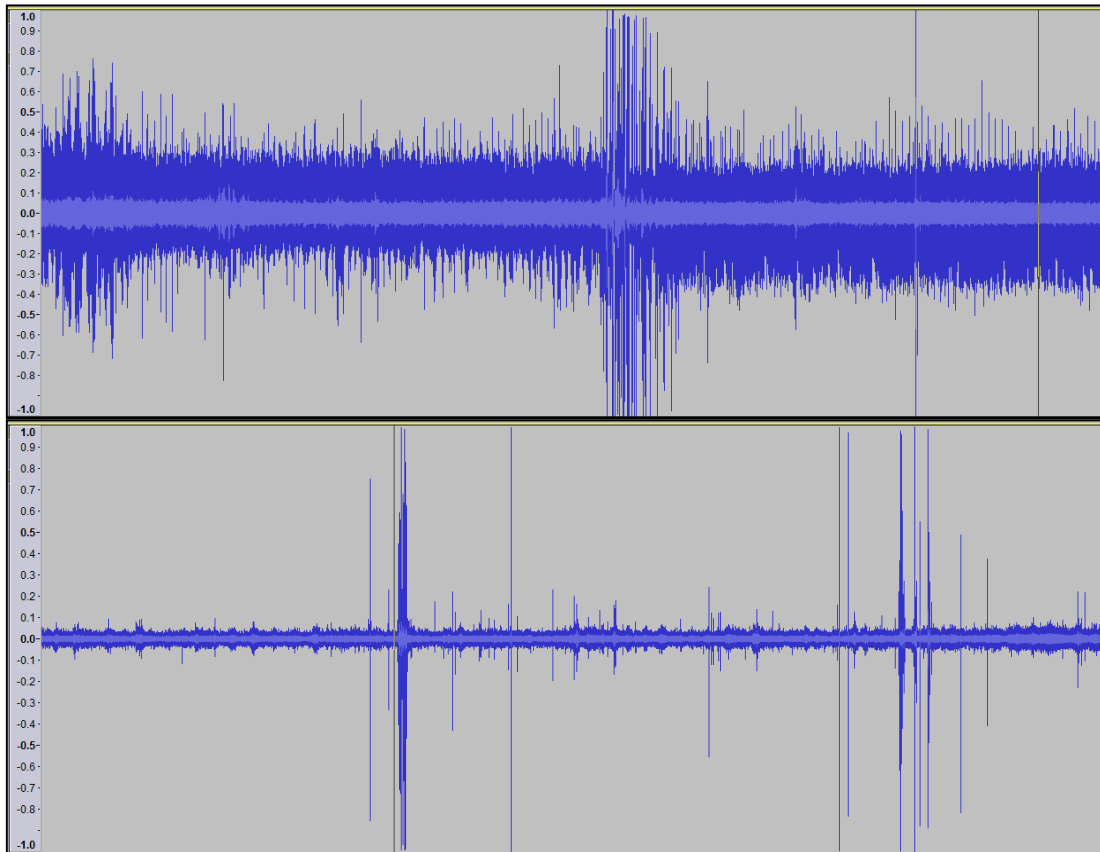
- |   |   |
|---|---|
| 1 | calm, no noises or pecking attempts                     |
| 2 | some lunges or pecking                                  |
| 3 | repeated pecks/lunges, some vocalisations               |
| 4 | consistent aggressive pecking/lunging, quacks or growls |

## 2.5 Data extraction

Heart rate was recorded onto voice recorders as MP3 audio files. In total, 368 hours of audio files were recorded. Data were extracted from these using one or both of two methods, depending on the quality of the each file.

### 2.5.1 Manual Analysis using Audacity

The free audio editor programme ‘Audacity’ (Audacity 2.0.0®) was used to visualize, rate and label heart rate files. The default setting of Audacity displays sound files as waveforms in time, with a linear vertical scale running from -1.0 (negative values) to +1.0 (positive values), centred on zero. The waveforms take on a distinctive pattern where a HR signal is present (Fig. 2.2).



**Figure 2.2:** Penguin heart rate (HR) data viewed through Audacity with audible HR signal (above) and no audible HR signal (below).

Viewing the files through Audacity allowed me to evaluate how much of each file contained useful HR signal. File names were listed in Microsoft Excel and categorized in relation to their quality: high if HR signal was clear throughout the recording, medium if sections of signal were present, and poor if no signal was located. Many files contained periods where the penguin was not in physical contact with the ED, so HR signal was not recorded. In some case the entire file failed due to a lack of signal. From this visual analysis using Audacity, 13 researcher presence recordings and 19 tourist presence recordings were discarded due to lack of signal, leaving me with 27 researcher and 48 tourist recordings with some sections of usable HR signal.

Manual heart beat counts were then used to calculate heart rate. These were performed in Audacity by selecting a segment of the sound recording where the signal was audible and uninterrupted for 30 seconds. I listened to each segment and counted the number of heart beats it contained. This datum and the start and finish times of the segment were recorded in Microsoft Excel.

## 2.5.2 Matlab Analysis

Medium and high quality files were further analyzed by Ursula Ellenberg using a program custom-written for MATLAB® (MATLAB 2007). This program is not publicly available and requires a considerable time commitment to gain operational knowledge, so I did not undertake this analysis myself. This program identified heart beats and calculated the rate of each beat, using a 12 second moving average of the surrounding beats. This method of analysis enabled the heart rate to be known at a much finer temporal scale than through manual analysis, so that the exact maximum heart rate during disturbance events could be known rather than inferred. The output was an Excel chart giving a heart rate with measures of confidence for every second of each HR recording. Using this chart, the time segment of interest could be selected and averaged.

Given that two methods were used, the outputs of each method of data analysis were compared to ensure the validity of manual analyses. Baseline HR (bpm), maximum HR (bpm) and time to recovery (s) were calculated using both Matlab® and manually for 11 researcher actions (five ‘human lifting nestbox lid’, three ‘speech playback’, three ‘penguin call playback’). Pairwise comparisons using paired t-tests were conducted in R to compare data derived from each source (Table 2.1). The same or very similar heart rates were calculated when recordings were analyzed with both methods. This gave me confidence in the accuracy of the manual analyses.

**Table 2.1:** *p* values resulting from comparison of Little Penguin Heart Rate outputs from manual and Matlab® analyses.

	Baseline HR	Maximum HR	Recovery time (s)
<i>p</i> value	0.912	0.912	0.214

For the tourist disturbance recordings, heart beat counts were performed at 15 minute intervals throughout the four hour recording period. These were taken from Matlab® output if files had been analyzed with Matlab®, or counted manually if not. If the time increment for a particular count did not have clear signal, a clear segment was sought on either side of the particular increment until one was located or five minutes either side of the increment was reached, e.g. if there was no clear signal at 30 minutes in a recording, the heart beat count of a clear segment anywhere between 25 and 35 minutes could be substituted in. Sound files needed to contain at least three heart beat counts for their data to be included in statistical analyses. This requirement meant that 11 data files from the ‘low’ zone of the penguin colony, 12 from the ‘control’ zone and 17 from the ‘medium’ zone were used in the statistical analyses, with the



remainder being excluded due to a lack of data. This resulted in a total sample size of 40 penguins. No files from the ‘high’ disturbance zone were usable.

For the researcher disturbance recordings, firstly the four disturbance events were labelled on the sound files in Audacity, and their exact recording times noted. Heart beat counts (as described above) were then performed at two minute intervals, beginning ten minutes before each event, and continuing until 20 minutes afterwards. This was so that the heart rate leading up to, during, and after each event could be recorded. If there was not a clear signal at a particular time increment, any clear 30 second segment within the two minute window would be counted. Maximum HR was taken from Matlab® if an output had been produced, or otherwise taken from a ten second heart beat count of the fastest section of HR.

## 2.6 Further Analyses

### 2.6.1 Researcher Disturbance

Heart beat counts taken at two-minute intervals during the ten minute period immediately before each stimulus were averaged to obtain resting HR (RHR). The increase in HR from this baseline to the maximum HR recorded during or immediately after each stimulus was calculated and presented as a percentage increase of the baseline value (%RHR increase). Linear models produced using R were used to relate %RHR increase across stimulus types (lid, human speech playback, penguin call playback, and weighing).

I defined the metric ‘time to baseline’ as the amount of time in seconds for penguin heart rate to return to and remain within RHR  $\pm$  two standard deviations for three consecutive heart beat counts following a stimulus. I also used this to compare the magnitude of the effect of the different stimuli on Little Penguin HR. I used a linear model to relate ‘time to baseline’ to stimulus type (lid, human speech playback, penguin call playback, and weighing).

#### 2.6.1.1 Energy Expenditure Calculations

The oxygen consumption ( $\text{VO}_2$ ) of Little Penguins resting in their thermo-neutral zone (typical conditions during incubation) can be calculated when the heart rate is known, and this can be converted into energy usage (kJ) (Green *et al.*, 2008; Stahel *et al.*, 1984). The higher the HR, the higher the  $\text{VO}_2$ , and the greater the metabolic rate. The field metabolic rate (FMR) of foraging and incubating Little Penguins is  $661 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (Gales & Green, 1990). Thus, a 1kg Little Penguin would require 661 kJ of energy per day during incubation. However, if this penguin experiences elevated HR for an extended period of time, this would alter its energy needs. The HR responses of Little Penguins being weighed were used to determine the amount of energy used in this response (see Appendix B for calculations).

## 2.6.2 Relationship between Heart Rate and Corticosterone Response

Endocrinologist John Cockrem (Massey University) took blood samples from 100 Little Penguins at the Oamaru Blue Penguin Colony in winter 2012, outside of their breeding season. The penguins sampled were ranked according to the amount of corticosterone (CORT), a stress hormone, present in their blood, following the standard CORT sampling procedure. Ten of the penguins sampled by J. Cockrem were previously sampled by me for the ‘Researcher Disturbance’ component of my study. I assessed whether the relative magnitude of stress responses of individual penguins was consistent between two measures of stress (HR and CORT). To do this I ranked the ten penguins by magnitude of their HR responses, and compared my rankings with CORT response rankings using the Spearman rank-order correlation analysis.

Ideally a value for RHR percentage increase would have been obtained for each of the ten penguins in response to each of the four stimuli. In reality this was not the case, due to a loss of HR signal often occurring immediately after the presentation of stimuli to the penguins. For seven of these ten penguins, a usable heart rate response was obtained for only one of the four stimuli (see Table 2.2).

Penguin ID	Stimuli
24144	1
31756	2,3,4
31786	1
35175	2
36906	1,2,3,4
38584	2
38738	3
43448	1,3
44810	3
46004	4

**Table 2.2:** The stimuli for which response data were obtained from individual penguins. ‘Penguin ID’ lists the flipper band number of each penguin. Stimulus 1: ‘penguin call playback’, 2: ‘human speech playback’, 3: ‘nestbox lid lift’, 4: ‘weighing penguin’.

Response values were obtained for stimuli 1, 2, or both, for seven of the ten penguins (Table 2.4). In order to make use of the available data, a hypothetical response value was defined as the mean of the responses to stimuli 1 and 2 for each individual penguin. To calculate this value, the available data were altered with correction terms (see Table 2.5). Correction terms were taken from the differences between the group mean RHR percentage increase responses to each stimulus. The group mean response to stimulus two (31.5) was approximately ten percent greater than the group mean response to stimulus one (21.8). The midpoint between these

values (26.7) was the basis for the correction terms: if a response value for stimulus one was obtained, this would be corrected by adding five to reach the midpoint between stimuli one and two. If a response value for stimulus two was obtained, this would be corrected by subtracting five (see Table 2.3). The correction terms for stimuli three and four are based on the difference between the group mean responses to these stimuli and the hypothetical group mean response value of 26.7.

**Table 2.3:** Mean resting heart rate percentage increase in response to each of the four stimuli (1: ‘penguin noise playback’, 2: ‘human speech playback’, 3: ‘nestbox lid lift’, 4: ‘weighing penguin’), standard deviations (SD), and the calculations used to standardize the response values between stimuli in relation data obtained. In calculations ‘S’ refers to stimuli number.

Stimulus	Mean (%)	SD	Obtained				
1	21.8	19.0	✓	✓			
2	31.5	12.9	✓		✓		
3	41.9	31.0				✓	
4	54.4	29.8					✓
Calculation			= (S1+ S2)/2	= S1 + 5	= S2 - 5	= S3 - 15	= S4 - 27

Table 2.4 shows hypothetical response values calculated for five individual penguins, using the calculations given in Table 2. 3.

**Table 2.4:** Hypothetical mean resting heart rate percentage increase values calculated for individual penguins in accordance with available stimuli (1: ‘penguin noise playback’, 2: ‘human speech playback’, 3: ‘nestbox lid lift’, 4: ‘weighing penguin’, Response = response value). Penguin ID refers to flipper band numbers. Response values are obtained using the calculations in Table 2.3.

Stimulus	Penguin ID				
	36906	31786	35175	38738	46004
1	28.3	17.9			
2	39.3		38.6		
3				71.9	
4					70.6
Response	= 33.8	= 22.9	= 33.6	= 56.9	= 43.6

The ten penguins were ranked 1-10 according to the size of this hypothetical response, from smallest to largest.

Spearman’s rank-order correlation coefficient  $r_s$  was used to compare HR rankings and corticosterone rankings.

### 2.6.3 Tourist Disturbance

The average heart rate across the four-hour recording period was calculated for each penguin by averaging the heart beat counts of all 30s segments. Data were taken from Matlab® output if this was available, or manually counted sections of clear signal if the file had not been analyzed with Matlab®. I used one-way ANOVAs to determine which of the five variables investigated were related to HR. This modelling grouped penguins across the three disturbance zones, taking into account nest box location, sex, aggression rating, previous nesting experience (in years), and number of chicks fledged in the previous breeding season (entered as factors) (R Core Team 2012).

Weighted AIC<sub>C</sub> values were computed for models containing the factors which did influence HR, to determine which model best explained HR. AIC values were obtained from R, and AIC<sub>C</sub> values were calculated using the following formula:

$$AIC_C = \frac{AIC + 2K(K+1)}{N - K - 1}$$

where K = no. parameters in model  
N = sample size

AIC<sub>C</sub> weights were then calculated using the difference between the smallest AIC<sub>C</sub> value of all models (min AIC<sub>C</sub>) and the sum of AIC<sub>C</sub> of all models:

$$\omega_i(AIC_C) = \frac{\exp(-0.5\Delta_i)}{\sum \exp(-0.5\Delta_i)}$$

where  $\Delta_i$  = the difference between AIC<sub>Ci</sub> and minAIC<sub>C</sub>

The AIC<sub>C</sub> criterion takes into account the fit and complexity of competing models, with a bias correction term for small sample size (Anderson *et al.*, 2001). AIC<sub>C</sub> weights can be interpreted as probabilities – that is, each value gives the probability that a given model would be judged the best model based on repeated sampling (Burnham & Anderson, 2004).

### 2.6.4 Chick Growth Modelling

Little Penguin chick weight data from the 2011-2012 breeding season were provided by Philippa Agnew. Chicks were weighed by colony staff at least once a week from one week old until they left the nest. Chicks from the OCPR were weighed an average of 6.3 times each over 55.4 days (n = 13). Chicks from the OBPC were weighed more frequently, on average 11.6 times each over 58.3 days (n = 60). Data were processed in Microsoft Excel.

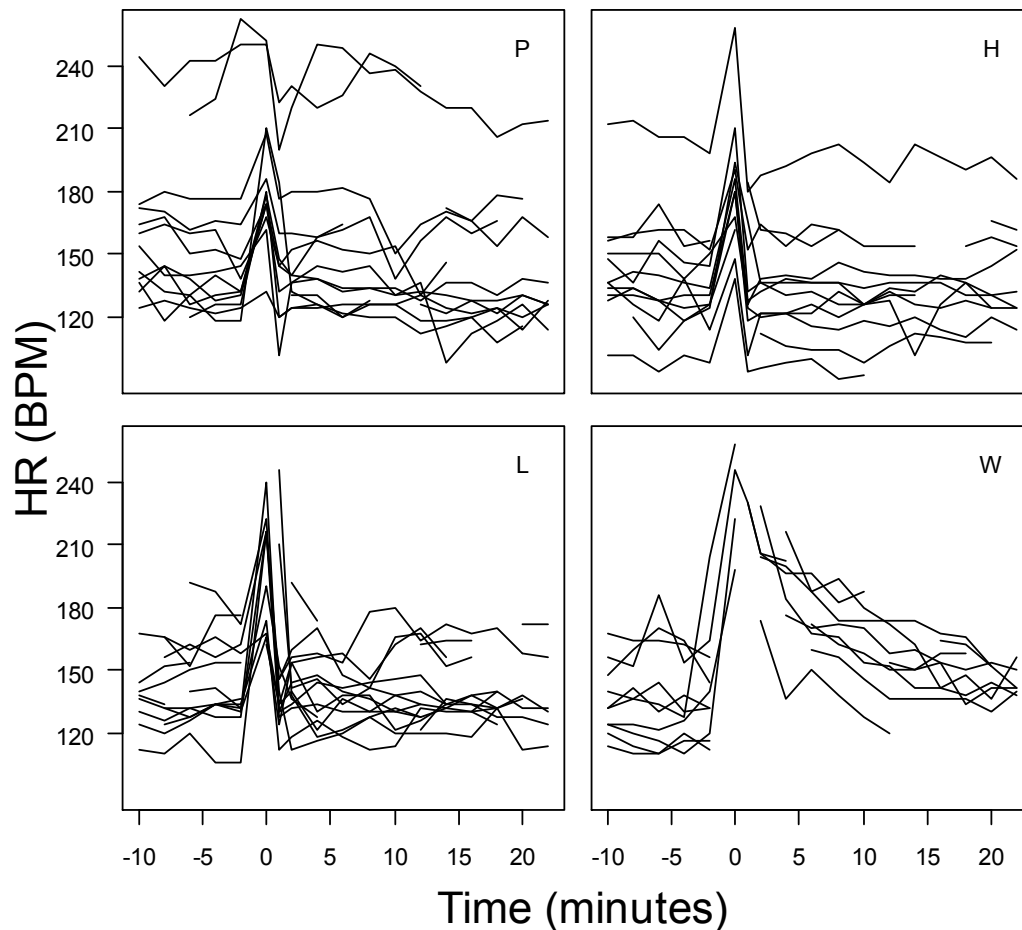
Chick growth was modelled in the R package ‘FlexParamCurve’ following the methodology of Oswald *et al.* (2012). This package includes functions to estimate parameters for nonmonotonic curves and select the models that best fit datasets. It is especially useful for describing the growth pattern of seabird chicks, as they typically reach a peak weight near to fledging, then lose weight as adult feathers are produced. The growth of chicks from the OPBC and OCPR was compared. For the modified R script used to model data and produce graphs, see Appendix A.

## Results

### 3.1 Researcher Disturbance

Nesting Little Penguins were presented with each of four stimuli (penguin call playback, human speech playback, lifting the nest box lid, and weighing the penguin) at thirty minute intervals.

Heart rate (HR) responses tended to follow similar patterns of increase in response to each stimulus, regardless of prior resting heart rate (Fig. 3.1). Maximum HR as a percentage of pre-stimulus resting heart rate (RHR) was thus considered a valid metric for comparison of response between stimuli.



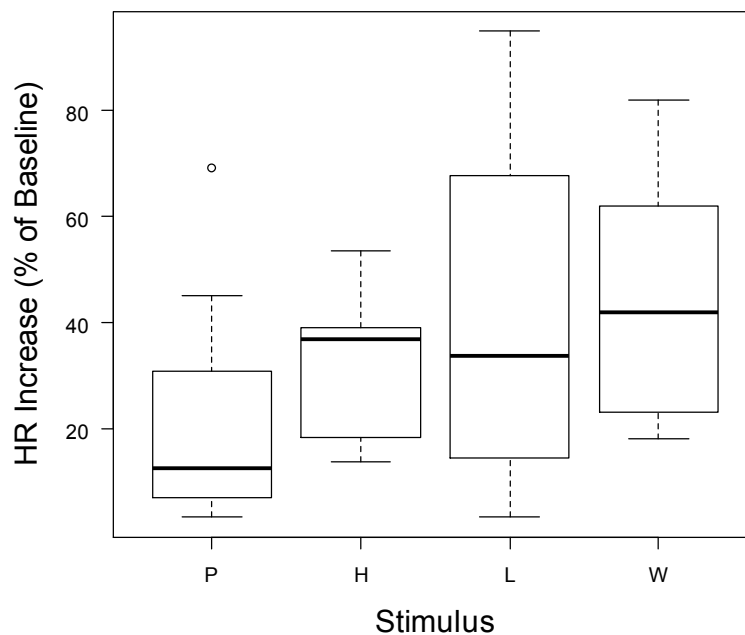
**Figure 3.1:** Little Penguin heart rate (HR) in beats per minute (bpm) over time in response to four stimuli: penguin call playback ('P', n=13), human speech playback ('H', n=12), lifting nest box lid ('L', n=14), weighing penguin ('W', n=11). Stimuli were presented to penguins at time = 0. Each line represents the heart rate of an individual penguin. Gaps in lines are a result of loss of heart rate signal due to penguin movement.

The mean effects of the four stimuli increased in magnitude in the following order: penguin call playback, human speech playback, lifting nest box lid, weighing penguin (Table 3.1). This order was consistent for both the magnitude of heart rate increase (%RHRinc) and the amount of time taken for HR to return to baseline (TimeBase(s)).

**Table 3.1:** Mean and standard deviation (SD) values for Little Penguin heart rate increase as a percentage of resting heart rate (%RHRinc), and time taken for heart rate to return to baseline following a stimulus (TimeBase(s)) in response to each of the four stimuli (penguin call playback, ‘Penguin’; human speech playback, ‘Human’; lifting nest box lid, ‘Lid’; and weighing penguin, ‘Weigh’).

	Penguin		Human		Lid		Weigh	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
%RHRinc	21.8	19.0	31.5	12.9	41.9	31.0	54.4	29.8
TimeBase(s)	70.8	99.5	108.4	89.5	290.0	247.8	1018.6	833.1

The recorded increases in maximum Little Penguin HR, presented as a percentage of the RHR, were significantly greater in response to weighing the penguin than in response to the penguin call and human speech playbacks (Fig. 3.2, Table 3.2). There were no significant differences between the other stimuli.

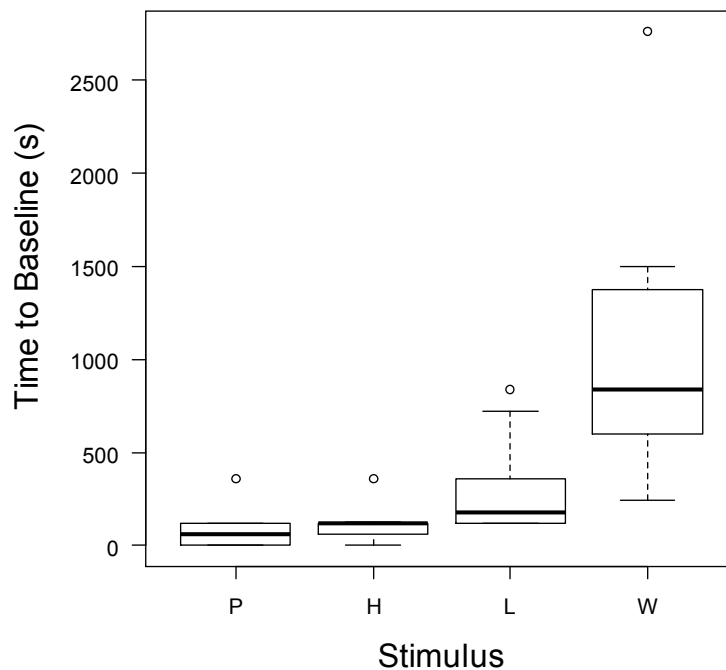


**Figure 3.2:** Little Penguin heart rate (HR) increase as a percentage of baseline heart rate in response to four stimuli: penguin call playback (P, n=13), human speech (H, n=12), lifting nest box lid (L, n=14), and weighing penguin (W, n=11). The plot shows group medians as bold lines, the first and third quartiles as the bottom and top of the ‘boxes’, and the lowest and highest data within 1.5 times the inter-quartile range of the lower and upper quartiles as the ‘whiskers’. Outliers are shown as circles.

**Table 3.2:** Output from linear models evaluating the magnitude of Little Penguin heart rate increase in relation to pairs of the four stimuli (penguin call playback, ‘Penguin’; human speech playback, ‘Human’; lifting nest box lid, ‘Lid’; weighing penguin, ‘Weigh’).

	Penguin		Human		Lid	
	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>
Human	0.152	2.19 <sub>(1, 23)</sub>				
Lid	0.055	4.046 <sub>(1,25)</sub>	0.291	1.168 <sub>(1, 24)</sub>		
Weigh	0.004	10.57 <sub>(1,22)</sub>	0.024	5.92 <sub>(1, 21)</sub>	0.319	1.04 <sub>(1, 23)</sub>

The amount of time taken for penguin HR to return to within pre-stimulus baseline values was significantly longer after weighing the penguin than after each of the other three stimuli (Table 3.3), and after lifting the nest box lid than both the sound playbacks (Fig. 3.3, Table 3.3). There were no significant differences between the other stimuli.



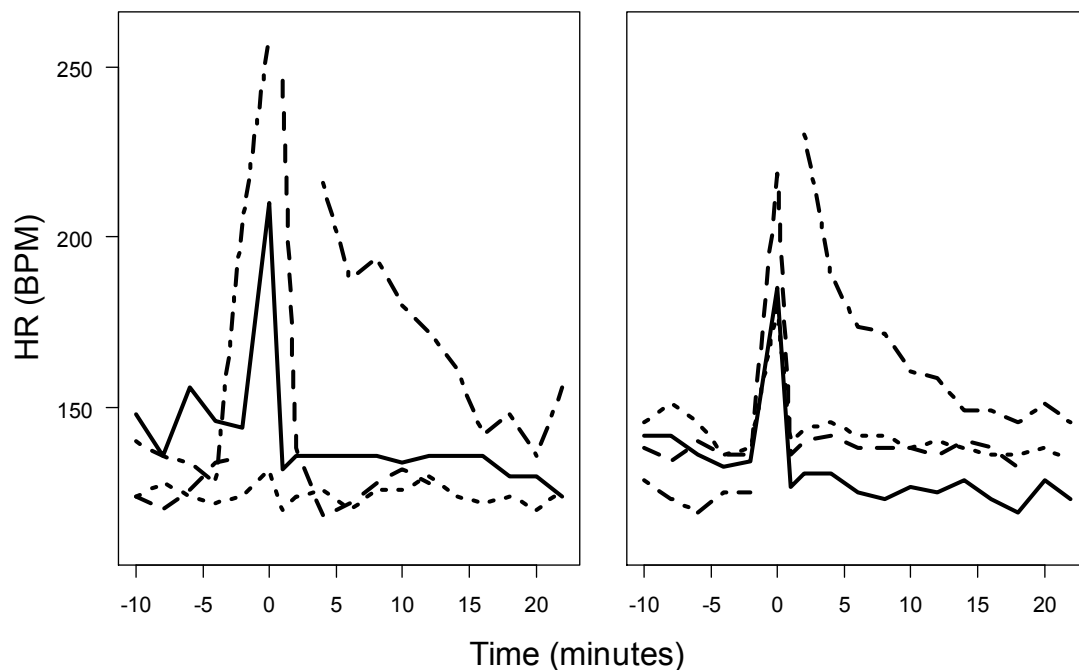
**Figure 3.3:** Time (s) for Little Penguin heart rate (HR) to return to pre-stimulus baseline in response to four stimuli: penguin call playback (‘P’, n=13), human speech playback (‘H’, n=12), lifting nest box lid (‘L’, n=14), and weighing penguin (‘W’, n=11). The plot shows group medians as bold lines, the first and third quartiles as the bottom and top of the ‘boxes’, and the lowest and highest data within 1.5 times the inter-quartile range of the lower and upper quartiles as the ‘whiskers’. Outliers are shown as circles.



**Table 3.3:** Output from linear models evaluating the length of stimuli response time in relation to pairs of the four stimuli (penguin call playback, ‘Penguin’; human speech playback, ‘Human’; lifting nest box lid, ‘Lid’; weighing penguin, ‘Weigh’).

	Penguin		Human		Lid	
	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>
Human	0.333	0.979 <sub>(1,23)</sub>				
Lid	0.007	8.677 <sub>(1,23)</sub>	0.026	5.699 <sub>(1,22)</sub>		
Weigh	<0.001	21.31 <sub>(1,19)</sub>	0.000	18.26 <sub>(1,18)</sub>	0.004	10.66 <sub>(1,18)</sub>

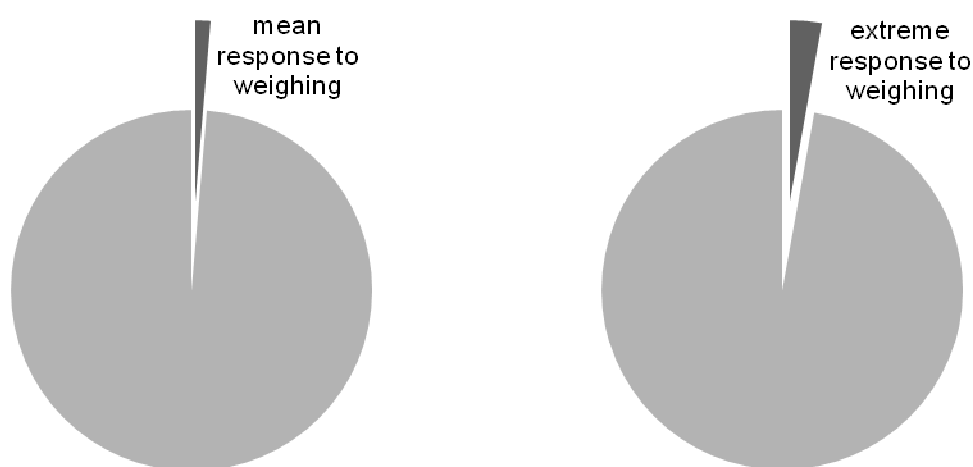
Figure 3.4 illustrates this pattern in two individual penguins. The penguin call playback stimulus prompted the smallest increase in HR. The human speech playback stimulus prompted a brief response. Lifting the lid of the nest box prompted a larger HR increase, and weighing the penguin caused the greatest increase in penguin HR. After the first three stimuli, heart rate quickly stabilized. However, HR took much longer to return to the pre-stimulus baseline after the penguin was weighed.



**Figure 3.4:** Heart rate of penguins #36906 (left panel) and #24922 (right panel) over time in response to each of four stimuli (penguin call playback = dotted line, human speech playback = solid line, lifting nestbox lid = dashed line, weighing penguin = dot/dash line). The penguins were presented with each stimuli at time = 0. Gaps in lines are due to loss of heart rate signal resulting from penguin movements.

### 3.1.1 Energy Expenditure

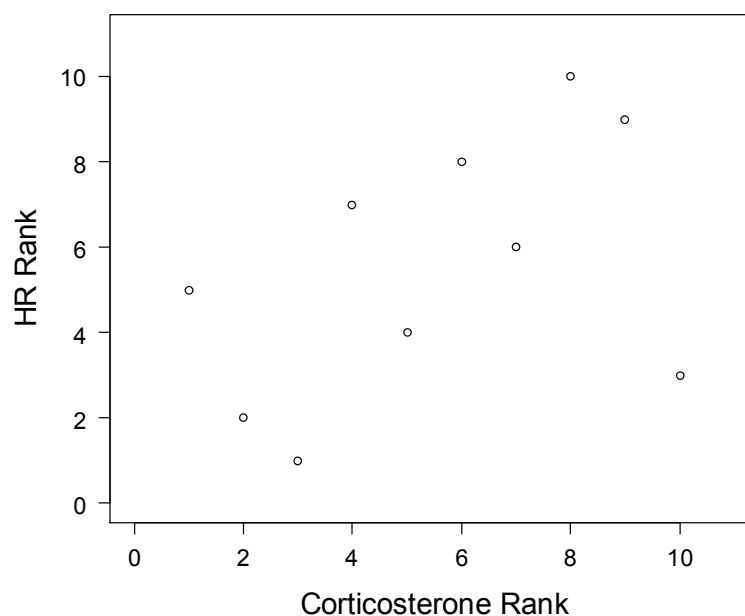
Of the four disturbance stimuli, weighing the penguin caused the greatest mean response in both increase in HR and recovery time. The mean amount of energy used in response to being weighed (17min recovery time), and the amount of energy used in an extreme response (31min recovery time) were related to the daily energy budget during incubation (Fig 3.5). The mean response used 1.3% of the penguin's daily energy budget, and the extreme response used 2.8% of the penguin's daily energy budget (see Appendix B for calculations).



**Figure 3.5:** Little Penguin energy expenditure in response to weighing as a fraction of daily energy budget during incubation. Left: mean response, 1.3% of daily energy budget (n=11); right: extreme response of individual penguin (#45167), 2.8% of daily energy budget.

### 3.2 Relationship Between Heart Rate and Corticosterone Response

Ten Little Penguins were sampled both in this study and by John Cockrem. These penguins were ranked by each of us according to the magnitude of their responses, i.e. according to HR increase as a percentage of pre-stimulus baseline, and by corticosterone levels present in blood samples (Fig. 3.6). A Spearman's Correlation ( $R_s = 0.467$ ) indicated that there was a moderate correlation between the two rankings.



**Figure 3.6:** Little Penguin rankings of magnitude of response to blood sampling (corticosterone levels) and human intervention (heart rate, HR) are displayed. Circles represent individual penguins (n=10).

### 3.3 Tourist Disturbance

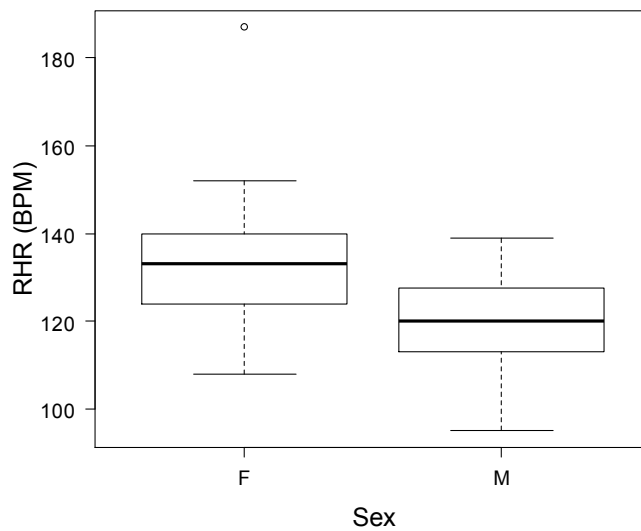
Five variables were investigated in relation to variation in Little Penguin resting heart rate (RHR). Four did not have any effect on RHR: aggression rating, number of chicks fledged in 2012, years of breeding experience, and level of disturbance (Table 3.4). However, RHR had a strong correlation with sex.

**Table 3.4:** Input variables in relation to Little Penguin resting heart rate. ‘Aggression’ = aggression rating (1-4) (n=35); ‘BreedExp’ = previous breeding experience (years) (n=40); ‘Chicks12’ = number of chicks fledged in the 2012 breeding season (n=40); ‘Sex’ = male or female (n=40); ‘Zone’ = location of nest box with proximity to human activity (control, low disturbance, or medium disturbance) (n=40).

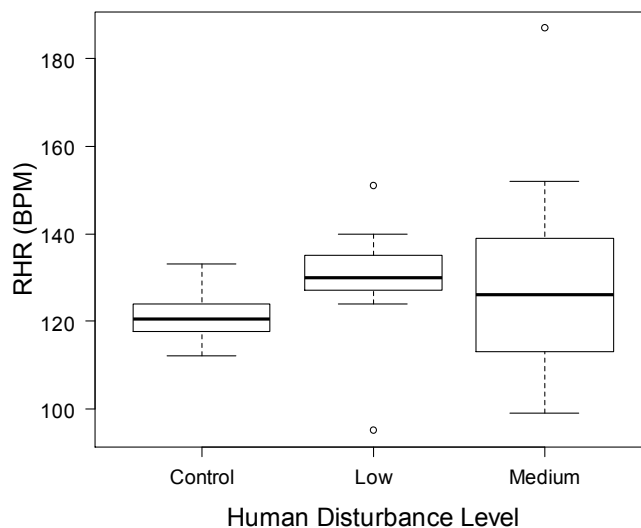
Variable	<i>p</i>	F	R <sup>2</sup>
Aggression	0.593	0.291 <sub>1,33</sub>	-0.021
BreedExp	0.113	2.628 <sub>1,38</sub>	0.040
Chicks12	0.923	0.009 <sub>1,38</sub>	-0.026
Sex	0.002	10.90 <sub>1,38</sub>	0.202
Zone	0.419	0.890 <sub>2,37</sub>	-0.006

RHR showed a significant difference between sexes (Fig. 3.7): the mean heart rate of female Little Penguins was 135.4 beats per minute (bpm) (SD = 18.3), whereas the mean heart rate of male Little Penguins was 119.7 bpm (SD = 11.8). Nest box zone (control, low disturbance, medium disturbance) did not explain any of the variation in Little Penguin RHR when analysed on its own (Fig.3.8).

The simplest model, ‘RHR by Sex’, best explained the data ( $\omega_i(\text{AIC}_C) = 0.681$ ). ‘Zone’ as a lone input variable was not likely to be the best model ( $\omega_i(\text{AIC}_C) = 0.004$ ), but when combined with ‘Sex’ and with both ‘Sex’ and the interaction between ‘Zone’ and ‘Sex’, this variable became slightly more informative ( $\omega_i(\text{AIC}_C) = 0.153$  and  $0.162$  respectively) (Table 3.5).



**Figure 3.7:** Mean Little Penguin resting heart rate (RHR) in beats per minute (bpm) in relation to penguin sex: female (F,  $n=17$ ) and male (M,  $n=23$ ). The plot shows group medians as bold lines, the first and third quartiles as the bottom and top of the 'boxes', and the lowest and highest data within 1.5 times the inter-quartile range of the lower and upper quartiles as the 'whiskers'. Outliers are shown as circles.

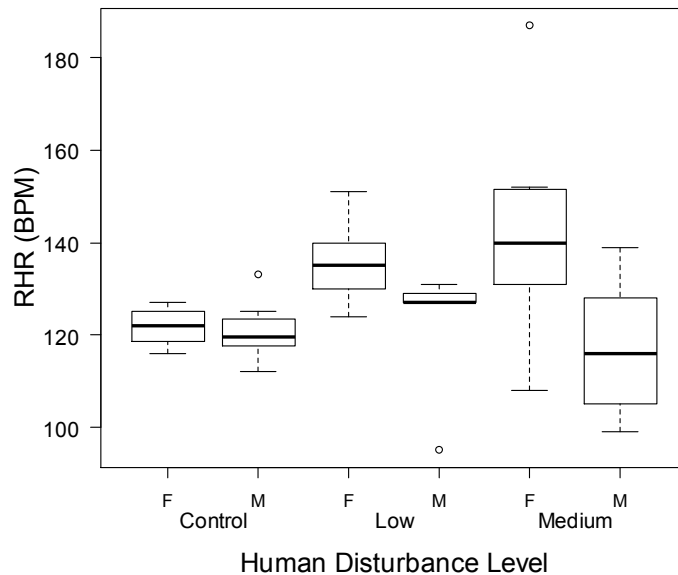


**Figure 3.8:** Mean Little Penguin resting heart rate (RHR) in beats per minute (bpm) in relation to human disturbance level (control ( $n=12$ ), low disturbance ( $n=11$ ), medium disturbance ( $n=17$ )). The plot shows group medians as bold lines, the first and third quartiles as the bottom and top of the 'boxes', and the lowest and highest data within 1.5 times the inter-quartile range of the lower and upper quartiles as the 'whiskers'. Outliers are shown as circles.

**Table 3.5:** Candidate set of models explaining variation in Little Penguin heart rate (HR) in relation to Sex, Zone (location of nest box within breeding colony in relation to tourist disturbance: low, medium, or control), and the interaction between these.  $K$  = number of estimated parameters for model  $i$ ;  $\omega_i(\text{AIC}_C)$  = weighted  $\text{AIC}_C$  value.

Model	$K$	$p$	$F$	$R^2$	$\omega_i(\text{AIC}_C)$
HR by Sex	1	0.002	10.90 <sub>1,38</sub>	0.202	0.681
HR by Zone	1	0.419	0.89 <sub>2,37</sub>	-0.006	0.004
HR by Sex + Zone	2	0.016	3.95 <sub>3,36</sub>	0.185	0.153
HR by Sex + Zone + Sex:Zone	3	0.013	3.41 <sub>5,34</sub>	0.236	0.162

Within each zone the mean RHR was higher in female penguins than in male penguins (Fig. 3.9). A significant difference in RHR between sexes was seen in the medium disturbance zone, where male RHR = 117.9 (SD=14.1, n=10) and female RHR = 142.9 (SD=24.8, n=7) (Table 3.6). Differences between sexes in the low disturbance and control zones were not significant.



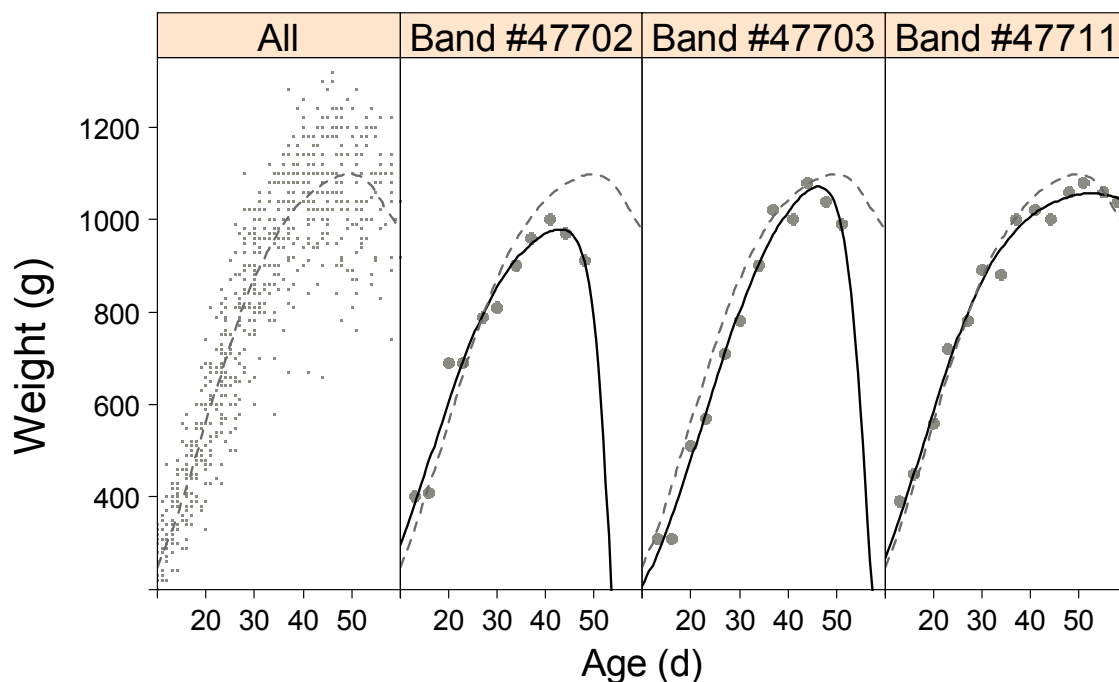
**Figure 3.9:** Mean Little Penguin resting heart rate (RHR) in beats per minute (bpm) in relation to human disturbance level (control, low, medium) and sex (F = female, M = male). For female penguins n=4, 6 and 7 and for male penguins n=8, 5 and 10 for control, low and medium respectively. The plot shows group medians as bold lines, the first and third quartiles as the bottom and top of the ‘boxes’, and the lowest and highest data within 1.5 times the inter-quartile range of the lower and upper quartiles as the ‘whiskers’. Outliers are shown as circles.

**Table 3.6:** Output from linear models comparing resting heart rates of male and female penguins at different levels of human disturbance (HD) (control, low disturbance, medium disturbance).

HD Level	<i>p</i>	<i>F</i>
Control	0.783	0.08 <sub>(1, 10)</sub>
Low	0.090	3.61 <sub>(1,9)</sub>
Medium	0.018	7.00 <sub>(1,15)</sub>

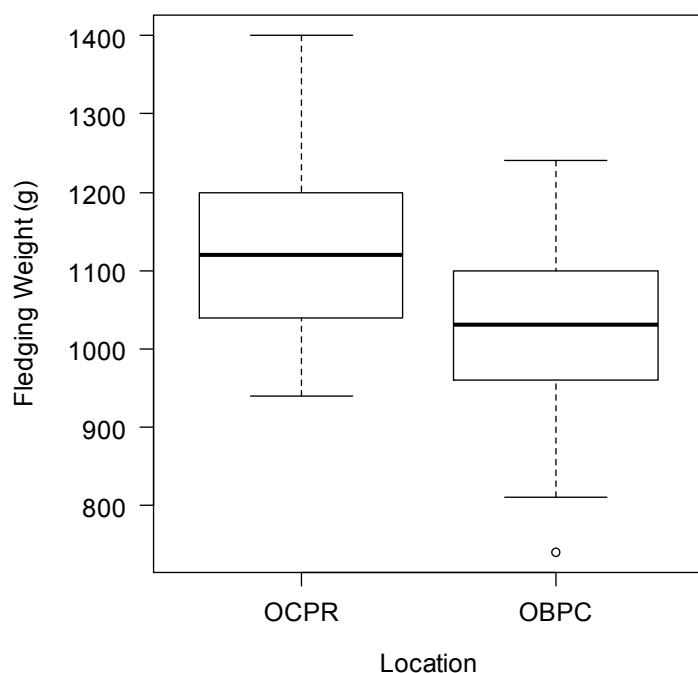
### 3.4 Chick Growth Modelling

The nonmonotonic Nonlinear Least Squares Richards curve ‘R11’ described the mean growth pattern of Little Penguin chicks in the 2011-12 breeding season (Fig. 3.10, panel 1). The growth patterns of individual chicks were also described by individually fitted curves (Fig. 3.10, panels 2-4). The growth curves fitted for penguins #47702 and #47703 are unrealistic as the masses plummet towards 0g at around day 50. However, the growth of penguin #47711 appears to be better described by an individually-fitted curve than by the group mean.



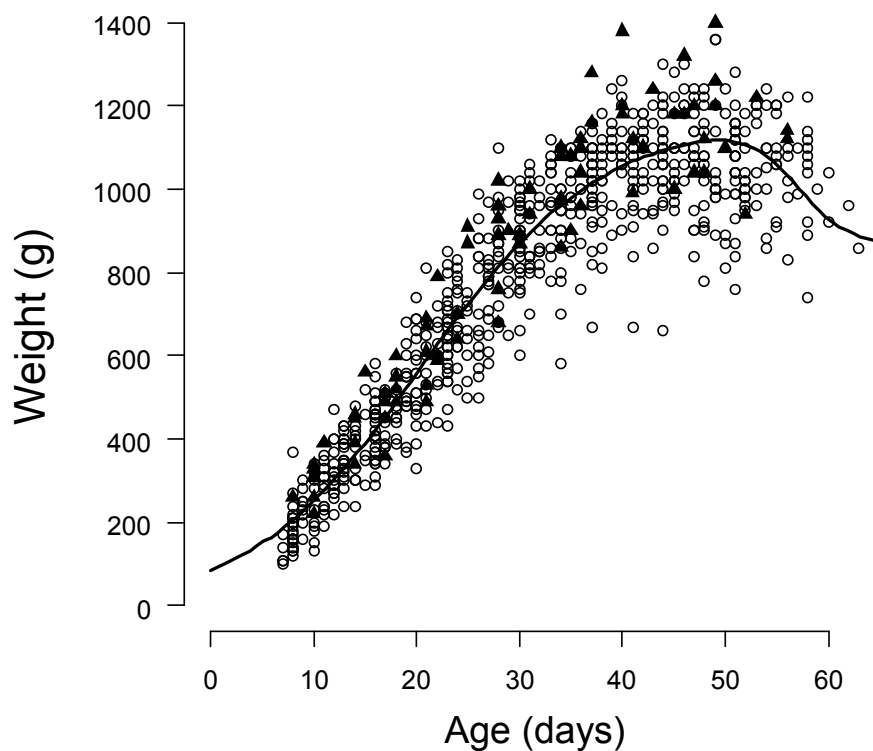
**Figure 3.10:** Little Penguin chick weight (g) in relation to age (days). Left panel: all data; three right panels: data for representative individual chicks. Dotted curve: Nonlinear Least Squares (NLS) fit to all data (Richards curve 11, same curve in each panel); solid curves: NLS fit to data for individuals (modified from Oswald *et al.* 2012).

Chicks that had no exposure to tourist presence (OCPR) seemed to attain equal or greater weights than chicks exposed to tourist presence (OBPC) at the same age. Fledging weights of chicks from the ‘unexposed’ group were significantly higher than those from the ‘exposed’ group (1131g vs. 1028g,  $p = 0.003$ , Fig. 3.11). Chicks of the lowest weights were consistently from the ‘exposed’ group (Fig. 3.12).



**Figure 3.11:** Little Penguin fledging weight (g) in relation to colony location: Oamaru Creek Penguin Refuge (OCPR, n=13), or Oamaru Blue Penguin Colony (OBPC, n=60).

The plot shows group medians as bold lines, the first and third quartiles as the bottom and top of the 'boxes', and the lowest and highest data within 1.5 times the inter-quartile range of the lower and upper quartiles as the 'whiskers'. Outliers are shown as circles.



**Figure 3.12:** Little Penguin chick weight (g) in relation to age (days). Open circles = chicks exposed to tourist presence (n=60), solid triangles = chicks with no exposure to tourist presence (n=13). Solid line = Nonlinear Least Squares curve fit to all data.



## Discussion

### 4.1 Researcher Disturbance

The heart rate (HR) responses of incubating Little Penguins to audio playbacks of a penguin call (a control noise) and of human speech were of similar magnitude. HR returned to within baseline levels in less than two minutes in response to each stimulus. This result shows that the sound of human speech nearby was not perceived as a substantial threat by Little Penguins in the Oamaru Blue Penguin Colony (OBPC). These penguins may have become habituated to the sound of human speech, as it is a common occurrence at their nesting area. However, this would not mean that these penguins were less affected by all forms of disturbance: King Penguins (*Aptendytes patagonicus*) were found to habituate to the sound of human footsteps, but not to more invasive forms of disturbance, such as handling (Viblanco *et al.*, 2012).

Lifting the lid of the nest box caused a more pronounced HR response than the audio playback stimuli, and penguins took a significantly longer period of time to recover from this stimulus. Of the six individual penguins for which HR recordings were obtained for both the ‘lid lifting’ and ‘weighing penguin’ stimuli, three had a greater maximum HR recorded in response to the ‘lid lifting’ stimuli than to being weighed. This was unexpected, as the ‘weighing penguin’ protocol was more invasive and of a longer duration, typically lasting 1-2 minutes. While a penguin was being weighed, the connection between the penguin and artificial egg was temporarily broken, so the maximum HR used in this instance was recorded as the penguin was being removed from the nest box. Because of this gap in HR signal, it is unlikely that the true maximum HR was recorded.

However, the potential disturbance effects of ‘lid lifting’ should not be dismissed. Disturbance events subjectively defined by humans as being low-impact may be interpreted quite differently by wildlife: Yellow-eyed Penguins showed a stronger HR response to a simulated ‘wildlife photographer’ than they did to a routine nest-check (Ellenberg *et al.*, 2013). Experimental protocol for ‘lid lifting’ involved making eye contact with the penguin for ten seconds from approximately one metre of distance. This interaction may have caused the penguin to perceive the researcher as a predator, causing a stress response comparative to that caused by handling (Beale & Monaghan, 2004b; Frid & Dill, 2002).

Weighing Little Penguins caused a HR increase that was significantly greater than the response to either of the audio playbacks. The amount of time taken for HR to return to baseline after a penguin was weighed was longer than the response time for each of the other three stimuli. This result was to be expected, as weighing was the most invasive of the four stimuli, and had the

longest duration. Although some penguins did not show behavioural signs of stress while being weighed, all underwent a physiological response. The minimum HR increase was 21.7% above RHR, and minimum recovery time was four minutes.

#### 4.1.1 Little Penguin Energy Budget

Differences between natural (penguin call playback) and human-induced (lifting nest box lid, weighing penguin) disturbance stimuli were statistically significant, but the issue to be concerned with is that of biological significance. Does the fact that staring at and handling Little Penguins, causing them to have a rapid increase in HR and spend some time recovering from the interaction, have the potential to have long-term consequences? To answer this question, the amount of energy used in a HR response must be calculated, and related to a Little Penguin's daily energy budget. The relationship between HR and metabolism is well-defined, with an increase in HR leading to increased oxygen consumption ( $VO_2$ ), and thus a more rapid use of energy (Froget *et al.*, 2001; Green *et al.*, 2008; Groscolas *et al.*, 2010; Weimerskirch *et al.*, 2002). This relationship is linear for Little Penguins (Green & Frappell, 2007; Green *et al.*, 2008).

Ellenberg *et al.* (2013) found that extrapolating the response of a single approach to a nesting Yellow-eyed Penguin by a simulated 'wildlife photographer' resulted in the use of energy equivalent to a large proportion of the penguins' daily energy budget. I followed this approach to determine the impact of weighing a Little Penguin on its daily energy budget. The field metabolic rate (FMR) of foraging and incubating Little Penguins is  $661 \text{ kJ.kg}^{-1}.\text{d}^{-1}$  (Gales & Green, 1990). To compensate for the mean HR response to weighing observed in my study, a 1kg Little Penguin would require an additional 8.9kJ of energy, or 1.3% of its daily energy budget (see Appendix B for calculations). This amount is likely to be negligible. However, of the eleven penguins weighed in my study, four did not return to RHR within the 30min recording period, with one stabilizing at a HR lower than the previous RHR. Penguin #45167 happened to remain on the ED for twenty minutes beyond the recording period, and RHR was not reached until 47 minutes after the stimulus. For this individual penguin, a minimum estimate of energy expenditure resulted in 2.8% of the daily energy budget being used (see Appendix B). In comparison, extrapolation of the response to a one minute visit of a simulated photographer to a Yellow-eyed Penguin nest into a twenty minute visit resulted in the energy expenditure of approximately one third of this penguin's daily energy budget (Ellenberg *et al.*, 2013). For a Little Penguin to use up this much energy in a similar response, HR would need to be sustained at 934.2 bpm for one hour, or elevated to 256 bpm for 4.25 hours, which is biologically unlikely (see Appendix B).

### 4.1.2 Response and Personality

As well as species-specific responses to disturbance events, the concept of individual response or ‘personality’ among animals is firmly established (Cockrem, 2007; Coppens *et al.*, 2010; Ellenberg *et al.*, 2009; Koolhaas, 2008; Reale *et al.*, 2007). When assessing the impact of human disturbance on penguins, rather than taking the mean response of a group of individuals, factors such as sex, previous experience with humans, and individual personality need to be taken into account. The context of an individual bird at a particular point in time will affect its stress response: if the bird is in poor body condition, it may be better off conserving energy than visibly responding to a threat; whereas a conspecific in better body condition may benefit from displaying a response (Beale & Monaghan, 2004a). For example, Little Penguins at Oamaru were less likely to desert their nests than those at Motuara Island, possibly because the Oamaru penguins generally have better body condition (Numata *et al.*, 2000). King Penguin chick stress response is linked to body condition, rather than stage of moult, implying that body condition dictates presence of response (Corbel *et al.*, 2010). Furthermore, the personality of the individual may affect its interactions with its environment, and influence reproductive success (Patrick, 2014, Ellenberg 2009). For this reason I believe that more detailed long-term records of individual personality and body condition are necessary for accurate determination of the impact of human disturbance on individual Little Penguins.

## 4.2 Corticosterone and Heart Rate Responses

Individual penguins were ranked according to the magnitude of both their corticosterone (CORT) and HR responses, to see if the magnitudes of response were correlated and thus could indicate the influence of individual personality on magnitude of response. There was a moderate correlation between the rankings. Nine out of the ten penguins sampled fitted the trend (i.e. comparatively high CORT response is correlated with comparatively high HR response). If the outlying data point (penguin #38584) were to be removed, the Spearman’s rank correlation coefficient could be upgraded to 0.764, which indicates a strong correlation. A larger sample size would help determine if this is a true relationship.

If Little Penguins can be ranked by the magnitude of their response to human presence with two different methodologies, and each ranking system produced a similar answer, this presents evidence for both 1) a relationship between HR and CORT; and 2) the persistence of individual personality or temperament in this species over an extended timeframe.

### 4.2.1 Comparing Heart Rate and Corticosterone

Recording HR and corticosterone (CORT) responses are both means of investigating the response of a Little Penguin to human disturbance. If there is a true relationship between CORT response and HR response, this means under some circumstances recording HR (a much less invasive procedure) to gauge the magnitude of response of Little Penguins to human disturbance may be an appropriate substitute for CORT sampling.

However, using HR response as a proxy for CORT may not be entirely appropriate if the effects of more than one stressor are to be considered: Nephew *et al.* (2003) found that captive European starlings (*Sturnus vulgaris*) exhibited stressor-specific HR responses, whereas CORT responses were not stressor-specific. HR responses attained pre-defined maximal levels, whereas CORT responses did not, suggesting that maximal responses may be stimulated by different aspects of a stressor for each of these two pathways. They proposed that starlings exhibit a standardized CORT response to sudden, acute stressors (Nephew *et al.*, 2003).

CORT responses to human disturbance may decrease over time with repeated exposure to humans, such as in Galapagos Marine Iguanas (*Amblyrhynchus cristatus*) and Magellanic Penguins, as the animals become habituated (Romero & Wikelski, 2002; Walker *et al.*, 2006). Habituation to human disturbance is sometimes looked upon favourably, as a decreased stress response may allow animals to avoid negative consequences of repeated elevated glucocorticosteroids; but potential negative effects include a reduced ability to adequately access stored energy in times of need (Walker *et al.*, 2006). Alternative explanations for a decreased CORT response include exhaustion, life history stage, and physiological desensitization without habituation (Cyr & Romero, 2009). Also, frequent disturbance may cause sensitive individuals to leave the colony or habitat, so that the animals which remain are those which exhibit a smaller response (Fowler, 1999).

Conversely, repeated unpredictable human disturbance may result in a magnified CORT response. Regularly disturbed Yellow-eyed Penguins had much higher CORT levels than their undisturbed conspecifics, suggesting that sensitization to frequent under-managed visitation was occurring (Ellenberg *et al.*, 2007). Long-term elevation of CORT may lead to decreased breeding success: male Adélie Penguins with artificially elevated CORT levels were much more likely to abandon their nests than those without artificially elevated CORT levels (Thierry *et al.*, 2013).

My research does not provide adequate data on the CORT response of Little Penguins to assess whether this species becomes habituated or sensitized to human disturbance; although the latter is unlikely given the current success of the OBPC. Regardless of trends in the magnitude of

Little Penguin CORT responses, my data suggest that the comparative magnitude of response between individual penguins is worthy of further investigation.

#### 4.2.2 Persistence of Personality

It is important to recognize variation in responses as an aspect of personality, rather than variance around a mean value, as genetic variation is important for the ongoing persistence of a species (Cockrem, 2005). Bird personalities fall somewhere on a continuum from proactive to reactive, with proactive individuals having relatively low CORT stress responses and reactive individuals having relatively high responses (Cockrem, 2007). These glucocorticoid responses of individual animals are repeatable (Cockrem, 2005).

The two periods of data collection for my investigation occurred around six months apart, and at different phases of the Little Penguin life cycle. Blood samples were collected to obtain CORT values in winter 2012 while penguins were finding mates, and HR was recorded in summer 2012-13 during incubation. The correlation between HR and CORT responses in my study support the idea that Little Penguins have individual personalities or consistencies in how they react to stressors. Variations in personality or coping style are essential for the maintenance of genetic diversity within a population, and enhance the ability to change with a changing environment (Koolhaas, 2008; Viblanc *et al.*, 2012). Different personality traits may be favoured under different environmental conditions (Patrick & Weimerskirch, 2014).

### 4.3 Tourist Disturbance

The Oamaru Blue Penguin Colony (visitor colony) has had similar success to the Oamaru Creek Penguin Reserve (control colony) in terms of long-term breeding parameters (hatching probability, number of fledglings per pair) since monitoring began in 1993 (Johannesen *et al.*, 2003; Perriman *et al.*, 2000). However, overall comparisons such as these may fail to detect impacts experienced by small groups of individuals exposed to higher levels of disturbance, or in the case of comparisons of long-term datasets, disturbance instigated by recent changes in management. My study took these comparisons a step further by separating the OBPC into ‘zones’ of visitor disturbance intensity. This zoning served to investigate whether resting heart rate (RHR) varied between incubating penguins which had moderate exposure to human disturbance during the day, as opposed to nests *in the same colony* which were less likely to be disturbed by human presence due to their location out of view of tourist pathways and at several metres distance. Hypothetically, penguins nesting nearby to human pathways could be adversely impacted by human disturbance, but the effects of this could be masked by the overall measurements of success from the colony, in which the majority of penguins are not

exposed to this intensity of disturbance. My data showed that the RHR of penguins nesting in the ‘medium disturbance’ and ‘low disturbance’ zones did not differ significantly from each other, or from the control zone ( $p = 0.49$ ). Unfortunately, measurements from the ‘high disturbance’ zone were largely unsuccessful due to low rates of nest occupation and poor quality signal during HR recordings, so data from this zone were not able to be included in my analyses. This in itself indicates that penguins nesting in the ‘high disturbance’ zone were more restless than those in other areas of the colony, which could be attributed to the more intrusive presence of tourists in this area.

The sex of the penguin was a significant predictor for RHR across all zones. In the medium disturbance zone, mean RHR was significantly higher for female penguins (143 beats per minute (bpm)) than male penguins (118 bpm) ( $p = 0.018$ ). Differences in RHR between sexes are common in sexually dimorphic seabirds, with males typically being heavier and having lower RHR (Ellenberg *et al.*, 2011; Green *et al.*, 2001; Groscolas *et al.*, 2010; Holmes *et al.*, 2005). However, other studies have found that the mean RHR for undisturbed Little Penguins in their thermo-neutral zone (10-30°C) ranges between 112.5-117 bpm, with no mention of sex-based differences (Green *et al.*, 2008; Green *et al.*, 2005; Stahel & Nicol, 1982). This indicates that the observed elevated female RHR may be biologically relevant.

The HR response of Wandering Albatrosses (*Diomedea exulans*) was found to differ between males and females, with male albatrosses experiencing elevated HR more quickly, and female albatrosses taking longer to return to RHR after a disturbance event (Weimerskirch *et al.*, 2002). The authors propose that males could be more prone to stress, and therefore be more reactive than females. The HR response of Yellow-eyed Penguins (*Megadyptes antipodes*) to human disturbance differed in relation to previous experience with humans and individual character, as well as by sex (Ellenberg *et al.*, 2009). However, many studies investigating the effect of human disturbance on penguin HR do not report differences between the sexes (Snares Penguins (*Eudyptes robustus*) (Ellenberg *et al.*, 2011), Adélie Penguins (*Pygoscelis adelia*) (Culik *et al.*, 1990), Humboldt Penguins (*Spheniscus humboldti*) (Ellenberg *et al.*, 2006). In King Penguins (*Aptenodytes patagonicus*), HR was found to vary in accordance with individual personality, rather than sex (Viblanco *et al.*, 2012). The high RHR I observed in female Little Penguins may be related to other factors such as individual personality or body condition, rather than sex; or a combination of all of these factors. Repeating this study with a larger sample size would enable me to determine if the larger difference in RHR between sexes in the medium disturbance zone was due to female Little Penguins responding differently to human disturbance than male Little Penguins.

## 4.4 Modelling Chick Growth

In many species of seabird, parents will stop feeding their chicks before the chicks have fully fledged, so the chicks lose weight in the days before fledging (Teixeira *et al.*, 2014; Corbel *et al.*, 2010). The use of linear models to describe seabird chick growth does not accurately describe this important drop in weight at fledging, and may therefore produce misleading fledging weight values (see Gales, 1987; Numata *et al.*, 2004).

My modelling compared growth in chicks from the two main penguin colonies at Oamaru – OPBC (tourism presence) and OCPR (no tourism presence). Chicks from the undisturbed colony were weighed less frequently than those from the tourist colony, and attained a significantly greater fledging weight (1131g vs. 1028g,  $p = 0.003$ ). The greater fledging weight of chicks from the OCPR may have been due to the fact that they were weighed less frequently, and thus were not weighed as close to the time of fledging as chicks from the OPBC. The data from the OPBC thus gave a more accurate indication of fledging weight than at OCPR. If the difference in fledging weights between colonies is a true difference, some possible explanations for this finding are as follows: more frequent weighings could have stressed the OPBC chicks, leading to less weight gain; alternatively, the presence of tourists could have affected the parents of these chicks, leading to less food being provided, leading to less weight gain. However, a range of factors, such as the number of nest mates and experience of parents may also affect fledging weight.

Fledging weight is thought to be a predictor of first year survival for many species of seabird, including the Little Penguins of Phillip Island (Reilly & Cullen, 1982). However, the year and number of nest mates (one or zero) were found to be the most important predictors of Little Penguin survival at the Oamaru Colony, with fledging weight surprisingly not having an effect (Johannesen *et al.*, 2003). In some Little Penguin colonies, fledging weight has been related to seasonal fluctuations in food availability, such as at Phillip Island (Chiaradia *et al.*, 2012). As this relationship was not found in the south-east region of NZ, food is not thought to be a limiting factor for Little Penguins in this area (Agnew *et al.*, 2014).

Some authors propose a minimum critical weight for chick fledging, above which probability of first-year survival increases with increasing weight (Magrath, 1991; Sagar & Horning, 1998). All of the Little Penguin chicks in an investigation by Johannesen *et al.* (2003) may have reached this critical weight, effectively eliminating fledging weight as a predictor at Oamaru. A study of chick quality and food availability in Gentoo Penguin (*Pygoscelis papua*) chicks found that in poor food years, there was no relationship between chick fledging weight and first-year survival, as all the chicks that survived to fledge were of above average quality; whereas in

good food years both low quality (lighter) and high quality (heavier) chicks all survived to fledge, but there was a positive relationship between fledge weight and ongoing survival (Williams & Croxall, 1990). Dann (1988) artificially manipulated Little Penguin clutch size at Phillip Island so as to alter chick weights. This study found that first year survival increased with weight at banding until 1100-1299g (85.1% survival), but that chicks in the next weight class (1300-1499g) were less likely to survive (57.1% survival), with clutch size being irrelevant to survival (Dann, 1988). One chick that was less than 699g at banding survived its first year (Dann 1988). However, chicks were banded at c.40-50 days of age, so those banded at 40 days may have increased in weight before fledging. These data suggests that fledging weight is an important predictor of first-year survival in Little Penguins until a weight of 1100g is attained (Dann, 1988). In my study, the mean weight of Little Penguins from the tourist colony did not exceed this value, indicating that these chicks may have a lower chance of survival than those from undisturbed colony.

## 4.5 Conclusions and Recommendations

In my eyes, an ideal world would be one in which well-behaved visitors were free to approach penguins on their nests, play with their pungent fluffy offspring, and perhaps get a few scratches as souvenirs of their experience, all with no detriment to the penguins. This would be a fantastic educational experience, and one that would surely persuade even the most miserly of tourists to empty their pockets. The closer the interaction between person and animal, the more likely the person will care about the animal's future wellbeing (Curtin, 2010; Schänzel & McIntosh, 2000).

However, this is not the world we live in. Coming into close contact with humans may cause penguins to undergo a stress response. Responses of penguins to human presence are species-specific, so that generalizations cannot be made about all penguin species in terms of reactions to human presence (Ellenberg *et al.*, 2007). Responses are also very context-specific, as individual penguins may respond in accordance with the energy they have available, so one set of guidelines may not be applicable year-round (Beale, 2007).

While conducting my investigation I encountered technological hitches which led to smaller sample sizes than those I had planned, and poor quality data that needed to be analysed manually. This limited the conclusions I could draw from my research. Artificial eggs are a useful tool in recording HR data only when the subject is co-operative – in my case, this meant that the penguin had to be lying in one spot for several hours in order for its HR to be recorded. HR measuring apparatus which attach directly to the test subject may simplify the data collection process and allow data to be collected across life-history stages, although the initial



stress of the attachment of the device must be taken into account (Weimerskirch, 2002). Technological progress towards ever smaller and more robust HR recorders should see the collection of such data become less invasive for the animal and more streamlined for the researcher.

My study provided some evidence that incubating Little Penguins at Oamaru exposed to a medium level of human disturbance had a sex-based HR response. Long-term HR elevation could have negative consequences such as decreased reproductive output, particularly in years of low food supply. Future monitoring work should take into account the physiological response of individual birds in relation to their location within the colony, rather than relying on overall colony measures to quantify reproductive success. Measures of overall success may mask detriment to certain individuals.

Two common research interactions – band checking (lid lifting) and weighing the penguin – were found to cause a significantly greater stress response than a natural stimulus (penguin call playback). Although the time taken for penguin HR to recover after weighing was greater than for band checking, the subjectively more invasive of these stimuli – weighing – did not always provoke the greater maximum HR response. This outcome shows that the intensity of a stimulus may be perceived quite differently by individual penguins.

Future investigations should seek to quantify the effects of human disturbance on Little Penguins across different stages of the breeding cycle, not just during incubation. The effects of handling on chick survival and a comparison of male and female responses at different times of year would inform researchers and tourist managers of how best to minimize human disturbance at all stages of the breeding cycle. A focus should be placed on individual personality, and whether human disturbance may be acting as a selection pressure against penguins of a particular character.

Guidelines around Little Penguin visitation must ensure that incubating Little Penguins are not suffering any detrimental effects of human disturbance. Current management practices at the Oamaru Blue Penguin Colony are satisfactory in minimizing any negative effects of the penguins being in close proximity with humans. Researcher interactions with Little Penguins at the Oamaru Blue Penguin Colony are short-lived and infrequent enough to not significantly impact a penguin's energy budget, and thus are believed to not result in long-term changes. Tourist visitation may promote the welfare of penguin colonies through generating revenue which can be used to provide resources for the penguins, so well-managed tourism benefits both the people and the penguins. Trends indicate that visitor numbers at penguin colonies will continue to increase. At Oamaru, current management practices should allow Little Penguin

numbers to continue to increase along with their visitors. In well-managed colonies, Little Penguins will continue to be a big attraction far into the future.

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## Appendix A

The following code is adapted from (Oswald *et al.*, 2012). It was used to model penguin chick growth, and to produce a graph to compare chick growth at the Oamaru Blue Penguin Colony (OPBC) and Oamaru Creek Penguin Refuge (OCPR).

#Model Selection Code

```
#load data
data(penguin.data)
data <- penguin.data
```

```
#group data
data <- groupedData (weight ~ ckage | bandid, data = data)
```

```
#run model selection to select best model parameters
```

```
#generate values for curve
```

```
modpar(data$ckage,data$weight, pn.options = "myoptions.2")
#the step above is optional - both model selection functions will call modpar if necessary
```

```
my.modselect.step <- pn.modselect.step (my.data$ckage, my.data$weight, my.data$bandid,
pn.options = "saved.options",
existing = TRUE)
```

```
#run stepwise model selection, saving results and options
```

```
my.mod.compare <- pn.mod.compare (data$ckage, data$weight, data$bandid, pn.options =
"myoptions.2") #run all model selection
# saving results and options
```

```
my.mod.compare # view the results of model selection
```

```
my.modselect.step # view the results of stepwise model selection
```

```
#Fit most suitable model from model selection
richardsR31.lis <- nlsList(weight ~ SSposnegRichards(ckage , Asym = Asym , K = K , Infl =
Infl, RAsym = RAsym, modno = 31, pn.options = "myoptions.2"), data = data)
```

```
#Fit nls model analogous to most suitable nlsList from model selection
richardsR31.nls <- nls(weight ~ SSposnegRichards(ckage , Asym = Asym , K = K , Infl =
Infl, RAsym = RAsym, modno = 31, pn.options = "myoptions.2"), data = data)
```

```
#Extract mean coefficients for nls curve
nlspams <- coef(richardsR31.nls)
```

```
#Drawing Comparative Graph
```

```
#plot points using different symbols
```

```
plot(creekgrowth$weight~creekgrowth$ckage, pch=17, xlab = "Chick Age (days)", ylab =
"Chick Weight (g)", xlim = c(5,65), ylim = c(50, 1500))
points(quarrygrowth$weight~quarrygrowth$ckage, pch = 1)
```

```

#specifying curve
curve(posnegRichards.eqn(x,modno = 31, pn.options = "myoptions.2"), add = TRUE, lwd =
0.5)

#formatting plot space, adding curve
panel.xyplot(x,y,cex = 0.3, pch = 16, col = "ivory4", cex.lab = 2)
  panel.curve(posnegRichards.eqn (x, Asym = nlspams[1], K = nlspams[2],
    Infl = nlspams[3], RAsym = nlspams[4], modno = 31, pn.options =
"myoptions.2"), lwd = 2, col = "gray42", lty = 2)

```



## Appendix B

### 7.1 Energy Consumption Calculations

The field metabolic rate (FMR) of foraging and incubating Little Penguins is 661 kJ.kg<sup>-1</sup>.d<sup>-1</sup> (Gales & Green, 1990). Little penguins consume 1mlO<sub>2</sub> for every 19.7J of energy used (CD Stahel & Nicol, 1982).

Clupeid fish provide 3.98kJ/g of metabolizable energy (Gales & Green, 1990), so a 1kg Little Penguin during incubation would require 661 kJ of energy per day, or 166.1g of fish.

While being weighed, Little Penguins had an average recovery time of 17 minutes, mean pre-stimulus resting heart rate (RHR) of 138.2 beats per minute (bpm) and mean maximum heart rate (HR) of 209.8bpm. As a crude measurement of energy used in this response, I calculated the requirement for a 17 minute period of HR at 174bpm, this HR being the midpoint between maximum HR and RHR.

The oxygen consumption (VO<sub>2</sub>) of blue penguins resting in their thermo-neutral zone (typical conditions during incubation) can be calculated when the heart rate is known:

$$VO_2 = (0.21 * HR) - 9.88 \quad (\text{Green } et al., 2008)$$

This meant a penguin with a HR of 174bpm consumed 26.7ml O<sub>2</sub> per minute.

If 1ml O<sub>2</sub> requires 19.7J of energy, this penguin uses 526J/min.

In a 17 minute HR response, this is 8.9kJ, or 2.25g of clupeid fish, or 15% of a 15g fish.

This equates to 1.3% of the estimated Daily Energy Expenditure (DEE) of 661kJ.

## 7.2 Specific Example: Penguin #45167

Little Penguin #45167 had a pre-stimulus RHR of 118bpm, and attained a maximum HR of 222bpm. After the stimulus, HR dropped to an average of 157.7bpm for the following 16 minute period, then fluctuated between 130 and 140 BPM for the next 31 minutes, with a mean HR of 136.6bpm.

The energy used by penguin #45167 in response to being weighed was calculated as follows:

HR of 157.7bpm for 16 minutes:

23.2 ml O<sub>2</sub>/min

457.8J/min

16mins = 7.3kJ

HR of 136.6bpm for 31 minutes:

18.8mlO<sub>2</sub>/min

370.5J/min

31mins = 11.5kJ

Total = 18.8kJ

This equates to 2.8% of the Daily Energy Expenditure (DEE) of a Little Penguin during incubation.

(but see (Green & Frappell, 2007) for issues with individual variability/accuracy)

### 7.3 Hypothetical Significant Response

The HR response of a Yellow-eyed Penguin (*Megadyptes Antipodes*) to a theoretical 20-minute interaction with a photographer was estimated to use 1/3 of its daily energy budget (Ellenberg *et al.*, 2013).

For a Little Penguin to respond to human disturbance to a comparable degree, 220.3KJ of energy (1/3 DEE) would need to be used in a response. This could be achieved by elevating HR over a one hour period as follows:

A one hour response would mean that 3.67KJ of energy were being used per minute. This equates to 186.3mlO<sub>2</sub>/min, or a HR of 934.3bpm.

Alternatively, if Little Penguin HR were elevated by 1.85xRHR (the maximum response of YEPs), HR would need to be sustained at 255.7bpm for 4.25 hours:

The mean RHR of Little Penguins before weighing was 138.2bpm. This would result in a maximum HR of 255.7bpm. VO<sub>2</sub> would equal 43.8ml/min, or 863.2J/min. To use 220.3kJ, energy would need to be consumed at a rate of 51.8kJ/hr, which would equate to a 4.25 hour response.