*Investigating the physiological underpinnings of proactive and reactive behavioural types in grey seals (Halichoerus grypus): Trial deployment of a minimally invasive data logger for recording heart rate and heart rate variability in a wild free-ranging breeding pinniped species*

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# Investigating the physiological underpinnings of proactive and reactive behavioural types in grey seals (*Halichoerus grypus*)

## Trial deployment of a minimally invasive data logger for recording heart rate and heart rate variability in a wild free-ranging breeding pinniped species

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# Abstract

Individuals differ non-randomly in their responses to stressors, exhibiting consistent individual differences (CIDs) in behavioural and physiological coping mechanisms commonly referred to as coping styles. Grey seals (*Halichoerus grypus*) are one of the few mammal species in which CIDs in stress responses have been documented in wild populations, though evidence thus far has been purely behavioural. Physiologically, coping styles can be distinguished by differences in the autonomic regulation of cardiac activity, which can be measured using heart rate variability (HRV).

The objectives of this study were two-fold. First, to assess the suitability of Polar® RS800CX monitors and H2/H3 sensors for conducting HRV analyses in grey seals. Second, to quantify inter-individual variation, repeatability, and reproductive performance correlates of baseline HRV.

Polar® devices were deployed successfully during the 2013 breeding season on female grey seals ($N$ = 15) on the Isle of May, Scotland, and were capable of recording HR patterns that characterise phocid seals at rest on land. However, artefacts were widespread and biased HRV metrics. Filtration and correction protocols were able to counteract the effects of artefacts, but severely limited the amount of data available for analysis.

There were significant inter-individual differences in baseline HRV, which could not be explained by factors associated with the breeding season (e.g. percentage mass loss, day of lactation), diurnal rhythms (e.g. time of day), or stressors (e.g. days since capture). These differences in baseline HRV showed consistency across early and late lactation. Individuals appeared to separate into two groups: those with consistently lower or higher baseline HRV, characteristic of proactive and reactive coping styles, respectively. Furthermore, females with lower baseline HRV showed greater maternal transfer efficiency – though there were no associations between baseline HRV and maternal expenditure (i.e. maternal mass loss, $kgday^{-1}$) or fitness outcomes (i.e. pup mass gain, $kgday^{-1}$). These findings build upon previous studies on behavioural CIDs in female grey seals by providing the first preliminary evidence for physiological CIDs that are associated with maternal investment. However, due to small sample

sizes, further studies are required to determine whether these findings are truly indicative of coping styles.

In their current form, the use of Polar® devices requires several caveats and further studies are needed to fully realise their potential. Future research should focus on validation against simultaneously recorded ECGs to improve artefact detection and correction, and modification to minimise the occurrence of artefacts. Despite their limitations, Polar® devices have immense potential as a minimally invasive research tool for conducting HRV analyses in the field.

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# Contents

# List of Abbreviations

| | |
|---|---|
| **ACTH** | Adrenocorticotropic hormone (p7) |
| **ANS** | Autonomic nervous system (p1) |
| **BPM** | Beats per minute (p23) |
| **CID** | Consistent individual difference (p1) |
| **CRF** | Corticotropin-releasing factor (p7) |
| **DOL** | Day of lactation (p46) |
| **DSC** | Days since capture (p46) |
| **DSG** | Days since electrode gel application (p45) |
| **ECG** | Electrocardiograph/electrocardiogram (p23) |
| **HFP** | High feather pecking (p12) |
| **HPA axis** | Hypothalamic-pituitary-adrenal axis (p5) |
| **HR** | Heart rate (p2) |
| **$HR_{COR}$** | Corrected heart rate (p39) |
| **HRV** | Heart rate variability (p2) |
| **IBI** | Inter-beat interval (p2) |
| **LAL** | Long attack latency (p10) |
| **LFP** | Low feather pecking (p12) |
| **MDML** | Maternal daily mass loss rate (p30) |
| **MPPM** | Maternal post-partum mass (p30) |
| **MPR** | Ratio of MDML to PDMG (p30) |
| **PDMG** | Pup daily mass gain rate (p30) |
| **PNS** | Parasympathetic nervous system (p16) |
| **RMSSD** | Root-mean square of successive differences between RR intervals (p32) |
| **$RMSSD_{COR}$** | Corrected root-mean square of successive differences between RR intervals (p39) |
| **SA node** | Sino-atrial node (p16) |
| **SAL** | Short attack latency (p10) |
| **SAM pathway** | Sympatho-adreno-medullary pathway (p6) |
| **SNS** | Sympathetic nervous system (p5) |
| **TIME** | Time of day (p46) |

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# Chapter 1: Introduction

## 1.1. General introduction

Research over the past two decades has revealed that individuals within populations differ non-randomly in their ability to cope with stressors, exhibiting distinct behavioural and physiological stress responses that are stable over time (Koolhaas 2008; Koolhaas *et al.* 1999, 2007, 2010). These responses are often classified into two distinct *coping styles* (see *Glossary*) that represent alternative strategies for coping with stressors (Koolhaas *et al.* 1999; Sih *et al.* 2004a, 2004b). Behaviourally, individuals that adopt a "proactive" coping style are characterised by aggression, risk-taking, reduced responsiveness to environmental stimuli, and relatively little flexibility. Conversely, "reactive" individuals are comparatively non-aggressive and risk-aversive, exhibiting more flexibility that permits greater responsiveness to environmental stimuli (Koolhaas *et al.* 1999; Sih *et al.* 2004a, 2004b; Carere *et al.* 2010).

Grey seals (*Halichoerus grypus*) are one of the few mammal species in which *consistent individual differences* (*CIDs*) for behavioural stress responses have been documented in free-living wild populations (Twiss et al. 2011; Twiss et al. 2012). Female grey seals show CIDs in vigilance behaviour that are stable both within and between breeding seasons. When presented with a standardised auditory stressor, some individuals will significantly increase pup-checking rates, whereas others will maintain a similar rate through disturbed and undisturbed *situations*. Females who modulate their pup-checking rates also display greater variation in reproductive performance (i.e. rates of maternal mass loss and pup mass gain, $kgday^{-1}$) and putatively lower levels of aggression (Twiss et al. 2012). Accordingly, breeding female grey seals can be classified into proactive or reactive behavioural types, wherein reactive individuals are less aggressive, more variable in their reproductive performance, and more responsive to stressors than their proactive counterparts. Whether these behavioural types are indicative of coping styles has yet to be determined.

Underpinning the behavioural characteristics of coping styles are physiological differences reflected in the functioning of the autonomic nervous system (ANS). When presented with a stressor, proactive individuals exhibit greater sympathetically-mediated responses, whereas reactive individuals exhibit greater parasympathetically-mediated responses (Koolhaas *et al.* 1999). In some cases, these differences are also present in the

absence of a stressor, such that proactive individuals show greater sympathetic activity under baseline conditions (Fokkema *et al.* 1988; Koolhaas *et al.* 1999; Sgoifo *et al.* 2005). One way autonomic activity can be measured is through *heart rate variability* (*HRV*), which refers to variation in both instantaneous heart rate (HR) and the interval between heartbeats, also known as *inter-beat intervals* (*IBIs*) or *RR intervals* (von Borell *et al.* 2007). Consequently, HRV can be used to infer coping styles, wherein proactive individuals exhibit lower HRV and higher HR in response to a stressor than reactive individuals.

### 1.1.1. Thesis objectives

The aim of this study was to develop a methodology for quantifying physiological CIDs concurrently with behavioural CIDs in a wild free-ranging pinniped species. Therefore, the primary objective was to:

(1) Assess the suitability of a commercially available, minimally invasive HR logger (Polar® RS800CX monitors and Polar® H2/H3 sensors) for conducting HR and HRV analyses in grey seals.

Furthermore, this study aimed to expand on previous studies on behavioural CIDs in female grey seals (Twiss *et al.* 2011, 2012) by examining physiological CIDs. More specifically, by:

(2) Quantifying inter-individual variation in baseline HR and HRV during the breeding season.

(3) Providing preliminary evidence for repeatability in baseline HR and HRV within the breeding season.

(4) Determining whether baseline HR and HRV are associated with proxies of maternal reproductive performance.

Ultimately, it is hoped that this study will be the first step towards elucidating the proximate physiological underpinnings of proactive-reactive behavioural types in grey seals.

### 1.1.2. Thesis outline

Chapter 1 provides background information on CIDs in behaviour (*Chapter 1.2. An introduction to consistent individual differences in behaviour*) and the vertebrate stress response (*Chapter 1.3. An introduction to the vertebrate stress response*), and how they form coping styles (*Chapter 1.4. Coping styles*). A brief overview of HRV and its relevance to studying stress

responses and coping styles is provided (*Chapter 1.5. Heart rate variability*), followed by a rationale for the present study (*Chapter 1.6. Thesis rationale*). Chapter 2 presents the methodology used, with focus on data processing, filtration, and correction protocols required for HRV analyses. Results from statistical analyses are presented in Chapter 3. Overall findings are discussed and concluded in Chapter 4.

## 1.2. An introduction to consistent individual differences in behaviour

The concept of *animal personality* has been used to refer to CIDs in behaviour that are stable over time and across different *contexts* and situations, regardless of age, sex, or physical and social environmental conditions (Dall *et al.* 2004; Sih *et al.* 2004a, 2004b; Réale *et al.* 2007; Carere *et al.* 2010). Animal personality has been reviewed extensively within the past decade (Gosling 2001; Dall *et al.* 2004; Sih *et al.* 2004a, 2004b; Dingemanse and Réale 2005; Réale *et al.* 2007, 2010; Dingemanse *et al.* 2009; Carere *et al.* 2010; Dingemanse and Wolf 2010; Wolf and Weissing, 2010, 2012; Carere and Maestripieri 2013; Carter *et al.* 2013) and there is now evidence of personality in a diverse range of taxa, from invertebrates (Kjalj-Fišer and Schuett 2014) to non-human primates (Freeman and Gosling 2010). The proliferation of research on animal personality, whilst fuelled by its appeal to public opinion, has been driven primarily by its far-reaching implications in ecology and evolution (Dall *et al.* 2004; Wolf and Weissing 2012; Carere and Maestripieri 2013).

Five ecologically relevant *behavioural traits* of animal personality have been identified: (1) boldness (reaction to risky situations); (2) exploration (reaction to novel situations); (3) activity; (4) aggression (agonistic reactions to conspecifics); and (5) sociability (non-agonistic reactions to the presence or absence of conspecifics) (Réale *et al.* 2007; Menzies *et al.* 2013). Consistent differences in these behavioural traits become apparent when individuals are faced with environmental or social challenges (Carere *et al.* 2010), revealing two distinct behavioural phenotypes distributed in a non-random, typically bimodal manner along an axis (e.g. bold–shy, explorative–avoidant, fast–slow, aggressive–non-aggressive) (Gosling and John 1999; Gosling 2001; Dall *et al.* 2004; Réale *et al.* 2007). For example, individuals often vary in their willingness to take risks. Whilst all individuals will alter their boldness in a context-dependent manner, some will consistently take more risks than others (i.e. the rank order between individuals is maintained) (Sih *et al.* 2004a, 2004b; Dall *et al.* 2004). Individuals who are consistently bolder

are said to have a bold *behavioural type* (Sih *et al.* 2004a, 2004b; Dall *et al.* 2004). Behavioural traits often form correlated suites, wherein consistently bolder individuals are also consistently more aggressive or exploratory. These correlated suites, referred to as *behavioural syndromes*, can be described as continuums – the most widely studied of which has been the proactive-reactive continuum (Koolhaas *et al.* 1999; Sih *et al.* 2004a, 2004b; Réale *et al.* 2007). Proactive individuals are more aggressive, bold, and exploratory than their reactive counterparts; show relatively little behavioural flexibility; and form routines more readily (i.e. behaviours are intrinsically driven). Conversely, reactive individuals show greater behavioural flexibility and greater responsiveness to their surroundings, adjusting accordingly to changes in the environment (i.e. behaviours are extrinsically driven) (Koolhaas *et al.* 1999; Sih *et al.* 2004a, 2004b). Fundamentally, the two behavioural types are distinguished by behavioural flexibility and the degree to which behaviours are modulated by environmental stimuli (Koolhaas *et al.* 1999; Sih *et al.* 2004a, 2004b).

Initially, intraspecific variation in behaviour was widely assumed to be non-adaptive "noise" surrounding a presumed adaptive average, as classical theory in behavioural ecology predicts individuals should optimise their behaviour to their environment and favour plasticity (Krebs and Davies 1978; Lott 1991; Wilson 1998; Dall *et al.* 2004; Brommer and Kluen 2012). Whilst it does not preclude *behavioural plasticity*, animal personality challenges classical theory as it implies individuals are limited in their ability to behave optimally across contexts (Sih *et al.* 2004a, 2004b; Réale *et al.* 2007; Twiss *et al.* 2012; Brommer and Kluen 2012). Multiple models explaining the existence and maintenance of animal personality have been suggested – e.g. frequency and/or state dependent mechanisms (Dall *et al.* 2004), niche specialisations (Bergmüller and Tarborsky 2010), or life-history trade-offs (Biro and Stamps 2008) – at the core of which is a general consensus that animal personalities represent alternative, but equivalent, adaptive response strategies to ecological challenges (Koolhaas *et al.* 1999; Dall *et al.* 2004; Sih *et al.* 2004a, 2004b). These strategies can experience vastly different fitness consequences that vary with temporal, spatial, and/or social environmental conditions (Coppens *et al.* 2010; Bokony *et al.* 2012). For example, consistent boldness may be adaptive in contexts that favour risk-taking (e.g. foraging), but maladaptive in others where caution is more appropriate (e.g. foraging in the presence of predators) (Dall *et al.* 2004). Similarly, proactive individuals may be

more successful in stable environments, whereas reactive individuals may be more successful within highly variable or stochastic environments (Koolhaas *et al.* 1999). Furthermore, animal personalities have been shown to have a genetic basis with a moderate heritability (Drent *et al.* 2003; van Oers *et al.* 2004a, 2004b), and subject to sexual (Schuett *et al.* 2010) and natural selection (Dingemanse *et al.* 2004; Smith and Blumstein 2008; Quinn *et al.* 2009; Baugh *et al.* 2013). Consequently, animal personality can be a critical determinant of fitness correlates, such as resource acquisition (e.g. foraging behaviour: David *et al.* 2011), resource defence (e.g. territorial behaviour: Amy *et al.* 2010), reproductive success (e.g. mate choice and parental care: David and Cézilly 2011; Mutzel *et al.* 2013), anti-predator behaviour (e.g. Blake and Gabor 2014), sociality (e.g. group behaviour and dominance hierarchies: Aplin *et al.* 2014; Colleter and Brown 2011), immunity (e.g. parasite load: Boyer *et al.* 2010), and dispersal (e.g. Brodin *et al.* 2013; Carvahallo *et al.* 2013).

## 1.3. An introduction to the vertebrate stress response

The term *stress* encompasses three concepts: (1) stimuli that cause "stress" (referred to as *stressors*); (2) the behavioural and physiological responses to those stimuli (i.e. *stress responses*); and (3) pathology that results from the overstimulation of those physiological responses (i.e. *chronic stress*) (Romero and Butler 2007). Stressors are often defined as threats – real or perceived, intrinsic or extrinsic, physiological or psychological – to the homeostatic integrity of an organism (McEwen and Wingfield 2002; Romero 2004; Charmandari *et al.* 2005). Organisms cope with stressors by mounting a stress response: a suite of complex behavioural and physiological mechanisms that re-establish homeostatic integrity (McEwen and Wingfield 2002; Romero 2004). These mechanisms are modulated primarily by neuroendocrinology associated with the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS), which secrete the main components of the vertebrate stress response: catecholamines and glucocorticoids (Chrousos and Gold 1992; Johnson *et al.* 1992; Sapolsky *et al.* 2000; Habib *et al.* 2001; Tsigos and Chrousos 2002; Charmandari *et al.* 2005; Romero and Butler 2007).

The primary catecholamines released in response to a stressor are adrenaline (epinephrine) and noradrenaline (norepinephrine) (Axelrod and Reisine 1984; Charmandari *et al.* 2005; Schmidt-Nielsen 2007). Following the detection of a stressor, the hypothalamus

initiates activation of the SNS (Axelrod and Reisine 1984; Charmandari *et al.* 2005). Adrenaline and noradrenaline are secreted from both the sympathetic nerve terminals (i.e. the general sympathetic pathway) and the adrenal medulla (i.e. the sympatho-adreno-medullary (SAM) pathway) (Figure 1.1) (Axelrod and Reisine 1984; Charmandari *et al.* 2005).

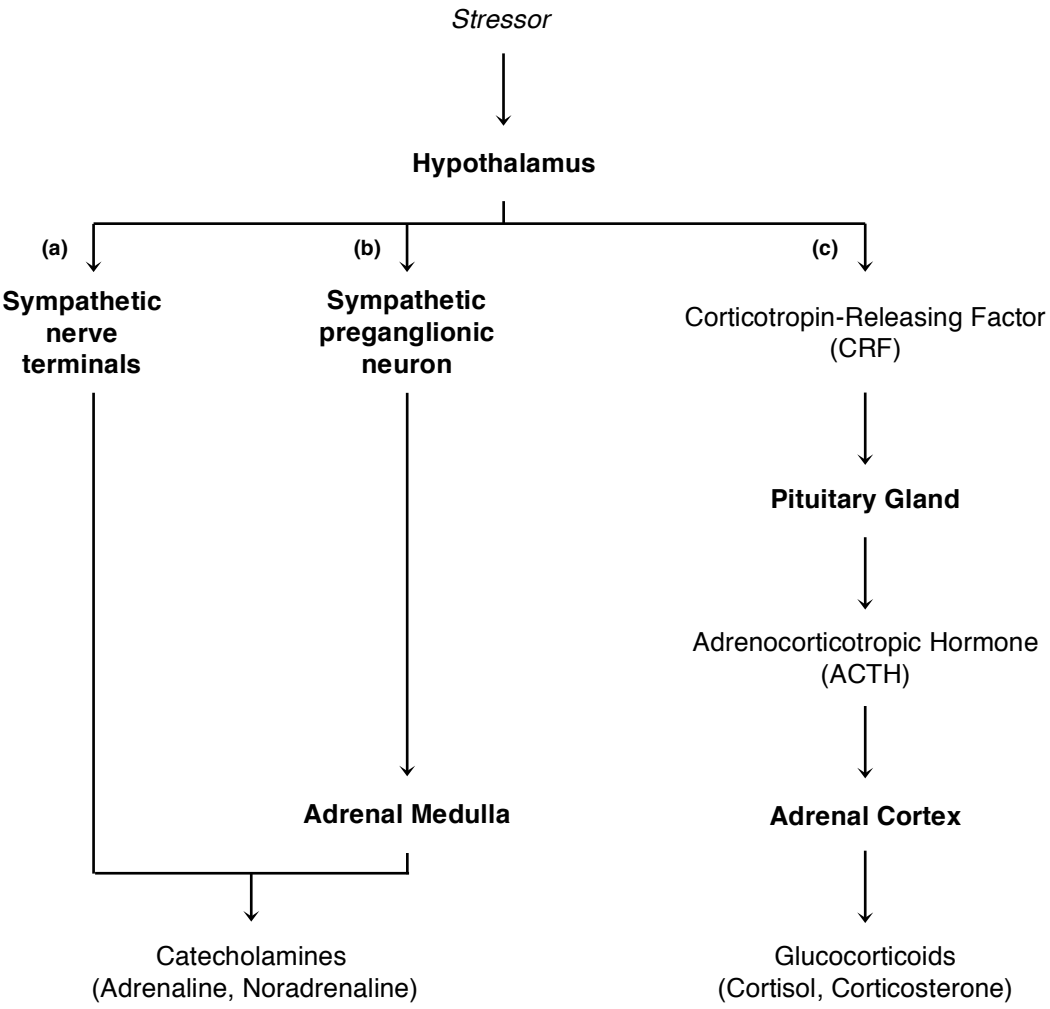


**Figure 1.1.** A brief overview of the vertebrate stress response. **(a)** General sympathetic pathway. **(b)** Sympatho-adreno-medullary (SAM) pathway. **(c)** Hypothalamic-pituitary-adrenal (HPA) axis. *Taken and adapted from* Sapolsky *et al.* 2000, Seaward 2006, *and* Romero and Butler 2007.

The SNS innervates the heart, skeletal muscles, digestive tract, and many other organs (Charmandari *et al.* 2005; Seaward 2006; Schmidt-Nielsen 2007). Consequently, catecholamines from sympathetic nerve terminals act immediately, within 2-3 seconds of exposure to a stressor (Table 1.1). Catecholamines from the adrenal medulla have a longer latency to act – approximately 20-30 seconds – since they are released into the bloodstream, but serve to reinforce and prolong the effects of the general sympathetic pathway (Seaward 2006). Both adrenaline and noradrenaline initiate organism-level responses within seconds of

detecting a stressor (Romero and Butler 2007), including increased HR, blood pressure, breathing rate, and visual acuity; decreased visceral activity; piloerection; vasodilation in skeletal muscle, lungs, and heart; vasoconstriction in the periphery (e.g. smooth muscle, skin); and conversion of glycogen into glucose (i.e. glycogenolysis) (Charmandari *et al.* 2005; Romero and Butler 2007; Schmidt-Nielsen 2007). These responses comprise the canonical *fight-or-flight* stress response (Cannon 1915), which serves to promote survival from an acute threat by preparing the body for rapid metabolism and locomotion; increasing vigilance; diverting endogenous resources to skeletal muscle; and suppressing processes that are superfluous during an emergency (e.g. digestion) (Sapolsky *et al.* 2000; Charmandari *et al.* 2005; Romero and Butler 2007).

**Table 1.1.** Summary of catecholamine and glucocorticoid responses to stressors.

| | **Response pathway** | **Secretory source** | **Time** |
|---|---|---|---|
| Catecholamines<br>• *Adrenaline*<br>• *Noradrenaline* | General sympathetic pathway | Sympathetic nerve terminals | Immediate<br>*(2-3 seconds)* |
| | Sympatho-adreno-medullary (SAM) pathway (i.e. sympathoadrenal response) | Adrenal medulla | Immediate<br>*(20-30 seconds)* |
| Glucocorticoids<br>• *Cortisol*<br>• *Corticosterone* | Hypothalamic-pituitary-adrenal (HPA) axis (i.e. adrenocortical response) | Adrenal cortex | Prolonged<br>*(Minutes/Hours)* |

*Taken and adapted from* Allen (1983), Seaward (2006) *and* Romero and Luke (2007).

The primary glucocorticoids that are released in response to a stressor are cortisol and corticosterone (Sapolsky *et al.* 2000; Tsigos and Chrousos 2002; Romero 2004; Romero and Butler 2007). Most species rely primarily upon either cortisol (e.g. fish, most mammals) or corticosterone (e.g. birds, reptiles, amphibians, rodents), though both can be found in most species (Romero and Butler 2007; Cockrem 2013a). The secretion of glucocorticoids results from a hormonal cascade, known as the HPA axis, which originates in the hypothalamus and culminates in the adrenal cortex (Sapolsky *et al.* 2000; Romero and Butler 2007). Following the detection of a stressor, the hypothalamus releases corticotropin-releasing factor (CRF), the main neuropeptide regulator that activates the HPA axis, which stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Sapolsky *et al.* 2000; Romero and Butler 2007). In turn, ACTH stimulates the secretion of glucocorticoids from the adrenal cortex (Sapolsky *et al.* 2000; Romero and Butler 2007). Glucocorticoids modulate a diverse array of responses, whose effects can be broadly classified into five groups: (1) increased blood glucose; (2) regulation of behaviours that control energy intake and expenditure; (3) inhibition of growth; (4) inhibition of reproduction; and (5) inhibition of immune function (Wingfield *et al.*

1998; Sapolsky *et al.* 2000; Tsigos and Chrousos 2002; McEwen and Wingfield 2002; Charmandari *et al.* 2005; Romero and Butler 2007). Collectively, these responses promote survival from a stressor by regulating the storage and mobilisation of endogenous resources, and postponing processes that can be affordably delayed (e.g. growth, reproduction) until the stressor has passed or the organism has recovered (McEwen and Wingfield 2002; Romero 2004; Romero and Butler 2007).

When compared to catecholamine-mediated responses, glucocorticoid-mediated responses are delayed, as the hormonal cascade of the HPA axis results in a time lag between the onset of a stressor and glucocorticoid secretion (Table 1.1) (Sapolsky *et al.* 2000; Romero and Butler 2008). Significant elevations of plasma glucocorticoid concentrations typically occur after three minutes, with peak plasma glucocorticoid concentrations 20 to 30 minutes following exposure to a stressor – though times depend on the nature of the stressor (i.e. duration, intensity) and vary between individuals and species (Sapolsky *et al.* 2000; Romero 2004; Romero and Reed 2005; Romero and Butler 2007; Cockrem 2013a). Assuming the stressor does not continue, glucocorticoids initiate a negative feedback loop that inhibits the secretion of CRF and ACTH, thereby reducing glucocorticoid concentrations within 30 to 60 minutes, and effectively terminating the stress response (Charmandari *et al.* 2005; Romero and Luke 2007). Although glucocorticoid-mediated responses are delayed, their effects are sustained for a longer period of time as they involve the production or inhibition of proteins (Romero and Butler 2007). Sympathetic activation and catecholamine-mediated responses are comparatively short-lived and wane quickly due to reflexive parasympathetic activation (Yvonne and Herman 2009). Consequently, the vertebrate stress response involves two “waves” of hormones and their effects (Sapolsky *et al.* 2000; Romero and Butler 2007). The first wave, mediated by catecholamines, occurs within seconds and initiates transient short-term effects. The second wave, mediated by glucocorticoids, occurs over the course of minutes or hours and initiates more prolonged effects.

The effects of catecholamines and glucocorticoids are largely protective and adaptive, enhancing fitness by promoting short-term survival from acute stressors (Sapolsky *et al.* 2000; Charmandari *et al.* 2005; Cockrem 2013a). However, hyperactivation of the stress response due to chronic stressors can be maladaptive. Prolonged secretion, overproduction, or dysregulation

of both catecholamines and glucocorticoids ultimately decreases fitness by increasing susceptibility to cardiovascular pathologies, such as hypertension, myocardial infarction, and cardiac arrhythmias (Rupp 1999; Dickens and Romero 2009); and inhibiting growth, reproduction, and immunity (Sapolsky *et al.* 2000; McEwen and Wingfield 2002; Charmandari *et al.* 2005), respectively. Therefore, longer-term survival requires balancing acute stress responses whilst minimising chronic exposure to stressors and subsequent hyperactivation of the stress response (Romero and Luke 2007).

## 1.4. Coping styles

### 1.4.1. Defining coping styles

Stress responses have been studied in almost all vertebrate groups and show highly conserved patterns (Cockrem 2013a). Following exposure to a stressor, mean plasma catecholamine and glucocorticoid concentrations increase (Cockrem 2013a; Baugh *et al.* 2013). The quality and magnitude of stress responses is highly variable, both between species and between groups within species – the latter often attributed to differences in sex, life-history stage, development, age, physiological condition, and social status (Breuner *et al.* 2008; Cockrem 2013a). However, there is also widespread variation between individuals irrespective of these differences. For example, some individuals may show little or no response to a stressor that evokes a relatively large response in others (Cockrem 2013a). Alternatively, some individuals may respond to stressors with greater catecholamine concentrations, whereas others may respond with greater glucocorticoid concentrations (Koolhaas *et al.* 1999). Comparable to animal personalities, individuals show consistent differences in their physiological responses to stressors that are stable over time (Koolhaas *et al.* 1999, 2007, 2010; Koolhaas 2008; Carere *et al.* 2010). When these distinct physiological responses are correlated with behavioural traits, they are referred to as *coping styles* (Koolhaas *et al.* 1999, 2007, 2010; Koolhaas 2008).

### 1.4.2. Evidence for coping styles

There is evidence for coping styles in a wide range of domesticated, captive, and wild taxa. Most of what is known about physiological characteristics of coping styles comes from research on the reactivity of the HPA axis and the SAM pathway – and to a lesser extent, the

functioning of the hypothalamic-pituitary-gonadal (HPG) axis and oxidative status (Koolhaas *et al.* 1999, 2007, 2010; Koolhaas 2008; Carere *et al.* 2010).

Much of the pioneering research on coping styles has been conducted on genetic selection lines of mice (*Mus musculus*) and rats (*Rattus norvegicus*). Male mice selected for either short attack latency (SAL) or long attack latency (LAL) when challenged by an intruder in their home cages differ in their behavioural and physiological responses to both social and non-social challenges (van Oortmerssen and Bakker 1981; Benus *et al.* 1987). During social challenges, SAL mice are more aggressive towards conspecifics than LAL mice (Benus *et al.* 1990). Following social defeat, SAL mice have a greater tendency to flee, whereas LAL mice are more likely to freeze (Benus *et al.* 1992). During non-social challenges, SAL mice show less behavioural flexibility and are more amenable to routine formation (Benus *et al.* 1987). Consequently, SAL mice are slower at reacting to changes in formerly stable environments, whereas LAL are better at reacting within stochastic environments (Benus *et al.* 1987). For example, SAL mice perform poorer in mazes following a single configuration change, or in mazes with continuously changing configurations, than LAL mice (Benus *et al.* 1987). When presented with aversive stressors (i.e. mild electric shocks), SAL mice are better at avoiding controllable shocks, whereas LAL mice are better at reacting to uncontrollable shocks (Benus *et al.* 1991). LAL mice adapt faster to changes in light-dark cycles than SAL mice, suggesting circadian rhythmicity is driven primarily by external factors (i.e. the zeitgerber), rather than internal factors (i.e. the pacemaker) (Benus *et al.* 1988). Plasma corticosterone concentrations are significantly higher in LAL mice when challenged with exogenous ACTH and introduced to novel environments (i.e. higher HPA axis reactivity) than SAL mice (Veenema *et al.* 2003, 2004, 2005a, 2005b). LAL mice also show comparatively higher circadian peak plasma corticosterone concentrations (i.e. higher HPA axis activity) (Korte *et al.* 1996; Veenema *et al.* 2003).

Wild-type rats exhibit considerable inter-individual variation in aggressive behaviour comparable to SAL and LAL mice (Koolhaas *et al.* 1999), as shown by responses to electrified prods (de Boer and Koolhaas 2003). Following a mild aversive shock, individuals can avoid further shocks either by actively burying or avoiding the electrified prod (Koolhaas *et al.* 1999). More aggressive males tend to engage in defensive burying, which is characterised by higher plasma catecholamine concentrations (i.e. greater sympathetic reactivity) (de Boer *et al.* 1990;

Sgoifo *et al.* 1996; Koolhaas *et al.* 1999). Conversely, non-aggressive males tend to become immobile, which is characterised by greater plasma corticosterone concentrations (i.e. greater HPA axis reactivity) (de Boer *et al.* 1990; Sgoifo *et al.* 1996; Koolhaas *et al.* 1999). Similar differences in plasma catecholamine and corticosterone concentrations are also observed following social defeat (Fokkema *et al.* 1994, 1998; Sgoifo *et al.* 1997, 1998). Furthermore, more aggressive males show higher plasma catecholamine concentrations than non-aggressive males at rest under baseline conditions (i.e. greater sympathetic activity) (Fokkema *et al.* 1988).

Overall, studies on genetic selection lines in rodents suggest inter-individual variation in behavioural traits (i.e. aggression) is related to the way in which individuals react to and cope with stressors (Koolhaas *et al.* 1999). Aggressive individuals tend to respond to stressors with an "active" or proactive strategy (e.g. fleeing, defensive burying) driven by internal cues, which is associated with more rigid routine-formation (Benus *et al.* 1987; Koolhaas *et al.* 1999). Non-aggressive individuals tend to adopt a "passive" or reactive strategy (e.g. freezing/immobility) driven by external cues, which is associated with greater behavioural flexibility and responsiveness to environmental stimuli (Benus *et al.* 1987; Koolhaas *et al.* 1999). Differences in these behavioural traits correspond with differences in physiological responses to stressors. Proactive coping styles are characterised by greater sympathetic activity/reactivity (i.e. higher plasma catecholamine concentrations), whereas reactive coping styles are characterised by greater HPA axis activity/reactivity (i.e. higher plasma corticosterone concentrations) (Table 1.2).

**Table 1.2.** Summary of the physiological characteristics associated with proactive and reactive coping styles.

| Trait | Measurement | Proactive | Reactive | Context |
|---|---|---|---|---|
| HPA axis activity | Glucocorticoids | Low | High | Baseline |
| HAP axis reactivity | Glucocorticoids | Low | High | Acute stressor |
| Sympathetic activity | Catecholamines<br>HR<br>HRV | High | Low | Baseline |
| Sympathetic reactivity | Catecholamines<br>HR<br>HRV | High | Low | Acute stressor |
| Parasympathetic reactivity | HRV | Low | High | Latency to return to baseline (following acute stressor) |

*Taken and adapted from* Koolhaas *et al.* (1999).

Similar behavioural and physiological characteristics have been observed in livestock (Koolhaas *et al.* 1999; Carere *et al.* 2010). Pigs (*Sus scrofa*) can be classified into distinct

behavioural types based on the number of escape attempts made during a back test (i.e. manual restraint whilst supine) (Hessing *et al.* 1994; Ruis *et al.* 2000). These behavioural types are detectable at an early age, persist into adulthood, and are associated with behavioural responses to social challenges, novel objects, and novel environments (Hessing *et al.* 1994; Ruis *et al.* 2000). High-resistant pigs are characterised by more escape attempts, greater aggression during group feeding competitions, shorter latencies to approach a novel object, and shorter times spent exploring a novel environment than low-resistant pigs. When startled, high-resistant pigs show substantially increased HR (tachycardia) indicative of greater sympathetic reactivity, whereas low-resistant pigs show only slightly increased HR or even decreased HR (bradycardia) (Hessing *et al.* 1994 Koolhaas *et al.* 1999). High-resistant pigs also show lower salivary cortisol than low-resistant pigs when challenged with exogenous ACTH, indicative of lower HPA axis reactivity (Ruis *et al.* 2000; Koolhaas *et al.* 1999). Consequently, high- and low-resistant pigs are thought to be representative of proactive and reactive coping styles, respectively. Comparable inter-individual variation in behavioural, cardiac, and adrenocortical responses to novel objects and environments has also been document in cattle (*Bos taurus*) (Hopster 1998; van Reenen *et al.* 2005) and horses (*Equus ferus callabus*) (Visser *et al.* 2002, 2003).

There is substantial evidence for coping styles in avian species, which compliment studies in mammalian species. As in rodents, much of the research has been performed using divergent selection lines of chickens (*Gallus domesticus*) and great tits (*Parsus major*). Chickens from two lines with high (HFP) or low (LFP) propensity to feather peck (Blockhuis and Beutler 1992) show physiological responses to stressors comparable to those observed in SAL/LAL mice, aggressive/non-aggressive rats, and livestock. When subject to manual restraint, plasma catecholamine concentrations are higher in HFP hens (i.e. greater sympathetic reactivity), whereas plasma corticosterone concentrations are higher in LFP hens (i.e. greater HPA axis reactivity) (Korte *et al.* 1997). LFP hens also show higher basal plasma corticosterone concentrations (i.e. greater HPA axis activity) than HFP hens (Korte *et al.* 1997; van Hierden *et al.* 2002). Furthermore, HFP hens are characterised by greater resistance under restraint and longer tonic immobility during open field tests (Blockhuis and Beutler 1992). Comparable inter-individual variation in behavioural and adrenocortical responses have also been documented in

HFP/LFP grey parrots (*Psittacus erithacus erithacus*) (van Zeeland *et al.* 2013) and Japanese quails (*Coturnix japonica*) selected for short or long tonic immobility (Satterlee and Johnson 1988; Mills and Faure 1991; Jones *et al.* 1994a, 1994b).

Great tits show CIDs in their propensity to explore a novel environment and can be categorised as slow but thorough explorers, or fast and superficial explorers (Verbeek *et al.* 1994). These exploratory traits are not only heritable (Dingemanse *et al.* 2002; Drent *et al.* 2003) but also correlate with various behavioural and physiological traits analogous to proactive and reactive coping styles described in mammalian species. Fast explorers tend to be bolder, more aggressive, form routines more readily, and show lower HPA reactivity in response to a stressor than when compared to slow explorers (Verbeek *et al.* 1994, 1996, 1999; Carere *et al.* 2003; Drent *et al.* 2003; Groothuis and Carere 2005; Stowe *et al.* 2010; van Oers *et al.* 2011; Baugh *et al.* 2012, 2013). Correlations between behavioural traits (e.g. boldness, exploration) and physiological responses to stressors have also been found in other avian species, such as zebra finches (*Taenopygia guttata*) (Martins *et al.* 2007), greylag geese (*Anser anser*) (Pfeffer *et al.* 2002; Kjalj-Fišer *et al.* 2007, 2010a, 2010b; Wascher *et al.* 2008, 2009, 2010, 2011), and Nazca boobies (*Sula granti*) (Grace and Anderson 2014).

### 1.4.3. Quantifying the physiological characteristics of coping styles

Coping styles are determined by measuring the reactivity of the HPA axis or the SAM pathway, which typically involves comparing glucocorticoid and/or catecholamine concentrations following exposure to a standardised stressor with baseline concentrations. Glucocorticoids and their metabolites can be sampled from plasma, salivary, urinary, or faecal samples (Kirschbaum and Hellhammer 1989; Millspaugh and Washburn 2004; Keay *et al.* 2006; Luecken and Gallo 2008). Direct measurement of glucocorticoids from plasma or saliva is easily achieved in the laboratory and/or captivity, but difficult in the wild. Biological sampling from free-ranging species in the wild necessitates capture and subsequent restraint or chemical immobilisation. For tractable species, restraint is often used as a standardised stressor (e.g. Carere *et al.* 2001; Carere and van Oers 2004). Since there is a delay between the presentation of a stressor and an increase in glucocorticoid concentrations (see *Chapter 1.3. An introduction to the vertebrate stress response*), glucocorticoid measurements obtained within three minutes of capture are often considered representative of true or close to baseline values (Romero and Reed 2005).

However, there is some debate on the robustness of the “three minute rule” (Cockrem 2013a). In some species, plasma glucocorticoid concentrations are capable of increasing just one minute after a stressor commences (e.g. Sgoifo *et al.* 1996), revealing the potential for upwardly biased (i.e. stress contaminated) estimates of baseline glucocorticoid concentrations. Stress contamination may also occur for a study sample containing individuals with consistently greater HPA axis reactivity (i.e. reactive individuals) (Baugh *et al.* 2013). Anaesthetisation has been shown to reduce or ameliorate the stress impacts of handling in some species, such as northern elephant seals (*Mirounga angustirostris*) (Champagne *et al.* 2012) and Weddell seals (*Leptonychotes weddellii*) (Harcourt *et al.* 2010), but differences in glucocorticoid responses between conscious and chemically immobilised animals are not clear (Cockrem 2013a).

Indirect measurement of glucocorticoid metabolites from faeces and urine provides a non-invasive alternative that reduces the impact on study animals, avoids stress contamination associated with capture, and allows for greater sample sizes (Millspaugh and Washburn 2004; Constable *et al.* 2006). Despite these advantages, there are caveats associated with the methodology and the interpretation of results (Millspaugh and Washburn 2004). Metabolites in faecal or urinary samples reflect the cumulative concentration of plasma glucocorticoids over a period of time (typically an hour or more) prior to sampling, making them more suitable for evaluating chronic stress rather than responses to acute stressors. Faecal corticosterone metabolites have previously been used to infer coping styles in avian species under captive condition – e.g. great tits (Carere *et al.* 2003) and greylag geese (Kralj-Fišer *et al.* 2007). Under wild conditions, however, repeated sampling from free-ranging individuals is near impossible for many species.

Similar to glucocorticoids, catecholamines and their metabolites can be measured from plasma or urine (Weinkove 1991; Peaston and Weinkove 2004). Plasma catecholamines have been used to determine coping styles successfully in laboratory species (e.g. Fokkema *et al.* 1994; Korte *et al.* 1997; Sgoifo *et al.* 1996, 2005). However, the short-term nature of the SAM pathway, coupled with the strict protocols necessary for sampling catecholamines, generally precludes their application beyond the laboratory (Goldstein *et al.* 1983; Peaston and Weincove 2004; Schmidt-Nielsen 2007). Furthermore, obtaining baseline measures of catecholamines are difficult and require invasive techniques such as cannulation (e.g. Fokkema *et al.* 1988). Indirect

measurement of catecholamine metabolites from urine carries the same logistical difficulties and limitations associated with sampling faecal and urinary glucocorticoid metabolites.

Alternatives to the direct measurement of catecholamines involve measuring proxies of sympathetic activity – e.g. HR, breathing rate, and peripheral or core body temperature – though results can be ambiguous. For example, attempts have been made to determine physiological differences between fast and slow great tits using breathing rates and body temperature (Carere *et al.* 2001; Carere and van Oers 2004; Fucikova *et al.* 2009). Sympathetic activation increases breathing rate and leads to peripheral vasoconstriction (Schmidt-Nielsen 2007). In turn, peripheral vasoconstriction reduces conductive heat loss and contributes to stress-induced increases in core body temperature (Nakamori *et al.* 1993). Consequently, elevated breathing rates and core body temperature would be expected for fast great tits when confronted with a stressor. Some studies have found a positive correlation between exploratory behaviour and breathing rate during capture (Fucikova *et al.* 2009), whereas others have found slow individuals showed higher breathing rates and higher cloacal temperatures than fast individuals when handled (Carere *et al.* 2001; Carere and van Oers 2004). Similarly, capture stress in farmed American mink (*Neovison vison*) is associated with elevated plasma cortisol and body temperature that persists for longer in shy individuals (Damgaard and Hansen 1996; Korhnen *et al.* 2000). Although confounding, these results may in part be explained by the potentiating effect of glucocorticoids on sympathetically-mediated responses, such as peripheral vasoconstriction (Romero 2004; Yvonne and Herman 2009). For example, stress-induced hyperthermia is also accompanied by increased plasma corticosterone in mice (Groenink *et al.* 1994) and increased plasma cortisol in domesticated silver foxes (*Vulpes vulpes*) (Moe and Bakken 1997).

Evidently, there is a clear need for non-invasive methodologies that are capable of quantifying physiological indicators of stress attributed to coping styles in wild free-ranging species. Ideally, these methodologies should allow for repeated sampling of individuals, avoid stress contamination (i.e. handling or capture), assess baseline conditions, and directly measure physiological responses to acute stressors. One such promising methodology is HRV analysis, which can be used to measure the activity of the parasympathetic and sympathetic branches of the ANS (Task Force 1996; von Borell *et al.* 2007).

## 1.5. Heart rate variability

### 1.5.1. Defining HRV

HRV refers to the variation in instantaneous HR or the intervals between heartbeats, also known as RR intervals or IBIs (Malik and Camm 1995; Task Force 1996; von Borell *et al.* 2007; Kamath *et al.* 2012). HRV was first recognised as a promising marker of autonomic function in the 1960s and has since been incorporated into a wide array of clinical research examining various physical, psychological, and pathological conditions (Malik and Camm 1995; Task Force 1996; von Borell *et al.* 2007; Kamath *et al.* 2012). HRV has also been applied extensively within veterinary and animal research, where it has been well established as a reliable indicator of both acute and chronic stress (von Borell *et al.* 2007).

### 1.5.2. Regulation of cardiac activity: HRV as an indicator of sympathovagal balance

Cardiac activity is largely under control of the ANS (Task Force 1996). The sino-atrial (SA) node, the primary regulator of HR, is innervated by both sympathetic and parasympathetic branches of the ANS (Task Force 1996; Berntson *et al.* 1997; von Borell *et al.* 2007). Parasympathetic influence, which decelerates HR, is mediated by acetylcholine secreted from the vagus nerve. Conversely, sympathetic influence, which accelerates HR, is mediated predominantly by adrenaline and noradrenaline secreted from the sympathetic nerve terminals – but also circulatory catecholamines secreted from the adrenal medulla (Task Force 1996; Charmandari *et al.* 2005; von Borell *et al.* 2007). HR represents the net effect of parasympathetic and sympathetic activity (Malik and Camm 1995; von Borell *et al.* 2007). Although the parasympathetic nervous system (PNS) and SNS are mutually exclusive (i.e. simultaneous relaxation and arousal cannot occur) they do not function on a continuum (Malik and Camm 1995; von Borell *et al.* 2007; Schmidt-Nielsen 2007). Increasing activity in one branch does not result in decreasing activity in the other (Berntson *et al.* 1997; Malik and Camm 1995; von Borell *et al.* 2007). Instead, both branches may function synchronously or independently of one another (Berntson *et al.* 1997; Malik and Camm 1995; von Borell *et al.* 2007). For example, the PNS can either assist or antagonise sympathetic activity by withdrawing or increasing parasympathetic input (Sapolsky *et al.* 2000; Tsigos and Chrousos 2002; Charmandari *et al.* 2005). An increase in HR is typically caused by an increase in sympathetic activity, but may also result from reduced parasympathetic activity or a combination

of both (Malik and Camm 1995; von Borell *et al.* 2007). Thus, HR alone cannot be used to accurately assess either parasympathetic or sympathetic activity (von Borell *et al.* 2007).

HRV measures the fluctuations in parasympathetic and sympathetic activity (i.e. sympathovagal balance) at the SA node (Malik and Camm 1995; Task Force 1996; von Borell *et al.* 2007). Consequently, it can be used to measure the balance of autonomic control, both under baseline conditions and following exposure to an acute stressor (von Borell *et al.* 2007). At rest, both sympathetic and parasympathetic branches are tonically active, though parasympathetic activity is dominant (Malik and Camm 1995; Task Force 1996; von Borell *et al.* 2007). Cardiac activity is maintained within a homeostatic range, which is regulated by various control and feedback mechanisms (Malik and Camm 1995; von Borell *et al.* 2007). Fluctuations in these regulatory components result in fluctuations in cardiac activity. As a result, the time intervals between consecutive heartbeats are highly variable and irregular at rest (Malik and Camm 1995; von Borell *et al.* 2007). HRV under baseline conditions can be used as an indicator for stress vulnerability (Johnson *et al.* 1992; Porges 1995). High vagal tone (i.e. high parasympathetic activity/reactivity as indicated by high basal HRV) has been associated with increased responsiveness to stressors and/or environmental challenges (Johnson *et al.* 1992; Porges 1995). Conversely, low vagal tone (i.e. low parasympathetic activity/reactivity as indicated by low basal HRV) has been associated with increased susceptibility to stressors (Johnson *et al.* 1992; Porges 1995). Physical activity or stressors, both physiological and psychological, are capable of shifting sympathovagal balance (Task Force 1996; von Borell *et al.* 2007). Generally speaking, a decrease in HRV (and a concurrent increase in HR) indicates reduced parasympathetic activity (and increased sympathetic activity) – and vice versa (Malik and Camm 1995; Task Force 1996; von Borell *et al.* 2007).

### 1.5.3. HRV as an indicator of coping styles

HRV has been applied primarily within stress research to improve animal welfare, veterinary research as an indicator of various pathologies, and biomedical research using animal models of human disease (von Borell *et al.* 2007). Almost all animal research studies using HRV have been conducted on laboratory or domesticated animals such as rats, chickens, Japanese quail, pigs, goats (*Capra aegagrus hircus*), sheep (*Ovis aries*), cattle, and horses. A handful of studies have examined HRV using non-laboratory or non-domesticated species, such

as European starlings (*Sturnus vulgaris*), northern elephant seals, and harbour seals (*Phoca vitulina*). HRV in wild-caught European starlings have been used to investigate how transport into captivity alters basal sympathetic tone and sympathetic reactivity in response to acute stressors (Dickens and Romero 2009), cardiovascular responses to acute and chronic stress (Cyr *et al.* 2009), and cardiovascular responses to chronic stress during different life-history stages (e.g. moult) (Kostelanetz *et al.* 2009). HRV in pinnipeds has been used to estimate breathing frequencies of wild juvenile northern elephant seals (Andrews *et al.* 2000) and to investigate the development of diving bradycardia in wild (Greaves *et al.* 2004) and rehabilitated (Fonfara and Casamian-Sorrosal 2014) harbour seal pups. Overall, however, few studies within animal research have focused on inter-individual variation in HRV.

To date, only four studies have used HRV concurrently with catecholamines to infer coping styles. Sgoifo *et al.* (1998, 2005) demonstrated that aggressive and non-aggressive rats show differences in HRV following exposure to acute stressors that are concomitant with differences in HR and plasma catecholamine concentrations. When challenged with restraint or social defeat, aggressive rats show comparatively greater plasma catecholamine concentrations, higher HR, and lower HRV that persists for longer – suggesting greater sympathetic reactivity and reduced parasympathetic rebound following sympathetic activation. Similar cardiac and catecholamine responses to restraint have been documented in HFP and LFP hens (Korte *et al.* 1997, 1999).

Accordingly, HR and HRV can be used to distinguish underlying differences in autonomic function between coping styles (Table 1.2). Proactive individuals are characterised by greater sympathetic reactivity and reduced parasympathetic reactivity compared to reactive coping styles (Korte *et* al. 1997, 1999; Sgoifo *et al.* 1998, 2005; Koolhaas *et al.* 1999). Greater sympathetic reactivity manifests as higher HR and lower HRV following exposure to an acute stressor, whereas reduced parasympathetic activity manifests as longer latency for HR and HRV to return to baseline values.

## 1.6. Thesis rationale

### 1.6.1. Why are coping styles important?

The survival of all organisms requires the ability to respond appropriately to challenges by maintaining behavioural and physiological stability in the face of environmental instability (Sapolsky *et al.* 2000; McEwen and Wingfield 2002; Yvonne and Hermann 2009). Many of these challenges are predictable, following daily or annual rhythms associated with fluctuations in environmental conditions (e.g. temperature, precipitation, tides) and life-history (e.g. breeding, migrating, moulting, hibernating) (McEwen and Wingfield 2002). Superimposed upon these predictable fluctuations are unpredictable challenges (e.g. predators, inclement weather) (McEwen and Wingfield 2002). Organisms must cope with these challenges by displaying behavioural and physiological adaptations (Wingfield *et al.* 1998; McEwen and Wingfield 2002). When an organism is challenged beyond its adaptive capacity to cope, its survival is compromised (Koolhaas *et al.* 1991).

Anthropogenic influence on natural systems is increasing (Wikelski and Cooke 2006). More than ever, species are being subjected to challenges that are novel or more unpredictable and severe (Wikelski and Cooke 2006; Cockrem 2013b). One of the most pressing requirements for contemporary biologists is to understand how natural systems cope with these additional challenges (Wikelski and Cooke 2006; Cockrem 2013b). Stress response studies often compare mean responses between species, populations within species, or groups within populations (e.g. disturbed–undisturbed, male–female, breeding–non-breeding, young–old, dominant–subordinate) (Cockrem 2013a). However, research into coping styles has revealed individuals often differ non-randomly in their susceptibility and response to stressors (Koolhaas *et al.* 1999). General patterns observed at the level of species, population, or group often hide the diversity of individual strategies (Carter *et al.* 2009; Favreau *et al.* 2014). The ubiquity of this inter-individual variation, the extent to which it is adaptive or non-adaptive, and its fitness consequences are relatively unknown in most wild systems (Cockrem 2013a).

There is substantial evidence within the animal personality literature that behavioural CIDs are widespread and confer differential fitness benefits that vary with temporal, spatial, and/or environmental conditions (Coppens *et al.* 2010). For example, bolder individuals that are better at dispersing may be able to locate new resources (e.g. Brodin *et al.* 2013). Aggressive

individuals may be better at competing for resources as they become more limiting (e.g. Boon *et al.* 2007). Reactive individuals that are more responsive to environmental stimuli may be able to exploit novel resources more effectively by being more amenable to innovation (e.g. Pfeffer *et al.* 2002). Consequently, behavioural CIDs may permit or facilitate the adaptation or persistence of species subject to anthropogenic influence (Sih *et al.* 2004a, 2004b; Dall *et al.* 2004).

Covariation between behavioural traits associated with personality and physiological traits associated with stress responses implies mechanistic and functional links (Veenema *et al.* 2003; Øverli *et al.* 2005; Carere *et al.* 2010). Precisely how they are linked remains unclear (Carere *et al.* 2010; Koolhaas *et al.* 2010). From a proximate perspective, there are three ways in which they might be linked: (1) stress physiology determines behaviour; (2) behaviour determines stress physiology; and (3) stress physiology and behaviour are jointly regulated (Sih *et al.* 2004a, 2004b; Carere *et al.* 2010). Assuming coping styles underpin behavioural CIDs, quantifying coping styles may be critical to understanding species' capacity to cope with anthropogenic pressure (Carter *et al.* 2009; Denver 2009; Favreau *et al.* 2014).

### 1.6.2. Why conduct field studies?

The majority of studies on coping styles have been conducted using laboratory or domesticated animals from genetic selection lines, which are not likely to be representative of coping styles in the wild (Réale *et al.* 2007; Smith and Blumstein 2008; Bell *et al.* 2009). Studies that have used non-laboratory or non-domesticated animals typically sample individuals from a natural population and subject them to standardised tests within a captive environment. There is some evidence CIDs in captivity reliably reflect CIDs in the wild (Herborn *et al.* 2010). However, interpretation of results from captive studies should be made with caution and ideally tested in the wild (Adriaenssens and Johnsson 2011). Behavioural and physiological patterns observed in the field may be absent in captivity, or limited to local populations within a species (Wilson *et al.* 1993; Minderman *et al.* 2009; Herborn *et al.* 2010). There is also the potential for sampling bias when conducting experiments using wild-caught animals (Wilson 1998; Biro and Dingemanse 2007). A relationship between behavioural syndromes and probability of capture has been reported in fish (Wilson *et al.* 1993), mammals (Réale *et al.* 2000; Malmkvist and Hansen 2001; Montiglio *et al.* 2012), and birds (Mills and Faure 2000). For example, Garamszegi *et al.* (2009) found bolder, more exploratory collared flycatchers (*Ficedulla*

*albicollis*) were more likely to be trapped. Consequently, sampling protocols may unintentionally select for risk-taking individuals that are not representative of the natural population. Furthermore, introduction to captivity has also been shown to elevate baseline glucocorticoid concentrations, alter functioning of the HPA axis, and diminish the magnitude of sympathetically-mediated stress responses (Coddington and Cree 1995; Davidson *et al.* 1997; Romero and Wingfield 1999; Nilsson *et al.* 2008). For example, wild-caught European starlings show elevated baseline HR and diminished HR responses to stressors following transport into captivity than when compared to starlings kept in captivity for several months (Cyr *et al.* 2008; Dickens and Romero 2008).

Evidently, there is a clear need to quantify coping styles within natural populations in the wild, though few field studies have integrated inter-individual variation in behaviour and physiology (but see Montiglio *et al.* 2012; Ferrari *et al.* 2013; Clary *et al.* 2014; Grace and Anderson 2014). Grey seals possess several characteristics that make them an ideal study system for investigating both behavioural and physiological CIDs in the wild. First, grey seals have unique and stable pelage patterns that can be readily identified in the field (Vincent *et al.* 2001), allowing individuals to be studied within a population. Second, grey seals are long-lived mammals that show inter-annual site fidelity (Pomeroy *et al.* 1994). Individual females return to approximately the same location within a breeding colony each year to give birth, where they remain within close proximity of their pups (approximately two body lengths) until weaning (Pomeroy *et al.* 1994). Consequently, grey seals can be reliably sampled repeatedly throughout their reproductive lifetime, allowing for long-term longitudinal studies of identified individuals. As with most phocid species, grey seals are capital breeders (i.e. they temporally separate reproduction and foraging) (Pomeroy *et al.* 1999). Females fast during the breeding season and rely on stored resources to provision their pups, providing a closed study system that allows accurate measurement of maternal reproductive performance (Pomeroy *et al.* 1999). Therefore, behavioural and physiological data (e.g. HR and HRV) can be linked to measures of reproductive success. Furthermore, grey seals – and pinnipeds in general – are a good model system for piloting telemetry required for recording HR and HRV, as they are tractable and large enough to carry instrumentation (Costa *et al.* 2001).

# Chapter 2: Methodology

## 2.1. Study site and study animals

Data were collected during the 2013 breeding season on the Isle of May (56.1856° N, 2.5575° W), situated at the mouth of the Firth of Forth, 8 km off the east coast of Scotland, UK (Figure 2.1). The Isle of May is approximately 1.9 km long and 0.5 km wide with a rugged rocky topography broken by patches of flat terrain suitable for breeding grey seals (Pomeroy *et al.* 2000). The breeding season on the Isle of May lasts from mid-October to early December, during which individual females come ashore to give birth to and nurse a single pup for an average of 18 days (Kovacs *et al.* 1987; Pomeroy *et al.* 2000). Females on the Isle of May fast and remain close to their pups throughout lactation, but may occasionally commute to freshwater pools, wallows, or the shore (Twiss *et al.* 2000). Towards the end of lactation, females come into oestrus and are mated, soon after which they return to sea; pups are weaned abruptly (Kovacs *et al.* 1987; Pomeroy *et al.* 1999; Twiss *et al.* 2000). Grey seals on the Isle of May have been part of a long-term study on reproductive performance and behaviour since 1987 (Pomeroy *et al.* 2000), providing a pool of known individuals that can be identified using brands, flipper tags, and pelage markings. A total of 40 females were captured on the Isle of May during the 2013 breeding season, of which 15 were selected for this study.

## 2.2. Ethics

Grey seals in the UK are currently protected under the Conservation of Seals Act 1970 and the Marine (Scotland) Act 2010, and fall under the Animals (Scientific Procedures) Act 1986. All animal handling for the application of telemetry devices was approved by a Durham University ethics committee and conducted by qualified experienced personnel under the Sea Mammal Research Unit (SMRU) UK Home Office Project License (PPL 70/7806). Observational protocols were designed to conform to the ASAB/ABS guidelines for the treatment of animals in teaching research.

## 2.3. Recording HR and HRV

RR intervals were recorded using Polar® RS800CX HR monitors and Polar® H2/H3 sensors (*Polar Electro Oy*, Kempele, Finland). Polar® devices have been validated for the recording of HRV in cattle (Hopster and Blokhuis 1994), pigs (Marchant-Forde *et al.* 2004),


**Figure 2.1.** The Isle of May, Scotland. The location of the Isle of May, relative to the rest of the UK, is shown in the inset.

horses (Parker *et al.* 2010; Ille *et al.* 2014), and dogs (Jonckheer-Sheehy *et al.* 2012; Essner *et al.* 2013; Essner *et al.* 2015), where they have been shown to produce results comparable to ECGs. Polar® sensors have also been successfully modified for deployment on free-ranging juvenile harbour seals (Greaves *et al.* 2004), albeit with subcutaneous electrodes. The recording range and resolution of the Polar® monitors, 15–240 beats $min^{-1}$ (bpm) and ± 1 ms, respectively, was deemed appropriate for the study species and objectives. Documented HRs in free-living grey seals range from 4 to 120 bpm (Fedak *et al.* 1988; Thompson and Fedak 1993). Although 4 bpm is well below the minimum recording threshold of Polar® RS800CX monitors, such extreme bradycardia has only been observed during prolonged dives and was assumed unlikely to occur on land (Kooyman *et al.* 1981; Hindell and Lea 1998).

Polar® sensors were attached to Polar® soft strap electrodes and housed within neoprene sleeves (Figure 2.2, Figure 2.3). The whole apparatus – the "sensor strap" – was mounted externally to individuals for the duration of lactation. Where feasible, sensor straps were attached during initial early lactation captures, as close as possible to an individual's first

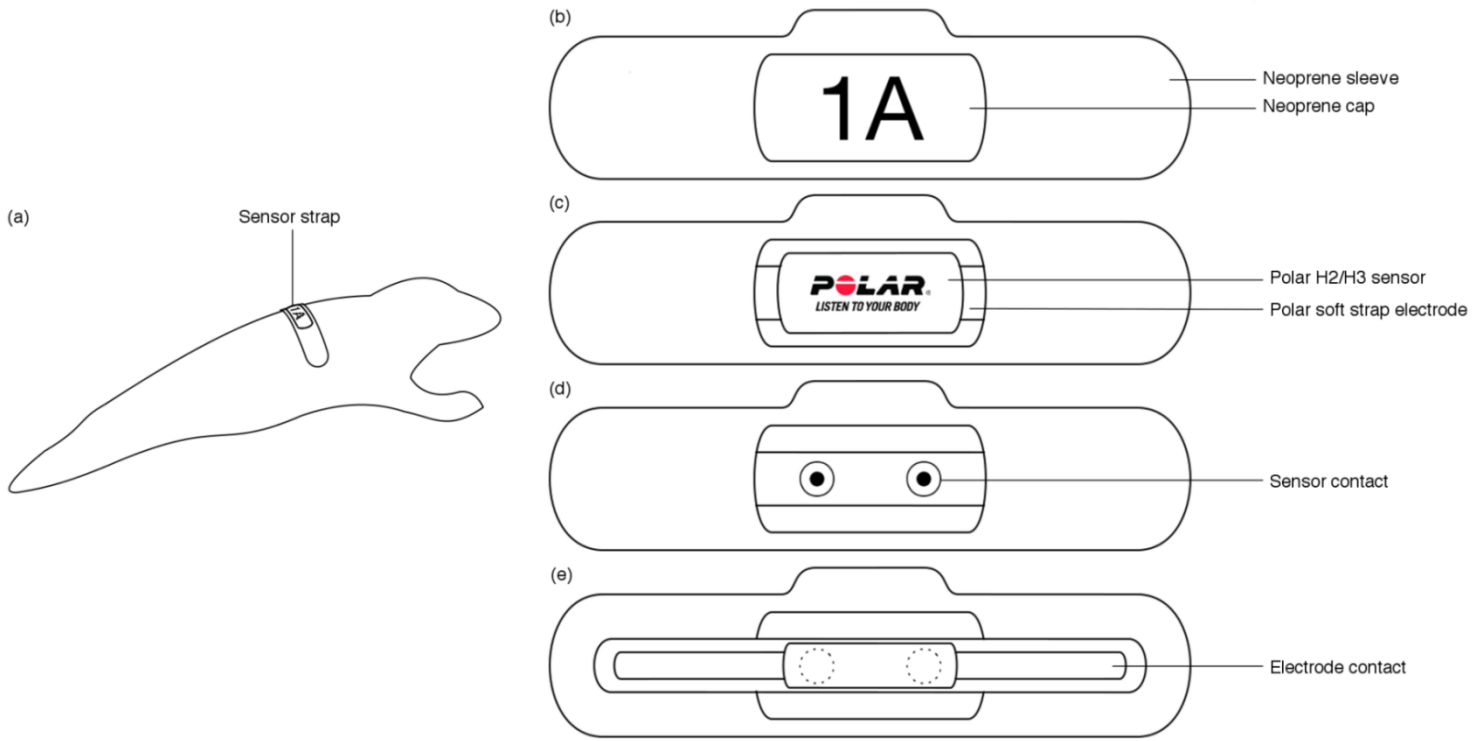


**Figure 2.2.** Basic schematic of the sensor strap apparatus used to transmit RR intervals of female grey seals. **(a)** Sensor straps were placed dorsally, just behind the right shoulder blade, where they would be least likely to interfere with behaviour and best positioned to transmit a signal to the monitor. **(b)** Complete sensor strap, as viewed from above. Only the neoprene sleeve and cap can be seen, which protect the Polar® H2/H3 sensor and Polar® soft strap electrode underneath. Sensor straps were labelled alphanumerically according to the Polar® RS800CX monitors they were paired with (e.g. data transmitted from Sensor A was recorded using Monitor 1). **(c)** Partially complete (functioning) sensor strap, as viewed from above. The protective neoprene cap has been removed, showing the sensor and soft strap electrode underneath. **(d)** Partially complete (non-functioning) sensor strap, as viewed from above. The protective neoprene cap and sensor have been removed. The sensors were glued to the sensor contacts on the soft strap electrode using fast-drying adhesive (Loctite 422 Instant Adhesive; *Loctite*, Hertfordshire, UK). **(e)** Complete sensor strap, as viewed from below. Adhesive was placed along the neoprene border surrounding the electrode contact, leaving the electrode contacts adhesive-free and forming a seal for the electrode gel (Spectra 360; *Parker Laboratories*, Fairfield, New Jersey, USA), which was inserted under the sensor strap. Electrode gel saturated the fur in contact with the electrodes and optimised signal conductance of the skin.


**Figure 2.3.** The sensor strap apparatus used to transmit RR intervals of female grey seals deployed in the field. **(a)** A grey seal female with a sensor strap containing a Polar® H2/H3 sensor and Polar® soft strap electrode on the Isle of May, Scotland. The sensor transmits RR interval data to the Polar® RS800CX monitor (out of shot). Complete **(b)** and partially complete **(c-e)** sensor straps, post-retrieval, as viewed from above **(b-d)** and below **(e)**. *See Figure 2.2. for full description*.

sighting on the island with a pup, and retrieved during final late lactation captures before individuals departed the breeding colony. In the event a female departed before final capture, sensor straps would have fallen off during the annual moult, approximately two months after the breeding season. Early and late lactation captures were, on average, 14 days apart. Mean sensor strap deployment time was 12 days, ranging from 4 to 17 days, providing between 5.9 to 22.2 hours of RR interval data per individual (Table 2.1). Of the 15 females selected for study, seven females were part of a quadruple capture programme and were subjected to two additional mid-lactation captures approximately every 4 days (Table 2.1). Three females were subjected to an additional single capture to check sensor straps when they were suspected to be malfunctioning (*ID = 72448/9, 74323/4, 50216*) (Table 2.1). Three females departed before final capture (Table 2.1). 12 females were caught in early lactation, whereas three females were caught in late lactation in an effort to increase sample size towards the end of the breeding season (ID = *73736, 50216, 49745*) (Table 2.1). Successful weaning was observed in all but two females (ID = *50216, 49745*), whose sensor straps were removed before the end of lactation.

Females were immobilised with a mass-specific intramuscular dose of zolazepam-tiletamine (*Zoletil,* Virbac, UK) and handled according to the capture protocol outlined in Pomeroy *et al.* (2000). At each initial capture, sensor straps were positioned dorsally behind the right shoulder blade and glued to the fur using fast-drying adhesive (Loctite 422 Instant Adhesive: *Loctite*, Hertfordshire, UK). Adhesive was placed around the neoprene bordering the electrode contacts (Figure 2.2, Figure 2.3), leaving the electrode contacts adhesive-free. Electrode gel (Spectra 360: *Parker Laboratories*, Fairfield, New Jersey, USA) was inserted underneath the sensor straps to optimise conductance between the skin and the electrode contacts. Transmission between the monitors and the sensors, which were paired prior to the start of capture procedures, was checked before release. Sensor straps were retrieved at each final capture by cutting the fur glued to the neoprene. Capture procedures lasted 30 to 60 minutes, after which females were monitored until they had regained mobility. No pup desertions as a result of the capture protocol were observed. Sensor straps were approximately 30 x 4 x 0.5 cm and weighed less than 0.5% of total body mass (Figure 2.2, Figure 2.3). They did not appear to interfere with usual behaviour (e.g. resting, comfort movements, vigilance or

**Table 2.1.** Summary of capture procedures, observation days, and recordings for study females on the Isle of May, Scotland ($N = 15$).

| ID | Days after pup birth until initial capture | Number of captures | Days tagged | Days observed | $N_R$ | Duration of recordings (hours) |
|---|---|---|---|---|---|---|
| 49450 | 1 | 4 | 16 | 4 | 11 | 6.50 |
| 58038 | 1 | 4 | 11 | 7 | 43 | 20.63 |
| 5B | 0 | 4 | 16 | 7 | 17 | 7.55 |
| 6L | 1 | 4 | 15 | 6 | 17 | 9.01 |
| 72159 * | 3 | 1 | 15 | 7 | 38 | 13.81 |
| 72448/9 † | 2 | 3 | 17 | 6 | 33 | 11.04 |
| 72900 * | 6 | 1 | 11 | 4 | 15 | 5.94 |
| 73736 * | 16 | 1 | 5 | 3 | 27 | 10.30 |
| 73743/4 | 7 | 2 | 11 | 6 | 27 | 9.30 |
| 74323/4 † | 4 | 3 | 11 | 5 | 32 | 11.85 |
| 50216 † | 8 | 3 | 8 | 6 | 31 | 16.05 |
| 49745 | 21 | 2 | 4 | 4 | 32 | 9.28 |
| OH | 2 | 4 | 14 | 5 | 26 | 17.36 |
| OJ | 1 | 4 | 15 | 12 | 38 | 22.20 |
| PFT | 2 | 4 | 17 | 7 | 44 | 20.43 |
| **Total** | - | 44 | 186 | 89 | 431 | 191.26 |
| **Mean** | 5.00 | 2.93 | 12.40 | 5.93 | 28.73 | 12.75 |
| **S.E.** | 1.56 | 0.32 | 1.08 | 0.55 | 2.62 | 1.39 |

** Departed before final capture. † Subjected to additional capture to check sensor strap. Days tagged refers to the number of days individuals were mounted with sensor straps, including the day sensor straps were attached. Days observed refers to the number of days females were subjected to behavioural observation, not the number of days females were observed on the breeding colony. $N_R$ = number of recordings.*

aggressive interactions with neighbours), nor did females make conspicuous efforts to remove the sensor straps. The Polar® monitors, which record RR intervals and instantaneous HR transmitted by the sensors, were not deployed with the sensor straps due to memory constraints. Assuming an average HR of 70 bpm, the monitors only had the capacity to store 40.5 hours. Instead, they were deployed concurrently during periods of behavioural observation. The monitors were mounted onto 1 m wooden stakes, positioned within 20 m of the focal female (Figure 2.4), and checked every 30 to 60 minutes. Females showed no adverse behavioural reactions indicating acute stress (e.g. locomotion or aggressive displays) towards the deployed monitors or when a researcher quietly approached the monitors, but typically increased vigilance before returning to (apparent) rest. RR interval data stored on the monitors were downloaded to a laptop using the Polar® IrDA USB adapter and Polar® ProTrainer 5 software. Recordings were visually examined and pre-processed in Polar® ProTrainer 5, in that they were "trimmed" where it was evident the signal between the monitor and the sensor had either been lost (Figure 2.5a) or corrupted (Figure 2.5b).

**Figure 2.4.** A female grey seal with a sensor strap on the Isle of May, Scotland. The Polar® RS800CX monitor, which is recording RR intervals transmitted by the Polar® H2/H3 sensor in the sensor strap, can be seen attached on the wooden pole positioned to the left of the female.

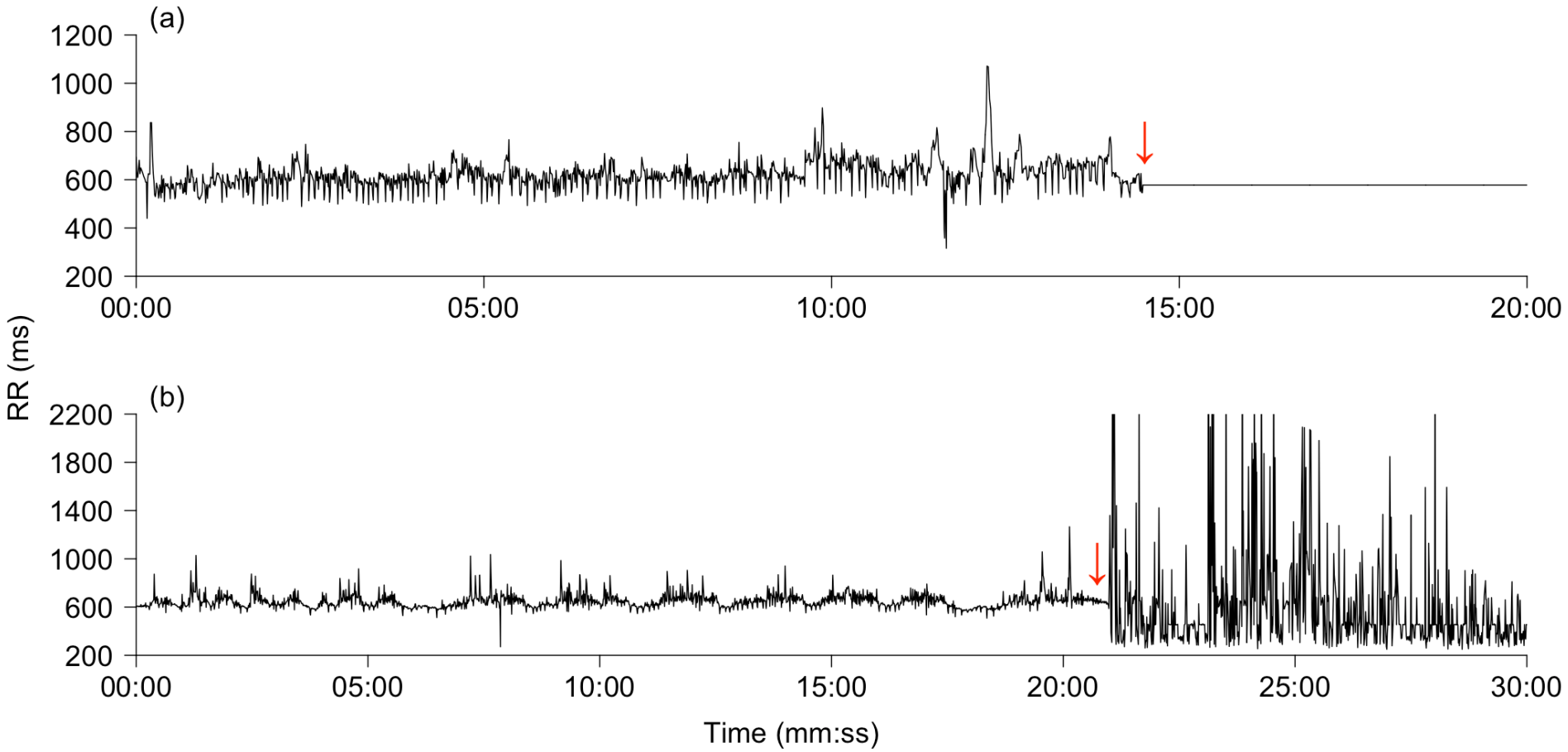


**Figure 2.5.** Examples of RR interval recordings before pre-processing "trimming" in Polar® ProTrainer 5 software, where it was evident the signal between the Polar® RS800CX monitors and Polar® H2/H3 sensors had either been **(a)** lost or **(b)** corrupted, as indicated by the red arrows. In these two examples, recordings would have been trimmed at **(a)** 14 minutes and **(b)** 21 minutes.

## 2.4 Measuring proxies of maternal reproductive performance

Females and their pups were weighed at each capture, as described in Pomeroy *et al.* (2000). Mass data were used to calculate three measures of maternal reproductive performance: (1) maternal daily mass loss rate (MDML, $kgday^{-1}$), (2) pup daily mass gain rate (PDMG, $kgday^{-1}$), and (3) maternal post-partum mass (MPPM, kg) (Pomeroy *et al.* 1999). Maternal expenditure can be measured using MDML; fitness outcomes of maternal expenditure can be measured using PDMG; and maternal transfer efficiency can be estimated as the proportion of MDML converted into PDMG (i.e. MDML:PDMG ratio or MPR). MPPM represents a standard reference point for female mass, and is an index of realised somatic growth and prior foraging success (Pomeroy *et al.* 1999). In general, females with a larger post-partum mass are able to expend more resources on their pup and attain higher pup growth rates (Pomeroy *et al.* 1999). From these measures of maternal reproductive performance, it was possible to calculate estimated mass loss on each day of observation as a percentage of MPPM (percentage mass loss, % kg), which was extrapolated from last known capture mass using MDML.

## 2.5. Behavioural data collection

The behaviour of each female, once mounted with a sensor strap, was recorded throughout lactation using 6 to 8 hour focal videos on alternating days until the sensor strap was retrieved. At the start of each focal video, camera times were synchronised to the deployed monitor, allowing for behavioural data to be matched with the corresponding RR interval data (± 1 second). Each female was observed for an average of 6 days (Table 2.1). Behavioural states from focal videos were extracted *post-hoc* using an Excel VBA-implemented ethogram, which were then grouped into five broader behavioural categories used for data processing and analyses: Rest, Vigilance, Comfort Movement, Locomotion, Aggression, and Mother-Pup Interaction (Table 2.2) (see *Chapter 2.9. Segment filtration and correction*).

**Table 2.2.** Ethogram used for breeding female grey seals on the Isle of May, Scotland.

| Category | Behaviour | Description |
|---|---|---|
| Rest | Rest | Female is motionless and lying prone or supine. Head is in contact with the ground. Eyes can be open or closed. |
| Comfort Movements | Comfort movement | Female performs low-intensity activities such as weight shifting, scratching, stretching or rolling. Often performed during *Rest*. |
| Vigilance | Alert | Female elevates head and upper body with the eyes open, looking in several directions or in one particular direction. |
| | Pup check | Female elevates head and upper body with the eyes open, as in *Alert*, but gaze is directed towards her pup. |
| Locomotion | Locomotion | Female travels in a particular direction, moving on her ventral or lateral surface using her fore- and hind-flippers. |
| | Re-orientate | Female moves "on the spot", using her fore- and hind-flippers to change her orientation without traveling in any particular direction. |
| | Explore | Female uses her nose, mouth or fore-flipper to investigate and/or manipulate the substrate and/or an object (e.g. a rock). |
| Aggression | Open mouth threat | Female opens her mouth and bares her teeth at a potential threat. Often done in conjunction with *Vocalisation*. |
| | Vocalisation | Female opens her mouth and makes a "growling" or "wailing" sound. Often done in conjunction with *Open mouth threat*. |
| | Slap | Female lies on her side and repeatedly slaps her flank with her fore-flipper. |
| | Aggressive flippering | Female rapidly moves her fore-flipper up and down in a "raking" motion at a potential threat. Females often flipper the air, but can also physically flipper an opponent (e.g. on the neck or head). |
| | Scratch ground | Female scratches at the ground with fore-flippers. |
| | Lunge | Female extends her head and neck rapidly towards an opponent without making physical contact. |
| | Bite | Female extends her head and neck rapidly towards an opponent, as in *Lunge*, but makes physical contact with her teeth with a biting action. |
| | Scrap | Female physically "grapples" with an opponent, using any combination of aggressive behaviours (typically *Aggressive flippering, Lunge* and/or *Bite*) in quick succession. |

*Taken and adapted from* Culloch (2012).

**Table 2.2.** Ethogram used for breeding female grey seals on the Isle of May, Scotland (continued).

| Category | Behaviour | Description |
|---|---|---|
| Mother-Pup Interactions | Nosing | Female makes physical contact with her pup, nose-to-nose or nose-to-body. Nostrils can be seen to flare. Often performed in conjunction with *Pup check*, when the pup is in close proximity. |
| | Pup flippering | Female uses her fore-flipper to lightly stroke and/or scratch her pup, often before and after *Nursing* or during *Play*. Female will also flipper her pup in a more rapid or "aggressive" manner to encourage it to move. |
| | Play | Female uses a combination of behaviours to interact with her pup, such as flippering, nosing and "gentle" biting, in quick succession. |
| | Present | Female lies on her side and "postures" so the pup has access to the nipples. Pup must be within 1 body length of the female (approximately 2 m). Often performed with *Pup flippering* to encourage the pup to suckle. |
| | Nipple nosing | Female is lying motionless and rolled onto her flank. Pup is visible and noses the nipple(s). Typically preludes *Nursing*. |
| | Nursing | Female is lying motionless and rolled onto her flank. Pup is visible and clearly suckling from a nipple. Time spent moving between nipples after the first nursing bout is considered part of the nursing sequence. |

*Taken and adapted from* Culloch (2012).

## 2.6. Calculating metrics of HRV

HRV was evaluated using time-domain analyses (Table 2.3) (Task Force 1996). Comparison of HRV metrics from recordings that differ in their duration is considered inappropriate (Task Force 1996; Kamath *et al.* 2012). However, discrepancies during monitor deployments in the field were unavoidable. RR interval recording durations, once they had been pre-processed and trimmed, ranged from 3.2 to 61.3 minutes. HRV analyses were therefore performed on successive segments extracted from each recording that were a standardised length of 300 RR intervals. Shorter segments are often more stationary and reliable, and allow analysis of progressive changes in short-term (i.e. high-frequency) changes of HRV (Kamath *et al.* 2012). Numerous studies have demonstrated that analysing "short-term" recordings (i.e. 5 minutes) produces results comparable to, or even better than, "long-term" recordings (i.e. 24 hours) (Task Force 1996; von Borell *et al.* 2007). Since segments extracted for analysis were short-term in nature (ranging from 1.7 to 7.2 minutes) and short-term time-domain measures of HRV are highly correlated (Task Force 1996; Kamath *et al.* 2012), only the root mean square of successive beat-to-beat differences (RMSSD, ms) is reported (Table 2.3).

**Table 2.3.** Common time-domain measures of HRV.

| Variable | Units | Variability Estimation (Frequency) | Description |
|---|---|---|---|
| SDNN | ms | Long-term (LF) | Standard deviation of all RR intervals. |
| SDANN | ms | Long-term (LF) | Standard deviation of the averages of RR intervals in all 5 min segments of the entire recording. |
| RMSSD | ms | Short-term (HF) | The root mean square of successive differences between RR intervals. |
| SDNN index | ms | Long-term (LF) | Mean of the standard deviations of all RR intervals for all 5 min segments of the entire recording. |
| SDSD | ms | Short-term (HF) | Standard deviation of differences between adjacent RR intervals. |
| NN50 count | – | Short-term (HF) | Number of pairs of adjacent RR intervals differing by ≥ 50 ms in the entire recording. |
| pNN50 | % , Ratio | Short-term (HF) | NN50 count divided by the total number of all RR intervals. |

*Taken and adapted from* Task Force (1996). *HF = high frequency. LF = low frequency.*

The RMSSD was chosen as it has better statistical properties and less sensitive to varying recording durations than other time-domain measures of HRV, such as the NN50 or pNN50 (Table 2.3) (Task Force 1996; Kamath *et al.* 2012). Furthermore, RMSSD values from "ultra short-term" recordings (i.e. less than 5 minutes) have been shown to be a reliable measure of HRV (Nussinovitch *et al.* 2011; Salahuddin et al. 2007) and consistent with measures taken from short-term segments (Thong *et al.* 2003). Average HR (bpm) of each segment is also reported.

## 2.7. Artefact detection

The importance of accounting for artefacts in RR interval data cannot be underestimated, as just a single spurious or missed beat can significantly bias the outcome of HRV analyses (Cheung 1981; Malik and Camm 1995; Berntson *et al.* 1990; Berntson and Stowell 1998; Berntson *et al.* 1997; Salo *et al.* 2001; Storck *et al.* 2001; Wilson 2001). Artefacts have many origins, both intrinsic (e.g. stress-induced arrhythmias, noise from muscle action potentials) and extrinsic (e.g. electromagnetic interference, equipment malfunction), which generate spurious beats that cause large deviations in or between RR intervals (Berntson *et al.* 1997; von Borell *et al.* 2007). Conventional artefact detection is performed manually to a very high standard and involves the assessment of every QRS complex from ECGs, since it is unknown how precise editing should be to overcome biases caused by artefacts (Task Force 1996). However, manual detection can introduce a large degree of inconsistency as it relies on subjective judgements (Berntson *et al.* 1997; Berntson and Stowell 1998). Manual detection also becomes less reliable and logistically demanding with large data sets or long-term

recordings, particularly where there is a high level of basal variability (Berntson *et al.* 1997; Berntson and Stowell 1998). Under these circumstances, the incorporation of an automated detection procedure is favourable, as they are replicable and reduce the need for arbitrary decisions (Berntson *et al.* 1997 Berntson and Stowell 1998). However, automated detection procedures should not replace manual detection entirely, as they are known to behave unsatisfactorily when left unchecked (Task Force 1996). A combination of both manual and automated approaches can be considered best practise for large data sets and/or long-term recordings (Berntson *et al.* 1997; Berntson and Stowell 1998).

Since Polar® monitors only record RR intervals, there is no absolute method for detecting artefacts in Polar® recordings after data collection. Validation studies comparing ECGs and Polar® devices have shown there are eight types of artefacts commonly found in Polar® recordings (Marchant-Forde *et al.* 2004; Jonckheer-Sheehy *et al.* 2012). Artefact Types 1–5 are visually represented by anomalous spikes and troughs (ranging from 1–3 RR intervals in length), whereas Types 6–8 are visually represented by flat lines or slopes (ranging from 3–8 RR intervals in length). Unlike Type 1–5 artefacts, Type 6–8 artefacts cannot be distinguished from one another graphically, as they are characterised by invariable sequences that differ in the number of RR intervals recorded. Without a corresponding ECG, it is not possible to determine if an invariable sequence is identical in length (Type 7), anomalously short (Type 8 – i.e. due to a missed beat), or anomalously long (Type 6 – i.e. due to a spuriously detected beat) (Jonckheer-Sheehy *et al.* 2012). It was assumed all invariable sequences were representative of Type 7 artefacts; detection was therefore limited to Type 1–5 and Type 7 artefacts. An additional distinction was made for Type 7 artefacts that were visually represented by a flat line (Type 7a) or a slope (Type 7b). Collectively, these seven artefact types were classified as "Peaks" (Type 1–5), "Flats", (Type 7a), and "Stairs" (Type 7b) (Figure 2.6).

Although software to process RR interval data for HRV analysis (e.g. *Kubios*: Tarvainen *et al.* 2014; *ARTiiFACT*: Kaufmann *et al.* 2011; and *RHRV*: Mendez *et al.* 2014) and distribution-based detection algorithms (e.g. Berntson *et al.* 1990; Linden and Estrin 1988) are widely available and able to accommodate Polar® data, preliminary processing revealed they were unable to reliably identify artefacts known to occur in Polar® recordings – namely Flats

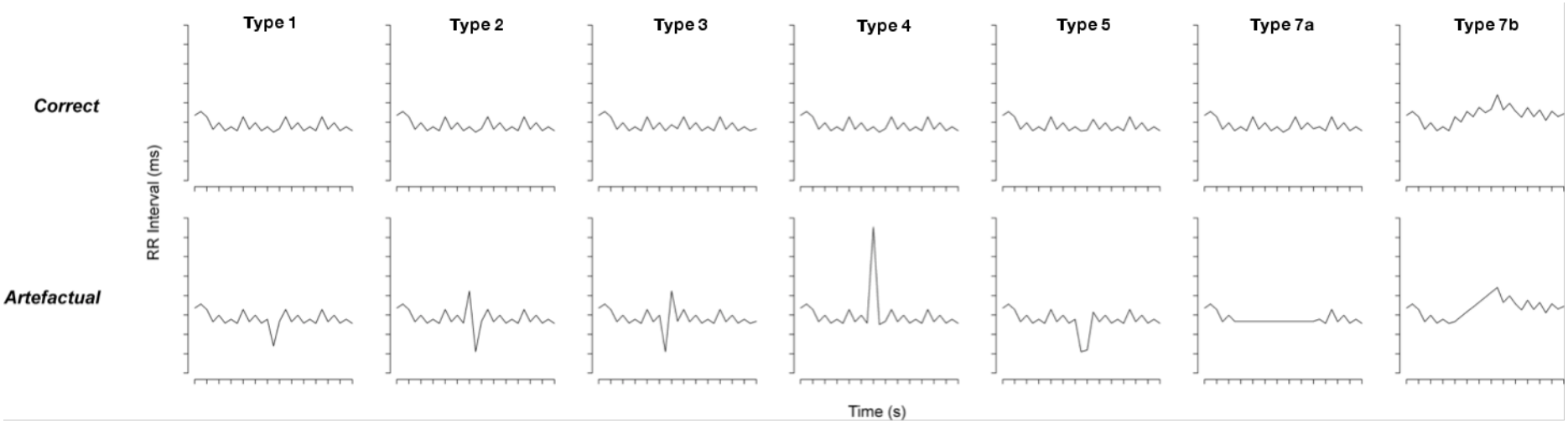


**Figure 2.6.** Common artefacts in Polar® RR interval recordings. Type 1 to 5 artefacts are categorised as “Peaks”. Type 1 artefacts are single point discrepancies that can be either positive or negative. Type 2 artefacts are characterised by a long RR interval (i.e. a “spike”) followed by a compensatory short RR interval (i.e. a “trough”). Type 3 artefacts are similar to Type 2 artefacts, except they are characterised by a short RR interval followed by a compensatory long RR interval. Type 4 artefacts are characterised by 2–3 extremely long RR intervals, often two or three times longer than the surrounding RR intervals. Type 5 artefacts are characterised by a flat-bottomed trough, consisting of two short RR intervals. Type 7a and Type 7b artefacts are categorised as “Flats” and “Stairs”, respectively. Type 7a artefacts occur when an RR interval is the same as the interval preceding it. Type 7b artefacts occur when the difference between successive intervals is the same. *Taken and adapted from* Marchant-Forde *et al.* (2004) *and* Jonckheer-Sheehy *et al.* (2012).

and Stairs. Instead, recordings were subjected to automated detection criteria derived from Cheung (1981), which has been validated for use on Polar® data (Marchant-Forde *et al.* 2004).

Briefly, each RR interval ($RR_n$) was compared to the preceding ($RR_{n-1}$) and the following ($RR_{n+1}$) interval. RR intervals were categorised as Flats (Type 7a) when they were the same as the preceding interval ($RR_n = RR_{n-1}$). RR intervals were categorised as Stairs (Type 7b) when the relative difference from the preceding ($D_{n-1} = RR_n - RR_{n-1}$) and following ($D_{n+1} = RR_{n+1} - RR_n$) intervals were the same ($D_{n-1} = D_{n+1}$). RR intervals were categorised as Peaks (Type 1–5) when the absolute percentage difference from a preceding interval ($DP_{n-1} = (D_{n-1} / RR_{n-1}) \times 100$) was greater than, or equal to, a critical percentage, $C$ ($DP_{n-1} \geq C$). Critical percentages reported in the literature differ depending on the study species, ranging from 20% (pigs; Marchant-Forde *et al.* 2004) to 30% (human infants: Schechtman *et al.* 1998). However, there are some difficulties obtaining references for critical percentages for pinnipeds.

Phocid seals are capable of abrupt and profound changes in HR, which help them cope with the extreme physiological demands of diving (Kooyman *et al.* 1981; Cummings *et al.* 2015). At the onset of apnoea (breath-holding), seals exhibit a strong, vagally-induced bradycardia known as the dive response (Greaves *et al.* 2004; Lapierre *et al.* 2004). This occurs not only at sea, but also on land – following a characteristic pattern of bradycardia during apnoea and tachycardia during eupnoea (regular breathing) (Castellini *et al.* 1994a, 1994b; Andrews *et al.* 1997; Falabella *et al.* 1999). Although these cardiorespiratory patterns have been studied extensively (Scholander 1940; Kooyman and Campbell 1972; Thompson and Fedak 1993; Castellini *et al.* 1994a, 1994b; Andrews *et al.* 1997; Falabella *et al.* 1999; Lapierre *et al.* 2004), data are often based on recordings where HR is sampled discretely (e.g. every 2–30 seconds) or instantaneous HR is derived from RR intervals and presented as an average across periods of behavioural interest (e.g. apnoea, eupnoea). Values for changes between successive RR intervals themselves are not explicitly reported. Consequently, these data are not entirely suitable for determining appropriate critical percentages.

Recordings of instantaneous HR by Thompson and Fedak (1993) demonstrated grey seals are capable of decreasing their HRs from above 100 bpm to well below 20 bpm within several heartbeats; estimates from figures suggest the greatest change between successive heartbeats was 66%. It was assumed changes of this magnitude represented an upper limit and

therefore unlikely to occur in this study, as such extreme bradycardia is typically only observed during prolonged dives (Kooyman *et al.* 1981; Hindell and Lea 1998). Apnoeic bradycardia in northern elephant seals, for example, is not only more profound when diving, but also more abrupt (Andrews *et al.* 1997). HRs of northern elephant seals have been observed decreasing 50-80% from surface rates immediately following submersion, but only 31% from eupnoeic rates gradually at the onset of terrestrial apnoeas.

For the purposes of this study, a critical percentage of 20% was chosen. Since Peaks are defined as anomalous spikes and troughs of variable magnitudes (Figure 2.6), larger critical percentages permit more data, but have a higher likelihood of missing artefacts. Smaller critical percentages are more restrictive, but have a higher likelihood of detecting artefacts. However, they may also have a higher likelihood of false alarms and exclude data that contain high levels of basal variability. Given the preliminary nature of the study, priority was given to minimising the likelihood of missed artefacts. Although the chosen critical percentage may have been conservative for phocid seals, it was not overly restrictive and retained 94.4% of all recorded RR intervals (*N* = 578,568). Furthermore, critical percentages greater than 20% did not cause a marked increase in the number of retained RR intervals (Figure 2.7) at the presumed cost of a higher likelihood of missed artefacts.

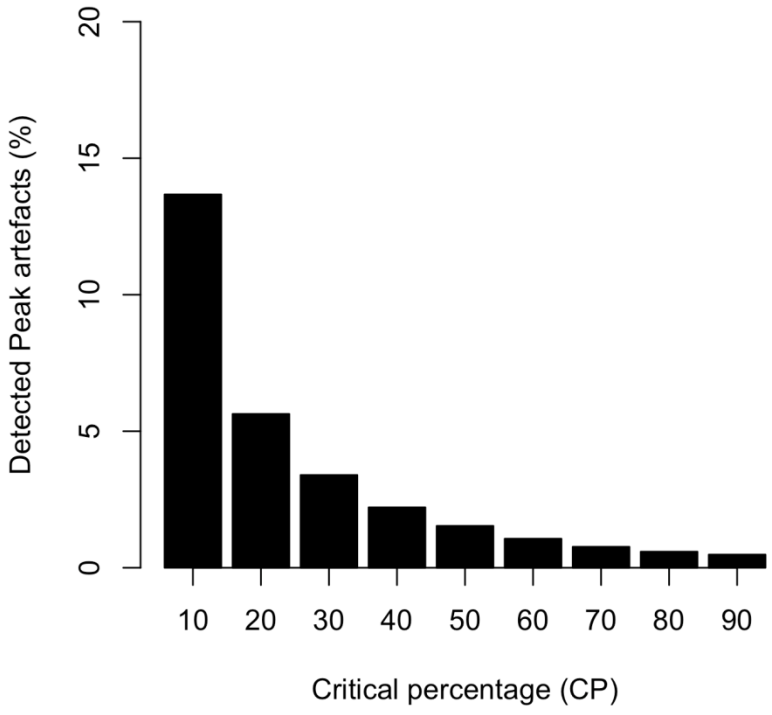


**Figure 2.7.** The percentage of RR intervals detected as Peak artefacts across different critical percentages (*CP*). Total *N* of RR intervals recorded = 578,685.

All recordings were subjected to the automated detection criteria and additional recommended checks by Schechtman *et al.* (1988) to ensure genuine physiological changes in RR intervals were not detected as artefactual. Overall, the artefact detection procedure was used to determine the quality of segments used for analyses (see *Chapter 2.9. Segment filtration and correction*).

## 2.8. Data processing summary

All recordings were processed as described below using *R* 3.0.3 (R Core Development Team 2013). First, recordings were matched with mass data, such that percentage mass loss on the day of observation for each recording was known (see *Chapter 2.4. Measuring proxies of maternal reproductive performance*). RR interval data from each recording were then paired with behavioural data, such that each interval was assigned a behavioural category (see *Chapter 2.5. Behavioural observation*). From these paired data, it was possible to extract the discrete segments of a standardised length (300 RR intervals) required for HRV analyses (see *Chapter 2.6. Calculating metrics of HRV*). Extracted segments were then subjected to the automated detection criteria described in *Chapter 2.7. Artefact detection*, such that the percentage of Flat, Stair, and Peak artefacts present in each segment was known.

Frequency distributions of RMSSD and HR in extracted segments revealed outliers containing extreme values upward of 900 ms and 800 bpm. These outliers were beyond the 95$^{th}$ and 99$^{th}$ percentiles for RMMSD (161 ms) and HR (135 bpm), respectively. Segments containing RMSSD or HR values greater than 161 ms or 135 bpm were therefore presumed erroneous and removed from the data set. Although there are no representative data on RMSSD for pinnipeds, the threshold for HR was conservative, given that the highest reported HR for adult grey seals swimming both at sea and in a flume is 120 bpm (Fedak *et al.* 1988). Once outlier segments had been removed, the remaining extracted segments (herein referred to as *Unfiltered* segments) were subject to filtration and correction before being used for statistical analyses.

## 2.9. Segment filtration and correction

Since artefacts are known to bias HR and HRV metrics derived from RR interval data (Task Force 1996), two Linear Mixed Models (LMMs) were used to quantify the effect of Flat, Stair, and Peak artefacts on RMSSD and HR in Unfiltered segments (for details on LMM

structure, fitting, and selection, see *Chapter 2.11. Statistical analysis*). A series of iterative LMMs were then used to determine the maximum permissible percentage of each artefact type within Unfiltered segments – i.e. the percentage at which Flats, Stairs, and Peaks could no longer be used to predict RMSSD or HR (see *Chapter 3.2. Determining filtration thresholds and obtaining parameter estimates for correction)*. Segments containing artefact percentages beyond these thresholds were then filtered out of the data set and excluded from analysis. The maximum permissible percentage for Flats or Stairs was 5% (see *Chapter 3.2. Determining filtration thresholds and obtaining parameter estimates for correction*). Threshold percentages for Peaks could not be determined, as they shared a strong linear relationship with RMSSD and HR. Peaks exerted a strong effect, even when present in small percentages, which could not be compensated for with filtration. Instead, the effect of Peaks in each segment was corrected for using parameter estimates obtained from LMMs quantifying the effect of artefacts on RMSSD and HR using segments containing less than 5% Flats or Stairs (herein referred to as *Filtered* segments) (see *Chapter 3.2. Determining filtration thresholds and obtaining parameter estimates for correction*). Corrected RMSSD ($RMSSD_{COR}$) and HR ($HR_{COR}$) were then calculated by:

$$RMSSD_{COR} = RMSSD - (E_R \times P)$$

$$HR_{COR} = HR - (E_H \times P)$$

Where $E$ = the parameter estimates for Peaks obtained from LMMs predicting RMSSD ($E_R$) or HR ($E_H$) and $P$ = the percentage of Peaks in each segment. All subsequent analyses requiring RMSSD and HR have been corrected for Peak artefacts, unless stated otherwise.

Baseline metrics were obtained from Filtered segments corrected for Peaks, where more than 90% of intervals were spent in Rest and confounding behaviours (i.e. Locomotion, Aggression) were absent (herein referred to as *Baseline* segments). A summary of data processing, filtration, and correction is provided in Figure 2.8.

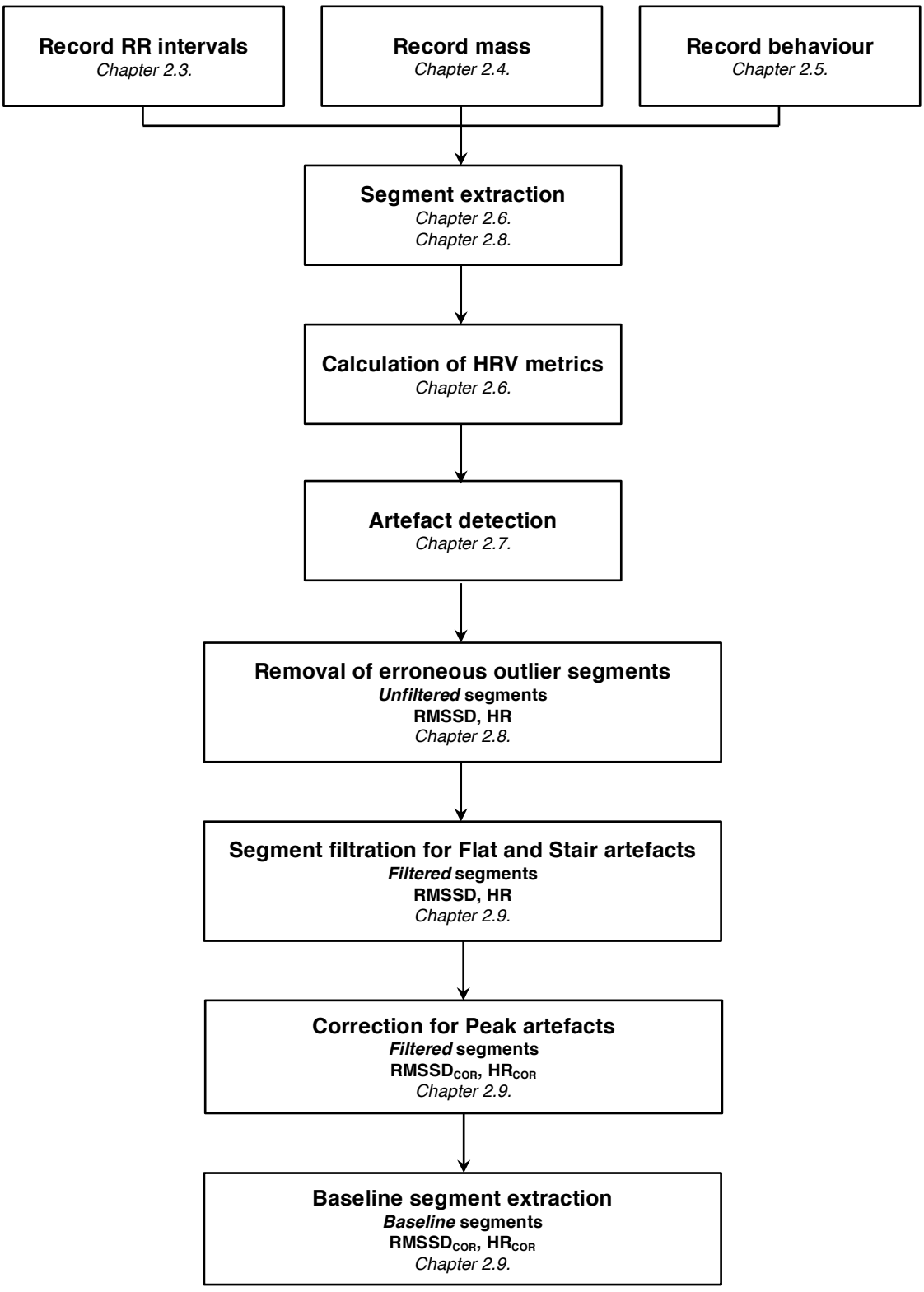


**Figure 2.8.** Summary of data collection, processing, filtration, and correction. Details are provided in the referenced chapters. *Filtered* segments are segments containing less than 5% Flats or Stairs artefacts. *Baseline* segments are segments where more than 90% of intervals were spent in Rest and confounding behaviours were absent (i.e. Locomotion, Aggression). $RMSSD_{COR}$ = RMSSD corrected for Peak artefacts. $HR_{COR}$ = HR corrected for Peak artefacts.

## 2.10. Summary of data available for analysis

2615 segments were extracted, of which 550 passed through filtration (Table 2.4). Whilst segments were extracted for all study females (*N* = 15), only 13 had Baseline segments (Table 2.4). Of those 13 females, a further two were excluded from analyses requiring baseline RMSSD and HR (*N* = 11, Baseline[1]: Table 2.4), as one female nursed three pups for the duration she spent ashore on the breeding colony (ID = *OH*) and another female had an insufficient number of Baseline segments (ID = *49450*: Table 2.4). Final capture data were missing for three females (ID = *72159, 73736, 49745*) and three pups (Mother ID = *73736, 74323/4, 49745*) due to early departure of females from the breeding colony. These females were therefore excluded from analyses incorporating baseline RMSSD and HR with percentage mass loss (*N* = 9, Baseline[2]: Table 2.4, Table 2.5). They were also excluded from analyses requiring measures of maternal reproductive performance alone (e.g. MDML: *N* = 11), combined (e.g. MPR: *N* = 10), or incorporated with baseline RMSSD and HR (*N* = 8).

**Table 2.4.** Summary of recordings and segments from study females on the Isle of May, Scotland (*N* = 15).

| ID | Unfiltered (*N* = 15) | | | Filtered (*N* = 14) | | | Baseline (*N* = 13) | | | Baseline[1] (*N* = 11) | | | Baseline[2] (*N* = 9) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_R$ | $N_S$ | Duration (h) | $N_R$ | $N_S$ | Duration (h) | $N_R$ | $N_S$ | Duration (m) | $N_R$ | $N_S$ | Duration (m) | $N_R$ | $N_S$ | Duration (m) |
| 49450 * † | 2 | 29 | 1.34 | 1 | 7 | 0.33 | 1 | 2 | 5.47 | - | - | - | - | - | - |
| 58038 | 16 | 188 | 10.50 | 14 | 80 | 4.39 | 8 | 25 | 81.59 | 8 | 25 | 81.59 | 8 | 25 | 81.59 |
| 72159 | 25 | 274 | 16.82 | 13 | 46 | 3.00 | 5 | 8 | 33.59 | 5 | 8 | 33.59 | - | - | - |
| 7290 | 11 | 57 | 3.56 | 4 | 5 | 0.38 | 3 | 3 | 15.08 | 3 | 3 | 15.08 | 3 | 3 | 15.08 |
| 73736 † | 24 | 261 | 12.31 | 4 | 6 | 0.30 | 3 | 5 | 15.24 | 3 | 5 | 15.24 | - | - | - |
| 5B | 4 | 38 | 2.15 | 2 | 10 | 0.60 | 2 | 8 | 28.94 | 2 | 8 | 28.94 | 2 | 8 | 28.94 |
| 6L † | 8 | 86 | 5.52 | 8 | 57 | 3.65 | 3 | 7 | 27.45 | 3 | 7 | 27.45 | 3 | 7 | 27.45 |
| 72448/9 | 25 | 197 | 11.88 | 9 | 31 | 2.11 | 7 | 19 | 80.11 | 7 | 19 | 80.11 | 7 | 19 | 80.11 |
| 73743/4 | 24 | 199 | 11.67 | 5 | 7 | 0.36 | 3 | 5 | 14.85 | 3 | 5 | 14.85 | 3 | 5 | 14.85 |
| 74323/4 | 26 | 256 | 14.18 | 20 | 137 | 7.76 | 14 | 66 | 228.24 | 14 | 66 | 228.24 | 14 | 66 | 228.24 |
| 50216† | 30 | 373 | 18.42 | 8 | 21 | 0.99 | 4 | 10 | 29.70 | 4 | 10 | 29.70 | 4 | 10 | 29.70 |
| 49745 * † | 27 | 222 | 12.75 | 8 | 30 | 1.83 | - | - | - | - | - | - | - | - | - |
| OH * † | 19 | 137 | 7.81 | 12 | 31 | 1.78 | 9 | 18 | 61.44 | - | - | - | - | - | - |
| OJ * † | 2 | 5 | 0.29 | - | - | - | - | - | - | - | - | - | - | - | - |
| PFT | 24 | 293 | 17.24 | 12 | 82 | 4.88 | 11 | 30 | 104.55 | 11 | 30 | 104.55 | 11 | 30 | 104.55 |
| **Total** | 267 | 2615 | 146.44 | 120 | 550 | 32.36 | 73 | 206 | 726.22 | 63 | 186 | 659.32 | 55 | 173 | 610.51 |
| **Mean** | 17.8 | 174.33 | 9.76 | 8.57 | 39.29 | 2.31 | 5.62 | 15.85 | 55.87 | 5.73 | 16.91 | 59.94 | 5.50 | 17.30 | 67.83 |
| **S.E.** | 2.54 | 28.67 | 1.55 | 1.41 | 10.26 | 0.59 | 1.08 | 4.83 | 16.72 | 1.17 | 5.59 | 19.27 | 1.39 | 6.23 | 22.85 |

** Females excluded from analyses requiring baseline RMSSD and HR. † Females excluded from ICC analyses. N = number of individuals. $N_R$ = number of recordings. $N_S$ = number of segments. Baseline[1] = Baseline segments for all females, irrespective of available mass data. Baseline[2] = Baseline segments for females with available mass data.*

**Table 2.5.** Measures of maternal reproductive performance and mean baseline RMSSD and HR for study females on the Isle of May, Scotland ($N$ = 15).

| ID | MDML | PDMG | MPPM | MPR | RMSSD (ms) | HR (bpm) |
|---|---|---|---|---|---|---|
| 49450 * † | 3.59 | 1.61 | 173.19 | 0.45 | 15.89 | 111.23 |
| 58038 | 4.23 | 2.33 | 211.63 | 0.55 | 34.23 | 97.85 |
| 72159 | - | 2.58 | - | - | 38.09 | 88.36 |
| 72900 † | 2.89 | 1.83 | 160.75 | 0.63 | 55.97 | 73.75 |
| 73736 † | - | - | - | - | 44.52 | 105.17 |
| 5B | 2.67 | 1.34 | 168.00 | 0.50 | 43.35 | 90.42 |
| 6L † | 4.00 | 1.63 | 255.60 | 0.41 | 26.75 | 91.77 |
| 72448/9 | 3.75 | 2.19 | 190.71 | 0.58 | 49.06 | 84.15 |
| 73743/4 | 3.20 | 1.53 | 149.00 | 0.48 | 45.62 | 106.99 |
| 74323/4 | 4.86 | - | 179.82 | - | 40.20 | 93.71 |
| 50216 † | 2.45 | 1 | 132.60 | 0.41 | 31.41 | 106.40 |
| 49745 * † | - | - | - | - | - | - |
| OH * † | 4.77 | 1.53 | 183.34 | 0.32 | 34.61 | 90.45 |
| OJ * † | 3.45 | 1.84 | 198.25 | 0.53 | - | - |
| PFT | 3.80 | 1.91 | 209.40 | 0.50 | 33.56 | 95.04 |

* *Females excluded from analyses requiring baseline RMSSD and HR.* † *Females excluded from ICC analyses. MDML = maternal daily mass loss rate* (kgday$^{-1}$)**.** *PDMG = pup daily mass gain rate* (kgday$^{-1}$)*. MPPM = maternal post-partum mass (kg). MPR = MDML:PDMG ratio.*

## 2.11. Statistical analysis

All data analyses were carried out using *R* 3.0.3. Since the primary objective of this study was to assess the suitability of Polar® RS800CX monitors and H2/H3 sensors for conducting HR and HRV analyses in grey seals, initial analyses focused on:

(1) Quantifying the impact of artefacts on HR and HRV (see *Chapter 2.9. Segment filtration and correction*).

(2) Developing artefact filtration and correction protocols to account for the impact of artefacts on HR and HRV (see *Chapter 2.9. Segment filtration and correction*).

(3) Identifying sources of artefacts.

(4) Determining the impact of data collection protocols.

Using data that had been filtered and corrected, subsequent analyses focused on quantifying inter-individual variation, repeatability, and reproductive performance correlates of baseline HR and HRV. The majority of analyses were conducted using a series of LMMs and Generalised Linear Mixed Models (GLMMs), described in detail below and summarised in Table 2.6.

**Table 2.6.** Summary of LMMs and GLMMs used for analyses.

| Model type | Dependent variable | *Segments* | *N* | $N_R$ | $N_S$ | Fixed effect(s) | Random effects |
|---|---|---|---|---|---|---|---|
| LMMs | RMSSD, HR | Unfiltered | 15 | 267 | 2615 | Flats, Stairs, Peaks | ID/HRM |
| | | Filtered | 14 | 120 | 550 | | |
| | $RMSSD_{COR}$, $HR_{COR}$ | Filtered | 14 | 120 | 550 | Flats, Stairs, Peaks | ID/HRM |
| | | Baseline | 13 | 73 | 206 | | |
| | | Baseline[1] | 11 | 63 | 186 | | |
| | | Baseline[2] | 9 | 55 | 173 | | |
| | $RMSSD_{COR}$, $HR_{COR}$ | Baseline[2] | 9 | 55 | 173 | DSC, Percentage Mass Loss, TIME | ID/HRM |
| | | Baseline[1] | 11 | 63 | 186 | DSC, DOL, TIME | |
| GLMMs | Flats, Stairs, Peaks | Unfiltered | 15 | 267 | 2615 | DSG, REST | ID/HRM |

*$N$ = number of individuals. $N_R$ = number of recordings. $N_S$ = number of segments. $RMSSD_{COR}$ = RMSSD corrected for Peak artefacts. $HR_{COR}$ = HR corrected for Peak artefacts. $N_s$ = number of segments. Baseline[1] = Baseline segments for all females, irrespective of available mass data. Baseline[2] = Baseline segments for females with available mass data. ID = individual identity. HRM = recording number. DSC = days since capture, where DSC 0 is the day of capture. Percentage Mass Loss = estimated mass loss (kg) on day of observation as a percentage of maternal post-partum mass (MPPM), extrapolated from last known capture mass using rate of maternal daily mass loss (MDML). TIME = time of day (AM/PM). DOL = day of lactation, where DOL 1 is the day the pup was born. DSG = days since electrode gel application, where DSG 0 is the day of application. REST = the proportion of RR intervals spent in Rest.*

LMMs and GLMMs were applied with the lmer() and glmer() functions, respectively, in the *lme4* package (Bates *et al.* 2014). Since multiple segments could be extracted from a single recording and multiple recordings were taken for each individual, female identity (ID) and recording number (HRM) were included as random effects for all LMMs and GLMMs (Bolker *et al.* 2008), wherein recording number was nested within individual identity. All LMMs and GLMMs were fitted using maximum likelihood and checked for assumptions of residual normality and homoscedasticity using Shapiro Wilk and Levene's tests, respectively (Bolker *et al.* 2008). Model selection followed AIC minimisation criteria, wherein models are excluded if they have a ΔAIC value greater than six, or have a ΔAIC value less than six but are more complex versions of their nested counterparts (Richards 2008). Models that met the minimisation criteria formed the "confidence set" (Richards 2008).

The probability level for significance was set at $p = 0.05$. Where appropriate, means are reported with standard errors of the mean (S.E.). With regards to sample sizes, *N* refers to the number of individual females, $N_R$ refers to the number of recordings, and $N_S$ refers to the number of segments.

#### 2.11.1. Assessing the suitability of Polar® devices for conducting HR and HRV analyses in grey seals

Potential sources of artefacts were assessed using GLMMs. The proportions of Flats, Stairs, and Peaks in Unfiltered segments were modelled in three separate GLMMs using a binomial error distribution and a logit link. Non-stationary behaviour and impaired electrode conductance are two of most common sources of artefacts (von Borell *et al.* 2007). Accordingly, fixed effects for GLMMs were the proportion of intervals spent in Rest (as a measure of stationary behaviour) and days since electrode gel application (DSG), where DSG 0 represents the day of application.

To determine whether sensor strap attachment and behavioural observation had any detrimental impacts on study females and/or their pups, proxies of maternal reproductive performance between study (i.e. captured and tagged) and non-study (i.e. captured but not tagged) females were compared using Mann-Whitney tests and Spearman's rank correlations. Kendall's rank correlations were also used to determine if there were any associations between

proxies of maternal reproductive performance and the number of days mounted with a sensor strap, or the number of days subjected to behavioural observation.

### 2.11.3. Quantifying inter-individual variation, repeatability, and reproductive performance correlates of baseline HR and HRV

To determine whether baseline $RMSSD_{COR}$ and $HR_{COR}$ exhibited significant inter-individual differences, Kruskal-Wallis tests were performed. Since baseline HR and HRV can be influenced by a variety of factors such as age, sex, body condition, diurnal rhythms, and severe stressors (e.g. capture or handling) (von Borell *et al.* 2007; Dickens and Romero 2009), two LMMs were run to quantify the effects of days since capture (DSC), percentage mass loss (see *Chapter 2.4. Measuring mass and proxies of maternal reproductive performance*), and time of day (TIME, AM/PM) on baseline $RMSSD_{COR}$ and $HR_{COR}$. Time of day was determined using the photoperiod midpoint of the day of observation. Since mass data were not available for all females with Baseline segments, additional LMMs with day of lactation (DOL) in place of percentage mass loss, where DOL 1 represents the day the pup was born, were run. Day of lactation was highly positively correlated with, and assumed to be a reliable proxy of, percentage mass loss (*Kendall's rank correlation*: tau = 0.756, *p*-value < 0.001).

Repeatability of baseline $RMSSD_{COR}$ and $HR_{COR}$ within the breeding season was assessed using a two-way random effects Intraclass Correlation Coefficient (ICC) (Bell *et al.* 2009), which considers both the individual and sampling intervals as random effects ($ICC_2$ in Shrout and Fleiss 1979). ICCs were performed using the ICC() function in the *psych* package (Revelle 2015). Of the 11 females with Baseline segments available for analyses (Table 2.4), four were excluded from repeatability analyses (ID = *6L, 72900, 73736, 50216*) as they only had Baseline segments from late lactation and/or a single day of observation ($N$ = 7: Table 2.4, Table 2.5). For each individual, mean baseline $RMSSD_{COR}$ and $HR_{COR}$ were taken from two separate days as far apart as possible, comprising "early" and "late" lactation samples. Since the interval between samples varied considerably across individuals, ranging from 3 to 14 days, an additional test was performed to determine whether the inter-sample interval influenced repeatability (Twiss *et al.* 2011). First, residuals were extracted from a linear regression using early lactation samples to predict late lactation samples. These residuals represented the degree of deviation from the line of best fit, and therefore, a measure of the difference in

individuals' mean baseline $RMSSD_{COR}$ and $HR_{COR}$ between early and late lactation. A second linear regression was then performed to determine whether these residuals showed any significant relationship with the inter-sample intervals.

To determine if baseline $RMSSD_{COR}$ and $HR_{COR}$ throughout lactation were correlated with proxies of maternal reproductive performance, Spearman's rank correlations were performed to test for associations between mean baseline $RMSSD_{COR}$ and $HR_{COR}$ with: (1) MDML (i.e. maternal expenditure); (2) PDMG (i.e. fitness outcomes); and (3) actual and (4) absolute residuals from a linear regression using MDML to predict PDMG (i.e. maternal transfer efficiency). Positive residuals imply efficient maternal transfer, as pups gain mass at a rate greater than would be expected at the rate of maternal mass loss (i.e. efficient mothers are able to conserve resources during lactation). Conversely, negative residuals imply less efficient maternal transfer, as pups gain mass at a rate lower than would be expected. The distinction between actual and absolute residuals was used to determine whether mean baseline $RMSSD_{COR}$ and $HR_{COR}$ were correlated with higher or lower maternal transfer efficiency (i.e. positive or negative deviation from the line of best fit) or overall variation in maternal transfer efficiency (i.e. absolute deviation from the line of best fit).

# Chapter 3: Results

## 3.1. Patterns observed in HR and HRV recordings

There were two main patterns observed in RR intervals recorded during rest: steady, variable HR, and episodes of bradycardia (Figure 3.1a–c). Resting RR intervals had a bimodal distribution that is typical of phocid seals (Figure 3.2), with peaks around 600 ms (100 bpm) and 1100 ms (55 bpm). The higher modal peak at 600 ms was approximately 1.8 times greater than the lower peak.

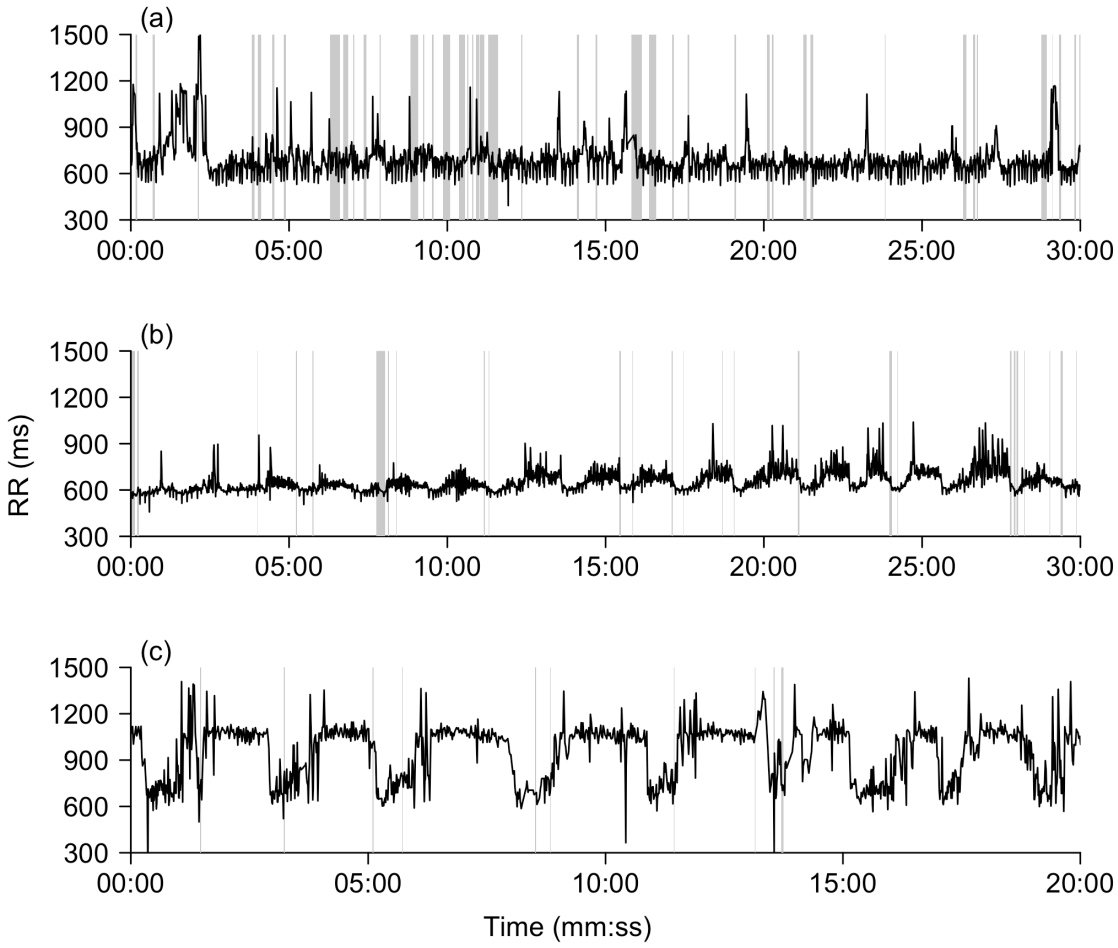


**Figure 3.1.** Examples of RR intervals recorded using Polar® devices from three different female grey seals on the Isle of May, Scotland during rest. The two main patterns observed were steady, variable HR **(a)**, and episodes of slight **(b)** and pronounced **(c)** bradycardia. Light grey blocks indicate periods of Vigilance.

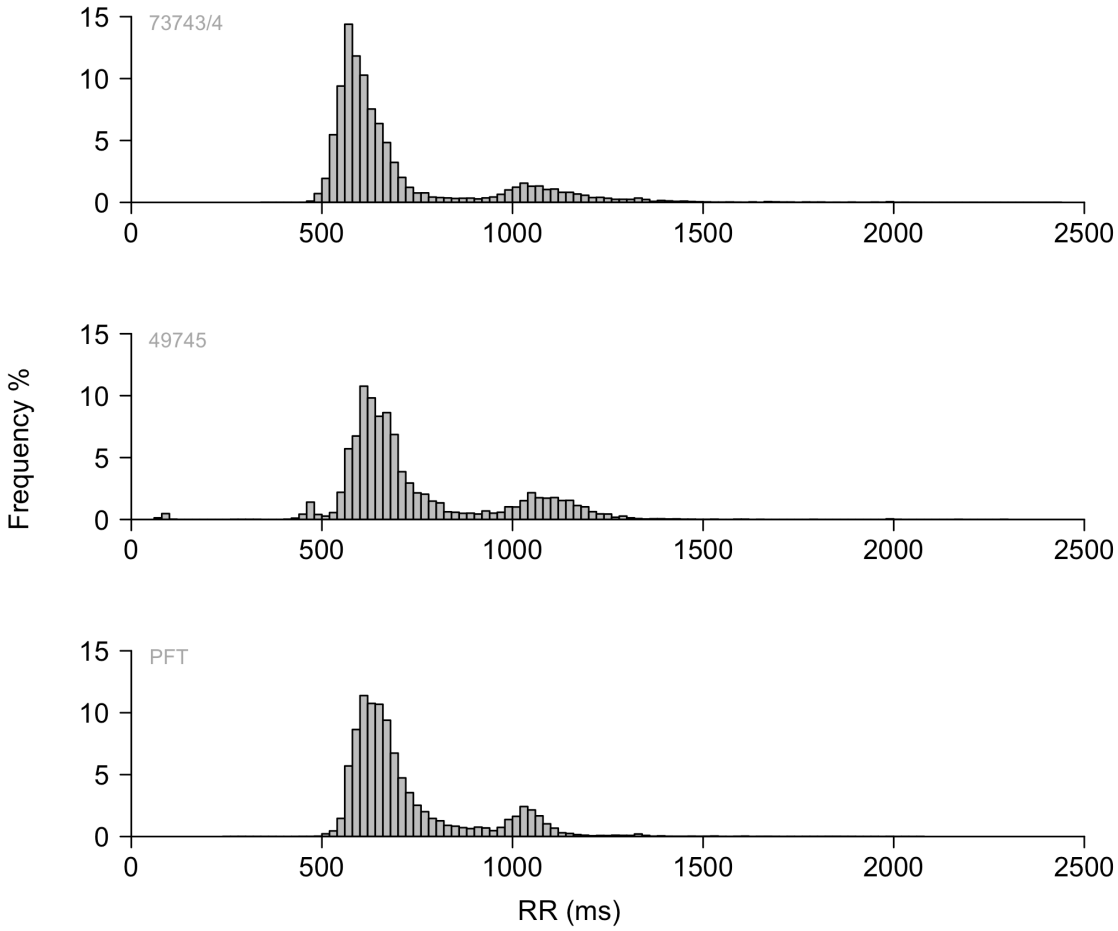


**Figure 3.2.** Examples of frequency distributions of resting RR intervals for three female grey seals on the Isle of May, Scotland. RR intervals have been collated from all available recordings for each female (73743/4: $N_R$ = 24, 5 days; 49745: $N_R$ = 30, 4 days; PFT: $N_R$ = 26, 5 days). Artefactual intervals have been removed.

## 3.2. Assessing the suitability of Polar® devices for conducting HR and HRV analyses in grey seals

### 3.2.1. The impact of artefacts on HR and HRV

Artefacts were widespread in Unfiltered segments – the majority of which (87.5%) contained all three artefact types (Table 3.1). RMSSD and HR were significantly effected by all three artefact types in Unfiltered segments (LMM (i): Table 3.2, Table 3.3). Stairs and Peaks increased RMSSD, whereas Flats decreased RMSSD (LMM (i): Table 3.2, Table 3.4, Figure 3.3). Artefacts had an opposite effect on HR than on RMSSD; Stairs and Peaks decreased HR, whereas Flats increased HR (LMM (i): Table 3.3, Table 3.5, Figure 3.4). Out of all the artefact types, Peaks had the strongest effects on RMSSD and HR (LMM (i): Table 3.4, Table 3.5). However, they did not affect RMSSD and HR equally. Effect sizes for Peaks were approximately seven times larger for RMSSD, suggesting RMSSD was far more sensitive to Peak artefacts than HR. Flats had similar effect sizes for both RMSSD and HR, whereas Stairs had a stronger effect on HR (LMM (i): Table 3.4, Table 3.5).

**Table 3.1.** The number and percentage of Unfiltered segments containing Flat, Stair, or Peak artefacts ($N_S$ = 2615).

| | $N_S$ | % |
|---|---|---|
| **Flats** | 0 | 0 |
| **Stairs** | 0 | 0 |
| **Peaks** | 0 | 0 |
| **Flats + Stairs** | 323 | 12.352 |
| **Flats + Peaks** | 4 | 0.153 |
| **Stairs + Peaks** | 1 | 0.038 |
| **Flats + Stairs + Peaks** | 2287 | 87.457 |
| ***Total*** | 2615 | 100 |

*$N_S$ = number of segments*

**Table 3.2.** Predictors of RMSSD as determined by LMMs, with recording number (HRM) nested within individual identity (ID) as random effects.

| Dependent variable | Segments | Fixed effects | Estimate | Standard error | *t* value | Pr(>\|z\|) |
|---|---|---|---|---|---|---|
| **RMSSD (ms)** | *(i) Unfiltered* | Intercept | 37.529 | 1.672 | 22.440 | **< 0.001*** |
| | | Flats | -0.373 | 0.043 | -8.624 | **< 0.001*** |
| | | Stairs | 0.113 | 0.043 | 2.600 | **0.009*** |
| | | Peaks | 7.489 | 0.123 | 60.944 | **< 0.001*** |
| | *(ii) Filtered* | Intercept | 41.416 | 2.851 | 14.529 | **< 0.001*** |
| | | Flats | 0.404 | 0.521 | 0.775 | 0.439 |
| | | Stairs | 0.302 | 0.459 | 0.657 | 0.511 |
| | | Peaks | 6.233 | 0.164 | 38.098 | **< 0.001*** |
| **$RMSSD_{COR}$ (ms)** | *(iii) Filtered* | Intercept | 41.420 | 2.850 | 14.529 | **< 0.001*** |
| | | Flats | 0.404 | 0.521 | 0.775 | 0.439 |
| | | Stairs | 0.302 | 0.459 | 0.657 | 0.511 |
| | | Peaks | 0.000 | 0.164 | 0.000 | 1.000 |
| | *(iv) Baseline[1]* | Intercept | 37.864 | 4.007 | 9.450 | **< 0.001*** |
| | | Flats | -0.607 | 0.781 | -0.777 | 0.438 |
| | | Stairs | 0.928 | 0.700 | 1.326 | 0.187 |
| | | Peaks | 0.182 | 0.235 | 0.773 | 0.441 |
| | *(v) Baseline[2]* | Intercept | 38.295 | 4.193 | 9.133 | **< 0.001*** |
| | | Flats | -0.674 | 0.798 | -0.844 | 0.400 |
| | | Stairs | 0.698 | 0.717 | 0.973 | 0.332 |
| | | Peaks | 0.196 | 0.248 | 0.789 | 0.431 |
| **$RMSSD_{COR}$ (ms)** | *(vi) Baseline[2]* | Intercept | 44.877 | 4.666 | 9.618 | **< 0.001*** |
| | | DSC | 0.723 | 0.637 | 1.136 | 0.266 |
| | | Percentage Mass Loss | -0.371 | 0.227 | -1.634 | 0.112 |
| | | TIME | -1.210 | 2.341 | -0.517 | 0.609 |
| | *(vii) Baseline[1]* | Intercept | 44.531 | 4.365 | 10.202 | **< 0.001*** |
| | | DSC | 0.602 | 0.580 | 1.039 | 0.306 |
| | | DOL | -0.570 | 0.372 | -1.533 | 0.133 |
| | | TIME | -0.369 | 2.248 | -0.164 | 0.870 |

** Asterisks indicate significant parameters. $RMSSD_{COR}$ = RMSSD corrected for Peak artefacts. Filtered = segments containing less than 5% Flat or Stair artefacts. Baseline[1] = Baseline segments for all females, irrespective of available mass data. Baseline[2] = Baseline segments for females with available mass data. DSC = days since capture, where DSC 0 is the day of capture. Percentage Mass Loss = estimated mass loss (kg) on day of observation as a percentage of maternal post-partum mass (MPPM), extrapolated from last known capture mass using rate of maternal daily mass loss (MDML). TIME = time of day (AM/PM). DOL = day of lactation, where DOL 1 is the day the pup was born. Unfiltered segments: N = 15, $N_R$ = 267, $N_S$ = 2615. Filtered segments: N = 14, $N_R$ = 120, $N_S$ = 2615. Baseline[1] segments: N = 11, $N_R$ = 63, $N_S$ = 186. Baseline[2] = N = 9, $N_R$ = 55, $N_S$ = 173.*

**Table 3.3.** Predictors of HR as determined by LMMs, with recording number (HRM) nested within individual identity (ID) as random effects.

| Dependent variable | Segments | Fixed effects | Estimate | Standard error | *t* value | Pr(>\|t\|) |
|---|---|---|---|---|---|---|
| **HR (bpm)** | *(i) Unfiltered* | Intercept | 95.756 | 1.803 | 53.110 | **< 0.001*** |
| | | Flats | 0.328 | 0.019 | 17.520 | **< 0.001*** |
| | | Stairs | -0.308 | 0.019 | -16.050 | **< 0.001*** |
| | | Peaks | -1.129 | 0.055 | -20.480 | **< 0.001*** |
| | *(ii) Filtered* | Intercept | 94.327 | 2.342 | 40.279 | **< 0.001*** |
| | | Flats | -0.052 | 0.167 | -0.310 | 0.756 |
| | | Stairs | -0.053 | 0.148 | -0.358 | 0.721 |
| | | Peaks | -1.004 | 0.057 | -17.702 | **< 0.001*** |
| **$HR_{COR}$ (bpm)** | *(iii) Filtered* | Intercept | 94.330 | 2.342 | 40.279 | **< 0.001*** |
| | | Flats | -0.052 | 0.167 | -0.310 | 0.756 |
| | | Stairs | -0.053 | 0.148 | -0.358 | 0.721 |
| | | Peaks | 0.000 | 0.057 | 0.000 | 1.000 |
| | *(iv) Baseline[1]* | Intercept | 95.757 | 3.073 | 31.163 | **< 0.001*** |
| | | Flats | -0.121 | 0.275 | -0.440 | 0.660 |
| | | Stairs | -0.352 | 0.256 | -1.376 | 0.171 |
| | | Peaks | -0.048 | 0.098 | -0.491 | 0.624 |
| | *(v) Baseline[2]* | Intercept | 95.124 | 3.371 | 28.219 | **< 0.001*** |
| | | Flats | -0.202 | 0.279 | -0.723 | 0.471 |
| | | Stairs | -0.275 | 0.261 | -1.056 | 0.293 |
| | | Peaks | -0.041 | 0.100 | -0.410 | 0.682 |
| **$HR_{COR}$ (bpm)** | *(vi) Baseline[2]* | Intercept | 93.009 | 3.875 | 24.000 | **< 0.001*** |
| | | DSC | 0.152 | 0.414 | 0.368 | 0.714 |
| | | Percentage Mass Loss | -0.068 | 0.146 | -0.462 | 0.646 |
| | | TIME | 1.919 | 1.328 | 1.446 | 0.155 |
| | *(vii) Baseline[1]* | Intercept | 92.153 | 3.485 | 26.440 | **< 0.001*** |
| | | DSC | -0.1115 | 0.387 | -0.297 | 0.768 |
| | | DOL | -0.062 | 0.242 | 0.254 | 0.800 |
| | | TIME | 2.622 | 1.319 | 1.988 | **0.052*** |

***** *Asterisks indicate significant parameters.* *$HR_{COR}$ = HR corrected for Peak artefacts. Filtered = segments containing less than 5% Flat or Stair artefacts. Baseline[1] = Baseline segments for all females, irrespective of available mass data. Baseline[2] = Baseline segments for females with available mass data. DSC = days since capture, where DSC 0 is the day of capture. Percentage Mass Loss = estimated mass loss (kg) on day of observation as a percentage of maternal post-partum mass (MPPM), extrapolated from last known capture mass using rate of maternal daily mass loss (MDML). TIME = time of day (AM/PM). DOL = day of lactation, where DOL 1 is the day the pup was born. DSG = days since electrode gel application, where DSG 0 is the day of application. REST = the proportion of RR intervals spent in Rest. Unfiltered segments: N = 15, $N_R$ = 267, $N_S$ = 2615. Filtered segments: N = 14, $N_R$ = 120, $N_S$ = 2615. Baseline[1] segments: N = 11, $N_R$ = 63, $N_S$ = 186. Baseline[2] = N = 9, $N_R$ = 55, $N_S$ = 173.*

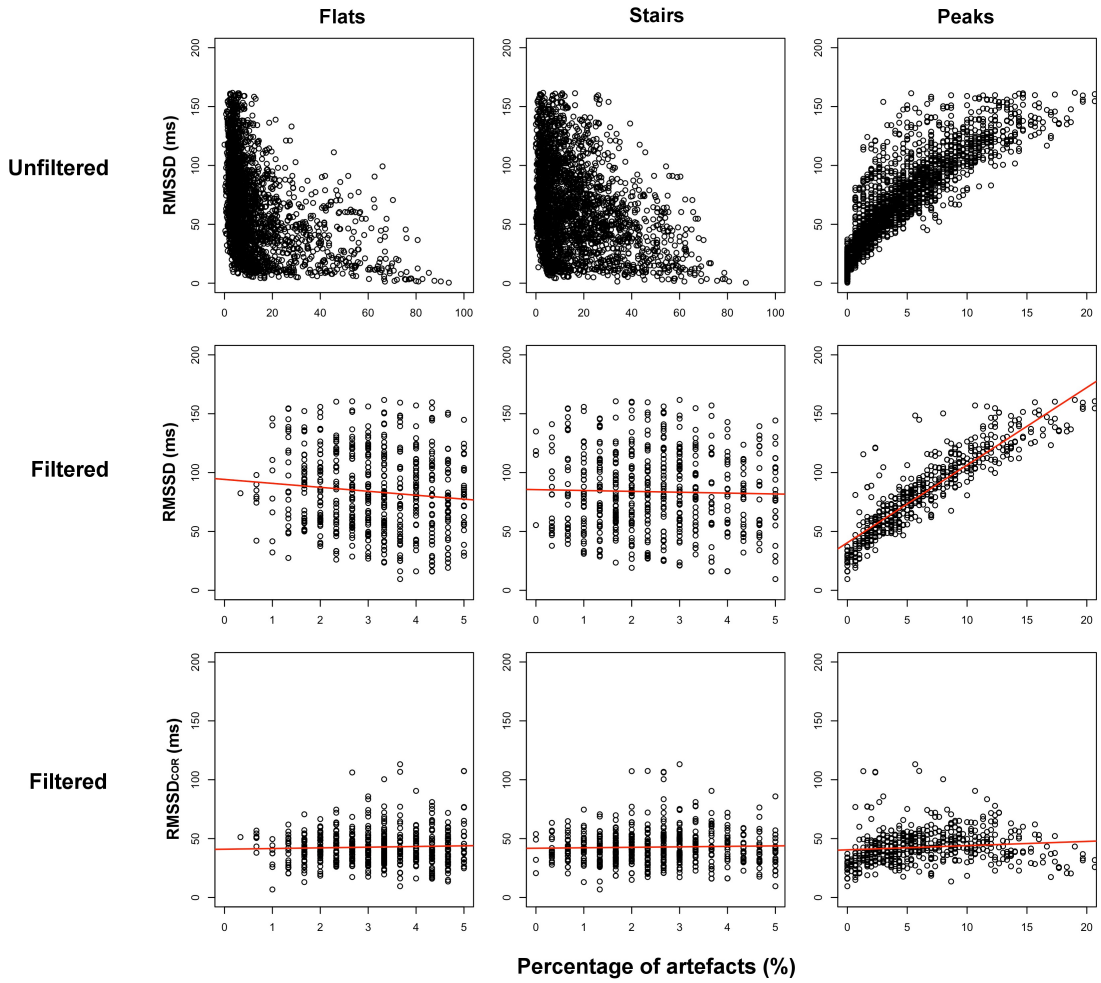


**Figure 3.3**. The effect of Flat, Stair, and Peak artefacts on RMSSD and $RMSSD_{COR}$ in Unfiltered and Filtered segments. Each data point represents RMSSD or $RMSSD_{COR}$ for a unique segment. The solid lines represent the lines of best fit.

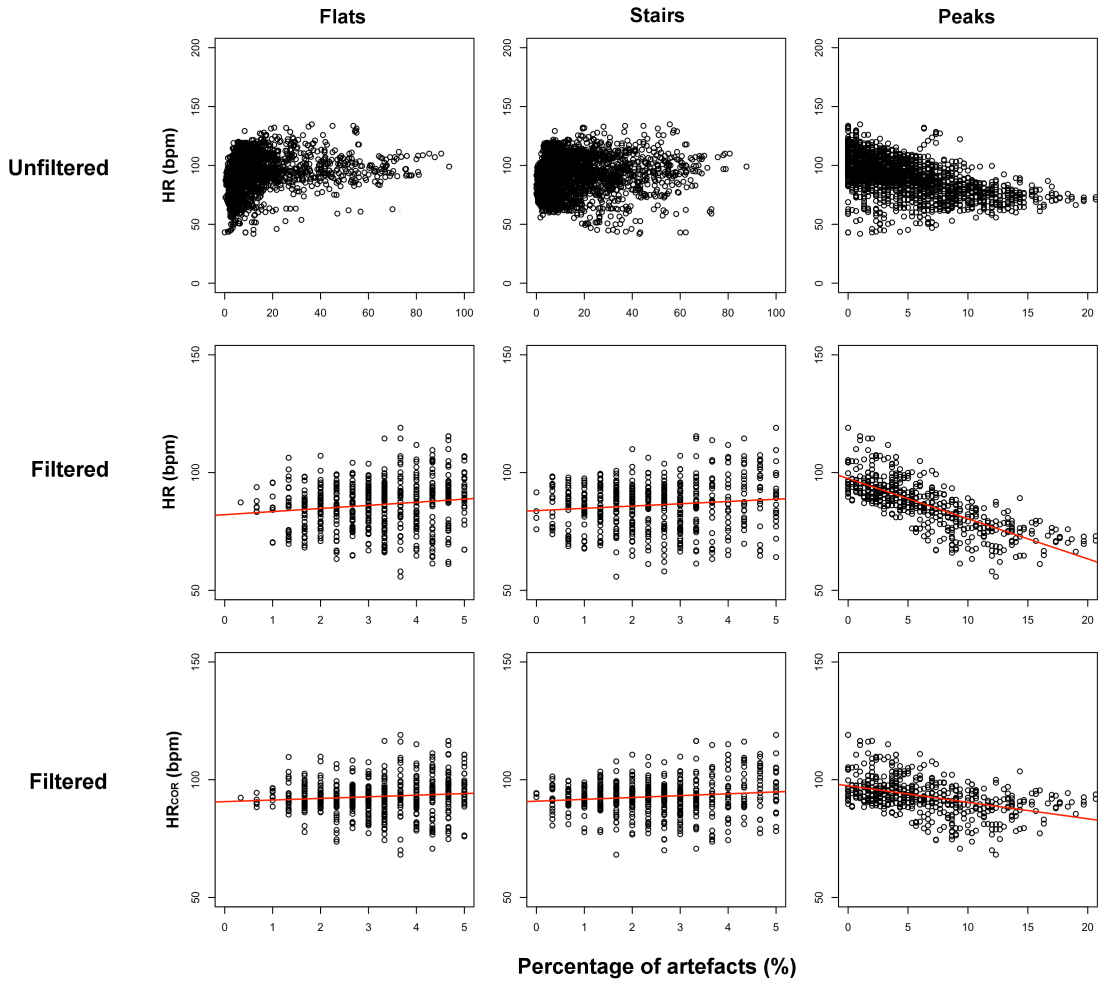


**Figure 3.4**. The effect of Flat, Stair, and Peak artefacts on HR and $HR_{COR}$ in Unfiltered and Filtered segments. Each data point represents HR or $HR_{COR}$ for a unique segment. The solid lines represent the lines of best fit.

**Table 3.4.** Parameter estimates for models retained within confidence sets from LMMs examining the influence of artefacts on RMSSD and $RMSSD_{COR}$ in Unfiltered, Filtered, and Baseline segments.

| Dependent variable | Segments | *N* | $N_R$ | $N_S$ | Model | AICc | ΔAICc | Intercept | Flats | Stairs | Peaks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RMSSD (ms)** | (i) Unfiltered | 15 | 267 | 2615 | Model 1 | 21832.167 | 0 | 37.529 | **-0.373*** | **0.113*** | **7.489*** |
| | | | | | Model 2 | 21836.827 | 4.660 | 38.802 | **-0.288*** | - | **7.392*** |
| | (ii) Filtered | 14 | 120 | 550 | Model 1 | 4324.544 | 0 | 43.793 | - | - | **6.208*** |
| **$RMSSD_{COR}$ (ms)** | (iii) Filtered | 14 | 120 | 550 | Model 1 | 4322.529 | 0 | 43.621 | - | - | - |
| | (iv) Baseline[1] | 11 | 63 | 186 | Model 1 | 1429.972 | 0 | 39.764 | - | - | - |
| | (v) Baseline[2] | 9 | 55 | 173 | Model 1 | 1327.768 | 0 | 39.466 | - | - | - |

** Asterisks indicate significant parameters. Random effects = (ID/HRM). N = number of individuals. $N_R$ = number of recordings. $N_S$ = number of segments. $RMSSD_{COR}$ = RMSSD corrected for Peak artefacts. Filtered = segments containing less than 5% Flat or Stair artefacts. Baseline[1] = Baseline segments for all females, irrespective of available mass data. Baseline[2] = Baseline segments for females with available mass data.*

**Table 3.5.** Parameter estimates for models retained within confidence sets from LMMs examining the influence of artefacts on HR and $HR_{COR}$ in Unfiltered, Filtered, and Baseline segments.

| Dependent variable | Segments | *N* | $N_R$ | $N_S$ | Model | AICc | ΔAICc | Intercept | Flats | Stairs | Peaks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HR (bpm)** | (i) Unfiltered | 15 | 267 | 2615 | Model 1 | 17573.265 | 0 | 95.756 | **0.328*** | **-0.308*** | **-1.129*** |
| | (ii) Filtered | 14 | 120 | 550 | Model 1 | 3157.171 | 0 | 93.966 | **-** | **-** | **-1.000*** |
| **$HR_{COR}$ (bpm)** | (iii) Filtered | 14 | 120 | 550 | Model 1 | 3155.138 | 0 | 93.989 | **-** | **-** | **-** |
| | (iv) Baseline[1] | 11 | 63 | 186 | Model 1 | 1092.941 | 0 | 94.058 | **-** | **-** | **-** |
| | (v) Baseline[2] | 9 | 55 | 173 | Model 1 | 1007.202 | 0 | 93.509 | **-** | **-** | **-** |

** Asterisks indicate significant parameters. Random effects = (ID/HRM). N = number of individuals. $N_R$ = number of recordings. $N_S$ = number of segments. $HR_{COR}$ = HR corrected for Peak artefacts. Filtered = segments containing less than 5% Flat or Stair artefacts. Baseline[1] = Baseline segments for all females, irrespective of available mass data. Baseline[2] = Baseline segments for females with available mass data.*

### 3.2.2. Determining segment filtration thresholds and obtaining parameter estimates for correction

As determined using a series of iterative LMMs, the maximum permissible percentage for Flats or Stairs was 5% (Table 3.6). Below this threshold, Flats and Stairs were no longer significant parameters and did not affect either RMSSD or HR (LMM (ii): Table 3.2, Table 3.3, Table 3.4, Table 3.5, Figure 3.3, Figure 3.4). Parameter estimates used to correct for the effect of Peaks on RMSSD (6.233, LMM (ii): Table 3.2) and HR (1.004, LMM (ii): Table 3.2) were obtained from LMMs using segments containing less than 5% Flats or Stairs (i.e. Filtered segments). Once corrected, $RMSSD_{COR}$ and $HR_{COR}$ in Filtered segments were no longer affected by any of the three artefact types (LMM (iii): Table 3.2, Table 3.3, Table 3.4, Table 3.5, Figure 3.3, Figure 3.4). Accordingly, filtration and correction protocols were effective in counteracting potential bias introduced by artefacts, even in Baseline segments (LMMs (iv-v): Table 3.2, Table 3.3, Table 3.4, Table 3.5).

**Table 3.6.** Significance of Flats and Stairs at different threshold percentages, as determined by LMMs examining the influence of artefacts on RMSSD and HR in Unfiltered segments.

| Threshold Percentage | | *N* | $N_R$ | $N_S$ | Pr(|z|) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | RMSSD (ms) | | HR (bpm) | |
| Flats | Stairs | | | | Flats | Stairs | Flats | Stairs |
| 5 | 5 | 14 | 120 | 550 | **0.439*** | **0.511*** | **0.756*** | **0.721*** |
| 10 | 5 | 14 | 146 | 729 | 0.003 | **0.243*** | < 0.001 | **0.786*** |
| 15 | 5 | 14 | 148 | 747 | < 0.001 | **0.293*** | < 0.001 | **0.707*** |
| 20 | 5 | 14 | 148 | 747 | < 0.001 | **0.293*** | < 0.001 | **0.707*** |
| 5 | 10 | 15 | 156 | 745 | **0.320*** | 0.003 | **0.999*** | < 0.001 |
| 10 | 10 | 15 | 198 | 1194 | 0.027 | 0.001 | < 0.001 | < 0.001 |
| 15 | 10 | 15 | 205 | 1306 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| 20 | 10 | 15 | 205 | 1314 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| 5 | 15 | 15 | 165 | 819 | **0.177*** | < 0.001 | **0.849*** | < 0.001 |
| 10 | 15 | 15 | 212 | 1416 | **0.078*** | < 0.001 | < 0.001 | < 0.001 |
| 15 | 15 | 15 | 223 | 1606 | 0.001 | < 0.001 | < 0.001 | < 0.001 |
| 20 | 15 | 15 | 223 | 1627 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| 5 | 20 | 15 | 175 | 859 | **0.068*** | < 0.001 | **0.574*** | < 0.001 |
| 10 | 20 | 15 | 225 | 1535 | 0.059 | < 0.001 | < 0.001 | < 0.001 |
| 15 | 20 | 15 | 239 | 1789 | 0.001 | < 0.001 | < 0.001 | < 0.001 |
| 20 | 20 | 15 | 240 | 1845 | 0.001 | < 0.001 | < 0.001 | < 0.001 |

** Asterisks indicate non-significant parameters. Random effects = (ID/HRM). N = number of individuals. $N_R$ = number of recordings. $N_S$ = number of segments,*

### 3.2.3. Sources of artefacts

The proportion of intervals spent in Rest and days since electrode gel application influenced the occurrence of almost all artefact types in Unfiltered segments (Table 3.7, Table 3.8). Flats and Stairs increased as individuals spent more time active (i.e. engaging in non-rest behaviours), whereas Peaks decreased (Table 3.7, Table 3.8). Flats were unaffected by days since electrode gel application, whereas both Stairs and Peaks increased (Table 3.7, Table 3.8). Models containing only the proportion of intervals spent in Rest were retained within the confidence sets for Stairs and Peaks, but with greater ΔAICc values (≥ 2) and no change in effect sizes (Table 3.8). When comparing effect sizes across all three artefact types, the proportion of intervals spent in Rest had the strongest effect on Flats and the weakest effect on Peaks (Table 3.8). They also had a far stronger effect on Stairs and Peaks than when compared to days since electrode gel application (Table 3.7, Table 3.8). Between Stairs and Peaks, days since electrode gel application had a stronger effect on Peaks, whereas the proportion of intervals spent in Rest had a stronger effect on Stairs (Table 3.8).

**Table 3.7.** Predictors of artefacts, as determined by GLMMs, with recording number (HRM) nested within individual identity (ID) as random effects.

| Dependent variable | Segments | Fixed effects | Estimate | Standard error | *z* value | Pr(>\|z\|) |
|---|---|---|---|---|---|---|
| **Flats** | *Unfiltered* | Intercept | -2.048 | 0.127 | -16.084 | **< 0.001*** |
| | | REST | -0.570 | 0.019 | -29.556 | **< 0.001*** |
| | | DSG | 0.021 | 0.019 | 1.094 | 0.274 |
| **Stairs** | *Unfiltered* | Intercept | -1.903 | 0.162 | -11.717 | **< 0.001*** |
| | | REST | -0.403 | 0.017 | -23.523 | **< 0.001*** |
| | | DSG | 0.046 | 0.021 | 2.154 | **0.031*** |
| **Peaks** | *Unfiltered* | Intercept | -3.848 | 0.152 | -25.317 | **< 0.001*** |
| | | REST | 0.360 | 0.032 | 11.206 | **< 0.001*** |
| | | DSG | 0.056 | 0.021 | 2.618 | **0.009*** |

** Asterisks indicate significant parameters. DSG = days since electrode gel application, where DSG 0 is the day of application. REST = the proportion of RR intervals spent in Rest. Unfiltered segments: N = 15, $N_R$ = 267, $N_S$ = 2615.*

**Table 3.8.** Parameter estimates for models retained within the confidence set from GLMMs examining the influence of Days Since Electrode Gel Application and the proportion of intervals spent in Rest on the occurrence of artefacts.

| Dependent variable | Model | AICc | ΔAICc | Intercept | DSG | REST |
|---|---|---|---|---|---|---|
| **Flats** | Model 1 | 62411.453 | 0 | 1.965 | **-** | **-0.569*** |
| **Stairs** | Model 1 | 64453.371 | 0 | 1.903 | **0.046*** | **-0.403*** |
| | Model 2 | 64456.003 | 2.632 | 1.719 | **-** | **-0.403*** |
| **Peaks** | Model 1 | 21210.955 | 0 | 3.848 | **0.056*** | **0.360*** |
| | Model 2 | 21215.631 | 4.676 | 3.627 | **-** | **0.361*** |

** Asterisks indicate significant parameters. Random effects = (ID/HRM). DSG = days since electrode gel application. REST = proportion of intervals spent in Rest. N = 15. $N_R$ = 267. $N_S$ = 2615.*

### 3.2.4. Determining the impact of data collection protocols

There were no significant differences in proxies of maternal reproductive performance between study and non-study females (Table 3.9). Accordingly, MDML was significantly positively correlated with PDMG in both study (*Spearman's rank*: $r$ = 0.769, $p$-value = 0.014) and non-study females (*Spearman's rank*: $r$ = 0.738, $p$-value < 0.001, $N$ = 10) (Figure 3.5). Within study females, there were no significant associations between proxies of maternal reproductive performance and days mounted with a sensor strap, or days subjected to behavioural observation (Table 3.10).

**Table 3.9.** Results from paired Wilcoxon signed-rank tests comparing measures of maternal performance between study and non-study females on the Isle of May, Scotland.

| | $N_{Study}$ | $N_{Non\text{-}Study}$ | W | $p$-value |
|---|---|---|---|---|
| **MDML** | 11 | 21 | 115.5 | 1.000 |
| **PDMG** | 11 | 21 | 115.5 | 0.836 |
| **MPR** | 10 | 21 | 102 | 0.948 |

*MDML = maternal daily mass loss rate ($kgday^{-1}$). PDMG = pup daily mass gain rate ($kgday^{-1}$). MPR = MDML:PDMG ratio.*

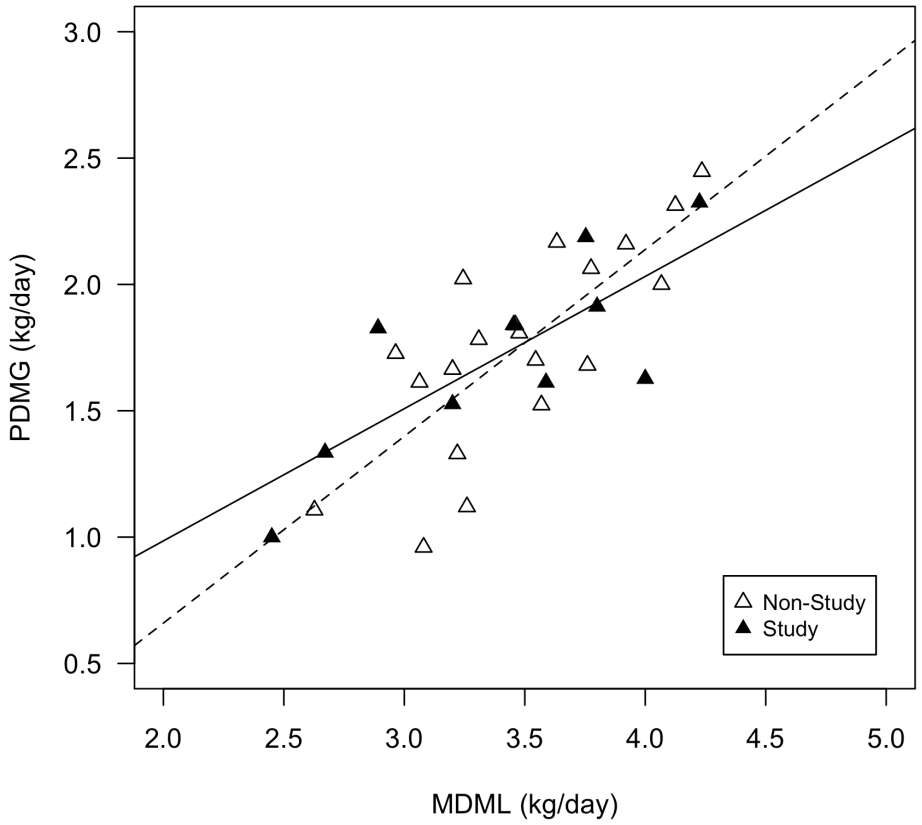


**Figure 3.5.** Scatterplot of Maternal Daily Mass Loss rate (MDML) and Pup Daily Mass Gain rate (PDMG) compared between study (*N* = 10) and non-study females (*N* = 19) on the Isle of May, Scotland 2013. The solid line represents the line of best fit for study females. The dashed line represents the line of best fit for non-study females.

**Table 3.10.** Results from Kendall's rank correlations testing the association between measures of maternal reproductive performance and days mounted with a sensor strap and subjected to behavioural observation.

| | | *N* | z | τ | *p*-value |
|---|---|---|---|---|---|
| **Days mounted with a sensor strap** | MDML | 11 | 0.731 | 0.181 | 0.465 |
| | PDMG | 11 | 0.893 | 0.221 | 0.372 |
| | MPR | 10 | 0.468 | 0.123 | 0.640 |
| **Days subjected to behavioural observation** | MDML | 11 | 0.081 | 0.020 | 0.935 |
| | PDMG | 11 | 0.991 | 0.250 | 0.322 |
| | MPR | 10 | 0.473 | 0.126 | 0.636 |

*MDML = maternal daily mass loss rate (kgday$^{-1}$). PDMG = pup daily mass gain rate (kgday$^{-1}$). MPR = MDML:PDMG ratio.*

## 3.3. Quantifying inter-individual variation, repeatability, and reproductive performance correlates of baseline HR and HRV

### 3.3.1. Inter-individual variation in baseline HR and HRV during the breeding season

Females on the Isle of May had an average baseline $RMSSD_{COR}$ and $HR_{COR}$ of 38.4 ± 0.9 ms and 93.9 ± 0.5 bpm, respectively ($N$ = 13). There appeared to be significant inter-individual differences in both baseline $RMSSD_{COR}$ (*Kruskal Wallis test:* $\chi^2_{(10)} = 43.895$, $p$-value < 0.001) (Figure 3.6a) and $HR_{COR}$ (*Kruskal Wallis test:* $\chi^2_{(10)} = 104.359$, $p$-value < 0.001) (Figure 3.6b). These differences could not be attributed to days since capture, percentage mass loss, day of lactation, or time of day. (Table 3.2vi–vii, Table 3.3vi–vii, Table 3.11, Table 3.12). There was some evidence that baseline $HR_{COR}$ was elevated in the afternoons, suggesting the presence of a diurnal rhythm (Table 3.3vii, Table 3.12). However, time of day was only retained as a significant parameter in the LMM examining variation in baseline $HR_{COR}$ with day of lactation as a proxy of percentage mass loss ($N$ = 11, Baseline[1]: Table 3.12).

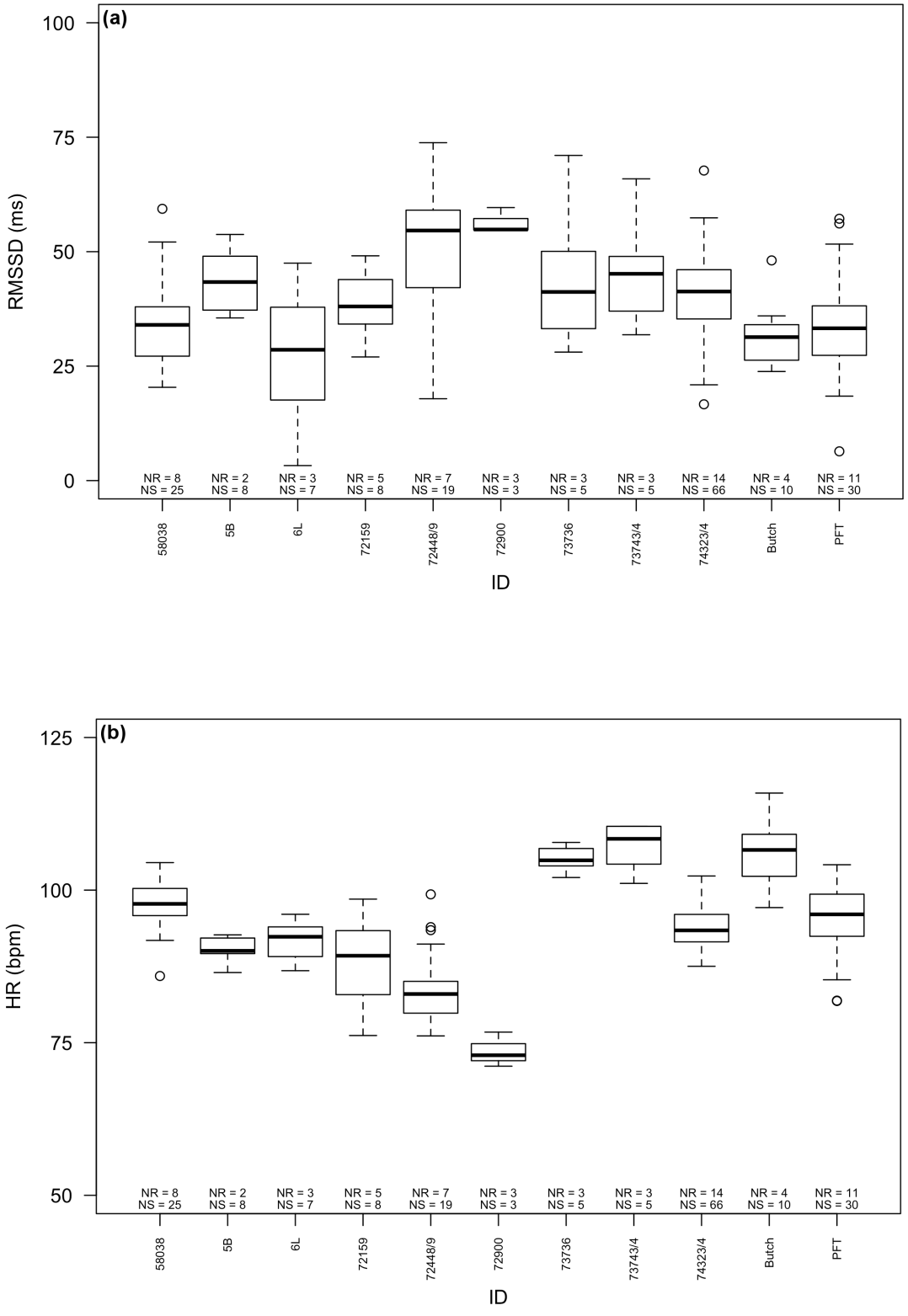


**Figure 3.6.** Baseline[1] **(a)** $RMSSD_{COR}$ and **(b)** $HR_{COR}$ across study females on the Isle of May, Scotland ($N = 11$). $N_R$ = number of recordings. $N_S$ = number of segments.

**Table 3.11.** Parameter estimates for models retained within the confidence set from LMMs examining the effect of Days Since Capture, Percentage Mass Loss, and Time Of Day on Baseline[2] $RMSSD_{COR}$ and $HR_{COR}$.

| Dependent variable | Model | AICc | ΔAICc | Intercept | DSC | Percentage Mass Loss | TIME |
|---|---|---|---|---|---|---|---|
| **$RMSSD_{COR}$ (ms)** | Model 1 | 1327.768 | 0 | **39.466*** | - | - | - |
| **$HR_{COR}$ (bpm)** | Model 1 | 1007.202 | 0 | **93.509*** | - | - | - |

*Random effects = (ID/HRM). DSC = days since capture. Percentage Mass Loss = estimated mass loss (kg) on day of observation as a percentage of maternal post-partum mass (MPPM). TIME = time of day (AM/PM). N = 9. $N_R$ = 55. $N_S$ = 173.*

**Table 3.12.** Parameter estimates for models retained within the confidence set from LMMs examining the effect of Days Since Capture, Day Of Lactation (as a proxy of Percentage Mass Loss), and Time Of Day on Baseline[1] $RMSSD_{COR}$ and $HR_{COR}$.

| Dependent variable | Model | AICc | ΔAICc | Intercept | DSC | DOL | TIME |
|---|---|---|---|---|---|---|---|
| **$RMSSD_{COR}$ (ms)** | Model 1 | 1429.972 | 0 | **39.764*** | - | - | - |
| **$HR_{COR}$ (bpm)** | Model 1 | 1090.183 | 0 | **92.366*** | - | - | **+ *** |
| | Model 2 | 1092.941 | 2.759 | **94.058*** | - | - | - |
| | Model 3 | 1094.497 | 4.315 | **94.948*** | -0.221 | - | - |
| | Model 4 | 1095.049 | 4.866 | **93.905*** | - | 0.012 | - |

*** Asterisks indicate significant parameters. Random effects = (ID/HRM). DSC = days since capture. DOL = day of lactation. TIME = time of day (AM/PM). N = 11. $N_R$ = 63. $N_S$ = 186.*

### 3.3.2. Repeatability in baseline HR and HRV within the breeding season

Baseline $RMSSD_{COR}$ showed a high degree of repeatability between early and late lactation ($ICC_2$ = 0.86, $F_{(6,6)}$ = 17, *p*-value = 0.002, 95% CI: 0.39–0.97) (Figure 3.7a), whereas baseline $HR_{COR}$ did not ($ICC_2$ = 0.46, $F_{(6,6)}$ = 2.5, *p*-value = 0.142, 95% CI: -0.46–0.89) (Figure 3.7b). Although the interval between early and late lactation samples varied considerably between individuals (Table 3.13), there was no evidence to suggest that it influenced repeatability of either $RMSSD_{COR}$ (adjusted $r^2$ = 0.005, $F_{(1,5)}$ = 0.706, *p*-value = 0.439) or $HR_{COR}$ (adjusted $r^2$ = 0.191, $F_{(1,5)}$ = 0.039, *p*-value = 0.852). Visually, individuals appeared to separate into two groups based on mean baseline $RMSSD_{COR}$ in early and late lactation (Table 3.13, Figure 3.7a) – i.e. those with baseline $RMSSD_{COR}$ "higher" (*N* = 3) or "lower" (*N* = 4) than 39.62 ms and 37.37 ms in early and late lactation, respectively. A small sample randomization test (Design 5a: Todman and Dugard 2001) revealed a significant difference between individuals with "higher" or "lower" baseline $RMSSD_{COR}$ (*t* = 12.161, 1000 permutations, *p*-value = 0.019).

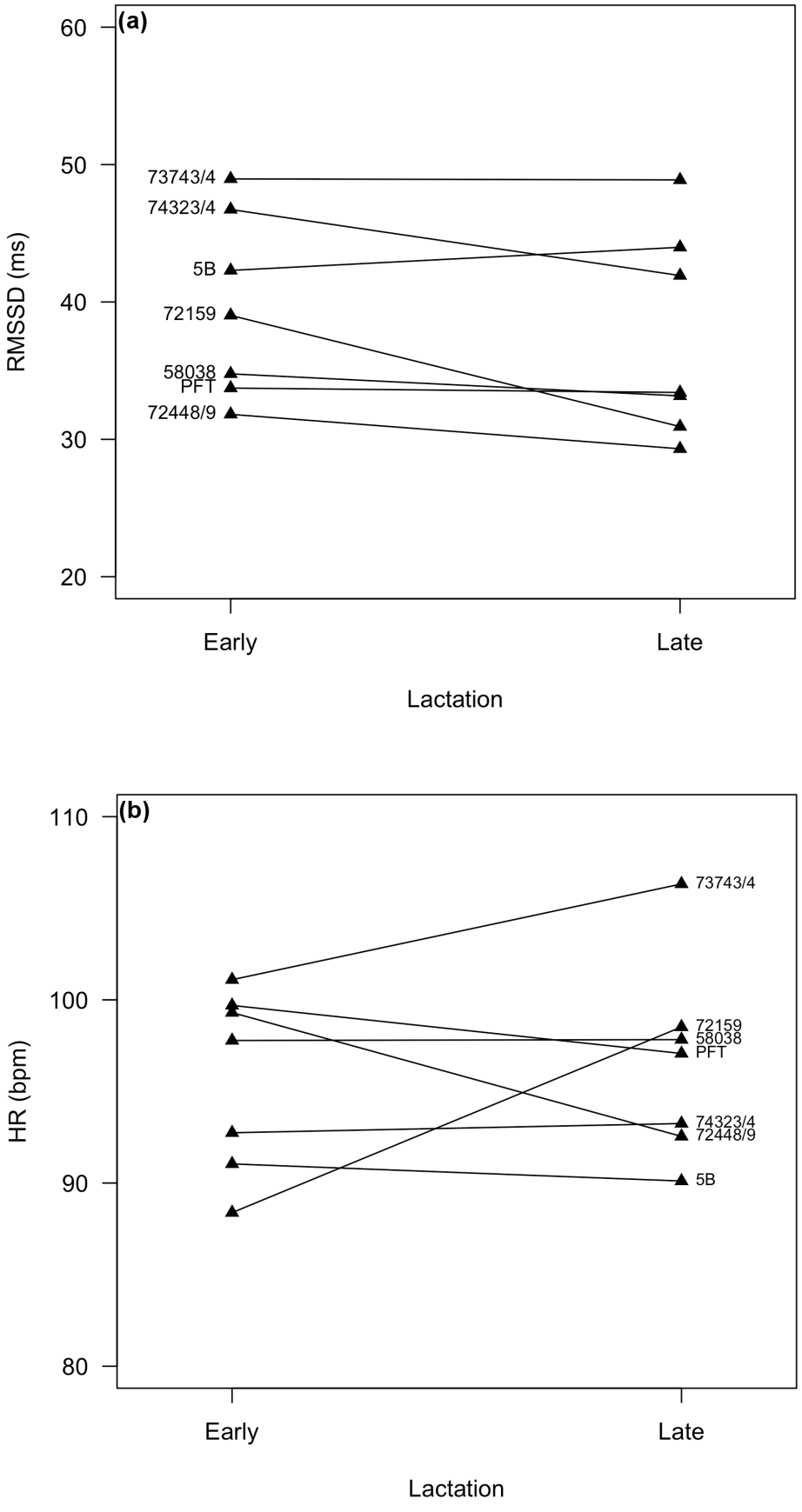


**Figure 3.7.** Mean baseline **(a)** $RMSSD_{COR}$ and **(b)** $HR_{COR}$ between early and late lactation for study females on the Isle of May, Scotland, 2013 ($N = 7$).

**Table 3.13.** Mean baseline $RMSSD_{COR}$ and $HR_{COR}$ in early and late lactation for study females on the Isle of May, Scotland ($N = 7$).

| ID | *Early Lactation* | | | *Late Lactation* | | | *Difference* | | |
|---|---|---|---|---|---|---|---|---|---|
| | **DOL** | **$RMSSD_{COR}$ (ms)** | **$HR_{COR}$ (bpm)** | **DOL** | **$RMSSD_{COR}$ (ms)** | **$HR_{COR}$ (bpm)** | **DOL** | **$RMSSD_{COR}$ (ms)** | **$HR_{COR}$ (bpm)** |
| 73743/4 | 9 | 48.96 | 101.10 | 16 | 48.88 | 106.33 | 7 | -0.08 | 5.23 |
| 74323/4 | 8 | 46.74 | 92.75 | 11 | 41.92 | 93.25 | 3 | -4.81 | 0.50 |
| 5B | 4 | 42.30 | 91.05 | 12 | 43.98 | 90.11 | 8 | 1.69 | -0.94 |
| 72159 | 5 | 39.03 | 88.38 | 17 | 30.93 | 98.52 | 12 | -8.10 | 10.14 |
| 58038 | 8 | 34.77 | 97.78 | 17 | 33.16 | 97.83 | 9 | -1.61 | 0.05 |
| PFT | 5 | 33.73 | 99.7 | 19 | 33.41 | 97.07 | 14 | -0.32 | -2.63 |
| 72448/9 | 4 | 31.83 | 99.31 | 16 | 29.31 | 92.55 | 12 | -2.51 | -6.76 |
| **Mean** | 6 | 39.62 | 95.72 | 15.43 | 37.37 | 96.52 | 9.29 | -2.25 | 0.80 |
| **S.E.** | 0.80 | 2.51 | 1.87 | 1.09 | 2.83 | 2.01 | 1.41 | 1.25 | 2.07 |

*DOL = day of lactation.*

### 3.3.3. Correlations between proxies of reproductive performance and baseline HR and HRV

There were no relationships between mean baseline $RMSSD_{COR}$ and $HR_{COR}$ with maternal expenditure (i.e. MDML) or fitness outcomes (i.e. PDMG) (Table 3.14). However, there was evidence $RMSSD_{COR}$ and $HR_{COR}$ were significantly associated with maternal transfer efficiency (i.e. actual or absolute residuals from a linear regression using MDML to predict PDMG) (Table 3.14). Mean baseline $RMSSD_{COR}$ was negatively correlated with actual residuals, suggesting that females with lower baseline $RMSSD_{COR}$ experienced greater maternal transfer efficiency (Figure 3.8). Conversely, mean baseline $HR_{COR}$ was negatively correlated with absolute residuals, suggesting females with lower baseline $HR_{COR}$ were more varied in their efficiency (Figure 3.9). Since mothers with larger post-partum mass are able to expend more resources on their pup, additional analyses were conducted to see whether greater maternal transfer efficiency was a result of greater maternal post-partum mass (i.e. MPPM). However, MPPM was not correlated with either actual (*Spearman's rank*: $r = 0.619$, $p$-value = 0.115) or absolute (*Spearman's rank*: $r = 0.524$, $p$-value = 0.197) residuals.

**Table 3.14.** Results from Spearman's rank correlations testing the association between proxies of maternal reproductive performance and mean baseline $RMSSD_{COR}$ and $HR_{COR}$ for study females on the Isle of May, Scotland ($N = 8$).

| | | *r* | *p*-value |
|---|---|---|---|
| **$RMSSD_{COR}$ (ms)** | MDML | -0.233 | 0.552 |
| | PDMG | 0.167 | 0.678 |
| | **Actual residuals** | **-0.762** | **0.037*** |
| | Absolute residuals | 0.119 | 0.793 |
| **$HR_{COR}$ (bpm)** | MDML | 0.050 | 0.912 |
| | PDMG | 0.400 | 0.291 |
| | MPR | -0.595 | 0.132 |
| | Actual residuals | 0.524 | 0.197 |
| | **Absolute residuals** | **-0.810** | **0.022*** |

** Asterisks indicate significant parameters. MDML = maternal daily mass loss rate* ($kgday^{-1}$). *PDMG = pup daily mass gain rate* ($kgday^{-1}$). *MPR = MDML:PDMG ratio. Actual and absolute residuals come from a linear regression using MDML to predict PDMG.*

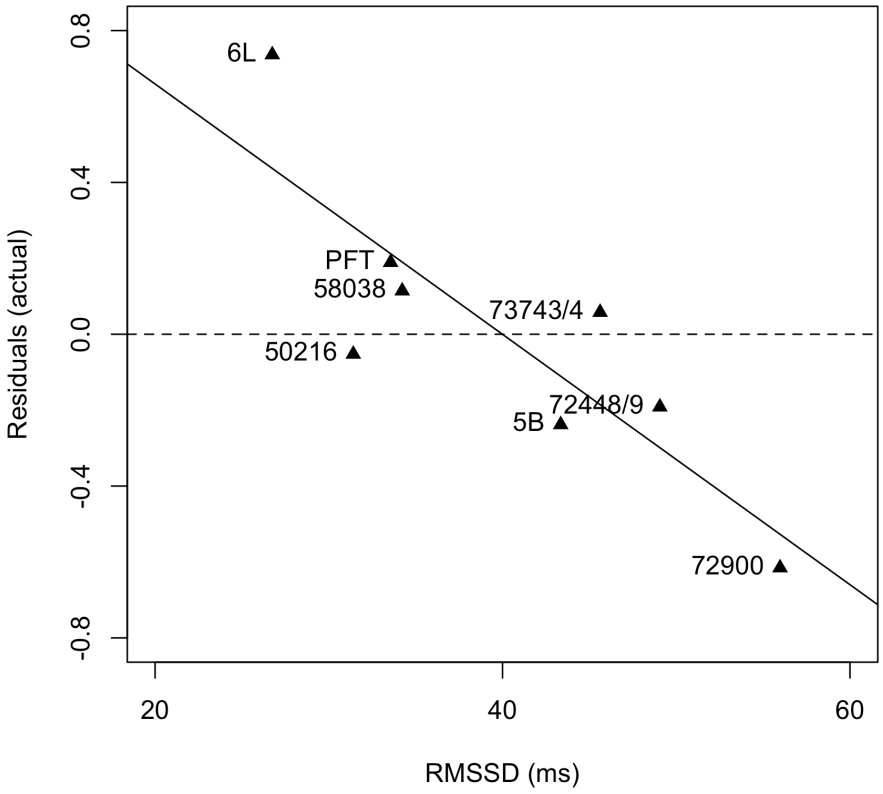


**Figure 3.8.** Mean baseline $RMSSD_{COR}$ against actual residuals from a linear regression using Maternal Daily Mass Loss rate (MDML) to predict Pup Daily Mass Gain rate (PDMG) (*N* = 8). The solid line represents the line of best fit.

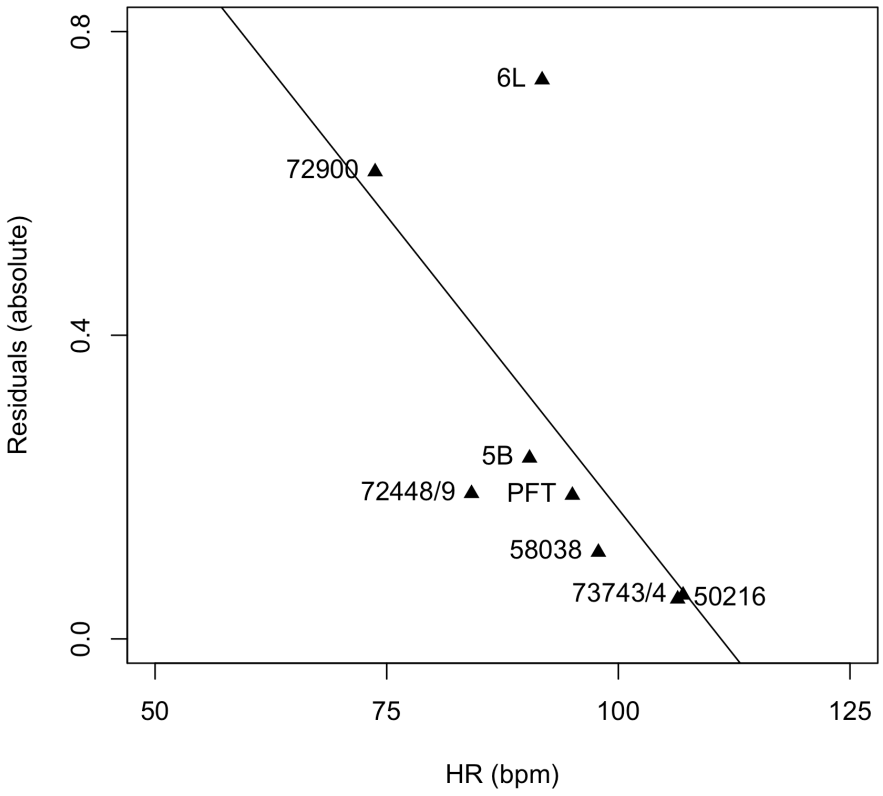


**Figure 3.9.** Mean baseline $HR_{COR}$ against absolute residuals from a linear regression using Maternal Daily Mass Loss rate (MDML) to predict Pup Daily Mass Gain rate (PDMG) (*N* = 8). The solid line represents the line of best fit.

# Chapter 4: Discussion

## 4.1. Summary of results

Polar® devices were piloted on female grey seals on the Isle of May, Scotland, during the 2013 breeding season. They were capable of recording characteristic HR patterns observed in phocid seals. However, artefacts (Flats, Stairs, and Peaks) were widespread in Unfiltered segments and biased RMSSD and HR. These effects were counteracted using filtration and correction protocols. The main sources of artefacts in Unfiltered segments were non-stationary behaviour and days since electrode gel application. Data collection protocols did not appear to have any detrimental effects on study females and/or their pups. Proxies of maternal reproductive performance were not associated with days mounted with a sensor strap or days subjected to behavioural observation – nor did they differ between study and non-study females. Both baseline $RMSSD_{COR}$ and $HR_{COR}$ varied significantly between individuals, but only baseline $RMSSD_{COR}$ showed a high degree of repeatability within the breeding season. Although baseline $RMSSD_{COR}$ and $HR_{COR}$ were not associated with proxies of maternal reproductive performance (i.e. MDML, PDMG), they were negatively correlated with maternal transfer efficiency and variation in maternal transfer efficiency, respectively.

## 4.2. Suitability of Polar® devices for conducting HR and HRV analyses in grey seals

### 4.2.1. The impact of artefacts on HR and HRV

Artefacts significantly affected HR and RMSSD in Unfiltered segments (Figure 3.1, Figure 3.2). Briefly, Stairs and Peaks increased RMSSD (and decreased HR), whereas Flats decreased RMSSD (and increased HR). The effects of Flats and Stairs were filtered out of the data, whereas the effect of Peaks was corrected within the data. These protocols seemed to counteract the bias caused by artefacts, though filtration greatly reduced the amount of data available for analyses (Before: $N = 15$, $N_R = 267$, $N_S = 2615$; After: $N = 14$, $N_R = 120$, $N_S = 120$) (Table 2.4). Filtration and correction protocols were implemented under the assumption segments from Polar® recordings free of artefacts were comparable to ECG recordings. Whilst validation studies have revealed a good level of agreement between Polar® and ECG recordings (Marchant-Forde *et al.* 2004; Jonckheer-Sheehy *et al.* 2012), some suggest Polar® devices may over- and/or under-estimate HRV parameters (Parker *et al.* 2010). Since Polar® devices only

record RR intervals, there is no absolute method for detecting artefcts in Polar® recordings after data has been collected (von Borell *et al.* 2007; Jonckheer-Sheehy *et al.* 2012). Ultimately, it is not possible to determine the efficacy of filtration and correction protocols without corresponding ECG recordings. However, filtered and corrected data produced HR and HRV metrics comparable to those previously reported in the literature. Average baseline $HR_{COR}$ of female grey seals (93.9 ± 0.5 bpm, *N* = 13) was within range of previously reported HR for breeding female grey seals resting on land (103 ± 36.1 bpm: Perry *et al.* 2002). There are no reports of RMSSD for pinnipeds, but average baseline $RMSSD_{COR}$ (38.4 ± 0.9 ms, *N* = 13) was within range of previously reported resting RMSSD for livestock – greater than in pigs (20.95 ± 3.07 ms: Marchant-Forde *et al.* 2004), but smaller than in dogs (56.46 ± 8.83 ms: Jonckheer-Sheehy *et al.* 2012) or horses (127.3 ± 96 ms: Parker *et al.* 2010).

#### 4.2.2. Sources of artefacts

Non-stationary behaviour and impaired electrode conductance are two of most common sources of artefacts (von Borell *et al.* 2007). Unsurprisingly, both the proportion of intervals spent in Rest and days since electrode gel application affected the quality of Polar® recordings. Movement likely caused uneven electrode contact, uneven distribution of electrode gel within the sensor straps, and noise from muscle action potentials, leading to a loss of signal transmission between the skin and electrodes. This loss was represented as Flats and Stairs, and precluded Peaks, explaining why movement increased the incidence of Flats and Stairs but decreased the incidence of Peaks. Movement also caused a loss of signal transmission between the sensors and monitors. Flats would occur when the loss of signal transmission was abrupt (Figure 2.5a) (e.g. when females rolled from a prone to supine position), whereas Stairs would occur when it was gradual (e.g. when females remained prone but orientated the sensor strap away from the monitor or moved beyond range of the monitors). Highly variable periods characterised by excessive Peaks (Figure 2.5b) may have been a result of sensors transmitting at the very limit of their range (20m). Similar periods have been observed in recordings from captive California sea lions (*Zalophus californianus*) and northern elephant seals (Green *et al.* 2007), where they occurred during high-intensity movement or when the electrodes were physically stimulated (e.g. by scratching or bumping). In this study, however, they were only observed when females were stationary, in the absence of any behavioural or environmental

changes. Electrode gel is necessary to facilitate conductance between the skin and electrodes. Although electrode gel was applied liberally under the sensor straps during attachment, the fur in contact with the electrodes was often dry or saturated with mud upon retrieval. Loss of electrode gel (e.g. due to evaporation, leakage, or contamination by mud) would have been compounded the longer it had been since application, increasing the incidence of Stairs and Peaks.

### 4.2.3. Limitations, advantages, recommendations, and alternatives to Polar® devices

Although Polar® devices were used to successfully conduct HR and HRV analyses on grey seals, the results of this study reveal several limitations. First, they are highly susceptible to artefacts, which were widespread in recordings. Second, Polar® monitors only record RR intervals; artefact detection, filtration, and correction protocols could not be fully validated (without corresponding ECG recordings). Second, Polar® monitors have small memory capacities (40.5 hours, assuming an average HR of 70 bpm) and had to be kept separate from the sensors to obtain multiple longer-term recordings from each study female. Ideally, monitors would be deployed within the sensor straps, eliminating many sources of artefacts. Instead, monitors were positioned at the very limit of the sensors' transmission range, where they required regular checking every 30 to 60 minutes. Monitors also often required repositioning – depending on the distance, body position, and orientation of the focal female – reducing the total available recording time. Study females showed temporary changes in behaviour indicating mild stress (i.e. increased vigilance) when the monitors were checked or repositioned by an observer – though proxies of maternal reproductive performance were not affected by the number of days mounted with a sensor strap or days subjected to behavioural observation. Additionally, there were no significant differences in proxies of maternal reproductive performance between study and non-study females, suggesting data collection protocols did not cause any detrimental effects on maternal expenditure (i.e. MDML), fitness outcomes (i.e. PDMG), or maternal transfer efficiency (i.e. MPR) (Figure 3.5). Third, Polar® sensors have small transmission ranges, which caused difficulties similar to small memory capacities. Finally, Polar® electrodes were highly sensitive to non-stationary behaviours and dependent on electrode gel to facilitate proper conductance between the skin and electrodes.

Despite their limitations, Polar® devices possess several advantages. They are cost-effective (i.e. permit a large sample size), minimally invasive (i.e. do not require shaving, sub-dermal electrodes, or surgically implanted components), commercially available, portable, easily maintained, readily modified for a variety of study species, and have been used extensively for animal research. Since sensor straps are glued to the fur, there is no risk of permanent attachment or injury upon failure to retrieve the device; sensor straps fall off during the annual moult following the breeding season. Furthermore, Polar® devices were capable of recording HR patterns that characterise phocid seals at rest on land (Figure 3.1a–c). Although respiratory data were not collected from focal videos, these patterns are highly similar to those that characterise arrhythmic breathing in phocid seals on land. Steady, variable HR (Figure 3.1a) likely occurred during periods of eupnoea, whereas episodes of bradycardia (Figure 3.1b–c) likely occurred concurrently with periods of apnoea. Peaks in the bimodal distribution of resting RR intervals were comparable to peaks observed in adult harbour seals (55 to 80 bpm: Perry *et al.* 2000) and northern elephant seal weanlings (50 to 100–105 bpm: Green *et al.* 2007).

Based on the results of this study, electrode gel should be applied liberally to minimise the occurrence of artefacts. Although shaving is recommended and thought to improve signal conductance (von Borell *et al.* 2007; Parker *et al.* 2010), fur may help retain electrode gel during long-term deployments (J. Clapp, *personal communication*). RR interval recording should focus on the days following sensor strap attachment before electrode gel is lost, to minimise the occurrence of Stairs and Peaks. Females towards the periphery of the breeding colony from lower density areas should be selected to accommodate repeated monitor deployments and minimise disturbance to non-study females. Monitors should be positioned within 20 m of focal females, preferably in an elevated position where they have a “clear line of sight” to sensor straps, to minimise the occurrence of Flats and Stairs. However, it should be noted that selection of peripheral females may confer selection bias, as there is evidence to suggest proactive females tend to be located within higher density areas (Twiss *et al.* 2012).

Alternatives to Polar® devices fall within three categories: (1) non-invasive portable ECG devices (2) invasive ECG devices; and (3) other non-invasive RR interval recorders. The predominant advantage of non-invasive portable ECG devices, such as Holter monitors, is the ability to record the entire QRS complex, which facilitates the detection and correction of

artefactual RR intervals. However, they are typically less robust, more expensive (i.e. reducing sample size), and only capable of short-term recordings (i.e. up to 48 hours), making them more suitable for recording RR intervals in captive or laboratory animals and livestock.

Invasive ECG devices, such as those requiring sub-dermal needle electrodes or implantation, have similar advantages and disadvantages as non-invasive portable ECG devices, with the exception of recording time and resolution. Some surgically implanted loggers, for example, can record up to 1 year of data, but only sample HR discretely (e.g. every 2–30 seconds) or instantaneous HR to the nearest 10-50 ms – rendering them unsuitable for conducting HRV analyses. Furthermore, invasive ECG devices often require surgical deployment procedures. Careful monitoring for infection or rejection is essential, particularly if devices are implanted. Responses to invasive procedures can differ markedly, even between pinniped species. Green *et al.* (2007) found implanted HR loggers tolerated by California sea lions caused a substantial inflammatory response in northern elephant seals, despite undergoing identical surgical procedures. There is also a risk of permanent attachment or injury upon failure to retrieve instrumentation.

Other non-invasive RR interval recorders from alternative manufacturers, such as Zephyr (*Zephyr*, Groningen, The Netherlands) or Firstbeat (*Firstbeat Technologies Oy,* Jyväskylä, Finland), offer improvements that address many limitations in the Polar® system – predominantly augmented transmission range and signal strength, as well as simultaneous recording from multiple sensors through a single, centralised monitor. However, they are often more costly and have not yet been as widely validated as Polar® devices for animal research.

## 4.3. Inter-individual variation, repeatability, and reproductive performance correlates of baseline HR and HRV

There was significant inter-individual variation in both baseline $RMSSD_{COR}$ and $HR_{COR}$ (Figure 3.6). These differences were not associated with days since capture, percentage mass loss, day of lactation, or time of day. However, since filtration protocols resulted in a small and uneven sample size, variation attributed to these factors may not have been detected.

$RMSSD_{COR}$ also showed a high degree of repeatability between early and late lactation. Furthermore, females could be separated into two groups based on mean baseline $RMSSD_{COR}$ in early and late lactation that differed significantly, wherein females exhibited “higher” or “lower”

baseline $RMSSD_{COR}$ (Figure 3.7a). There was no evidence to suggest baseline $HR_{COR}$ was repeatable between early and late lactation; neither could females be separated into two significantly different groups based on mean baseline $HR_{COR}$ (Figure 3.7b). Overall, these results suggest baseline $RMSSD_{COR}$ was consistent across lactation, whereas baseline $HR_{COR}$ was not, and that some females exhibited consistently higher or lower baseline $RMSSD_{COR}$. Underlying differences in ANS activity that distinguish coping styles are often only evident in HRV metrics (von Borell *et al.* 2007). Although females were not challenged with a standardised stressor, high sympathetic tone has been associated with greater sympathetic reactivity, and subsequently, with the proactive coping style (Sgoifo *et al.* 1998, 2005). It is tempting to consider females with consistently "lower" baseline $RMSSD_{COR}$ (i.e. higher sympathetic tone) as proactive individuals, and females with consistently "higher" baseline $RMSSD_{COR}$ (i.e. lower sympathetic tone) as reactive individuals. However, further studies are needed to substantiate these preliminary findings since sample sizes were small ($N = 7$).

Mean baseline $RMSSD_{COR}$ and $HR_{COR}$ were not associated with maternal expenditure (MDML) or fitness outcomes (PDMG). However, mean baseline $RMSSD_{COR}$ and $HR_{COR}$ were negatively correlated with maternal transfer efficiency (i.e. actual residuals) and variation in maternal transfer efficiency (i.e. absolute residuals), respectively (Figure 3.8, Figure 3.9). The performance of different coping styles can be determined by the stability of the environment (Koolhaas *et al.* 1999). Proactive individuals tend to be more successful under stable conditions, whereas reactive individuals tend to be more successful in highly variable, stochastic conditions (Dingemanse *et al.* 2004; Smith and Blumstein 2008). Assuming females with lower baseline $RMSSD_{COR}$ are putative proactive individuals, increased maternal transfer efficiency might be expected within stable environments favouring proactive coping styles. Reactive behavioural types are characterised by greater variation in reproductive performance (Twiss *et al.* 2012), which might explain why females with lower baseline $HR_{COR}$ were more varied in their maternal transfer efficiency – assuming females with lower baseline $HR_{COR}$ (i.e. lower sympathetic tone) were putative reactive individuals.

## 4.4. Future research

Future research should focus on using simultaneous ECGs to validate the use of Polar® devices for conducting HR and HRV analyses in grey seals. Validation studies should examine

the level of agreement between RR intervals (using Bland-Altman analysis) and differences in HR and HRV metrics obtained from Polar® devices and ECGs (Marchant-Forde *et al.* 2004; Parker *et al.* 2010; Jonckheer-Sheehy *et al.* 2012). They should also examine the reliability of HR and HRV metrics obtained from non-stationary segments, as there is evidence the agreement between Polar® devices and ECGs may be diminished under non-stationary conditions (Parker *et al.* 2010). Recent developments in portable non-invasive ECGs, such as Veterinary iPhone ECG Monitors (*Woodley Veterinary Diagnostics*), could make concurrent Polar® and ECG recordings easily achievable in captivity (e.g. Fonfara *et al.* 2014).

In addition to validation studies, ECGs should be used to refine artefact detection criteria and correction protocols. The critical percentage for the detection of Peaks (see *Chapter 2.4. Artefact detection*) was chosen based on two assumptions: (1) the uppermost limit of acceptable percentage change between successive RR intervals was 66%, as estimated from recordings of instantaneous HR in diving grey seals (Thompson and Fedak 1993); and (2) changes of this magnitude were unlikely to occur in this study, as they have only been observed during prolonged dives. As with filtration and correction protocols, it is not possible to determine the efficacy of the chosen critical percentage without corresponding ECG recordings. In this regard, ECGs of resting grey seals on land – particularly at the onset of apnoea, where the most abrupt and profound changes are likely to occur (Andrews *et al.* 1997) – would be invaluable for future studies. Detection criteria for Flats and Stairs cannot be refined further, as they do not vary in their manifestation – unlike Peaks, which can present as five different types (Figure 2.6). Simultaneous Polar® and ECG recordings could also be used to identify potential artefacts that are specific to grey seals. Artefacts in Polar® recordings have been rigorously examined and categorised, but only in domesticated animals such as pigs (Marchant-Forde *et al.* 2004) and dogs (Jonckheer-Sheehy *et al.* 2012). Phocid seals known for their characteristically arrhythmic HRs, which may generate artefacts not observed in other species. Equine ECGs, for example, have pronounced T waves that may be mistaken for R peaks by Polar® devices (Parker *et al.* 2010). Distribution-based detection algorithms (e.g. Kaufmann *et al.* 2011; Berntson *et al.* 1990; Linden and Estrin 1998) could provide an alternative to the Peak detection criteria used in this study. However, they are unable to identify all artefacts known to occur in Polar® recordings, and would need to be used alongside detection criteria for Flats and Stairs. Correction protocols

were used to counteract the effect of Peaks on HR and RMSSD in extracted segments. Future studies could correct the Peaks within the RR interval data, before HR and RMSSD are calculated, using published correction algorithms that have been validated for Polar® data (Cheung 1981) – though it is not known whether they can be used to correct Flats and Stairs.

Modifications to Polar® devices could be made to address limitations that cause artefacts, such as small memory capacity, small transmission range, or impaired electrode conductance. Greater memory capacities would allow monitors to be deployed with the sensors, minimising signal loss or interference, as well as disturbance to study females by removing the need for repeated monitor deployments. Augmented transmission ranges would provide similar advantages, and/or overcome the limitations of small memory capacities. Greater signal strength would allow monitors to be positioned further away from study females, reducing disturbance and the likelihood of artefacts caused by signal interference between the sensors and monitors (i.e. Flats and Stairs). Impaired electrode conductance caused by uneven electrode contact and electrode gel loss could be improved with the use of a non-soluble and/or conductive adhesive gel. Polar® soft strap electrodes could be modified into articulated electrodes positioned on either side of the body cavity, which would minimise noise from muscle action potentials (i.e. Stairs and Peaks). Articulation would also allow each individual electrode to be sealed, preventing uneven distribution of electrode gel, evaporation, leakage, or contamination from mud. Alternatively, the contact electrodes of the Polar® system could be replaced altogether by the standard electrodes used in ECG systems, which provide greater contact and better signal quality (Parker *et al.* 2010).

Ultimately, the aim of this study was to develop a methodology for quantifying physiological CIDs concurrently with behavioural CIDs in grey seals. Whilst preliminary findings were promising, HR and HRV metrics were only obtained from baseline segments. Future studies should introduce a standardised stressor (e.g. RCV: Twiss *et al.* 2011, 2012) to record the magnitude of change (i.e. sympathetic reactivity) and the time taken to return to pre-stressor values (i.e. parasympathetic reactivity). Coupled with established behavioural metrics (i.e. pup-checking, aggression: Twiss *et al.* 2011, 2012), sympathetic and parasympathetic reactivity could be used to substantiate putative coping styles, or elucidate the proximate physiological underpinnings of proactive-reactive behavioural types, in grey seals.

## 4.5. Conclusion

The primary objective of this study was to assess the suitability of a commercially available, minimally invasive HR logger (Polar® RS800CX monitors and Polar® H2/H3 sensors) for conducting HR and HRV analyses in wild free-ranging grey seals. Polar® devices were deployed successfully during the 2013 breeding season on female grey seals ($N$ = 15) on the Isle of May, Scotland, and were capable of recording characteristic HR patterns observed in phocid seals. The suitability of the Polar® monitors and sensors was assessed by quantifying the quality of the RR interval data obtained – i.e. the occurrence of artefacts and their effects on HR and HRV metrics (RMSSD). Artefacts in Polar® recordings are well-documented and can be broadly classified into three categories – Flats, Stairs, and Peaks – all of which were observed in this study and significantly biased HR and RMSSD. The main causes of artefacts were non-stationary behaviour and impaired electrode conductance, which interfered with signal transmission between the skin and electrodes or the sensors and monitors. Since artefacts were widespread, filtration and correction protocols were necessary, at the cost of severely limiting the amount of data available for analysis. Although it was not possible to determine the efficacy of protocols without corresponding ECG recordings, resulting HR and RMSSD were comparable or within range of previously reported values in the literature, suggesting they were able to counteract the effects of artefacts. Filtered and corrected data were used for subsequent analyses quantifying inter-individual variation, repeatability, and reproductive performance correlates of baseline HR and RMSSD.

There was significant inter-individual variation in baseline $RMSSD_{COR}$ and $HR_{COR}$, which could not be explained by factors associated with the breeding season (e.g. percentage mass loss, day of lactation), diurnal rhythms (e.g. time of day), or stressors (e.g. days since capture). Baseline $RMSSD_{COR}$ – but not baseline $HR_{COR}$ – was consistent across early and late lactation, and there was some evidence to suggest individuals showed consistently higher or lower baseline $RMSSD_{COR}$. Females with lower resting $RMSSD_{COR}$ were more efficient (i.e. their pups gained mass at a rate higher than would be expected at the rate of maternal mass loss), whereas females with lower resting $HR_{COR}$ were more varied in their maternal transfer efficiency. These findings build upon previous studies on behavioural CIDs in female grey seals (Twiss *et al.* 2011, 2012) by providing the first preliminary evidence for physiological CIDs that

are associated with maternal investment. However, due to small sample sizes, further studies are required to determine whether the inter-individual variation, consistency, and correlations with proxies of maternal reproductive performance observed in baseline $RMSSD_{COR}$ and $HR_{COR}$ are truly indicative of coping styles.

Despite their limitations, Polar® devices have immense potential as a minimally invasive research tool for conducting HR and HRV analyses in wild free-ranging pinniped species. Future research should focus on: (1) comparative ECG studies, to validate metrics obtained using Polar® devices, artefact detection criteria, artefact correction protocols, and the use of Polar® devices to record RR intervals under non-stationary conditions; (2) modifying Polar® devices, to minimise the occurrence of artefacts by increasing the memory capacity of monitors, augmenting transmission ranges of sensors, and improving electrode conductance; and (3) obtaining HR and HRV metrics concurrently with behavioural metrics following exposure to a standardised stressor.

## Glossary

| Term | Definition | Reference(s) |
|---|---|---|
| Animal personality (p3) | Consistent inter-individual differences in behaviour that are stable over time and across situations. Interchangeable with ***consistent individual difference***. | Sih *et al.* (2004a, 2004b) |
| Behavioural plasticity (p4) | The ability of individuals to change their behaviour in response to variation in environmental conditions. | Betini and Norris (2012) |
| Behavioural syndrome (p4) | A suite of correlated ***behavioural traits*** that are stable over time and across situations. For example, individuals that are consistently more bold, aggressive, and exploratory are considered to be "proactive" individuals. | Sih *et al.* (2004a, 2004b) |
| Behavioural trait (p3) | A distinguishing behavioural characteristic of ***animal personality*** that is quantifiable and stable over time and across situations. Five ecologically relevant traits have been identified in animals, including boldness, exploration, activity, aggression and sociality. | Sih *et al.* (2004a, 2004b); Menzies *et al.* (2013) |
| Behavioural type (p4) | A classifier of ***animal personality***. For example, individuals can be classified as "bold" or "shy" behavioural types based on their reaction to risky situations. | Sih *et al.* (2004a, 2004b) |
| Chronic stress (p5) | Pathology that results from overstimulation of ***stress responses***. | Romero and Butler (2007) |
| Consistent individual difference (p1) | A consistent inter-individual difference in behaviour that is stable over time and across situations. Interchangeable with ***animal personality***. | Sih *et al.* (2004a, 2004b) |
| Context (p3) | A functional behavioural category (e.g. feeding, mating). | Sih *et al.* (2004a, 2004b) |
| Coping style (p1) | A suite of correlated behavioural and physiological traits that are stable over time. | Koolhaas *et al.* (1999, 2007, 2010) |
| Heart rate variability (p2) | The variation in instantaneous HR or the intervals between heartbeats, also known as ***RR intervals*** or ***inter-beat intervals (IBIs)***. | ESC Task Force (1996)<br>von Borell *et al.* (2007) |
| Inter-beat intervals (p2) | *See* ***RR Intervals***. | |
| RR intervals (p2) | The interval between heartbeats, or more specifically, between the R peaks of QRS complexes in ECGs. Also known as ***inter-beat intervals (IBIs)***. | ESC Task Force (1996) |
| Situation (p1) | A given set of conditions that can be classified along a continuous gradient (e.g. levels of predation risk) or into discrete sets (e.g. breeding vs non-breeding). | Sih *et al.* (2004a, 2004b) |
| Stress (p5) | A term that encompasses, and often used to refer to, ***stressors***, ***stress responses***, or ***chronic stress***. | McEwen & Wingfield (2002)<br>Romero (2004)<br>Romero and Butler (2007) |
| Stressor (p5) | A threat – real or perceived, intrinsic or extrinsic, physiological or psychological – to the homeostatic integrity of an organism. | McEwen & Wingfield (2002)<br>Romero (2004)<br>Charmandari *et al.* (2005) |
| Stress response (p5) | A suite of behavioural and/or physiological mechanisms organisms mounted in response to a ***stressor*** that re-establish homeostatic integrity. | McEwen & Wingfield (2002)<br>Romero (2004) |

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