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IMPACTS OF CAPTURE AND HANDLING ON WILD BIRDS

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Summary of the Thesis

Bird ringing is a key ecological research technique that involves the capture and handling of birds. It is used extensively to obtain information on population dynamics of wild birds, and many aspects of avian behavior, physiology and life-history, which would otherwise be unfeasible to obtain. Despite millions of birds ringed every year, little is known about the short- or long-term impacts on birds, and whether there are negative welfare, conservation and scientific consequences, which can ultimately bias the interpretation of data from wild bird studies. In this thesis I study the type of intrusion that capture and handling causes to the bird, by analyzing their interlinked physiological and behavioral responses to capture stress, including hormonal and immunological responses, energy regulation decisions (feeding behavior and thermal regulation) and breeding effort. I further study the immediate effects that capture and handling has on birds through analyzing types of injuries and the rates at which injuries and mortality occur. I have focused mainly on mist-netting, which is the most widely used capture technique, and captures of passerine birds, which is the most frequently sampled taxon. However, I also demonstrate how the impacts of capture and handling can be studied in marine birds and applied to other capture methods. These studies reveal the range of short-term impacts that capture and handling may have on wild birds, and highlight aspects of methodology that have a strong effect on these impacts. The longer term consequences for lifetime fitness and demographic change require further study. This thesis demonstrated the importance for researchers to be aware of the potential effects of their activities on their study subjects, particularly for susceptible species and situations, and to continuously reasses their methods for effective improvement. I propose several guidelines, which aim to promote the birds' welfare in regards to data collection.

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Bander's Code of Ethics:

“1. Banders are primarily responsible for the safety and welfare of the birds they study so that stress and risks of injury or death are minimized (…)*”*

in North American Bander's Manual for Shorebirds

Chapter 1

Introduction

1.1 Relevance of the topic

Man's curiosity and knowledge of the natural world is as old as humanity itself. We can find remnants of an embryonic ecology, in the first hunting strategies, and in the understanding of movement patterns and distribution of animals. The first known documents related to natural science disciplines are those of Aristotle and other philosophers of the classical period, around 350bC. For many centuries the natural sciences were restricted to descriptions of the natural world based on the capture or observation of organisms.

Until the 20th century, the major goal of the natural sciences was the cataloging of new species and morphological descriptions of individuals. A proper species description was was often based on the collection of specimens, often to be preserved by taxidermy. With the awareness that species and habitats were under anthropological pressure and that some species were already at the risk of extinction, disciplines such as conservation of biodiversity and the study of environmental change arose. With this shift in paradigm it became important to understand species and their requirements, by studying living species. To achieve this, techniques to capture and mark individuals started being developed and from this newly acquired appreciation of preservation of life, another appreciation began to emerge: that of ethics in the scientific method. Balancing the outcomes of scientific activity and the potential impacts that it causes to wildlife has been a subject of ongoing debate, which is far from being resolved.

Every year, millions of wild birds are captured, handled, marked (usually with only an individually numbered ring or band) and released in the course of research studies and population monitoring. The European Union for Bird Ringing (EURING) estimates that approximately 4 million are ringed annually across Europe (Baillie *et al*. 2007), and over 115 million birds have been ringed in Europe since the advent of bird-ringing studies just over a century ago (Baillie *et al*. 2007). While in Britain and Ireland alone, the number of birds ringed increases every year, with over 1.15

million birds (adult and pulli) ringed in 2011 (Dadam *et al*. 2012), I estimate that globally, the total number of birds ringed, annually, could well be double the number provided by EURING for Europe alone.

The proportion of ringed individuals in a population varies widely; depending mainly on the conservation status and interest to science of the species. For example, all known wild individuals of the kakapo (*Strigops habroptilus*) have been ringed, and are continuously monitored.

The marking of birds as individuals has facilitated detailed field investigations of avian biology, including population dynamics, behaviour, ecology and physiology. Indeed, much of what we know about the lives of wild birds has been the direct result of marking studies. For example, of the 115 million birds already ringed in Europe, over 2 million have been "recovered" (re-captured or found dead) elsewhere, revealing in great detail the migratory journeys undertaken by many species (Wernham *et al*. 2002). Likewise, up to January 2011, of the 64 million birds that have been ringed in the United Sates, over 4 million were recovered (USGS 2001). Bird-ringing studies are undoubtedly important for their scientific and conservation value (e.g. Dadam *et al*. 2012). In some countries, such as the UK, they are an important integrating part of the national demographic monitoring of bird populations, for example, to assess productivity and abundance of some species and furthermore whether these changes are indicative of environmental change (Dadam *et al*. 2012). Because of this, bird ringing is rightly viewed as a "vital research tool" (Baillie *et al*. 2009), an "important technique" (Calvo & Furness 1992) and a "unique and essential method" (Bairlein 2001).

Birds may be captured by a variety of methods (table 1.I), though in terms of numbers, most birds are now caught using mist-nets (fine-gauge nets that are tethered vertically in position between poles), in widespread use by bird-ringers since the 1960s. On capture, birds are handled, measured, marked using rings, bands or other methods, and released back into the wild (table 1.II). Marking with metal leg-rings is the most widely used technique, although a range of other marking techniques may be used together with leg-rings, or as an alternative. Recent technological developments have also led to some birds being fitted with small electronic tracking devices such as geo-locators, radio-transmitters and satellite transmitters. During handling, tissue samples may also be collected, including feathers, blood, faeces or vomit (table 1.III).

Table 1.I: Methods used to capture wild birds for research purposes (excludes methods for non-research purposes such as pole-traps, sticky lime-traps etc.). Frequency of use is indicated using a 4-point scale; 1 = very common, 2 = common, 3 = rare, 4 = very rare.

Method	**Frequency of use**	**Key references**	**Studies examining impacts**
Mist nets	1	Recher *et al*. 1985; Jenni & Leuenberger 1996; Fair *et al*. 2010; NABC 2001; Ralph & Dunn 2004	Recher *et al*. 1985; Refsnider 1993; Dunn 1999; Ballard *et al*. 2004; Fair *et al*. 2010, Petronilho 2002
Submerged mist nets	4	Breault & Cheng 1990	
Heligoland traps	2	Bub 1995; Woodford 1959; Hussell & Woodford 1961; Brownlow 1952	NABC 2001
Other passive funnel traps-type traps	2	Bub 1995; Fair *et al*. 2010; Senar *et al*. 1997	Ponjoan *et al*. 2008; Fair *et al*. 2010
Actively triggered cage-type traps (e.g. clap traps)	2	Bub 1995	Leenen 2009
Trapping at nest	2	Fair *et al*. 2010; Kania 1992	Fair *et al*. 2010; Kania 1992
Whoosh / clap nets	3	Davis 1981; Bub 1995	
Cannon nets	3	Bub 1995; Fair *et al*. 2010	Ponjoan *et al*. 2008; Cox Jr & Afton 1998a; Fair *et al*. 2010
Nest traps	3	Davis 1981	Hill & Talent 1990
Leg nooses	4	Bub 1995	Ponjoan *et al*. 2008, Benson & Suryan 1999
Bal-chatri traps	3	Berger & Mueller 1959; Thorstrom 1996; Bloom *et al*. 2007; NABC 2001	Fair *et al*. 2010
Use of sedative chemicals (eg. Alpha-chloralose)	4	Caccamise & Stoufer 1994	Stoufer & Caccamise 1991; McGowan & Caffrey 1994

Table 1.II: Marks and devices used in studies of wild birds.

Method	**Key references**	**Studies examining impacts**[1]
Marks for recognition of individuals or groups		
Metal leg rings / bands	Fair *et al*. 2010 ; Varland *et al*. 2007	Amat 1999; Reed & Oring 1993; Regehr & Rodway 2003
Plastic coloured leg rings	Fair *et al*. 2010; Varland *et al*. 2007	Pierce *et al*. 2007; Reed & Oring 1993; Gratto-Trevor 1994; Cresswell *et al*. 2007
Nasal marks	Fair *et al*. 2010	Regehr & Rodway 2003; Evrard 1996; Pelayo & Clark 2000
Patagial (wing) tags/flags and Flipper bands	Fair *et al*. 2010 ; Varland *et al*. 2007	Culik *et al.* 1993; Bellebaum & Buchheim 2008; Saraux *et al*. 2011a
Neck rings	Fair *et al*. 2010	Menu *et al*. 2000; Schmutz & Morse 2000; Castelli &. Trost 1996; Reed *et al*. 2005
Plumage dyes	Fair *et al*. 2010; Varland *et al*. 2007; Wendeln *et al*. 1996	White *et al*. 1980
Leg flags	Fair *et al*. 2010	Regehr & Rodway 2003
Geo-locators	Stutchbury *et al*. 2009	Rodríguez *et al*. 2009
Satellite transmitters	Meyburg & Fuller 2007	
Radio transmitters	Kenward *et al*. 1999; Newman *et al*. 2005 ; Mech & Barber 2002; Southern 1965; Dwyer 1972; FAO 2007, Fair *et al*. 2010	Ponjoan *et al*. 2008; Massey *et al.* 1998; Whittingham 1996; Sharpe *et al*. (unpublished); Schmutz & Morse 2000; Demers *et al*. 2003; Mech & Barber 2002; Pietz *et al*. 1993; Rotella *et al*. 1993; Houston & Greenwood 1993; Vaughan & Morgan 1992; Phillips *et al*. 2003; Sohle 2003, Anich *et al*. 2009; Hiraldo *et al*. 1994; Igual *et al*. 2005 ; Reynolds *et al*. 2004; Steenhof *et al*. 2006; Whidden *et al*. 2007; Naef-Daenzer *et al*. 2001

Erro! A origem da referência não foi encontrada.**:** (continued)

Method	**Key references**	**Studies examining impacts**[1]
Attached**[2] **devices for remotely tracking location and/or behaviour		
PIT tags	Boisvert & Sherry 2000; Ottosson *et al*. 2001 and Keiser *et al*. 2005 *in* Nicolaus *et al*. 2008	Nicolaus *et al*. 2008

[1] Calvo & Furness (1992) provide a general review of impacts of marks and devices on birds, as do Boitani & Fuller 2000 (pp.27-31) and Nietfeld *et al*. 1994.

[2] Tracking methods, that do not require the attachment of a device to individual birds, are excluded.

Table 1.III: Tissue samples collected from birds.

Method	**Key references**	**Studies examining impacts**
Blood 1. Whole blood 2. Plasma 3. Platelets 4. Red blood corpuscles	Harvey *et al*. 2006; FAO 2007; Fair *et al*. 2010	Sheldon *et al*. 2008; Watson *et al*. 2004; Cockrem *et al*. 2009
Feathers	Harvey *et al*. 2006; Taberlet & Bouvet 1991; Segelbacher 2002; Horvath *et al*. 2005; Fair *et al*. 2010	Donald & Griffith 2011
Vomit	Branco *et al*. 2007 ; Barrett *et al*. 2007; Diamond *et al*. 2007; Fair *et al*. 2010	Verkuil 1996; Diamond *et al*. 2007
Pellets	Johansen *et al*. 2001; Votier *et al*. 2001, 2003, 2007; Fair *et al*. 2010	
Faeces	Barrett *et al.* 2007; Idaghdour *et al*. 2003; FAO 2007; Palme 2005	

Although capture and marking is clearly an invaluable and sometimes essential method for detailed field studies of wild birds, there is widespread acknowledgement that capture and handling themselves are stressors (e.g. Laiolo *et al.* 2009, Le Maho *et al*. 1992, Marco *et al*. 2006). Specifically, capture and handling potentially induces a range of stress-related responses, including hormonal, behavioural, and physiological responses (Le Maho *et al*. 1992). It is known from studies of stress responses in general that such responses may affect the welfare, behaviour, fitness, and survival of individuals, which may ultimately impact on the population (e.g. Laiolo *et al*. 2009, Angelier *et al*. 2009, Costantini *et al*. 2007, Millspaugh & Washburn 2004). Thus, the impacts of capture and handling could have demographic and conservation implications (e.g. Spée *et al*. 2011). Furthermore, at the individual level, the welfare of the subjects should be an important consideration in any research programme (e.g. Cuthill 1991, Redfern & Clark 2001), with recent studies demonstrating that even though the impacts are often considered small, they still exist (e.g. Spotswood *et al*. 2012). Any negative effects of capture and handling on individual birds might have a substantial impact on the population as a whole. From a scientific perspective, most research programmes that use capture and handling as a tool make the assumption (explicitly or, more often, implicitly) that the behaviour, biometrics, ecology and demography of the captured animals are broadly representative of the whole population (Redfern & Clark 2001). However, if capture and handling has appreciable effects on the bird's behaviour and physiology then this assumption may not hold, with implications for the accurate interpretation of such datasets.

The methods used for capturing and handling wild birds deserve a rigorous examination, not least because they are often the topic of informal yet heated debates that are frequently conducted largely in the absence of objective data (e.g. [ca. 2012] online discussions within the *Facebook* group [Stop Bird Banding]). Anecdotal evidence is not only used to draw attention to individual incidents where bird ringing has had a negative impact, but has sometimes been used to support the view that capture and handling does not have marked adverse effects on birds. However, it is difficult to draw strong conclusions from this kind of information. For example, birds are sometimes observed to begin feeding (themselves or their offspring) immediately following release, but this could be because they urgently need to recoup lost energy reserves rather than because they are unaffected by the experience and in any case, other individuals may not begin to feed so promptly. What is needed is a comprehensive, evidence-based overview of the existing data on the avian stress response, broadly defined but specifically applied to the context of capture and handling of wild birds.

1.2 What is stress and how do animals respond to stress?

Discussion of the effects of stress on organisms requires clear working definitions of what stress is, its causes (the "stressor"), and of how organisms respond to such stressors (the "stress-response"). Numerous definitions of stress exist in the literature, however some are very unclear in distinguishing the stress response from stress itself, and others focus in different challenges, such as the organism's perception and physiological responses. In table 1.IV, I present such definitions categorising them as pertinently as possible however throughout the thesis I will refer to stress, stressor and stress response by using Selye's (1963) terminology: (stress) describes an "animal's defence mechanism in the presence of a stimulus (stressor)", that can be "any situation that elicits defensive response" (the stress response) (*in* Siegel 1980).

Table 1.IV: Some commonly used definitions of (i) stress, (ii) stressors, and (iii) stress response.

Definitions of stress	**References**
Stress denotes the magnitude of forces external to the bodily system which tend to displace that system from its resting or ground state	Lee 1965
Stress describes the animal's defence mechanism in presence of a stimulus-stressor	Selye 1963
Emergency life history stage (ELHS), that directs the individual away from normal life history stages into a physiological and behavioural state that will allow survival in the best condition possible.	Wingfield & Kitaysky 2002
(...) can result in an acute and substantial elevation of plasma levels of glucocorticoids (...) which can subsequently have marked effects on physiology and behaviour	Lynn & Porter 2008
Definitions of stressor	
Any situation that elicits defensive responses	Selye 1963
An environmental condition that is adverse to the well being of an animal	Stott 1981
Relatively brief events termed "labile perturbation factors", that potentially reduce long-term fitness	Wingfield *et al*. 1998
Stressors are real or perceived challenges to an organism's ability to meet its real or perceived needs	Greenberg *et al*. 2002
Definitions of stress response	
Various reactions undertaken by organisms to restore physiological integrity referred to as 'biological stress'	Stott 1981

Table 1.IV**:** (continued)

Definitions of stress response	**References**
The stress response is manifested through physical, behavioural and physiologic changes (…)	Harvey *et al*. 1984
Responses (...) evoked that enable the organism to cope by either removing the stressor or facilitating coexistence with it	Antelman & Caggiula 1990
Biological response elicited when an individual perceives a threat to its homeostasis	Moberg & Mench 2000
Physiologic response to stimuli perceived as a threat (real or anticipated)	Newman et al. 2005
Mechanisms that allow individuals to survive or cope with a stressor	Martin 2009

As Selye's definition implicates, stress, is the body's behaviour towards a stressor that requires a stress response. Such a definition implies that a stressor will be anything that can stimulate the stress response, be it an adverse environmental condition that is detrimental to the well being of the bird (Stott 1981), or a simple encounter with a predator, whether real or perceived (Greenberg *et al*. 2002).

Similarly, the avian stress response can be defined at a variety of levels from general definitions of its function, to specific definitions of its manifestation at the individual level, i.e. hormonal, physiological and behavioural responses, which in turn may lead to population-level ecological responses (table 1.V). Specifically the stress response is "the physiological and behavioural responses, mediated by hormones and neural mechanisms, that birds use when they are facing an aversive stimulus" [such as capture and handling]. The stress response will be therefore, "the mechanisms that allow individuals to survive or cope with the stressor" (Martin 2009) by redirecting their behaviour and physiology to immediate life-saving actions (Moberg & Mench 2000). Such a definition implies that the stress response is in general adaptive, though it may not always result in the survival of the individual exhibiting a stress response (Angelier *et al*. 2009).

Table 1.V: Some commonly observed manifestations of the avian stress response.

Manifestations	**References**
Hormonal	
Increased circulation of glucocorticoids [e.g. corticosterone]	Axelrod & Reisine 1984; Schwabl *et al*. 1991; Romero *et al*. 1997; Silverin 1998; Sapolsky *et al*. 2000; Möstl & Palme 2002; Cockrem *et al*. 2008
Increased production of catecholamine hormones [epinephrine and norepinephrine]	Koolhaas *et al*. 1999; Romero & Butler 2007; Martin 2009
Physiological	
Increased heart and respiratory rate	Newman *et al*. 2005; Cabanac & Guillemette 2001; Straub *et al*. 2003; Siegel 1980; Cyr *et al*. 2008
Muscular exertion	Abbott *et al*. 2005; Ponjoan *et al*. 2008; Spraker *et al*. 1987; FAO 2007; Dabbert *et al*. 1993
Changes in heat flux / body temperature	Hiebert *et al*. 2000; Møller 2010; Cabanac& Guillemette 2001; Nord *et al*. 2009; Carere & van Oers 2004; Davenport *et al*. 2004; Siegel 1980
Changes in immunocompetence	Bourgeon & Raclot 2006; Martin *et al*. 2005; Butler & Dufty Jr 2007; Cirule *et al*. 2012; Martin 2009
Increased blood pressure, muscle tone and nerve sensibility	Siegel 1980
Increase blood sugar levels	Siegel 1980

Table 1.V**:** (continued)

Manifestations	**References**
Behavioural	
Increased vigilance	Gosler 2001
Aggression towards the handler/ringer, screaming	Laiolo *et al*. 2009
Alarm/distress calls	Laiolo *et al*. 2009
Changes in time spent on foraging activity	MacLeod & Gosler 2006; Angelier *et al*. 2011; Gosler 2001
Decrease in time spent on reproductive activity	Jennings *et al*. 2009
Regurgitation of food	Branco *et al*. 2007; Barret *et al*. 2007
Increased dispersion away from capture site	Lee & McDonald 1985; Silverin 1997; Wingfield *et al*. 1997
Feather loss	Møller *et al*. 2006; NABC 2001
Ecological (population-level effects)	
Survival rates	Cox & Afton 1998a; Holt *et al*. 2009
Breeding success	Perfito *et al*. 2002; Burtt & Tuttle 1983; Thorup 1995, Angelier *et al*. 2011; Van den Brink & Pigott 1996; Hull & Wilson 1996a; Ortega *et al*. 1997; Olsen & Schmidt 2001
Mate choice	Roberts *et al*. 2007
Population size	Holt *et al*. 2009
Other potential impacts of stressors, not part of the adaptive stress response	
Disruption of feathering / increase in preening	Greenwood & Sargeant 1973; Gilmer *et al*. 1974; Siegfried *et al*. 1977

Table 1.V**:** (continued)

Manifestations	**References**
Other potential impacts of stressors, not part of the adaptive stress response	
Hypo/ hyperthermia	Nord *et al*. 2009; FAO 2007; Carere & van Oers 2004
Capture myopathy	Ponjoan *et al*. 2008; Spraker *et al*. 1987; Dabbert *et al*. 1993; Nicholson *et al*. 2000
Physical injury (e.g. cuts, strains, fractures)	NABC 2001; Spotswood *et al*. 2012
Predation	Spotswood *et al*. 2012; NABC 2001
Mass loss/ gain	Perry 1981; Macleod & Gosler 2006; Senar *et al*. 2002; Carrascal & Polo 1999
Haemorrhage	NABC 2001
Thermal exaustion	NABC 2001
Strangling	NABC 2001

Physiologically, the stress response is mediated by two endocrine systems: the adrenal medulla, which releases the catecholamine hormones epinephrine and norepinephrine, and the adrenal cortex, that releases glucocorticoid hormones (Romero & Butler 2007). The first acts on the immediate response designated by the “fight-or-flight” response, and the second acts over a longer time scale. In the presence of a stressor the adrenal medulla will release epinephrine, arousing the body to action, through: increasing heart rate, breathing rate and blood pressure, whilst releasing norepinephrine that will divert the blood flow to skeletal muscles and away from the gut to promote either escape behaviour (the “flight” part of the response) or enhancing the bird’s levels of aggression (the “fight” part of the response) (Siegel 1980; Koolhaas *et al*. 1999; Purves *et al*. 2001). The fight-or-flight response is characterised by rapid increases in blood pressure, muscle tone, nerve sensibility, respiration rate, and blood sugar levels, which are made available by the norepinephrine, and utilized by the epinephrine (Siegel 1980). Epinephrine promotes the relaxation of the smooth muscle in the airways to dilate the bronchial tubes, increasing oxygen intake. In contrast birds can also demonstrate a passive stress response characterized by a reduced skeletal blood flow, lowered heart rate and reduced respiration (to hinder detection by a predator) (Gabrielsen & Smith 1995, Criscuolo *et al*. 2001)

Behaviourally, when coping with stress, Koolhaas *et al*. (1999) have determined that the birds can display two types of behaviour: in the first the bird exhibits a typical fight-or-flight behaviour, becoming more agitated, and a second and alternative type, which is behaviourally characterized by immobility and low levels of aggression. These two very different types of response (which may be two ends of a spectrum) allow the bird either to remove the stressor (by escaping it), or to endure it (“facilitated coexistence”), respectively.

Adaptive responses to stressors may involve a trade-off between responses favouring immediate survival and those favouring longer term reproductive success (Buchanan 2000, Gosler 2001, Wingfield & Silverin 2002). How a wild bird perceives, interprets and responds to capture and handling by humans may therefore potentially affect its long-term fitness as well as its immediate survival and welfare.

It is questionable whether stress responses are always adaptive, for example if a bird promotes the “agitated” type of behaviour and tries to escape a capture device it can clearly cause further harm to itself. The best way to understand whether a stress response is always adaptive is to analyze and fully comprehend the effects of capture and handling. Clearly the most obvious potential negative impact of capture and handling on birds may be direct mortality, but a wide range of other impacts are possible. I will review the main types of impact that are described across the literature, to provide a better understanding of the outcomes of the stress response. It is worth mentioning beforehand that some types of impact are not mutually exclusive, and their net effects may be additive or even synergistic. For example, a bird suffering wing-strain may be more prone to predation following its release.

1.2.1 The role of the endocrine system in the adaptive stress response

In terms of Selye’s (1963) definition of the stress response as “the animal’s defence mechanism in presence of a stimulus-stressor”, capture and handling are likely to be perceived as stimulus-stressors by birds. Indeed, standardised capture-stress protocols are used to induce a stress response, in order to investigate the hormonal changes underlying the physiological response to stressful events (Wingfield *et al*. 1995, 1997, Silverin 1998, Canoine *et al*. 2002, Canoine & Gwinner 2002, Cockrem & Silverin 2002).

As mentioned previously, the first response a bird will demonstrate when in stress, is mediated by the endocrine system, and relies on the release of hormones. The immediate action is mediated by the release of epinephrine (the same as adrenaline) and norepinephrine that will provide in conjunction, an immediate behavioural response, that can either be translated into an “agitated” or a “calm” behaviour. Over the longer time scale, glucocorticoids are released by the adrenal cortex, and by interacting with intracellular receptors will cause the production of new proteins. The type of response that the glucocorticoids give is more sustained, and provides a wider array of responses to the stressor than an immediate “fight-or-flight” response.

The main glucocorticoid hormone associated with stress responses in birds is corticosterone. The primary function of glucocorticoids in birds is to maintain energetic levels of glucose and free fatty acids. Thus, when there is an energetic challenge, circulating corticosterone levels rise and energy stores are mobilized in order to maintain basic functions (Dallman *et al*. 1993), or to facilitate emergency responses.

There are two different types of stress states that a bird can experience, and those are determined by the duration and intensity of stress. When a stress episode lasts for a period of a few minutes to a few hours (e.g. capture and handling) it is defined as an acute stress episode, but if there is a long-term exposure to the stress for periods of several hours per day for weeks or months it is designated as chronic stress (Dhabhar & McEwen 1997). While the immediate effects of stress hormones are mostly advantageous, long-term exposure to stress leads to a constant elevated level of corticosterone, that can have detrimental impacts such as heart disease (which results from the permanent high blood pressure), damage to the muscle tissue, inhibition of growth, suppression of the immune system, whilst down-regulating the corticosterone responses to acute stressors (Rich & Romero 2005, Martin *et al*. 2005). Because capture and handling is a recognized acute stressor, I will focus on effects that are a consequence of acute stressors.

Because of its role in the stress response, the level of circulating corticosterone in the blood are often used to define whether the bird is “stressed” or not. In an unstressed situation, birds display a baseline level of corticosterone, which is considered normal for daily function, and allows the bird to maintain basic energy and salt balance (Busch 2006). However, after being presented with a stressor, the corticosterone levels rise rapidly (over a few seconds-minutes), until it reaches a plateau in proportion to the magnitude of the stressor (e.g. Muller *et al*. 2006). At this point, the bird is considered to be in stress, and will use immediate life-saving strategies. Such a set of physiological and behavioural strategies are either: a) escaping the stressor; b) adapting to the stressor (e.g. Newman *et al*. 2005); c) suffering adrenal exhaustion; or d) undergoing pathological changes that can result in death (Newman *et al*. 2005 and references within, Silverin 1998, O'Reilly & Wingfield 2001). Overall these may affect the individual and/or disrupt ongoing activities (e.g. breeding) (Wingfield & Ramenofsky 1999, Sapolsky *et al.* 2000).

There is a consensus that the release of glucocorticoids will have direct impacts on behaviour (e.g. “agitated” or “calm”), metabolism, energetic regulation, reproduction, growth and the immune system, as summarised in table 1.VI. These are therefore the main fields of impact that capture and handling will have on the bird, as an outcome of the adaptive stress response (Tarlow & Blumstein 2007, Wingfield 2003, Romero & Butler 2007). However, as mentioned, the conjunction of these responses will determinate how well the bird copes with the stressor, the combined outcome could be maladaptive (Romero & Butler 2007). The consequences of the corticosterone response are however dependant on the duration of the stressful event; for example, brief periods of handling do not seem to affect immune or morphological development in American kestrels (*Falco sparverius*) or common starlings (*Sturnus vulgaris*), whereas 24hr of captivity resulted in suppressed cutaneous immune responses (Butler & Dufty Jr. 2007).

Table 1.VI: Studies examining the potential effects of increased circulating corticosterone levels.

Impacts of increased corticosterone	**References**
Behaviour	
[Free living passerines] Increase in activity and "escape" behaviour.	Astheimer *et al*. 1992
[Willow Tit (*Poecile montanus*)] Juveniles may leave the breeding area prematurely.	Silverin *et al.*1989
Metabolism	
[Chicken (*Gallus gallus domesticus*)] Loss of body mass, even and despite increases in food consumption.	Bartov *et al*. 1980
Mobilization of energy resources and inhibition of anabolic processes, such as growth and reproduction.	Siegel 1980; Harvey *et al*. 1984
[Free living European passerines] Catabolism of skeletal muscles.	Falsone *et al*. 2009
Reproduction	
[Free living passerines] Decrease in reproductive behaviour and disruption of reproductive hormone release.	Moore & Zoeller 1985; Sapolsky *et* al.1985; Silverin 1986; Wingfield & Silverin 1986; Wingfield 1988
[Barn swallows (*Hirundo rustica*)] Reduced hatchability of eggs, and smaller body size and slower plumage development of fledglings.	Saino *et al*. 2005
If corticosterone concentrations rise in phase III of Adélie penguin's (*Pygoscelis adeliae*) fasting, attentiveness to the nest or eggs will decrease, with further abandonment of the breeding attempt to return to the sea to feed.	Cockrem *et al*. 2006
Adélie penguins will abandon the egg when incubation is associated with a natural long fast, provided there's also a rise in proteolysis and prolactin level reaches a low threshold value.	Spée *et al*. 2011

Table 1.VI**:** (continued)

Impacts of increased corticosterone	**References**
Growth	
Exposure to stressful events during growth and development can have a significant phenotypic impact.	Blas *et al*. 2007; Spencer *et al*. 2009
[White-crowned sparrow (*Zonotrichia leucophrys*)] Altered both begging behaviour and growth of nestlings.	Wada & Breuner 2008
[Black legged kittiwake (*Rissa tridactyla*)] reduced growth efficiency and compromised cognitive abilities of chicks.	Kitaysky *et al*. 2003
[Common quail (*Coturnix coturnix*)] slower chick growth and higher activity of the hypothalamo-adrenal axis.	Hayward & Wingfield 2004
[Chicken (*Gallus gallus domesticus*)] Inhibition of skeletal calcification, inducing osteoporosis in adults.	Siegel 1980
[Eurasian kestrel (*Falco tinnunculus*) and barn owl (*Tyto alba*)] Suppressed growth of feather, bone and body mass.	Müller *et al*. 2009
Immune system	
[American kestrels (*Falco sparverius*) or Common starlings (*Sturnus vulgaris)*] Suppressed cutaneous immune responses.	Butler & Dufty Jr. 2007
Interference in the immune system (e.g. reduced number of circulating lymphocytes, and an increase in heterophil numbers).	Shapiro & Schechtman 1949; Siegel 1968; Råberg *et al*. 1998; Martin *et al*. 2005 ; Newman *et al*. 2005
Increased resistance to some bacterial diseases (due to heterophil increases)	Siegel 1980; Rhen & Cidlowski 2005

A complication in considering corticosterone/stress responses is that baseline levels of corticosterone vary seasonally and between species and sexes (Wingfield *et al*. 1995, Busch 2006), and are higher in periods of higher energetic demand for each individual. For example, baseline corticosterone levels are high during the breeding season (higher in the sex most responsible for the parental care, Wingfield *et al*. 1995, O'Reilly & Wingfield 2001), during moult, and before and during migration (higher before spring migration than autumn migration, Ashteimer *et al*.

1995, Romero *et al*. 1997, 2000, Falsone *et al*. 2009). Hence if a bird is exposed to a stressful event in any of these periods it may be less able, or even unable, to mount an adrenocortical response at all (Jenni *et al*. 2000, Romero & Romero 2002, Smith *et al*. 1994, Müller *et al*. 2006). Furthermore, both baseline and stressed levels of corticosterone vary according to latitude (Martin *et al*. 2005, Silverin *et al*. 1997), diel rhythm (Breuner *et al*. 1999), photoperiod (Pravosudov *et al*. 2002), weather (Romero *et al*. 2000), habitat (Busch 2006), body condition (Raja-Aho *et al*. 20010, Heath & Dufty 1998), genetics (Odeh *et al*. 2003), personality/ coping style/ behavioural syndrome (Cockrem & Silverin 2002, Cockrem 2007, Cockrem *et al*. 2009, Costantini et al. 2008, Sih *et al*. 2004, Groothuis & Carere 2005, Bell 2007, Wolf *et al*. 2007, Koolhaas *et al*. 1999), and age (Quillfeldt *et al*. 2009, Sims & Holberton 2000). In some cases, individual birds are even able to modulate their adrenocortical response, e.g. Jenni-Eiermann *et al.* (2009), Silverin & Wingfield (1998) and Ashteimer *et al*. (1995), making it very difficult to predict the level of stress response that a specific individual will demonstrate.

1.2.2 Consequences for the immune system

In periods of acute stress, the release of catecholamines and the increase in circulating corticosterone affects immunity by inducing a change in the abundance of leukocytes in the blood. The stress induced change in leukocyte profiles induces a re-distribution of cells within different body compartments (Welsh *et al*. 2006). Specifically, increased corticosterone leads to a decrease in blood circulating lymphocytes, by directing these cells to the skin thus suppressing the organisms' normal defense against viral infections of the depleted compartments (peripheral blood and spleen). This decrease is accompanied by an increase in the abundance of heterophils circulating in the blood (Shapiro & Schechtman 1949, Newman *et al*. 2005, Siegel 1980, Davis 2005) which come from the bone marrow and can protect the bird against infection or wounding (Siegel 1980, Dhabhar & McEwen 1997, 1999a, Martin 2009, Dhabhar 2002, 2009), that may result from the presence of the stressor (e.g. attack of a predator). Such a redistribution of leukocytes to target body compartments, enhances the bird's defences for potential immune challenges especially in those compartments to which immune cells migrate during stress, and results in a decrease of blood circulating leukocytes (Welsh *et al*. 2006). In the same manner as corticosterone, immune sensitivity to stressors is not fixed within (and amongst) species and lifetime, but may vary seasonally in relation to photoperiod (Martin 2009), between genders (e.g. male house sparrows (*Passer domesticus*) in Martin *et al*. 2006 and female house sparrows in Martin *et al*. 2006 and Greenman *et al*. 2005) and can be lower in periods of higher physical demand (e.g. reproduction) (Råberg *et al*. 1998). Furthermore the sensitivity of the immune system to corticosterone can be both “programmed” early in life (Butler & Dufty 2007) or simply non-existent, as is the case for some tropical species (e.g. Matson *et al*. 2006, Martin & Rubenstein 2008).

1.3 Consequences for energy regulation

From the perspective of an individual’s survival, the trade-off between gaining energy and avoiding predation is vital (MacLeod & Gosler 2006, Rogers & Smith 1993, McNamara *et al*. 2005), such that a bird can perceive capture and handling in two different ways: either (1) the event is treated as an encounter with a predator or (2) is treated as an interruption to foraging (Macleod & Gosler 2006). In the first case, birds would be predicted to lower their fat reserves to facilitate escape in subsequent predator encounters, but in the second case, they will try to increase their fat reserves, to buffer themselves against starvation during further interruptions to foraging. Such anti-starvation strategy may be enhanced if the food supply is variable (Rands & Cuthill 2001, Macleod & Gosler 2006). Which of the above responses are used by birds may differ with time of day. For example, in a study of great tits (*Parus major*), Gosler (2001) found that birds react differently to capture in the morning and afternoon, as their priority in the morning is to avoid predators (e.g. Eurasian sparrowhawk (*Accipiter nisus*)) and later in the day is to build up energy reserves to survive during the night.

There are two ways in which capture and handling could affect the energetic reserves levels of birds: either it can induce changes in body mass and/or changes in thermal regulation capacity.

1.3.1 Changes in body mass

Some studies report cases of mass loss in birds over the hours or days following capture and handling (e.g. Leberman & Stern 1977, Castro *et al*. 1991). The short term causes of this mass loss suggested by the authors are: loss of opportunity to feed (Clark 1979, Schwilch & Jenni 2001); excretion and water loss (Clark 1979, Dunn 1999), or even “handling shock” - a term referring to a situation in which the bird seems to get traumatized and not feed or metabolize normally for the first day or two after handling (Leberman & Stern 1977, Clark 1979, Rogers & Odum 1966). Excretion and water loss are, however, the most cited explanations for post-capture mass loss in the literature. Water loss may be detrimental, as dehydration might occur. This may be critical in dry regions, where it is more difficult to replace water lost (Dunn 1999), or during periods of higher energetic stress, such as fuelling for migration, because part of the migratory fuel mass is composed of water (Scott *et al*. 1994 and references within). Furthermore, excretion can also clear the gut of incompletely digested food, having a two-folded cost: non-absorbed energy and extra foraging time (Dunn 1999).

Post-capture mass loss can only be identified and confirmed if birds are re-captured, or remotely-weighed following release. Furthermore, body mass is strongly influenced by temperature, especially above a threshold of about 30º (Castro *et al*. 1991); depending on species (Castro *et al*. 1991, Refsnider 1993), individual traits (i.e. age, sex, reproductive state, personality and past lifetime events). Body mass loss can also depend on the duration of handling and timing of recapture, such that recovery periods for body mass loss can vary from a few hours to a few days (Schwilch & Jenni 2001, Clark 1979, Carere & van Oers 2004). Altogether, loss of mass and its effects are hard to predict, but potentially important effects have been suggested, such as a delay in the time of departure on migration of up to 5 days for migratory birds undergoing handling shock (Leberman & Stern 1977).

1.3.2 Changes in thermal regulation

Capture-induced changes in energy reserves such as those described above may have consequences for a bird’s thermal regulation, as part of a strategy to minimise energy expenditure in the hours or days following capture. When a bird’s energy reserves are lower than optimal at dusk, it may strategically reduce its body temperature in order to minimize the expenditure of energy overnight (Nord *et al*. 2009, Cooper & Gessaman 2005, Chaplin *et al*. 1984). Energetically this lowered body temperature presents advantages for the individual as it reduces energetic reserves expenditure, thus promoting survival. Because this strategy also has associated costs (particularly reduced responsiveness to predators), the strategy is avoided when not necessary (Nord *et al*. 2009). There are two states into which a bird can enter, in order to reduce thermoregulation costs: torpor and hypothermia (Schleucher 2004). There is some controversy in the definition of states, but generally it is assumed that during hypothermic stages, the bird remains attentive and can resort to its normal activity levels spontaneously, whilst during torpor, physiological mechanisms have to be triggered for the bird to be active, which usually takes an enlarged amount of time. The strategic use of hypothermia and torpor, however, varies among taxa (McKechnie & Lovegrove 2002): e.g. passerines are more prone to hypothermia (McKechnie & Lovegrove 2003, Dolby *et al*. 2004, Clemens 1989), whilst caprimulgiformes are more prone to torpor (e.g. common nighthawk (*Chordeiles minor*) Fletcher *et al*. 2004).

Birds may however respond to capture and handling protocols with the opposite of an hypothermic state: they can demonstrate a rapid rise in body temperature, which can be compared to a state of fever, and is frequently associated with an increase in respiration rate and heart rate (Carere & Van Oers 2004, Cabanac & Guillemette 2001). Such an increase in heart rate can lead to tachycardia (Cabanac & Guillemette 2001, Meyer *et al*. 2008) with lethal effects. Hyperthermia is also a typical consequence of a specific condition designated as “capture myopathy” and evidence has been gathered hyperthermia it is inversely related with weight, and varies with sex (females have slight higher body temperature) (Carere & van Oers 2004 and references within).

1.4 Capture myopathy

Capture myopathy is a special condition, very well documented for waders and waterfowl in particular. It is a pathological condition of birds that arises as a consequence of the extreme muscular exertion and trauma that can occur during capture, restraint, transport and handling. Physiologically, capture myopathy occurs when lactic acidosis and free radical production takes place, resulting in acute degeneration of muscle tissue (Cox & Afton 1998, Young 1967, Spraker 1982). The main symptoms of capture myopathy are: dyspnea, hyperthermia, weakness, muscle rigidity and collapse, and various forms of histological degeneration such as skeletal and cardiac muscle necrosis (e.g. Finlay & Jeske 1997, Abbott *et al.* 2005, Marco *et al*. 2006) (Dabbert & Powell 1993, Taylor 1994). Such complications can be fatal or enhance susceptibility to predation (Abbott *et al.* 2005, Rogers *et al.* 2004). The end result of the myopathy is generally a difficulty or inability to fly and/or walk (e.g. Marco *et al*. 2006), and can include irreversible leg paralysis (e.g. Young 1967, van Heerden 1977), a clearly non-adaptive stress response, and handicapping to the individuals survival.

Although I have found no documentation for this condition in passerines, capture myopathy has been documented for a range of different bird species (Nicholson *et al*. 2000), affecting them differently, as well as having variable effects on different individuals from within the same species. The following characteristics and traits appear to be associated with an increased risk of capture myopathy:

a) bigger birds (especially shorebirds and other long legged birds);
b) birds that are in an unusual physiological state (e.g. birds with very large pre-migratory fat loads or emaciated birds);
c) certain capture techniques (e.g. Bollinger *et al*. 1989). According to Minton (1993), mist netting causes proportionally more problems related to capture myopathy than cannon netting. Henschel & Louw (1978) indicate that the struggling in the net may also be exacerbated by further restraint in small bags or low keeping cages;
d) time duration of struggling (e.g. Carpenter *et al.* 1991);
e) higher ambient temperatures;
f) sex (e.g. Rogers *et al*. 2004, suggest male red knots *Calidris canutus* as being more susceptible);
g) species habits (e.g. cannon-netting of shorebirds involves a large number of captured birds as they are gregarious) (Rogers *et al*. 2004, Minton 1993, Clark & Clark 2002);
h) a high endoparasite burden (Melville 1982);
i) type of cage where birds are restrained (Bainbridge 1976), i.e. tallness of the box related to body size.

Bird ringers and veterinarians have experimented with various treatments to enable birds to recover from capture myopathy. These range from treatment of birds with vitamin E and selenium (Abbott *et al*. 2005), aggressive intravenous fluids, physical therapy (Smith *et al*. 2005, Rogers *et al*. 2004) along with oral anxiolytic and muscle relaxant drugs (e.g. Valium) (Smith *et al*. 2005, Piersma *et al.* 1991). However treatment is only possible if myopathy is diagnosed early (i.e. at the hyperthermia stage), which can be difficult, as its prognosis is poor, and an animal whose muscle has been destroyed cannot be saved, as the muscle will never regenerate. Nevertheless preventing capture myopathy might be the most effective way of minimising the impacts on birds.

Because, this condition has been thoroughly covered in the literature I will not refer to this condition further in the thesis. However it is a relevant impact of capture and handling and therefore important to be mentioned. For further references please refer to Minton (1993) and Green (1978), for guidelines on how to prevent the condition, and Smith *et al.* 2005, Abbott *et al.* 2005, Rogers *et al*. 2004 for thorough treatment guidelines.

1.5 Demographic impacts: breeding success

The ultimate goal of bird ringing is to monitor population demographics, and in this sense, nest success is a key factor in determining whether a bird population will grow, decline, or maintain stability. The breeding season is a period of high energetic demand for birds (Bryan & Bryant 1999, Bryant & Westerterp 1983, Drent & Daan 1980, Schnase *et al*. 1991, Merilä & Wiggins 1997, Cresswell *et al*. 2003). Although birds spend little energy keeping the eggs warm, they can spend most of the day inside the nest, and therefore foraging opportunities may be limited (Olson *et al*. 2006). The incubation of eggs and the rearing of nestlings have energetic costs that can be compensated by optimisation of foraging efficiency and reductions of energetic costs (Bryan & Bryant 1999). It is an important part of their annual cycle and determinant of their lifetime fitness. This season can be particularly critical for species in which mates do not provision, and because weather and foraging conditions sometimes are not optimal (Bryan & Bryant 1999). Capture and handling can not only expose the captured individual to an unnecessary risk, but can also affect its dependent progeny and hence the future of the population. If the parent is kept away from the nest by capture, some consequences can be predicted: such as eggs or young suffering from lack of incubation or brooding, parental and/or offspring energetic costs, parental behaviour or health modifications, increased time of exposure of the nest to predation and parasitism, damaging of eggs by a frightened parent (e.g. if catching at the nest), death of the nestling if it is frightened away from the nest (e.g. during nest capture of parents), or an increased probability of nest desertion (Davenport *et al*. 2004, Jennings *et al*. 2009, Kania 1992). Even if the birds are not captured, bird-ringing activities might influence reproductive success either positively, by discouraging predators from using the area around the nest (Churchwell & Barton 2006), or negatively (Olsen & Schmidt 2001), even if it is just because of the human activity associated with operating mist nets near nests (Nisbet 1981, Jennings *et al*. 2009). The disturbance impacts range through the whole nesting season, for example, mist netting a bird that has established a territory in the surrounding area, might cause it to relocate away from an area otherwise perfectly suitable, to an area of lesser quality. If the bird is in the laying stage, the stress event will lead to an increase in circulating corticosterone which is transferred into the yolk, producing offspring that will grow more slowly and demonstrate higher activity of the hypothalamo–adrenal axis in response to capture and handling (Hayward & Wingfield 2004). Capturing a parent during incubation might cause increased chilling of the nest, and thus the parent may have to spend considerably more energy in order to increase the nest temperature again (Spellerberg 1969). Also, capturing a parent during provisioning might lead to undernourished juveniles with a lower probability of survival to the next breeding season, or even, if a parent is captured at the nest it can cause the nestlings to leave the nest earlier and increase their risk of mortality (Kania 1992).

The extent of capture and handling interference with breeding activities will vary with method, handling time (Olsen & Schmidt 2001), environmental conditions (Ouyang *et al*. 2012), species and breeding stage (table 1.VII) (Kania 1992). In this sense, Kania (1992) has assessed that nest desertion probabilities decrease as the breeding season advances, and hence capture should be safer.

Table 1.VII: Studies examining the potential effects of capture and handling during the breeding season.

Breeding stage	**Example of studies examining effects**
Pre-nesting	Jamieson *et al*. 2005
Laying	Kania 1992[1]
Egg incubation (1st and 2nd half)	Olsen & Schmidt 2001; Ouyang *et al*. 2011; Webb 1987; Ortega *et al*. 1997; Ballard *et al*. 2001; Hull & Wilson 1996; Kania 1992
Offspring rearing (parents and nestlings)	Listøen *et al*. 2000; Quillfeldt *et al*. 2008 ; Ortega *et al*. 1997 ; Hull & Wilson 1996; Kania 1992
General trapping method	
At nest	Olsen & Schmidt 2001; Kania 1992; Ortega *et al*. 1997; Hill & Talent 1990; Lendvai & Chastel 2010; Dubiec 2011
Mist-netting	Jennings *et al*. 2009; Jamieson *et al*. 2005

[1] Kania (1992) is a general review of the safety at catching birds in the nest.

From the large spectrum of studies carried, there are some that have found that bird ringing *per se*, mostly, has no effect on the reproductive viability, e.g. Jennings *et al*. 2009 (wrentits (*Chamaea fasciata*) and song sparrows (*Melospiza melodia*)), Hill & Talent (1990) (least terns (*Sterna antillarum athalassos*) and western snowy plovers (*Charadrius nivosus*)) and Thorup (1995) (Dunlin (*Calidris alpina*)). Partially, this could be because an embryo's viability is not impaired by relatively short periods of chilling; for example, eggs can survive cooling well below 25°C (Olson *et al*. 2006 and references within). While some authors point out that a small amount of chilling can even improve hatching success (according to Oppenheim & Levin 1975 *in* Webb 1987) or promote the development of thermoregulation in birds (according to Landauaer 1967 *in* Webb 1987), others emphasize that even though embryos can tolerate cooling, it will have a two-fold cost in growth efficiency and rate of development (Olson *et al*. 2006). If the chilling periods are prolonged it might lead to the death of the embryo (Webb 1987). Also, the adaptive response towards the capture event can prioritize the value of current reproduction relative to the value of future reproduction and survival, in which case, the birds would display a mitigated stress response and give priority to breeding and maximum fitness (Bókony *et al*. 2009).

There are, however, others studies, that reveal drastic impacts, e.g. Olsen &. Schmidt (2001) describe nest desertion by hooded crows (*Corvus cornix*), as a consequence of trapping activities (whether or not successful), Kania (1989) demonstrated that capturing female great tits resulted in a desertion level of 29% nests, and Ouyang *et al*. (2011) observed great tit nest desertion resulting in the abandonment of eggs and nestlings. In these cases it could well be that the parents perceived capture as a predation event (Silverin 1998, Gosler 2001), and diverted their life-history strategies to an adaptive response towards immediate survival, which simultaneously inhibited reproduction (Székely *et al*. 1996, Bókony *et al*. 2009).

According to the "brood value hypothesis" (Bókony *et al*. 2009 and references within, Lendvai & Chastel 2008), the stress response should be modulated as a function of the relative importance of current reproduction. Specifically, species with few breeding opportunities, will tend to not abandon their brood, whilst "prudent parents", for example long-lived species, will prioritize their own survival, as their lifetime reproductive success relies largely on their survival rather than of seasonal fecundity (Bókony *et al*. 2009 and references within). In addition Székely *et al*. (1996), point that other factors such: as survival probabilities of the offspring and/or the parent's, the behaviour of the mate (e.g. male desertion), reproductive constraints, and the behaviour of other males and females in the population can play a role in the decision of whether or not to desert the nest.

Apart from simple standard capture and restraint procedure, many authors also take blood samples for corticosterone assessments in order to obtain relevant physiological measures (Angelier *et al*. 2011). While this adds to the cumulative effects of handling stress (Wingfield *et al*. 1997), and may therefore be considered detrimental (Angelier *et al*. 2010 and references), some authors have not found any detrimental effect, e.g. Angelier *et al*. (2011) with black-browed albatrosses (*Thalassarche melanophris*); others found it to be "minimal", e.g. Brown (1995), in which 2 out of 110 chicks of ring-billed gull (*Larus delawarensis*) that were blood sampled died, and Lendvai & Chastel (2010) found it affected male house sparrow parental care; while others found it to be detrimental, e.g. Criscuolo (2001) (nest desertion in common eider (*Somateria molissima*), Kania (1992) (who reviews datasets from nest captures and guidelines to avoid nest desertion), Ouyang *et al*.

(2011) (great tit nest desertion) and Kilgas *et al*. (2007) (great tit nest desertion, however body condition was not significantly different between deserting and non-deserting females).

Ultimately nest desertion or nest predation (Kania 19992, Verboven *et al*. 2001) are clearly the most handicapping outcomes. However, impacts on parents' fitness will also affect their ability to provide care to their nestlings (Lendvai & Chastel 2010).

Because of the role that an elevation of glucocorticoids plays in the mediation of immediate decision-making strategies, birds generally display an increased level of baseline corticosterone throughout the breeding season, which may enhance their tolerance to capture stress, as physiologically the bird is conditioned to discount stressful events, in favour of maximising breeding success.

Despite the role of corticosterone, during the breeding season another hormone has an important role in the response to acute stressors: prolactin, the "parental hormone" which assists and promotes the expression of parental behaviours (i.e. incubating, brooding and feeding) (Chastel *et al*. 2005, Angelier & Chastel 2009). Although these two stress response hormones are not related (Angelier & Chastel 2009, Angelier *et al*. 2013), they both display a role in the decision of nest desertion, as increased corticosterone levels depress prolactin levels ('the prolactin stress response'). However, prolactin levels can be attenuated as an hormonal tactic, thus prioritizing parental care over parental survival during stressful situations. For example, as long-lived birds age, the value of current reproduction increases towards parental fitness, as the prospects for future breeding opportunities decrease, and thus the bird's prolactin response to acute stress decreases (Angelier *et al*. 2007, Angelier & Chastel 2009).

However the trade-off between immediate survival and reproductive effort relies also on: the type of species, as those with higher constraints (e.g. having a larger number of broods, or species with female biased parental care) will display increased baseline level of corticosterone; predictability and availability of suitable habitat; environmental factors such as latitude; breeding stage (e.g. birds that can breed all year around or birds at the beginning of a breeding attempt); and personality/ individual quality (Burtt & Tuttle 1983, Bókony *et al*. 2009, Ouyang *et al*. 2011, Seltmann *et al*. 2012, Silverin *et al*. 1997). Also, as a result of their increased levels of corticosterone, birds can have a lower viral immune response throughout the breeding season (Martin *et al*. 2006, Greenman *et al*. 2005). Furthermore, Angelier *et al*. (2007) have demonstrated, in snow petrels (*Pagodroma nivea*), that parental age enhanced the probability of successfully fledging a chick.

Capture and handling of birds during the breeding season should always be optimised to prevent further impacts, even if the methodology to be used results in a lower capture rate (Kania 1992).

I have not found any study assessing the impacts of capture and handling in strictly polygynous species. Even though the majority of birds are socially monogamous (Koskimies 1984), Møller (1986) has assessed that of 122 European passerine species, 39% were polygynous. In this type of mating system, a male will have more than one female partner and consequently more than one nest to attend. It is to be expected that if the female is captured the risk of nest predation can become higher as the male may not be available to protect the nest in the forced extended absence of the female, and the nestlings may suffer from undernourishment. Whether the male is monoterritorial or polyterritorial will further influence this risk. Two examples of widely studied polygynous passerine species are: the blackbird (*Turdus merula*) and the blue tit (*Cyanistes caeruleus*) (Dhondt 1987, Hasselquist & Sherman 2001), even though polygyny is more frequently found among species breeding in open habitats (marshes, meadows, pastures and low shrubs) (Møller 1986).

Capture, handling and marking birds during the breeding season may have further impacts. For example, some authors have described behaviours such as parents trying to pull the ring from recently banded nestlings' legs, even twisting their legs (e.g. Bolen & Derden 1980, Lovell 1945, Berger 1953, Brackbill 1954). However such a matter is beyond the scope of this review.

1.6 Physical integrity

So far I have described the effects that bird ringing has on the bird's behaviour, physiology and on important stages of the annual cycle such as the breeding season. In table 1.I to 1.III I have demonstrated the large range of capture and handling techniques currently in use to capture different bird species in various circumstances, as well as marking and sampling techniques. When evaluating the impacts of capture and handling, the many ways in which birds can suffer from direct physical injury, and the types of injury that can occur have also to be considered. These impacts are clearly detrimental, independent of the adaptive stress response, and are mainly inherent to the fact that birds are being held in captivity and being handled.

1.6.1 Direct mortality

Occasionally, despite the best efforts of the researcher, birds may die or suffer injuries during capture and handling. Clearly, direct mortality is not an adaptive outcome of the stress response for the individual concerned, but whether it is an important effect at the population level depends on two key issues: (i) the mortality rate among captured birds of different species; and (ii) whether this mortality varies between age/sex classes within a population. However, a striking feature of direct mortality effects is the lack of available data on either issue. The few available data are summarised in table 1.VIII, and they indicate that mortality rates may vary substantially between studies (from 0.06% to 6.63% in the available datasets). Despite the few publications presented on table 1.VIII, with a highlight on that of Spotswood *et al*. (2012), the lack of available data is itself intriguing. Most ringing schemes do not have a compulsory and standardized format for collecting such infomation, despite its obvious importance. It is possible that some researchers are unwilling to report any negative impacts that they feel they may subsequently be blamed for, but it is also possible that some do not report bird-ringing mortalities simply because they don’t have a proper platform to do so.

Another intriguing issue is how one might decide on an ethically “acceptable” mortality rate. The Association for the Study of Animal Behaviour’s Guidelines for the Ethical Use of Animals in Behavioural Research (ASAB 2012) recommends that such decisions be viewed in a cost-benefit framework, in which the scientific benefits of proposed studies are weighed up against the anticipated risks to the animals concerned. Such decisions are inevitably to some degree subjective, but even limited data such as that presented in table 1.VIII or in Spotswood *et al*. (2012), is invaluable in beginning to quantify the risks in comparison to the well-known scientific benefits. Also, the “Bander’s Code of Ethics” (*in* NABC 2001), advocates that the bird bander (the American equivalent to bird ringer) should strive for a goal of zero casualties (any debilitating injury or death) by prioritizing the safety and welfare of the birds they study so that stress and risks of injury or death are minimized. It also advocates the need to continuously assess the ringer’s own work to ensure that it is beyond reproach.

By examining table 1.VIII, the most commonly reported causes of death are (in order of importance): predation, “unknown” causes, cold or heat exhaustion, and to a lesser extent, a result of injuries. It is striking that a substantial number of fatalities are attributed by Spotswood *et al*. (2012) to being the result of “stress”. This implies that even though the avian stress response is in general an adaptive response to stressors, mortality can however be a maladaptive result of the acute stress response, at least in some circumstances. It is a striking that the second most cited cause of death is “unknown”: This could be denoting some lack of awareness of the birds’ physiology, or that researchers are assuming capture and handling is not causing any damage to the bird.

Even though predation is a “natural phenomenon”, it shouldn’t be treated lightly, ultimately because it may be an indirect fault of the researcher. Although some researchers feel that the impact of predation during bird ringing activity is “minimal” (Collins 2007), predation can also have a two-fold cost for the birds, as the predator might damage the net, and if not repaired properly, it might cause further injury to subsequent birds (NABC 2001).

Additional to the causes of mortality summarised in table 1.VIII, the NABC (2001) also mentions that birds could be dying during capture and handling as a consequence of a punctured trachea (which would be the case of a seed-eating bird if a well-filled crop is pressed too hard against the windpipe in baited traps for example), or “natural causes” (e.g. bacterial infection like Salmonellosis).

It is not clear that mortality is necessarily due to error on the part of the bird ringer (we are not aware of any experienced ringer who would claim to no longer experience bird deaths during ringing). Nevertheless, it seems generally appreciated that mortality rates may be higher among birds handled by trainee bird ringers during their period of training. Therefore it is ultimately the trainer’s responsibility to monitor the birds’ safety whilst being handled by the trainee.

Table 1.VIII: The reported causes of death among birds captured in the course of bird-ringing studies.

Capture method	**Overall mortality rate (deaths/no. caught)**	**Causes of death (in order of relevance)**	**Reference**
Mist nets (mainly)	0.29% (12/4119)	Unknown Predation Hypothermia Dehydration Drowning Aggression	Petronilho 2002
Mist nets	1.3% (53/4184)	Predation	Recher *et al*. 1985
Mist nets (marine birds)	0.06% (4/6000)	Unknown Predation Collision	R.J. Thomas, unpublished data
Mist nets	0.23% (not provided)	Stress Predation Unknown Internal Strangled Cold Break Tangled Accident Heat Cut Wing Dislocation Tongued Band	Spotswood *et al*. 2012
Rocket nets (waterfowl)	6.63% (23/347)	Predation Unknown	Cox Jr & Afton 1998

1.6.2 Physical injury

In order to be ringed, a bird is captured, extracted from its capture device, transported to the ringing table and then handled for ringing, biometric measurements, tissue sampling (where relevant) and released. Whilst most ringers recognise that taking measurements of the bird should carry little to no risk of injury to the bird, factors like handling technique and time taken can pose some risk . It still has to be clarified which stage of the capture and handling process poses greatest risk to the birds,it seems more likely that the bird will suffer physical injury during the capture/extraction stage or that at least, the range of possible injuries is higher during this stage, as demonstrated in table 1.IX. Although the relevance of such injuries to the bird's fitness seems straightforward, this will depend largely on the severity of such injuries.

Table 1.IX: The reported causes of injury among birds captured as a result of mist-netting. Other injuries related with specific methods, such as forehead abrasion in wire traps or haematomas as a result of blood sampling are omitted in this table (Spotswood *et al*. 2012, NABC 2001, Sheldon *et al*. 2008).

Type of injury	Capture/ Extraction	Handling
Stress	X	X
Cuts (e.g. feather base)	X[1]	X
Wing (e.g. strain)	X	
Break	X[1]	X
Dislocation	X	
Internal / haemorrhage	X	
Entanglement related	X	
Strangling	X	
"Tongued"	X	
Cold (e.g. hypothermia)	X	
Heat	X	
Predation	X	
Tail loss	X	X
Damaged feathers	X[1]	X
Crushed tarsi / ring related		X
Accident	X	X
Unknown	X	X

[1] refers to injuries that can be/ are caused by human handling rather than the capture itself.

Whether injuries can be part of the adaptive stress response is again debatable. Concomitantly with the mortality casualty, injuries can be a consequence of the stress response, e.g. a bird getting “tongued” (i.e. its toungue becoming entangled and damaged) while trying to escape the capture device, but also the direct result of being captured and handled, e.g. when a researcher fails to notice that the ring is overlapping and thus damaging the birds’ tarsus (NABC 2001). Further, the possible implications that injuries have on the birds and on bird populations, are poorly reported, although Spotswood *et al*. (2012) haven’t found any evidence for increased mortality over time of injured birds compared with uninjured birds. Despite this, it is expected that the implications of injuries at a populations level, will depend on the species, individual traits (age, sex) (Spotswood *et al*. 2012, Sheldon *et al*. 2008) and life-history stage, as for example, “handling-shock” can cause delayed migration in some individuals (Leberman & Stern 1972). In their study of the effects of mist-netting on birds, Spotswood *et al*. (2012) demonstrated that the type of incident was positively correlated with body mass, with larger birds more prone to structural injuries (e.g. cuts and fractures) and smaller birds with stress related injuries (e.g. strain and tangling). Also, the types and rates of incidents vary according to external factors such as the type of capture device, the experience of the ringer and the year of capture (Spotswood *et al*. 2012, NABC 2001).

To some extent, the risk of physical injury can be minimised by the researcher, through appropriate training, awareness of the risk of injury, and carefulness in handling birds. However, physical injury does not necessarily imply bad practice, as some injuries (such as the ones resulting from the stress response) seem unavoidable and thus there seems nothing that the ringer can do to prevent these from happening.

1.7 Thesis outline

It is clear from the effects that I have described so far that there are two categories of impact that capture and handling can have on birds: a physiological impact (related to the physiological stress response), and a methodological impact (related to the capture and handling protocol and referring to injuries and mortality). For these reasons I have developed my research and thesis using two separate approaches: either investigating the physiological aspects of the birds’ response in order to provide clarification of aspects that I have found to not be clearly described in the literature, or focusing on the methodology itself. I believe this multi-perspective approach is the best way to further aid in understanding the wide range of impacts that can arise from capture and handling.

It is clear that there is a need to fully understand how a bird perceives capture and handling, and to quantify its intrusion into the bird's life. I have primarily focused my efforts on the method of capture using mist nets, due to its ubiquity relative to more specific capture techniques. I will then proceed to clarify the impacts on the bird's energy balance, by studying the effects that capture and handling has on body mass and thermal regulation. Subsequently I will focus on the impacts on breeding behaviour, to quantify the disruption that bird ringing causes to the incubation behaviour of birds.I will then explore the direct consequences of an increased level of corticosterone on the immune system, in marine birds (specifically European storm petrels *Hydrobates pelagicus*) that could likely present a different response than those of passerines. This was carried out as a part of a wider study assessing the capture and handling methodology of storm-petrels in which I will demonstrate that it is possible to continuously assess methodologies, and make improvements towards the prioritizing the birds' welfare.

After presenting the physiological approach I will analyze the injuries and mortalities rates resulting mainly from mist-netting in a European context, but I will also make reference to other capture methods, with the intention that this aids in better understanding the causes and consequences of capture and handling.

I will conclude with an analysis of bird-ringing as a tool itself, and provide insight into what still needs to be further investigated and improved.

Ethical Note

All of the studies described in this thesis were carried out under the licensing and regulations of the European Bird-Ringing Scheme (EURING), as implemented by the national bird-ringing schemes of Portugal (administered by Instituto de Conservação da Natureza e Florestas – ICNF) and the United Kingdom (administered by the British Trust for Ornithology (BTO) on behalf of the UK Government Home Office). The majority of the studies described in this thesis were carried out using entirely conventional capture and handling methods as part of standard bird-ringing studies for routine monitoring of populations, migration behaviour etc. Therefore, the birds studied were being captured and handled for reasons other than the purposes of this thesis, and my studies took advantage of these captures to investigate the impacts on the birds involved. Nevertheless, the numbers of individual birds captured and sampled was minimised wherever possible, balanced against the benefits of gathering sufficient data to achieve appropriate statistical power in the data analyses. Where specific unconventional procedures were required (i.e. for blood sampling and sampling of cloacal temperatures of birds caught in Portugal), specific permission was approved by ICNF (Instituto de Conservação da Natureza e das Florestas). The PhD was carried out with the knowledge and approval of Cardiff University's Home Office Liaison Officer, Dr Peter Hunt, following discussions of the project plans and licensing arrangements. The bird-ringing activities were carried out in collaboration with numerous licensed bird ringers in Portugal and the UK. The identities of these ringers were kept anonymous in this thesis (for reasons discussed below), but included highly experienced BTO and ICNF licensed ringing trainers.

Chapter 2

A time budget for mist netting and bird ringing, and the immediate responses of birds to capture and handling

2.1 Abstract

Passerine bird ringing with mist nets comprises four main stages: capture, extraction from the net, transportation (by keeping the bird in a cotton bag), and ringing/processing with variable time durations. This chapter aims to quantify the time disruption of the birds' daily routine, to obtain a comprehensive understanding of the immediate associated costs for birds, by looking at body mass and brood patch temperature changes, and to record how different individuals react physiologically and behaviourally during the procedure. This was achieved by taking target directed measurements during bird-ringing sessions. In this observational study, the variables recorded are the time durations of those procedures and the bird's coping responses. The captivity stages that are not directly dependent on the bird ringer's rapidity were those of longest duration, while the remaining time varied according to the expertise of the ringer, the individual species, type of capture, and when applicable, extraction technique used. Time disruption of the bird's routine varied from 7 min up to 1h 40 min, and throughout the procedure birds lost body mass. Incubating females showed a decreased brood patch temperature during handling. Behaviourally, birds were more likely to display agitated types of behaviour, including the utterance of distress and alarm calls, and escape behaviour. The importance of reducing the time birds spend in the net and in the bag is emphasized, as well as minimizing handling time by trainee bird ringers.

2.2 Introduction

Capture and handling of wild birds is an extremely useful tool for scientific research purposes, and despite the various techniques that can be used to serve this purpose (refer to table 1.I), the most widely spread and currently in use is mist netting (Spencer 1984). Mist netting has proven to be a versatile method of catching a wide range of small to medium sized birds (e.g. passerines and shorebirds (FAO 2007, chapter three), whilst providing a standard apparatus that is easy to set up, easy to run, mobile and economically accessible. In brief it consists of vertically suspending an inconspicuous mesh net attached to poles in target places where the birds' activity is assessed to be high, increasing the chances of intercepting them during their daily routines (for further details, please consult references listed within table 2.I).

A typical ringing session relies of opening the net(s), visiting them at regular intervals to collect any trapped birds (subsequently referred to as "net rounds"), transporting birds in cotton bags to the ringing table, fitting rings and taking measurements (subsequently referred to as "processing" birds). The ringing session ends by closing the nets and furling them so that no additional birds become entangled. Because mist netting is extensively described elsewhere, and precise guidelines are suggested for the method (table 2.I), I will not go into further detail about the procedure in itself, but only the points where this chapter will make a contribution.

Table 2.I: Some relevant and more recent literature describing the various bird capture methods, and handling techniques.

Reference	**Capture Methods**	**Handling Methods**
Davis 1981	X	
Ralph 1988	X	X
Ralph *et al*. 1993	X (only mist-netting)	X
Fair *et al*. 2010	X	X
Schemnitz 2005	X	X
Ralph 2005		X
Bub 1995	X	X
De Beer *et al*. 2001	X	X
Svensson 1992		X
NABC 2001	X	X
Keyes & Grue 1982	X (only mist-netting)	

There are three methods of extracting a bird from a mist-net: the feet first, body grasp and rollover methods (Ralph *et al*. 1993). The most regularly used is the feet first method (Ralph *et al*. 1993, Redfern & Clark 2001), which consists straightforwardly of untangling the feet first. This is done by immobilizing both tibiae to untangle hind limbs, and then releasing the tail, wings and often lastly the head (Ralph *et al*. 1993). The body grasp method is a more recent technique and involves removing the birds by immediately holding their body in the “ringers grip”. It is used when a) the bird isn’t too tangled and be immediately secured in the “ringer’s grip”, or b) if there is tangling around the neck and wings, slipping the fingers over the body and under the wings, and starting to remove threads whilst moving the hand into the ringer’s grip (Ralph 2005). The rollover method implies starting to remove the threads from one leg, then progressively remove threads from the same side of the body, turning the bird until reaching the last leg. All three methods aim to remove the bird from the net and secure it in the ringer’s grip (Ralph *et al*. 1993, Ralph 2005). There is discrepancy in opinions of which is the best technique to remove the birds, e.g. it is argued that the body grasp technique provides better rapidity in extraction and can help avoid injuries by minimizing the pressure that is put on the bird’s legs (Ralph 2005, NABC 2001).

Once extracted from the net, the bird is put inside a cotton bag (small – 23 x 13 cm, or large – 30 x 30 cm) in which it will be transported into the ringing table to be processed. The standard processing of a bird involves the following steps: 1) identify the species; 2) mark with a metal ring or register the ring number if it is a recapture; 3) age and sex it (if possible); and 5) collect biometric measurements (e.g. wing chord, tarsus, 8th primary length, etc). If the ringing is being carried as a part of a particular study, other additional measurements and/or additional marking systems can be used.

Despite the widespread and long-established use of mist netting and bird ringing, the way that they are perceived by the bird, and the impacts they can cause, has received little attention (MacLeod & Gosler 2006). Although there is concern that mist netting might physically injure birds (e.g. Spotswood *et al*. 2012), it is striking that across the literature the simple fact that the bird is interrupted in its daily routine is seldom mentioned. Similarly, the immediate responses of the bird (behavioural and physiological) during capture and handling (cf. after the event) have rarely been considered (e.g. Gosler 2001, Castro *et al*. 1991), nor have the effects of key factors such as the experience of the bird ringer and species of bird on the overall timing and response to capture been considered (Spotswood *et al*. 2012). These are important gaps considering that they could underpin improvements in bird welfare and lead to practical recommendations.

To my knowledge there are no published studies on the duration of mist netting/bird ringing for birds, although sometimes, guidelines are provided for the whole session (e.g. the British Trust for Ornithology's Constant Effort Sites Scheme advises for netting sessions of six hours). It is generally recognized that holding birds for extended periods of time (e.g. more than 1 hour) can be detrimental (e.g. Refsnider 1993, Leberman & Stern 1977), and thus researchers strive to process and release birds as soon as possible. Disrupting a bird's routine may be particularly important on winter mornings, when birds need to recoup energy faster due to longer, colder nights (e.g. Reinertsen 1996), or during the breeding season, when breeding females need their energetic reserves to further re-warm or maintain the nest temperature (Eikenaar *et al*. 2003). The experiments of Refsnider (1993) suggest that the physical impact of captivity upon birds is time sensitive. She assessed how birds lose weight over time during captivity, with the biggest losses being found during the first 30 minutes of a two hour period of captivity. There are a number of reasons that could explain such a situation, for example mass loss resulting from excretion, or accelerated metabolism leading to a higher energetic consumption (for the fight-or-flight response), without the chance of replenishing that fat consumption. In the context of mist netting and bird ringing, this 30 minute period is close to the maximum time interval recommended for visiting the mist nets by the British Trust for Ornithology (Redfern & Clark 2001), suggesting that alterations to the mist netting/bird ringing time budget could affect the extent of weight loss.

In the previous chapter I have acknowledged that individual birds respond differently during stressful events, and that their responses cannot simply be explained by species, age or sex. This variation may be indicative of differences in vulnerability to stress (Groothuis & Carere 2005, Carere *et al*. 2005), but also it could be due to the coping style of the bird. A coping style in this sense would refer to the coherent set of behavioural and physiological stress responses that an individual bird demonstrates consistently over time, much like a "personality" (Koolhaas *et al*. 1999). Across the literature there are many other different names for the "coping style" (e.g. Groothuis & Carere 2005, Wolf *et al*. 2007, Bell 2007) but for the purpose of consistency, and because I believe this is the most suitable label I will only refer to this designation.

The behavioural component of a coping style, the coping response, can be described as one of two possible responses: 1) the active response, characterized by a demonstration of territorial control and aggression, termed as "fight-or-flight" (Cannon 1915 *in* Koolhaas *et al*. 1999, Purves *et al*. 2001); and 2) the passive response, characterized by a demonstration of immobility and low levels of aggression, referred to as "conservation-withdrawal" by Engel & Schmale 1972 (*in*

Koolhaas *et al*. 1999). These two categories have been sub-divided by some authors e.g. the fight-or-flight response can be distinguished according to the magnitude of the aggression demonstrated (Koolhaas *et al*. 1999, Laiolo *et al*. 2009; refer to page 12 for their distinction). Behavioural responses to stressful events in birds can also include the utterance of fear screams (Högstedt 1983, Laiolo *et al*. 2009), alarm calls (Spotswood *et al*. 2012), "freezing" or "tonic-immobility" (Møller 2010), predator mobbing or the extreme manifestation of a condition that has been referred to as "handling shock", which refers to a change in the birds' behaviour that could render them incapable of normal feeding for a variable period of time (Leberman & Stern 1977). All of these could be species-specific (Nocera & Ratcliffe 2009, Leberman & Stern 1977).

Although Koolhaas *et al*. (1999 and references within) chose to designate these responses as "fight-or-flight" and "conservation-withdrawal" I will simply refer to them throughout the thesis as "agitated" or "calm" behaviour. This change in designations will serve two purposes: firstly I wish to avoid confusion in the text between the "fight-or-flight" type of behaviour and the "fight-or-flight" adrenal medulla mediated response, and secondly, to highlight that this type of behaviour is an outcome of the sum of both catecholamine- and glucocorticoid-mediated hormonal responses. Also, I wish to avoid ambiguity when referring using the term "withdrawal", so I have simply shortened the designation to "calm", although by no means implying that the bird is not stressed, despite the apparent calmness of the birds.

Although there is some information about the way birds immediately respond to capture and handling (e.g. Refsnider 1993, Criscuolo *et al*. 2001), I have not found a connection between studies, thus the present chapter is aimed at providing a comprehensive global approach. I intend to do so by integrating the following parameters: time disruption of the bird's daily routine, immediate body mass changes, utterance of calls and coping behaviour responses throughout the capture and handling event. There are two overall aims: i) to establish a time budget for mist netting and bird ringing, and investigate factors that affect that budget; and ii) to record the immediate responses of birds to capture and handling, and how that varies among and within species. In so doing, I hope to provide baseline data about the capture/ringing process and a deeper understanding of the factors affecting overall timing and avian response that could aid in the assessment of any improvements that could be made to the already existing guidelines. Specifically, this chapter aims to test the following hypotheses:

1. Different stages of the ringing process differ in their contribution to the overall time that a bird is retained.
2. Extraction from the net and handling times are influenced by the experience of the ringer.
3. Extraction from the net and handling times vary among species.
4. The body grasp technique accelerates the extraction stage.
5. The length of the ringing process influences the weight loss of the birds.
6. Bird ringing disruption will impart extra energetic costs to breeding females.
7. Each species has a typical coping response.
8. Coping responses are individually constant throughout the ringing procedure.
9. The utterance of calls differs between species, and between coping responses.

2.3 Methods

Data collection took place both in the United Kingdom (UK) and in Portugal. In the UK, data was collected in the winter of 2010/2011 from two sites: Parc Penalta near Ystrad Mynach and Parc Cwm Darran near Fochriw, in Caerphilly Borough, South Wales (51º34’57’’ N, 3º13’12’’ W). In Portugal, data was collected between the summer of 2011 and the spring of 2013 in four sites: Paúl de Tornada and Serra da Columbeira, both in Leiria district, Mexilhoeira Grande, South Portugal, and Évora, in the Alentejo region. The monitoring totals were as follows:

Table 2.II: Logistic details for bird ringing sessions carried in Portugal and South Wales.

	South Wales	Portugal
Number of sessions	3	46
Total monitored birds	53	356
Total monitored species	8	30
Total number of bird ringers	6	9

In the U.K., mist-netting of birds was performed following the guidelines of Redfern & Clark (2001), and in Portugal following Cardoso & Tenreiro (2006). Data were only collected with the consent of the bird ringers involved.

Data collection involved:

a) timing procedures to the nearest second in four stages: i) time spent in the net; ii) extraction duration; iii) time spent in the bag; and iv) handling time;
b) registering the degree of expertise of the ringer both in extraction and handling;
c) registering the technique used to extract the bird: body grasp or feet first;
d) registering coping responses both in the net and in the hand;
e) measuring mass to the nearest 0.01g at three occasions: i) after extraction; ii) beginning of handling; and iii) before release;
f) register if and when a bird defecated, and when possible measure the faeces weight to the nearest 0.01g;
g) whenever a bird vocalized, recording the stage and the vocalization with RememBird Mark II, Software Hothouse Ltd., East Sussex, United Kingdom; and
h) during breeding season measure body temperature of incubating females, with a contact digital thermometer, measured at the skin surface of the brood patch.

Time budget assessment

Data collection was carried out differently in both countries. In the United Kingdom, only procedures (a) and (b) were recorded. In order to do so, a few observers monitored the nets with binoculars, and selected the first 2-3 birds that they saw entering the net. From this moment a stopwatch was started, allowing the observer to assess to the nearest second the time length of each step. To differentiate between bags in which different birds were placed following extraction, a color band was used to mark the bag, so the followers would know exactly which bird they were monitoring, and the bird could wait in the normal queue for processing, without any type of priority.

In Portugal, timing the procedures for a), b) and c) revealed to be more difficult, as the nets were farther away sight, and there was only one follower. To cope with this handicap, digital video cameras were used to monitor the time the bird went into the net. When extraction began, the follower timed to the nearest second each stage, independently of whether it had been filmed or not, and the extraction technique. To differentiate between birds, different combinations of bird bags and string color, were used in order to avoid the awareness of the bird ringer. The technique, and extraction method utilized for each bird were registered when possible.

Response of the bird

The coping behaviour responses were discriminated between as mentioned in Koolhaas (*et al*. 1999): but under the names “agitated” and “calm”. Because within the “agitated” coping response the magnitude of behaviours varies (Koolhaas *et al*. 1999, Laiolo *et al*. 2009), the response was broken down into: “fight” (also termed as proactive and active, Koolhaas *et al*. 1999) when the bird was aggressive towards the ringer (i.e. pecking and biting) and struggling; and “flight” (also termed as reactive or passive, Koolhaas *et al*. 1999) when the bird only put effort into trying to escape. The response “calm” was used when the bird did not struggle or peck, and remained quiet.

Unlike the behavioural sample described above, body mass data were collected for a smaller sampling subset, and species with an average mass smaller than 6 g were not used (e.g. common firecrest *Regulus ignicapilla*), as the mass was considered to be too small to detect meaningful changes. The weighing balance used had a capacity of 100 g, and a sensitivity of 0.01g. When a bird defaecated, the maximum portion possible was collected and weighed using the same balance inside an Eppendorf tube (with a standard mass of 1.02 g ± 0.01 g).

In order to record bird calls, the Remembird digital recorder (Mark II, Software Hothouse Ltd., East Sussex BN8 4AP, United Kingdom) was used. The recorder was permanently recording when birds were being handled, in order to collect all the possible calls of the bird. These calls were further classified into: alarm, distress, flight call and simple call (contact call).

Data analysis

For the purpose of data analysis the length of time from when a bird entered the net to when the extraction began was referred to as “time in net”; the length of time from the start of extraction from the net beginning, to when a bird was put in a bag was referred to as “extraction time”; the time from when a bird was placed in a bag, to being removed was referred to as “time in bag”; and the time from when a bird was removed from the bag until release was referred to as “handling time”. “Total time” refers to the time from a bird hitting the net, to its eventual release following ringing and processing.

Due to logistic reasons, such as number of ringers and catch rate, the number of birds used in each stage varied, with a total of 403 birds used.

An 'experienced' ringer refers to a person who holds a full ringing license (BTO stages C and A); 'intermediate' experience refers to a person who holds a trainee license, and has undertaken a substantial amount of practice under supervision; 'beginner' refers to a person who has little ringing experience, no trainee license, and works under direct supervision at all times.

Also, data regarding extraction technique (i.e. feet first or body grasp) was collected only for intermediate bird ringers, who were the only ones that were willing to use both methods. All experienced bird ringers that participated in this project claimed that they were not confortable with the body grasp method, and preferred to use only the feet first method, claiming also it to be "faster".

Data analysis was performed in R 2.12.0 statistical software (R Core Development Team 2009). Box plots were created to allow for a visual comparison between time duration of stages, (accounting for bird ringer's experience, individual species, extraction method), and brood patch temperatures, assessing the variance, range and mean time, for addressing hypotheses 1,2,3,4 and 6. All box plots show the median (notch, dark line), and the 25th and 75th percentiles (lower and higher ends of box respectively). The ends of the whiskers showed 1.5 times the interquartile range (when outlier circles present) or the furthest value from the median (when no outliers present). Outlier circles represented values outside 1.5 times the interquartile range. References to the mean values of variables also include reference to the standard error of the mean, to provide an idea of the accuracy of the mean, unless otherwise stated.

To investigate the differences between stages in relation to time duration, bird ringer's experience, individual species and extraction techniques a set of negative binomial GLMs was performed, the following starting models were utilized:

Table 2.III: Models for GLM analysis, including dependent and independent variables and parameters.

Dependent Variable	**Independent variables**	**Model Family**	**Model Link Function**
Time duration	Stage	Gamma	Inverse
Extraction time duration	Bird ringer's Experience	Gamma	Inverse

Dependent Variable	**Independent variables**	**Model Family**	**Model Link Function**
Processing time duration of recaptures	Bird ringer’s Experience	Gamma	Inverse
Processing time duration of first captures	Bird ringer’s Experience	Gamma	Inverse
Extraction time duration	Species Body mass	Gamma	Identity
Handling/ Processing time duration	Species Body mass	Gamma	Identity
Extraction time duration	Extraction method	Gamma	Inverse
Body mass loss	Species Total time from extraction initiation to release Time in bag	Gamma	Inverse
Temperature loss	Species Handling/ Processing time duration	Gamma	Identity

To investigate whether there were differences in body condition (i.e. weight and superficial brood patch temperature) a set of one sample t-tests of changes between stages was carried out. Lastly, differences in coping responses and the utterance of calls was assessed through Chi-squared and Fisher’s exact tests.

2.4 Results

Overall a total of 403 birds were assessed: 53 birds were monitored in the UK, and 350 birds were monitored in Portugal, all of which were passerines (n = 29 species). Net-round intervals in the UK were shorter (20-30 min), than in Portugal (45-60 min).

Hypothesis 1: Different stages of the ringing process differ substantially in their contribution to the overall time that a bird is retained.

The longest stage of the bird ringing process was the time in the bag, with a mean of 19.32 min, and the shortest was the extraction from the net, with a mean of 1.31 min (table 2.IV). Overall the process took 33.55 min, ranging from 7.03 to 103.58 min (table 2.IV), and for 53.7 % of the birds the total duration of procedures was higher than 30 min.

Table 2.IV: The four stages of the ringing process both in the UK and Portugal.

	Mean time (min)	**Median time (min)**	**Minimum time (min)**	**Maximum time (min)**	**Standard error**
Time in net	16.55	7.34	0.18	76.33	1.800
Extraction time	1.31	0.95	0.07	10.28	0.076
Time in bag	19.32	16.26	1.42	76.1	0.788
Handling time	4.37	3.45	0.68	30.82	0.187
Total time	33.55	27.65	7.03	103.58	1.749

There was a highly significant difference in duration between the four stages of the mist netting and ringing process (figure 2.1; $F_{3, 108} = 50.21$, $p < 0.0001$). The time spent in the bag was longer than the other stages, with the greatest variation being in the capture stage (time in net). The two stages involving actually handling birds, the extraction process and the ringing process, were shorter and less variable, with the extraction time being shorter than any other stage (table 2.IV).

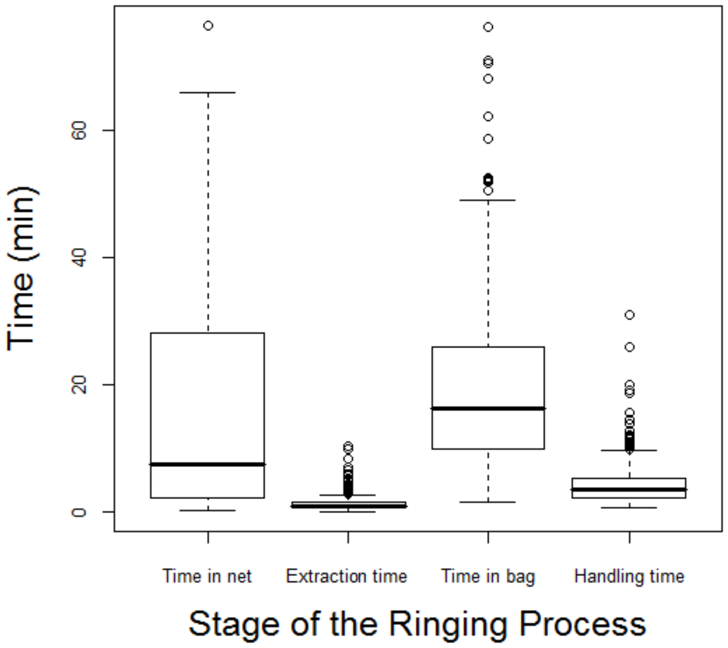

Figure 2.1: The time duration of each stage of the ringing process.

Hypothesis 2: Extraction from the net and handling times are influenced by the experience of the ringer.

As expected, beginners spent substantially more time than intermediate or experienced bird ringers extracting birds from nets ($F_{2, 257} = 15.014$, $p < 0.0001$) and subsequently handling them, whether they were first captures ($F_{2, 156} = 133.39$, $p < 0.0001$) or recaptures of ringed birds ($F_{2, 96} = 13.758$, $p < 0.0001$) (figure 2.2). Although intermediate and experienced bird ringers took similar time to extract birds, intermediate ringers took significantly longer to "process" either first captures (t = -5.019, $p < 0.0001$) or recaptures (t = -2.604, $p = 0.0107$).

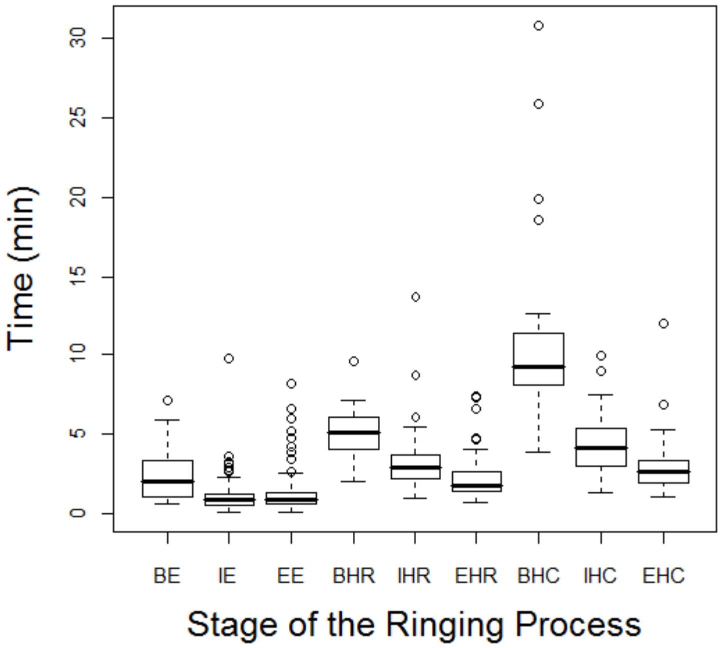

Figure 2.2: The time duration that beginners, intermediate and experienced ringers take for the extraction, handling of recaptures and handling of captures. BE – Beginner Extraction, IE – Intermediate Extraction, EE – Experienced Extraction, BHR – Beginner Handling Recapture, IHR – Intermediate Handling Recapture, EHR – Experienced Handling Recapture, BHC – Beginner Handling first Capture, IHC– Intermediate Handling first Capture, EHC – Experienced Handling first Capture.

Hypothesis 3: Extraction from the net and handling times vary among species.

In addition to the ringer's experience, extraction and handling times were variable among species (figures 2.3 and 2.4; tables 2.V and 2.VI).

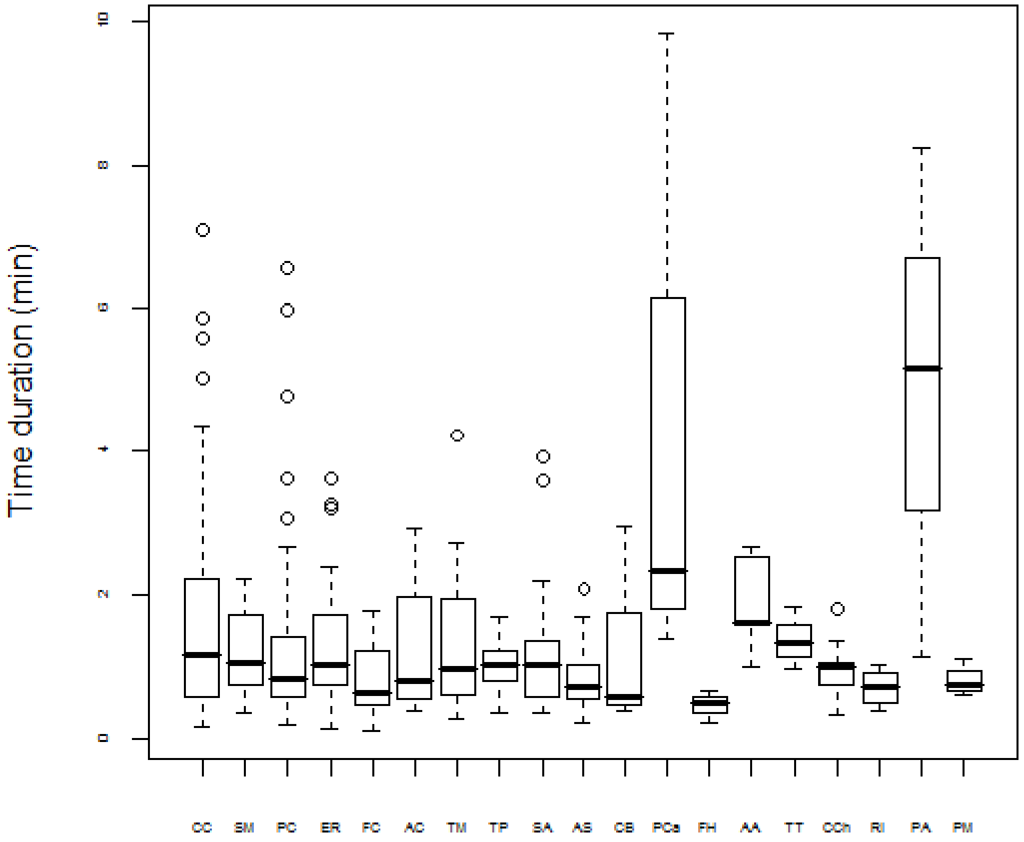

Figure 2.3: Time duration of extraction in minutes according to species: CC- Cetti's warbler *Cettia cetti*, SM- Sardinian warbler *Sylvia melanocephala*, PC- European chiffchaff *Phylloscopus collybita*, ER- European robin *Erithacus rubecula*, FC- common chaffinch *Fringilla coelebs*, AC- long-tailed tit *Aegithalos caudatus*, TM- common blackbird *Turdus merula*, TP- song thrush *Turdus philomelos*, SA- Eurasian blackcap *Sylvia atricapila*, AS- Eurasian reed warbler *Acrocephalus scirpaceus*, CB- short-toed treecreeper *Certhia brachydactyla*, PCa- blue tit *Cyanistes caeruleus*, FH- European pied flycatcher *Ficedula hypoleuca*, AA- common kingfisher *Alcedo atthis*, TT- Eurasian wren *Troglodytes troglodytes*, CCh- European greenfinch *Chloris chloris*, RI- common firecrest *Regulus ignicapillus*, PA- coal tit *Periparus ater* and PM – great tit *Parus major*. Individuals with less than 3 captures are not illustrated in the figure.

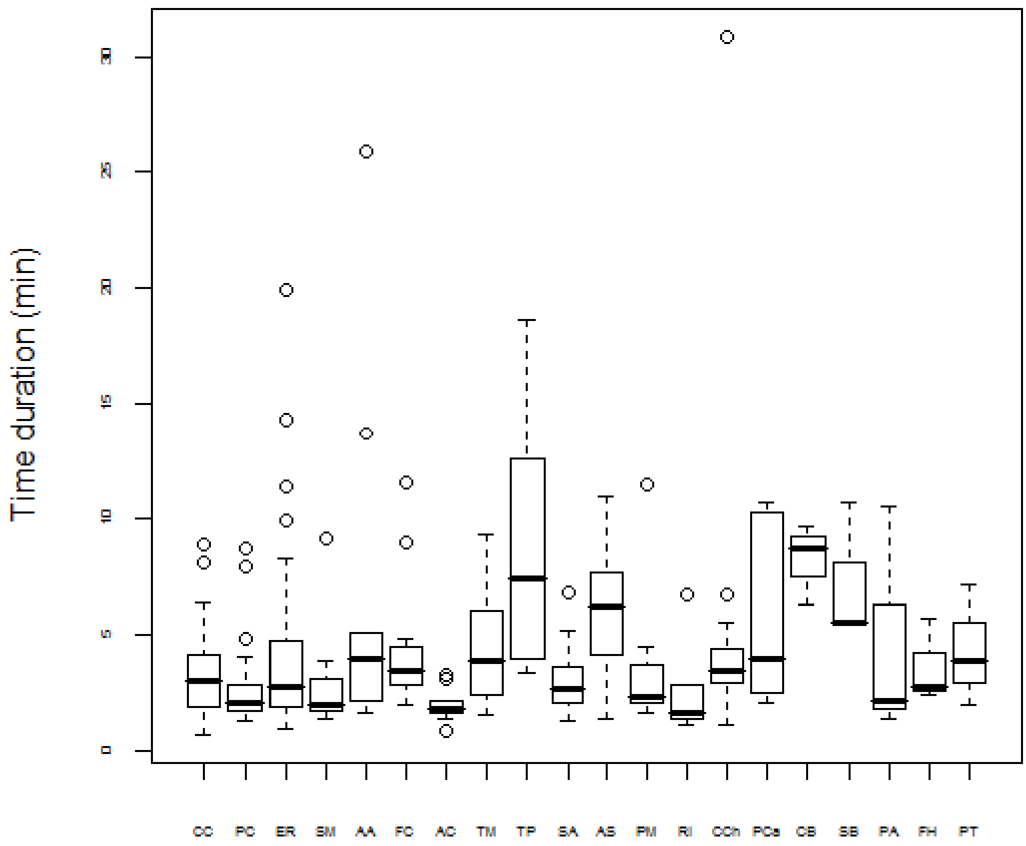

Figure 2.4: Handling time durations in minutes according to species: CC- Cetti's warbler *Cettia cetti*, PC- European chiffchaff *Phylloscopus collybita*, ER- European robin *Erithacus rubecula*, SM- Sardinian warbler *Sylvia melanocephala*, AA- common kingfisher *Alcedo atthis*, FC- common chaffinch *Fringilla coelebs*, AC- long-tailed tit *Aegithalos caudatus*, TM- common blackbird *Turdus merula*, TP- song thrush *Turdus philomelos*, SA- BEurasian blackcap *Sylvia atricapilla*, AS- Eurasian reed warbler *Acrocephalus scirpaceus*, PM- great tit *Parus major*, RI- common firecrest *Regulus ignicapillus*, CCh- European greenfinch *Chloris chloris*, PCa- blue tit *Cyanistes caeruleus*, CB- short-toed treecreeper *Certhia brachydactyla*, SB- garden Warbler *Sylvia borin*, PA- coal tit *Periparus ater*, FH- pied flycatcher *Ficedula hypoleuca*, PT- willow warbler *Phylloscopus trochillus*. Individuals with less than 3 captures are not illustrated in the figure.

Table 2.V: Final model of variables influencing time of extraction. Model adjusted $R^2 = 0.1003$, $F_{28,220} = 1.987$, $p = 0.0034$.

Model Term	**Test statistic F**	**D.F.**	***p***	**Parameter level**	**Coefficient**	**Standard error**
Species	2.2858	25	0.0008			
Ringer's experience	10.8239	2	< 0,0001	beginner	Reference level	
				intermediate	-83.7674	24.1087
				experienced	-80.0887	24.1597
Body mass	6.9598	1	0.009		-1.9260	0.9741

Table 2.VI: Final model of variables influencing duration of handling. Model adjusted $R^2 = 0.2908$, $F_{31,239} = 4.571$, $p < 0.0001$.

Model Term	**Test statistic F**	**D.F.**	***p***	**Parameter level**	**Coefficient**	**Standard error**
Species	3.3087	28	< 0.0001			
Ringer's experience	61.2482	2	< 0.0001	beginner	Reference level	
				intermediate	-231.0443	42.5944
				experienced	-301.2393	40.5626
Body mass	0.2142	1	0.6439		+0.5252	0.464

Extraction took longer for species with lower body masses, such as coal tits, blue tits and wrens than for heavier birds such as blackbirds or song thrushes (figure 2.3, table 2.V). During the handling stage the body mass of the bird had no additional influence on the time duration of handling. Figure 2.4 shows that species such as treecreepers, song thrushes, reed warblers and garden warblers took substantially more time to process compared to long-tailed tits, coal tits and firecrests.

Hypothesis 4: The body grasp technique accelerates the extraction stage.

On average, birds were extracted from mist nets in 58 ± 0.16 s less by using the body grasp extraction technique compared to the 'feet first' method ($F_{1,106}$ = 4.599, *p* = 0.034). In figure 2.5 it is also apparent that extraction by the feet first method has more outlier times.

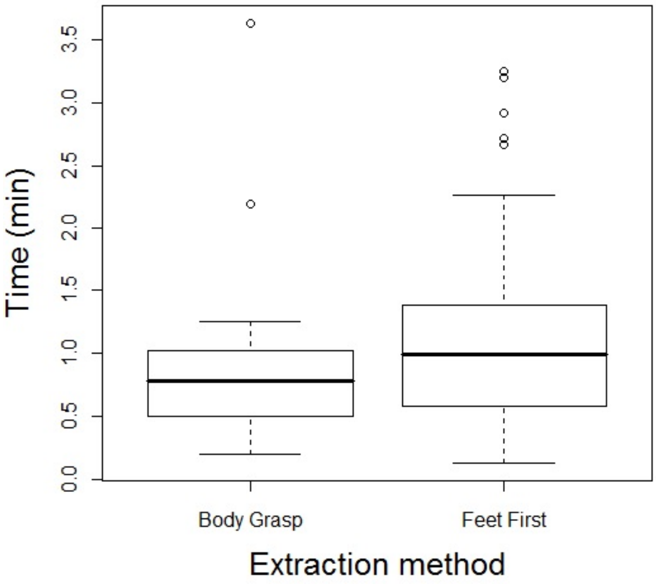

Figure 2.5: The time duration that an intermediate bird ringer takes to extract a bird either using the body grasp method or the feet first method (n = 33 and n = 75 birds respectively).

Hypothesis 5: The length of the ringing process influences the weight loss of the birds.

The mean duration of handling (including time in the bag) from the moment of extraction from the net until release was 23.69 ± 0.85 min, ranging from 2 min to 49.17 min. Throughout the whole period, birds lost an average of 0.19 ± 0.03 g (One sample t-test for a significant difference from zero mass change; t = 4.7997, d.f. = 49, *p* < 0.0001). The largest proportion of this weight loss occurred between the end of extraction and the beginning of handling (i.e. inside the bag). The mass change during this period was significantly different from zero (One sample t-test; t = 5.2531, d.f = 49, p < 0.0001). Although the time spent inside the bag was significantly greater than the processing (handling) time ($F_{1,88}$ = 198.02, p < 0.0001, also refer to figure 2.1), neither the time spent in the bag, or furthermore the total length from the moment of extraction till release, or the type of species had any influence on weight loss (table 2.VII).

Table 2.VII: Final model of variables influencing time of extraction. Model adjusted R^2 = 0.1760, $F_{12,28}$ = 1.711, p = 0.1178.

Model Term	**Test statistic F**	**D.F.**	***p***
Species	1.6396	9	0.1521
Time from extraction to release	0.7391	1	0.3973
Time in bag	0.6533	1	0.4258
Processing time	1.0739	1	0.3089

Hypothesis 6: Bird ringing imparts extra energetic costs to breeding females.

Brood patch temperature varied noticeably throughout the 2 stages measured (inside the bag and processing; figure 2.6). Inside the bag there was an average increase of temperature of 1.1 ± 0.23 ºC, with a subsequent mean drop in brood patch temperature during handling of 1.7 ± 0.18 ºC. This drop in temperature was significantly influenced by the processing time duration, but did not differ significantly between species (table 2.VIII).

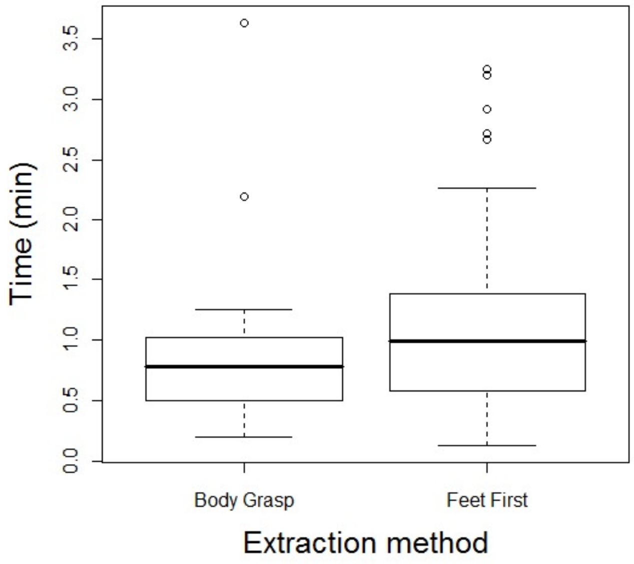

Figure 2.6: Temperature measured at the surface of the brood patch with an ear thermometer after extraction, beginning of handling and before (n = 46 birds). Mean temperatures are 40.7 ± 0.19 ºC, 41.8 ± 0.12 ºC and 40.1 ± 0.17 ºC respectively.

Table 2.VIII: Final model of variables influencing brood patch temperature decrease during the processing of birds. Model adjusted $R^2 = 0.3688$, $F_{12,26} = 2.851$, $p = 0.0123$.

Model Term	Test statistic F	D.F.	*p*
Species	1.7284	11	0.1228
Processing duration	11.4752	1	0.0023

Hypothesis 7: Each species has a typical coping response.

Of the 78 birds used for this particular study, the vast majority displayed an agitated type of behaviour (table 2.IX). During extraction 80% percent of the birds displayed an agitated behaviour, of which 55% exhibited the flight component, 13% the fight component by demonstrating aggression towards the researcher, whilst the remaining 12% did not demonstrate a tendency for any component and displayed both aggression and an escape behaviour. During processing, a slightly higher proportion of the birds (82.6%) were agitated, with 60% favouring flight, 12% favouring fight, and the remaining 10.6% displaying both to a similar extent.

During both extraction and handling, individuals of the same species display identical coping responses ($\chi^2 = 96.5428$, d.f. = 45, $p < 0.0001$ and $\chi^2 = 90.1666$, d.f. = 45, $p < 0.0001$ respectively).

Table 2.IX: Number of birds that displayed either an agitated or a calm coping response. Agitated has been broken down into 3 categories: overall agitated, agitated favouring fight, and agitated favouring flight.

Species	**Extraction**				**Processing**				**Total**
	Agitated	Fight	Flight	Calm	Agitated	Fight	Flight	Calm	
European chiffchaff *Phylloscopus collybita*		1	4	12	1		7	9	**17**
European greenfinch *Chloris chloris*		3	8	2	1		9	3	**14**
Eurasian blackcap *Sylvia atricapilla*	1		12		1		11		**13**
European robin *Erithacus rubecula*	2		6			1	7		**8**
Common chaffinch *Fringilla coelebs*	1		3		2		2		**4**
Sardinian warbler *Sylvia melanocephala*			4				4		**4**
Cetti's warbler *Cettia cetti*	1		2		1		2		**3**
Great tit *Parus major*	1	2			1	1	1		**3**
Long-tailed tit *Aegithalos caudatus*		1	1			2	1		**3**
Blue tit *Cyanistes caeruleus*		1	1			1	1		**2**
Coal tit *Periparus ater*	1	1			1	1			**2**
Eurasian reed warbler *Acrocephalus scirpaceus*	1					1			**1**
Common firecrest *Regulus ignicapilus*				1				1	**1**
Common kingfisher *Alcedo atthis*		1			1				**1**
Song thrush *Turdus philomelos*	1					1			**1**
European serin *Serinus serinus*			1				1		**1**

Hypothesis 8: Coping responses are individually constant throughout the ringing procedure.

Individuals tend to display the same coping response during both extraction and handling ($\chi^2 = 63.8505$, d.f. = 9, $p < 0.0001$).

Hypothesis 9: The utterance of calls is related to both species, and coping response.

Out of the 285 birds utilized to test this hypothesis, 122 birds, belonging to 20 different species, uttered calls (table 2.X). During extraction and handling, respectively, 93% and 91% of the calls were distress calls and the remainder alarm calls. At release, no birds uttered distress calls, but rather flight calls (56%), alarm calls (24%) and simple calls (20%). Also, substantially more birds uttered calls during extraction (76 birds) than during handling (43 birds) or release (50 birds).

The utterance of sounds was related to the type of behaviour a bird displayed during extraction and handling (Fisher's Exact Test, $p < 0.0001$ and $p < 0.0001$, respectively). Calm birds were less likely to utter alarm or distress calls during both extraction and handling, as well as birds that favour escape “flight”, when compared to the remaining agitated birds (table 2.X).

Table 2.X: Number of birds that uttered calls during capture and handling per specie.

Species	**No call**	**Called**	**Total**
European chiffchaff *Phylloscopus collybita*	32	17	49
European robin *Erithacus rubecula*	38	6	44
European greenfinch *Chloris chloris*	12	19	31
Eurasian blackcap *Sylvia melanocephala*	19	9	28
Cetti’s warbler *Cettia cetti*	15	8	23
Common blackbird *Turdus merula*	7	9	16
European chaffinch	5	7	12

Species	**No call**	**Called**	**Total**
Fringilla coelebs			
Common kingfisher *Alcedo atthis*	2	9	11
Eurasian reed warbler *Acrocephalus scirpaceus*	4	6	10
Long-tailed tit *Aegithalos caudatus*	2	8	10
Sardinian warbler *Sylvia melanocephala*	7	2	9
Song thrush *Turdus philomelos*	2	6	8
Common firecrest *Regulus ignicapillus*	5	1	6
Great tit *Parus major*	2	4	6
Eurasian wren *Troglodytes troglodytes*	2	1	3
Coal tit *Periparus ater*	1	2	3
Blue tit *Cyanistes caeruleus*	1	2	3
Willow warbler *Phyloscopus trochilus*	0	3	3
European pied flycatcher *Ficedula hypoleuca*	2	0	2
Short-toed treecreeper *Certhia brachydactyla*	2	0	2
Waxbill *Estrilda astrild*	0	2	2
Garden warbler *Sylvia borin*	1	0	1
Wryneck *Jynx torquilla*	1	0	1
Black redstart *Phoenicurus ochrurus*	1	0	1
European serin *Serinus serinus*	0	1	1
Total	163	122	**285**

2.5 Discussion

The results obtained from this study give a broader indication of how current ringing practices comply with guidelines and recommendations, and furthermore how birds behave during capture and handling. Although there are recommendations for how often nets should be checked and for the maximum time birds should be held before release, as far as I am aware this is the first time that these times have been quantified during routine bird ringing activities.

The mean total time a bird was held captive (from moment of capture to moment of release) was 34 minutes, which is a similar duration to the British Trust for Ornithology's recommended interval between net checks of 20-30 minutes (Spencer 1984, Redfern & Clark 2001). In other ringing schemes, in certain situations the allowable interval between net checks is up to 45 minutes (NABC 2001). Nevertheless, 42% of the birds were held captive for periods longer than the mean duration, and the longest time a bird was held captive was nearly two hours. The maximum time in the net was exactly 1 hour and 17 minutes, which indicates that some ringing stations were not always complying with these guidelines (table 2.IV). Although this situation could be a consequence of the specific catch rate in that net round, it is clear that the total retention time can be considerably higher than the length of the net round. There are no current guidelines for the maximum recommended total duration a bird is held out of its environment other than a "reasonable period of time" (Spencer 1984). Although no times are stated, a total time in captivity of nearly two hours is likely to exceed the time envisaged.

The longest stages of the ringing process are the ones that do not require handling, and the bird is simply restrained in the mist net or the bag. Extraction and handling times were significantly shorter. In figure 2.1 it is possible to see several outlier values, which are themselves of interest as they correspond to situations that do not conform to the relevant guidelines and are thus due to human error. For example, these incidents included birds processed out of order from capture, longer net rounds due to higher catch rates, or even longer processing of the birds due to the experience of the ringer, e.g. the maximum time a bird was kept during the handling stage (31 min) corresponded to the first bird a beginner ringer has processed. These lapses demonstrate that bird ringing is liable to human mistakes that greatly increase the time the bird is retained and could be potentially detrimental.

Clearly, and understandably, beginner bird ringers spend significantly more time in every stage as compared to intermediate and experienced bird ringers. Intermediate ringers take on average the same time to extract a bird as experienced bird ringers, but longer to process the birds, and therefore experienced ringers take significantly less time than the remaining two. This suggests that the rates at which ringers learn the different skills and gain the appropriate knowledge varies between extraction, which is a mechanical operation/manual skill, and processing, which involves different skills and more expertise (assessing the birds age, particularly requires good knowledge of the species). This difference in expertise is clearly associated with the total time that bird ringing takes, suggesting that senior bird ringers could account for this and use personnel effectively whilst closely supervising beginners.

There are a few important issues that bird ringers should bear in mind, and that this study allows me to highlight: firstly, according to Recher *et al*. (1985) when birds are in the net they are more susceptible to injuries and mortality, and thus net rounds that allow a bird to be in the net for long periods (e.g. more than 1 h) are not acceptable, and bird ringers should strive to check nets more regularly than this. Secondly, although beginners learn from practice, experienced bird ringers should prioritize the bird's welfare even if the means that beginners will have to extend their learning period. For example, instead of a beginner doing all the measurements with the same bird, they could start by learning to put a ring on some birds, then take measurements in others, in order to decrease handling time of the individual birds involved. Thirdly the results from hypothesis three demonstrated, that although the feet first method is the preferred method of extraction by the majority bird ringers, the body grasp technique may make extraction faster. This conclusion needs to be treated with caution for two reasons. The first is that most of the more experienced ringers that contributed to this study refused to use an alternative method of extracting birds from mist nets to their preferred approach and may be less familiar with the alternative method, tending to increase the time taken. Secondly, whilst the feet first method can be used in most situations, the body grasp cannot be used for example in situations that the bird is “double pocketed” or severely tangled which is a recurrent situation for “tit” (Parus) species (personal observation and NABC 2001). Nevertheless, the results of this study suggest that further work to identify the optimal extraction technique could reduce the time that this procedure takes. This advantage would be in addition to other welfare aspects, such as the NABC guidelines (2001) which suggest that feet first has the disadvantage of holding the legs, which if done incorrectly can cause injury.

Time durations of the different capture and handling stages were also influenced by the species. Smaller species took longer time to extract than bigger ones, which may be related to the degree of entanglement that different body sizes can involve; and during handling some species took longer to process than others. This could demonstrate, the need for experienced bird ringers to focus their beginner ringers on species that will generally take less time to handle, thus decreasing the average time duration that birds are held in captivity. Although the process of learning bird ringing is lengthy, the bird's welfare should always be a priority, thus any contribution to decrease the overall time in captivity should be accomodated.

I have found that mass loss was significant in the period between the moment of extraction and the release. Refsnider (1993) has undertaken a similar experiment in which she demonstrated that birds lost the biggest part of their body mass during the first 30 min of capture. In the subsequent 90 min the mass loss was much smaller, in most cases less than 1% of the initial mass on the subsequent period of 30 min. Because mean total time in captivity was 33.55 min, such a finding could imply that most birds lose a significant portion of their body mass. Also Rands & Cuthill (2001) warn that mass loss can be extremely quick in response to capture and handling from the moment birds enter the net. Due to logistical reasons, capture time was only recorded for 14 individuals for this particular experiment and time in the net ranged from as low as 1 min to 34 min before extraction started. Mean mass loss corresponded to 0.19 g for a mean body mass of 12.13 g, which corresponded to 1.6% of body mass, in a mean time of 29 min.

Although the biggest portion of mass is lost directly after capture, Refsnider (1993) was able to demonstrate that mass loss during capture and handling is continuous, which highlights the importance of minimizing the duration of these procedures. In this sense, adapting procedures might aid in accomplishing lower restraint times, for example by adapting the extraction technique and utilizing the one that provides swifter extraction (refer to figure 2.5, wich demonstrates that the body grasp method usually allows to extract a bird in less time than the feet first method).

Physiologically, nesting is a very demanding task for female birds, especially in passerines as females are usually the only ones developing a brood patch and are the most responsible for the brooding and keeping the nest warm. In order to do so they need to expend considerable energetic resources generating heat, which might be from allocated fat reserves or come directly from foraging (Rands *et al*. 2006). It is possible that females have increased their temperature inside the bag, for the sole reason that they were being kept inside cotton bags rather than entering a fever like state. This could explain why females had a lower brood patch temperature at release after having cooled off outside the bag. Each way the drop in temperature was significant when compared to initial measurement, which can be indicative that handling could have induced a reduction in blood flow to the skeletal muscles, to aid in the preservation of energetic resources (Criscuolo *et al.* 2001). If this is the case then females could be suppressing their fight-or-flight response favouring the passive response.

During both extraction and handling, birds demonstrated an agitated behaviour, favouring the escape (flight) response, although there was a small percentage of birds which remained calm, notably chiffchaffs. Because behaviour has been found to be consistent within species, it is possible that chiffchaffs (and possibly firecrest, for which n=1) favour the passive physiological response, thus demonstrating an “immobilized” behaviour (Criscuolo *et al*. 2001). Such a finding, that coping responses are species related have also been suggested elsewhere (e.g. Koolhaas *et al*. 1999, Bell 2007), but I have not found any study that links it to using capture and handling as a direct stressor. Also, when being handled, these birds utter significantly more distress calls than others. The only other time they utter an alarm call is for the purpose of signalling danger. Upon release some individuals still signal the danger, but the majority utter calls to signal their presence to others: simple calls (contact calls) and flight calls. Both distress and alarm calls are part of the antipredator defense, with distress calls being often a synonym for fear screams uttered when birds are either captured by a predator or in a mist-net and handled by a human (Caro 2005, Martin *et al*. 2011). Distress calls can serve many purposes, e.g. startling the predator, warning conspecifics about the presence and location of the predator or asking for help, or even providing honest signals to predators about the individual’s quality (Caro 2005, Martin *et al*. 2011, Laiolo *et al*. 2007). Although distress calls have been further linked to parasite load, body size and to be related to corticosterone levels (Martin *et al.* 2011, Laiolo *et al*. 2007), I have not explored this subject further, as the purpose of my study was to provide a description of the birds’ response to the bird ringing methodology.

The main conclusions of this chapter are that bird ringing can remove the bird from its daily routine through a period of time that can be surprisingly high, and that the handling part of the method is considerably shorter compared to the restraining it requires. Factors such as the type of extraction and the experience of the bird ringer have an effect on the length of the handling time, although the length of the extraction stage will still be comparatively brief part of the overall interruption to the bird’s time budget. From this study it is clear that capture and handling can have an energetic cost associated; either in terms of body mass loss, or heat loss in brooding females. Finally, the majority of birds seem to perceive capture and handling as a predation event, and constantly favour escape, whilst demonstrating behaving in a manner consistent with “fear”.

Chapter 3

Body mass regulation in response to capture and handling in European passerines

3.1 Abstract

Capture and handling interrupts a bird’s daily routine, inducing immediate physiological and behavioural changes that can disturb the bird’s foraging behaviour and subsequently its mass regulation. Regardless of the physiological basis of the stress response, the effects upon these traits will depend on how the bird perceives this interruption: whether as an interrupted foraging event, after which the bird is predicted to increase its body mass, or as an encounter with a predator, leading to a decrease in body mass. To examine the effects of capture on body reserves and the foraging behaviour of free-living passerines, individuals were pre-trained to forage from electronic balances and remotely monitored for foraging frequency and body mass. Experiments lasted three days, with birds experimentally captured (> 15 m from the feeder) and released on day 2. After capture, birds decreased their feeder attendance and 21% did not return again to the feeder. Body mass amongst those birds that did return did not decline significantly. Capture and handling induced immediate foraging behaviour changes that were still measurable on the subsequent day, but body mass was unchanged. Results did not allow for the measurement of the trade-off between starvation and predation, in light of capture and handling protocols. Implications of the experimental design in these results are discussed.

3.2 Introduction

Bird ringing is used extensively for the monitoring of populations (Spotswood *et al*. 2012). The data it provides can be used, in some instances, to assess body condition, which may allow conclusions to be drawn about the relative probability of survival of an individual bird and at a larger scale how it will affect population dynamics (Cresswell 2009).

In chapter one, I described how birds can perceive capture and handling as a stressor, similar to an encounter with a predator, causing them to respond behaviourally and physiologically. In chapter two, I demonstrated how it affected the bird's behaviour, disrupted its immediate routine and may have energetic costs. In addition, a variety of studies and anecdotal notes based on re-captures of ringed birds report cases of body mass loss over a period of hours to 2-3 days subsequent to the first capture (e.g. Leberman & Stern 1977, Castro *et al*. 1991, Collins 2007), whilst others report an increase in mass over an extended period of time (of several months) (e.g. Poole & Brown 2007). Nevertheless it is clear that bird ringing may affect the immediate body mass of the bird, and it is possible that this could be extended over a considerable period of time (e.g. Leberman & Stern 1977).

The most frequently reported causes for any loss of mass during bird ringing are: missed opportunities to feed during the corresponding period of time (Clark 1979, Schwilch & Jenni 2001), increased metabolic rate and tissue metabolism (e.g. fat) (Davidson 1984, Thomas 2000), or water loss and excretion (Clark 1979, Dunn 1999). Excretion may have a double cost, as it can clear the gut of incompletely digested food, further impeding energetic absorption (Dunn 1999). Davidson (1984) found evidence that mass can be lost in two phases, based on shorebirds held in captivity for extended periods of time: initially in the form of water loss, and later as fat and tissue metabolism. Additional to these reasons, "handling shock" is also mentioned in the literature, and it refers to a condition that would impair the normal feeding or metabolism of the bird during the first day or two after ringing, which could thus lead to mass loss (Leberman & Stern 1977, Clark 1979, Rogers & Odum 1966).

Usually birds maintain a body mass below their maximum capacity of fat storage, as the accumulation of fat can have associated costs (Witter & Cuthill 1993), e.g. increased fat loads can have an effect on flight performance such that it can slow down the escape from predators (Fransson & Weber 1997). Thus the body mass displayed at certain periods of a bird's life-stage and the rates at which fat is accumulated, will result of a trade-off between the risks of starvation and predation (Witter & Cuthill 1993, Fransson & Weber 1997 and references within).,The starvation-predation risk trade-off theory predicts that mass will be lower when foraging conditions are more favourable and when predation risk is increased (Macleod *et al*. 2008, Zimmer *et al*. 2011). This implies that the way birds cope with capture and handling will also be dependent on the quality of the foraging environment (determined furthermore by environmental changes) (Macleod *et al*.

2008, Cresswell *et al*. 2009). For example, despite the high abundance of predators, the bird may respond by risking predation and forage more in poorer foraging habitats (e.g. Macleod *et al*. 2008). Furthermore any weight loss may: a) be strongly influenced by temperature, especially above a threshold of about 30 ºC, as dehydration is more likely (Castro *et al*. 1991); b) vary amongst and within species (i.e. age, sex, reproductive state, personality, life history) (Castro *et al*. 1991, Refsnider 1993); and, c) be affected by the season of life-stage in which they are handled and recaptured, in a way that layover periods can vary from a few hours to a few days (e.g. Schwilch & Jenni 2001, Clark 1979).

As I have demonstrated in chapter two, bird ringing activities can remove the bird from its daily routine for periods of time that can last up to 100 minutes. In the trade-off between compensating for the lost food intake and increased risks of predation, this disruption may lead the bird to either compensate with extra foraging time, risking longer exposure to predators but increasing body mass, or restraining itself from feeding, which can lead to a decrease in body condition (Macleod & Gosler 2006). Either way, the direction and scale of their response will depend on how the bird perceives capture and handling, either as: 1) an interrupted foraging event, heightening the risk of starvation (Ekman & Hake 1990) or 2) a close encounter with a predator, heightening the perceived predation risk (e.g. Gosler *et al*. 1995, Macleod & Gosler 2006, Macleod *et al*. 2008). This duality in responses is well explained by the fact that predation risk is mass dependent, either due to higher body mass slowing the take-off and reducing manoeuvrability to escape from predators or due to the increased foraging time necessary to maintain a greater mass (Macleod *et al*. 2008, Witter & Cuthill 1993). However, other responses are possible, for example, Bautista & Lane (2000) have demonstrated that an increased perceived risk of predation can lead coal tits (*Periparus ater*) to increase their evening body mass thus coping with the extra energy they will have to spend to remain attentive.

Despite the fact that the trade off between starvation and predation has been studied for passerines and waterfowl (e.g. Cresswell *et al*. 2009, Gosler 2001, Zimmer *et al*. 2011), the potential impacts that capture and handling have on body mass are still to be fully understood. The main reason for this is that capture-induced impacts on body mass can normally only be studied in the small percentage of birds that are re-captured in order to assess if there is any change in body mass. Although some studies analyze data from re-captures, they do so primarily over extended periods of time (e.g. months, Poole & Brown 2007), with few studies looking at the first few days after capture (e.g. Collins 2007). An additional weakness of previous studies is that the re-capture process can bring bias to the data analysis, as it doesn't account for the induced stress of capture. A solution for this is to remotely-weigh the birds, by attracting them to a feeder on top of a balance, and allow the bird to weigh themselves (Thomas 2000, Rands *et al*. 2006).

Thus, the present study was designed to assess how birds respond to capture and handling in matters of body reserves regulation without the need to re-capture individual birds. The main goals were to assess: a) changes in foraging rate and body mass (by monitoring the bird remotely: prior, during and after capture); and b) differences in these impacts between geographic regions and time of capture.

3.3 Methods

The study was carried out as part of the routine bird ringing licensed by the UK (BTO) and Portuguese (CEMPA) ringing schemes. Between 2010 and 2013, individual birds were pre-trained to collect *ad libitum* food (mealworms; *Tenebrio molitor* larvae) from a bowl on top of electronic balances, during the winter in the UK, Senegal and Portugal (figure 3.1). The display of the balance was monitored using a video camera that recorded continuously throughout the day, allowing remote weighing of birds during daylight periods to the nearest 0.1 g. The body mass of the birds was calculated by subtracting the current bowl mass (containing mealworms, measured immediately before a bird hopped on to the bowl) from the total mass of the bowl, food and the perched bird. If a bird fed again within 5 minutes, only the first weighing was taken into consideration in order to clearly distinguish between foraging bouts.

Figure 3.1: Remote weighing of a male black redstart *Phoenicurus ochrurus*. Photo by Leila Duarte.

The protocol consisted of monitoring each bird’s foraging behaviour and body mass over three consecutive days, without needing to capture the bird repeatedly. The first day served as a normal (unstressed) body mass control, day 2 was the capture day, and the remainder of day two, plus day three (post-capture day), was used to quantify the subsequent changes that capture and handling can cause. In the UK, birds were experimentally captured in mist nets (> 15 m from the food supply), ringed and released, while in Portugal and Senegal, the birds were captured with spring traps baited with a single mealworm, in the area surrounding the feeder (2-5 m) and then released. In the UK and Senegal birds were color ringed to permit individual recognition without re-capturing. In Portugal, birds of the same species were rarely seen feeding together, thus the distinction between individuals relied on the birds’ territoriality. There were two cases when two individuals were seen feeding during the same period, the distinction was thus based in the presence of a metal ring, and afterwards the leg which had the ring and feather plumage differences (e.g. two individuals of black-redstart, were differentiated based on gender differences in plumage).

Across the three locations, 33 birds were included in the study. In Senegal, the monitoring was carried in Djoudj Park (16°30′00″N, 16°10′00″W) with bird-ringing licensed through the British Trust for Ornithology. The area selected was around the

Grand Lac, which is dominated by sandy dunes and patches of grassland habitat and the sample comprised five Northern wheatears *Oenanthe oenanthe* and one woodchat shrike *Lanius senator*. Data was collected in conjunction with Adam Seward. In Portugal, the monitoring was carried in a woodland area surrounded by orchards in Serra da Columbeira (39º18'37''N, 9º13'16''W), where six European robins *Erithacus rubecula*, two common blackbirds *Turdus merula*, two black redstarts and one great tit *Parus major* were monitored. In the UK the sampling was carried out in Cardiff (51º34'57'' N, 3º13'12'' W), where two European blackbirds, two Eurasian wrens *Troglodytes troglodytes* and 12 European robins were monitored in conjunction with Dr Rob Thomas and various other licensed bird ringers.

Data analysis

Data were analyzed with the statistical software R 2.12.0 (R Core Development Team 2009). Box plots were created to allow for a visual comparison between feeder attendance, and body mass across the three days, and before and after capture. All the box plots performed show the median (dark line), and the 25th and 75th percentiles (lower and higher ends of box respectively). The ends of the whiskers showed 1.5 times the interquartile range (when outlier circles present) or the furthest value from the median (when no outliers present). Outlier circles represented values outside 1.5 times the interquartile range. References to the mean values of variables include reference to the standard error of the mean, to provide an idea of the accuracy of the mean.

Feeder attendance is quantified under "number of visits". Because, sometimes, cameras failed, I used "number of visits per hour", in which I count number of visits and divide by the period of hours that it relates to, as a measure of feeder attendance to standardize comparisons.

To better understand the effects of capture and handling I will perform comparisons in which I use either the term "before capture time" and "after capture time", this corresponds to the time of day that each individual was captured, rather than a single fixed time point. By using this approach I can better distinguish if on the previous and subsequent days the bird's individual behaviour is equivalent to that of the capture day, whilst also having a shorter timeframe to measure the behavioural response (instead of comparing between whole days, I can thus compare part-days).

To investigate the difference in feeder attendance (coded as “Number of visits per hour”) across the fixed effect covariates: 1) between days, 2) between the “before capture time” periods of the 3 days, and 3) between the “after capture time” periods of the 3 days, a set of pairwise contrasts within a generalized linear mixed model (GLMM) were carried out. As these contrasts were carried out within the overall GLMs, corrections for multiple testing were not required (Crawley 2007). The individual birds’ identities (coded by their ring number) were used as a random factor. An identical analysis was performed but to investigate the difference in body mass: 1) at dawn (coded as body mass at dawn) and 2) at dusk (coded as body mass at dusk) between days, between the “before capture time” periods and between the “after capture time” periods.

Pie-charts were used to describe desertion rates from the feeder and a One Sample t-test was applied to the change of mass overnight (day 1-2, and day 2-3), to assess if this mass change was significant.

3.4 Results

3.4.1 Feeder attendance

Following capture and handling on day 2, seven of the 33 captured birds (21% of individuals) did not return to the feeder on day 3, and the remaining birds generally decreased their attendance at the feeder (figure 3.2 and 3.3). The difference in the rate of visits between day 1 and days 2 and 3 was substantial (figure 3.2), as birds significantly decreased their attendance on day 2 and 3 (pairwise contrasts within GLMM; day 1 vs. day 2, t = -3.281, d.f. = 60, *p* = 0.0017; day 1 vs. day 3, t = -3.347, d.f. = 60, *p* = 0.0014). Conversely, feeder attendance was similar between days 2 and 3 (pairwise contrasts within GLMM; t = -0.105983, d.f. = 60, *p* = 0.9159).

Figure 3.3 shows another prominent pattern in feeder attendance. In general across the 3 days, the birds attend the feeder more often before individual’s “capture time” and attended less frequently afterwards. However, although there is no significant difference in the attendance rate before “capture time” between day 1 and day 2 (pairwise contrasts in GLMM; day 1 vs. day 2, t = 0.1091, d.f. = 60, *p* = 0.9135), there is a significant difference between the “before capture time” of day 2 and day 3, and between day 1 and day 3 (pairwise contrasts in GLMM; day 2 vs.

day 3, t = -2.7797, d.f. = 60, *p* = 0.0073; day 1 vs. day 3, t = -2.6087, d.f. = 60, *p* = 0.0115).

A similar pattern indicative of the intrusiveness of the capture event can be noted on the feeding rate of the "after capture time" across the 3 days, as day 2 is significantly different from days 1 and day 3 (pairwise contrasts in GLMM; day 2 vs. day 1, t = 3.8484, d.f. = 60, *p* = 0.0003; day 2 vs. day 3, t = 3.3237, d.f. = 60, *p* = 0.0015), and rates of attendance on day 1 and day 3 are not significantly different from each other (pairwise contrasts in GLMM; day 2 vs. day 1, t = -0.5986, d.f. = 60, *p* = 0.5517).

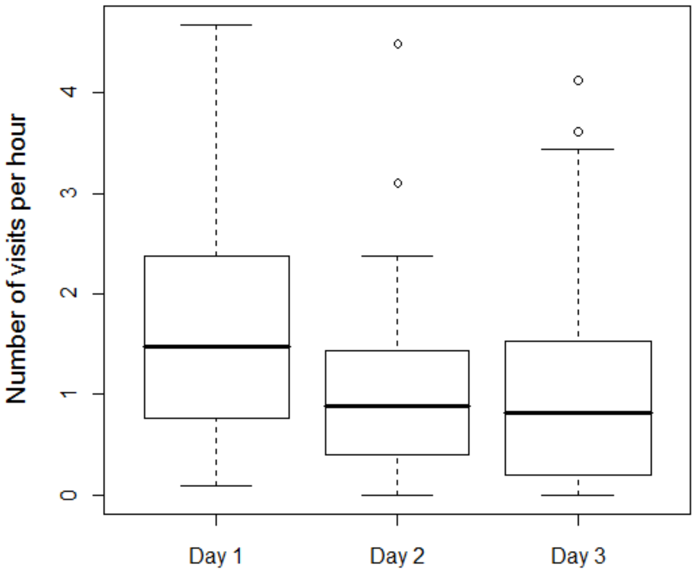

Figure 3.2: Total number of visits to feeder per hour from day 1 to day 3 (n = 24 individuals on each day).

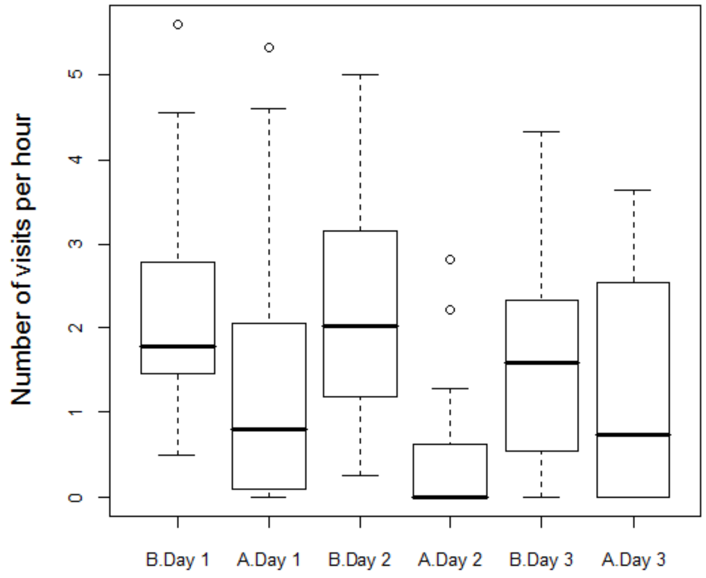

Figure 3.3: Visits to the feeder per hour before (B.) and after (A.) capture times from day 1 to day 3 (n = 24 individuals on each day).

62.5% of the birds sampled for feeder attendance did not return back to the feeder after capture on day 2 (figure 3.4), as compared to 25% not returning after the equivalent time on day 1 and 50% on day 3 (22% of which were birds that did not return throughout the whole day of 3 at all). Of the 62.5% of birds (n = 20) that did not return after capture on day 2, seven birds had not been to the feeder after the same time on the previous day (i.e. day 1), five birds were captured 1 hour before dusk, and seven (21% of individuals) did not return at all before the end of the study.

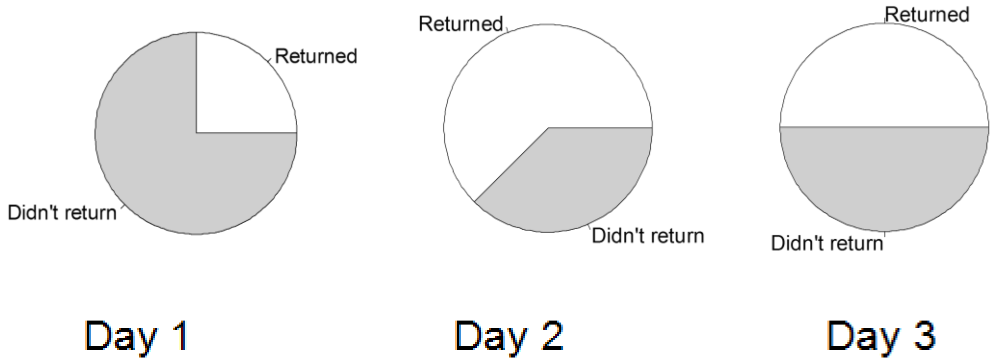

Figure 3.4: Return rates to the feeder after capture time across the days (n = 32). The proportion of birds that did not return were: on day 1; 25% (8), on day 2; 62.5% (20) and on day 3; 50% (16). Seven of these 16 birds on day 3, were individuals that also had not returned to the feeder after capture on day 2.

3.4.2 Mass regulation

Immediately before the capture event (day 2, n = 8 birds) birds' body mass averaged 20.41 ± 1.30 g and 20.14 ± 1.29 g after the capture event, at their next visit to the feeder. There was no statistical difference between the body mass before and after the capture event (t = -0.7, d.f. = 12, *p* = 0.4973).

Dawn body masses were not significantly different among the three days (pairwise contrasts in GLMM; day 1 vs. day 2, t = 0.9333, d.f. = 42, *p* = 0.3560; day 1 vs. day 3, t = -0.2494, d.f. = 42, *p* = 0.8043; day 2 vs. day 3, t = -1.1197, d.f. = 42, *p* = 0.2692) (figure 3.5). There was no statistical difference between dusk body mass across the the three days (pairwise contrasts in GLMM; day 1 vs. day 2, t = 0.3715, d.f. = 32, *p* = 0.7127; day 1 vs. day 3, t = -1.5046, d.f. = 32, *p* = 0.1422; day 2 vs. day 3, t = -1.8358, d.f. = 32, *p* = 0.0757) (figure 3.6).

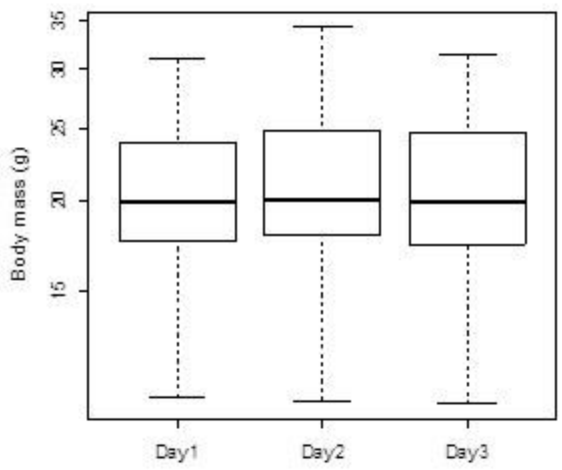

Figure 3.5: Dawn body mass (g) from day 1 till day 3 (n = 18, s = 4 species).

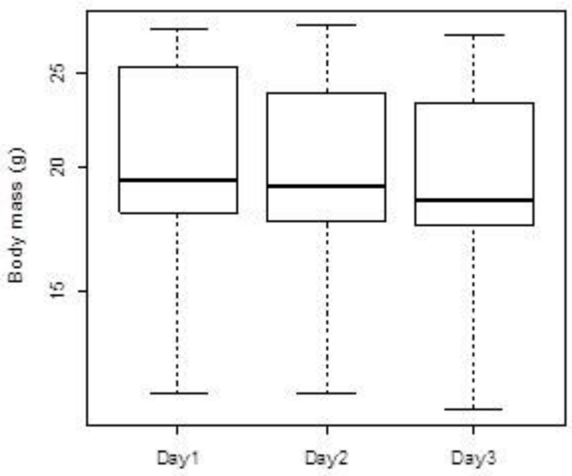

Figure 3.6: Dusk body mass (g) from day 1 till day 3 (n = 13, s = 2 species).

The difference in overnight mass loss between night 1-2 and 2-3 was not significant (t = -0.843, d.f. = 104.7, *p* = 0.4012).

3.5 Discussion

In this experiment, bird ringing did not seem to impart large detrimental energetic costs to the birds, as measured by changes in body mass. However bird ringing did cause alterations in the foraging behaviour of birds, as birds generally decreased their attendance to the feeder, with 22% of them not returning to the area after having been captured.

After being captured, birds seemed to avoid the feeder, with 62.5% not returning to the feeder at all on day 2, and 22% not returning at all on the subsequent day. Such a result could indicate that birds were indeed perceiving capture and handling as a predator attack, thus avoiding the area.

Alternatively, it could be due to trap shyness (Thomas 2000) as all of the birds that did not return to the feeder on day 3 were those captured with clap traps (in Portugal and Senegal) rather than mist nets (in the UK), and thus the capture was relatively close to the feeder (> 2-10 m away). In this case, birds could also be associating capture and handling with being fed *ad libitum,* with food that would otherwise not be available. Under such circumstances, birds may be more likely to shift feeding from the novel food to an alternative "safe" (natural) food source following capture, rather than in a more conventional bird ringing situation, where birds may only have alternative established food sources available (e.g. Whittingham & Evans 2004). Wild birds are often reluctant to forage on foods that are not "familiar", taking variable periods of time to adjust to it (or not adjusting at all), a behaviour that has been described as "dietary conservatism" (Marples *et al*. 1998, 2007, Marples & Kelly 1999). During the period of acceptance, the food is eaten but still recognized as less attractive, which might have been the case for these birds, and led them to switch to a more "familiar" source of food, abandoning the "unfamiliar" type that might have been associated with the capture event.

However, Gentle & Gosler (2001) conducted an experiment in which birds were repeatedly exposed to a model predator, and were never induced to abandon the feeder. Furthermore it can also suggest that alternative feeding areas availability might be higher in Portugal than in the UK.

Alternatively, the milder winters in Portugal may produce different responses from the UK, where accumulating reserves to endure the colder nights is essential (e.g. Polo *et al*. 2007, Blem 1990, Kullberg 1998). Because starvation risk is likely to be lower in Portugal than in the UK, birds in Portugal can possibly "afford" to lose mass to be able to escape a predator faster than in the UK. To support this rationale, in Portugal less than 30% of the birds returned to the feeder, of which all returning birds were captured in the morning as compared to 50% of the birds in UK returning to the feeder, of which 12.5% of these returning birds had been captured in the afternoon.

The fact that birds did not lose mass significantly from the moment of capture until dusk, and furthermore overnight (as reflected by dawn weight), despite what other authors have found (e.g. Gosler 2001), could be a result not of a trade-off between starvation and predation, but rather maintaining body reserves to endure the cold of the night (Merom *et al*. 2005). It could be the case that the bird perceives having reduced body reserves for maintaining body temperature through the night, or risking having to resort to hypothermia, as a higher risk than the possible risks associated with capture and handling. Although hypothermia presents energetic savings, when a bird enters hypothermia its attentiveness could become decreased, which could increase the risks from nocturnal predators (Bautista & Lane 2000).

Due to the limited sample size in the present study, hazards inherent for data collection and data available for each species, it was not possible make a thorough comparison between sampling sites (i.e. different latitudes), species or even circadian time of capture. However, these results indicate a possible role of the territory quality, and how environmental characteristics (e.g. ambient temperature) may influence the resulting behaviour of birds towards capture and handling.

The interpretation of how birds perceive capture and handling, i.e. as an encounter with a predator, or an interrupted foraging event, is not new (e.g. Gosler 2001, Macleod & Gosler 2006). However, previous work focussed on understanding how an individual species perceived capture and handling, over an extended period of time, whilst the present experiment extended it to multiple bird species (even though sometimes the sampling size would correspond to a single individual). Further, in the earlier experiments, birds did not abandon the feeder, which happened in the present experiment and which could thus reveal a more extreme reaction to the perceived predation risk.

If a wintering bird has lowered body mass reserves at dusk, resulting from an increased predation risk during the day, it might resort to hypothermia at night to cope with the depleted energetic reserves at dusk. Although hypothermia aids in reducing the overnight energetic expenditure, there could be associated costs to it, that could render the bird less responsive, decreasing its rest-time vigilance, and consequently increasing the risk of nocturnal predation (Pravosudov & Lucas 2000, Laurila & Hohtola 2005, Bautista & Lane 2000). Although no inference can be made in regards to potential nocturnal predators in the current experiment, this could be a hypothetical explanation for a lack of a significant decrease in body mass at dusk.

Chapter 4

Overnight thermal responses of migrating passerines in response to capture and handling

4.1 Abstract

Birds can enter a state of facultative hypothermia in circumstances where energy conservation is a priority, such as in the event of insufficient body mass reserves at dusk or a heightened perceived risk of starvation. However, facultative hypothermia can have important costs, and is avoided when not absolutely necessary. Because capture and handling induces increased energy consumption and mass loss, it is possible that birds subjected to capture stress can resort to hypothermia in certain periods of their annual cycle (e.g. migration, when fuel deposition is a priority). Stress-induced changes in metabolic rate are sometimes accompanied by a marked alteration in heart rate, which is a consequence of the cardiovascular component of the stress response. To investigate these responses, barn swallows *Hirundo rustica* and Western yellow wagtails *Motacilla flava*, two species with different coping responses to capture stress, were captured as they entered their roosts at dusk during the migration period, and their body temperature, mass and heart rate were measured at dusk and overnight before their release at dawn. In both species, overnight body temperatures following capture corresponded to those of the daytime active phase and there was no indication that either species resorted to facultative nocturnal hypothermia. There was no significant overnight variation in heart rate, and all birds lost mass during the trial. The invasiveness of the repeated measurements of temperature or mass, or taking blood samples, was associated with larger declines in body mass than birds only weighed at dusk and dawn, except for a single sampled group of yellow wagtails Barn swallows appeared to show a stronger response to invasive measurements than yellow wagtails did, indicating the possible role of coping mechanisms in determining the strength of the physiological stress response.

4.2 Introduction

A previous study has demonstrated how capture and handling during the day can induce a lower body mass at dusk (Gosler 2001), which in turn could lead birds to resort to nocturnal hypothermia in order to cope with reduced energetic reserves at dusk (e.g. Cooper & Gessaman 2005). The present chapter aims to investigate whether capture and handling could alter thermal regulation and heart rate in birds, leading to changes in overnight energy consumption and body mass regulation.

4.2.1 Bird's body temperature

Amongst the endothermic homeotherms, the highest body temperatures are found in the Class Aves. Within the Aves, bigger birds tend to have lower body temperatures (T_b), and smaller body temperature variations than smaller birds (Prinzinger *et al*. 1991, but see Butler & Woakes 2001 for an exception). Other factors that may influence the body temperature that a specific individual will demonstrate include: gender, hormones, circannual and circadian rhythms, and ambient temperature (T_a) (Prinzinger *et al*. 1991, Nord *et al*. 2009). Over the course of a day, T_b varies between three levels of activity: rest phase, active phase and high activity phase (Reinertsen 1996). In a review of avian T_b, Prinzinger *et al*. (1991) assessed that these activity levels corresponded to mean body temperatures of 38.54 ± 0.96 ºC, 41.02 ± 1.29 ºC and 43.85 ± 0.94 ºC, respectively. In terms of energy regulation, maintaining a high body temperature requires a high food intake. However, foraging is costly, and the amount of time spent foraging involves a precise trade-off between exposure to predation and foraging (Gosler 2001) for temperature maintenance. To cope with the costs of maintaining body temperature, birds resort to a set of behavioural and physiological mechanisms, such as microhabitat selection, communal roosting, facultative hypothermic responses, or adaptive changes in metabolic traits (e.g. body temperature regulation) (Dawson & Whittow 2000).

Facultative hypothermic responses are common in birds; these involve strategically decreasing their body temperature to a lower regulated level, in order to minimize energy expenditure (Butler & Woakes 2001). Strategic hypothermia has long been identified as a facultative response to periods of low food supply, and/or cold stress (Prinzinger *et al*. 1991, Schleucher 2004, Cooper & Gessaman 2005). There is controversy regarding how to define hypothermic responses and their categorization (see Schleucher 2004 and Nord *et al*. 2009 for distinctive opinions, and further details), however in order to describe boundaries between the extremes of the hypothermic responses, I will categorize them as two conditions: hypothermia and torpor.

4.2.2 Defining normothermia

To distinguish between hypothermia and torpor it is important to first define the T_b that is to be considered normothermia, i.e. the normal body temperature of the bird, and how this is assessed. Because birds maintain different body temperatures across the circadian cycle, it is important to measure hypothermic responses relative to the T_b of the corresponding phase of the circadian rhythm. For example, for describing nocturnal hypothermic responses, normothermic T_b values are those corresponding to the rest-phase (Schleucher 2004), when measuring T_b internally. But in cases where the T_b measurement is taken by measuring the surface skin temperature, Barclay *et al*. (2001) argue that it is more accurate to measure overnight drops in body temperature using the active phase temperature as the normothermic comparison value, because it accounts for the natural variability of T_b (e.g. Fletcher *et al*. 2004). Normothermic values of T_b can be assessed through a range of different methods. Some studies utilize values as presented by other authors (eg. Cooper & Gessaman 2005, Geiser 1988), others measure it over a short period in optimal conditions, such as captivity, for comparison (e.g. Nord *et al*. 2009); others monitor the birds over a long period of time (e.g. Butler & Woakes 2001), others establish a threshold of temperature, below which the bird is considered to be in torpor/hypothermia (e.g. Firman *et al*. 1993); while others define a specific drop in temperature to identify torpor/hypothermia (Fletcher *et al*. 2004). In this specific study, I will use T_b as measured in Prinzinger *et al*. (1991) as a threshold.

4.2.3 Distinction between hypothermia and torpor

Once normothermia has been defined, the subsequent definitions of hypothermia and torpor conditions are still controversial in the literature. For this reason, in their review of facultative hypothermic responses in birds, McKechnie & Lovegrove (2003) simply used the term hypothermia to include torpor, and rest-phase or controlled hypothermia. Despite their preference, it is pertinent for this thesis that I describe torpor and hypothermia separately, as they have different consequences in the energetic regulation metabolism.

Hypothermia and torpor are qualitatively similar, as both imply a decrease in T_b in relation to the normothermic range. The consensus seems to rest in defining rest-phase-controlled-hypothermia (that I simply refer to henceforth as “hypothermia”) as a relatively small nocturnal decrease in T_b (e.g. 3-8 ºC), whilst maintaining responsiveness to external stimuli, i.e. the bird can actively and spontaneously increase its temperature to resume activity (McKechnie & Lovegrove 2003, Prinzinger *et al*. 1991, Reinertsen 1996), by using either ambient heat or generating it metabolically (Fletcher *et al*. 2004). In contrast, torpor implies a more substantial decrease in body temperature (e.g. $T_b > 20$ ºC, in most passerines), rendering the bird into a lethargic state in which it is incapable of responding to external stimuli (Prinzinger *et al*. 1991, Nord *et al*. 2009, Schleucher 2004). A bird in hypothermia can resume activity almost immediately, but a bird in torpor can take up to 20 minutes to be able to resume activity (Reinertsen 1996). The physiological criteria to differentiate hypothermic from torpor responses relies, nowadays, on the extent of metabolic reduction (McKechnie & Lovegrove 2003, Brigham *et al*. 2011). Consequently, using metabolic rate (MR) to quantify heterothermy could be a more reliable tool than behaviour or temperature thresholds/intervals as it accounts for ecologically demanding external outputs, e.g. periods of low ambient temperatures, or increased food scarcity (Nord *et al*. 2009, Brigham *et al*. 2011, Boyles *et al*. 2011). Despite the usefulness of metabolic rates in defining metabolic responses, the measurement of real-time variation in MR is not feasible in field studies, thus the measurements in this study were made using measurements of core body temperature following previous successful experiments across the literature (Cooper & Gessaman 2005).

4.2.4 Which birds resort to hypothermia and why?

In the ecological context, controlling body temperature can be vital. The birds that are able to enter controlled hypothermia or torpor are usually the ones that exploit highly temporally variable food availability, such as occurs in frugivorous, nectarivorous, or insectivorous species (McKechnie & Lovegrove 2002). However, small body size is also associated with controlled hypothermia, and even granivorous birds of small body mass are able to down-regulate their body temperature (eg. Dolby *et al*. 2004, Schleucher 2004). The same applies to birds that practice energetically demanding activities such as hovering or aerial insectivory (Schleucher 2004).

Birds not only resort to torpor or controlled hypothermia in response to acute shortages of energy, but also in response to energetically challenging stages of the circannual rhythm such as when fuelling for migration (Butler & Woakes 2001, and see also references in Schleucher 2004), which can last several weeks. Nocturnal hypothermia during pre-migration fuelling periods enables birds to accumulate energy more rapidly, as the rate at which the fat deposits are consumed decreases, allowing for a quicker replenishment (rather than accelerating the instantaneous rate of fuelling). As a result, the bird can devote more energy to fat deposition than it would if it had to maintain a constant T_b (Butler & Woakes 2001), leading to more rapid fuel deposition over multiple days. The saved energy from hypothermia can then also be diverted to regeneration of other tissues such as the locomotory muscles and gastrointestinal tract, which can be metabolised during long migration journeys (Butler & Woakes 2001). Other birds may resort to nocturnal hypothermia all year round, to cope with high climatic variability. For example, facultative hypothermia is associated with higher latitudes or altitudes (e.g. Cooper & Gessaman 2005). This can be especially critical in smaller birds, which have larger surface area to volume ratio, yet still have to sustain a high metabolic rate and body temperature (Nord *et al*. 2009). Examples of such species are the mountain chickadee (*Poecile gambeli*) and juniper titmouse *Baeolophus ridgwayi*, from the family Paridae, which resort to year-round hypothermia (Cooper & Gessaman 2005). The depth of T_b decrease did not vary significantly between seasons, but was dependent on daytime temperature. For these two species, nocturnal hypothermia resulted in energy savings of 7-50% (mountain chickadee) and 10-28% (juniper titmouse) respectively. These ecologically important reductions in energy consumption allowed the birds to cope with the high metabolic demands of living in higher altitudes (e.g. 700-3300 m), which are characterized by harsh climatic conditions throughout much of the year. These members of the family Paridae resort to hypothermia more frequently than other members of the family, demonstrating that even closely related bird taxa may differ greatly in their use of hypothermia (Cooper & Gessaman 2005 and references therein).

Even within an individual species, there can be considerable variation in the use of controlled hypothermia. Haftorn (1992) found that wintering members of the Paridae family with body mass < 20 g, expend about 10% of their fat deposits on long, cold winter nights. These conditions, together with low food availability during the short daylight periods, favour the use of nocturnal hypothermia. For example, Nord *et al*. (2009) demonstrated that the use of hypothermia among wintering blue tits *Cyanistes caeruleus* was associated with low ambient temperature (T_a).

4.2.5 Capture and handling effects on thermal regulation

Capture-induced changes in energy reserves such as those described in the previous chapter may have consequences for a bird's thermal regulation, as part of a strategy to minimise energy expenditure in the hours or days following capture. When a bird's energy reserves are lower than optimal at dusk, it may strategically reduce its body temperature in order to minimize the expenditure of energy overnight (Nord *et al*. 2009, Cooper & Gessaman 2005). Switching to this strategy means: 1) reduced overnight energy consumption, 2) reduced exposure to predators by decreasing foraging time, and 3) increased time available for activities other than foraging (Pravosudov & Lucas 2000, McNamara *et al*. 2005).

Even though the strategy of nocturnal hypothermia appears beneficial, as it means expending substantially less energy overnight, birds generally do not use it, unless in the conditions described above. This suggests that there are costs involved that would counterbalance these potential benefits. Pravosudov & Lucas (2000) suggest that one of the costs could be a higher risk of predation at night, as birds would be less responsive to external stimuli (Reinertsen 1996). However, this cost seems to differ between species, as some birds take a longer time to increase their body temperature and become responsive than others (see Reinertsen 1996, for specific examples). In this case the trade-off would involve either 1) saving energy but being less responsive to predator attacks; or 2) decreasing body condition by burning energy reserves that have not been replenished, whilst remaining fully attentive. In both cases there is a risk of death. Haftorn (1992) demonstrated, in five species of the family Paridae, that the nocturnal metabolism was adjusted according to the evening body mass, and that hypothermia during sleep resulted from a trade-off between energy reserves and predation risk, suggesting that capture related-mass loss at dusk could expose birds to an extreme cost of exposure either to starvation or to predators. Furthermore, if a bird is left with very low body reserves, it might not even have sufficient energy reserves to increase its temperature back to the normothermic range.

Another possible explanation of why birds normally avoid hypothermia, is that birds that resort to hypothermia or torpor may get less of the restorative type of sleep (Reinertsen 1996), thus avoiding hypothermia when not absolutely necessary.

4.2.6 Stress hormones and temperature regulation

Because corticosterone (the primary stress hormone in birds) is known to have a role in the re-establishment of energy homeostasis (Belthoff & Dufty Jr. 1998), it is pertinent to consider whether increased levels of corticosterone in the blood following capture and handling could have a role in regulating hypothermic responses. However, very few studies have addressed this question. Using brown rats *Rattus norvegicus* Okuda *et al*. (1986) have demonstrated experimentally that a hypothermic decrease in temperature was correlated with an increase of circulating corticosterone levels. In contrast, Lange and Leimar (2004) found that great tits *Parus major* with elevated levels of stress hormones failed to resort to nocturnal hypothermia. This could be a taxonomic difference in the physiological stress response, or could depend upon how the animal perceives the event. If a bird was to interpret capture and handling as a continuing threat it might fail to resort to hypothermia on the day or subsequent days after the capture, leading to a decrease in body condition. A reduced capacity to use strategic hypothermia to maximise fuel deposition rate could also explain Leberman and Stern's (1972) observations of prolonged migration stopovers after capture and handling, rather than (or in addition to) simply the effect of handling shock delaying departure.

Hiebert *et al*. (2000), using rufous hummingbirds (*Selasphorus rufus*) as a model, also found that corticosterone is involved in the regulation of torpor. However they found that corticosterone concentrations *per se* cannot fully explain seasonal changes in the response to energetic challenges and that some other feature of the physiological effects of corticosterone could be playing a role in this mediation.

A short term response to a stressor (e.g. handling), concomitantly prolonged by the release of corticosterone, is that birds exhibit a rapid rise in body temperature which is usually associated with tachycardia (Singer *et al*. 1986, Carere & van Oers 2004, Cyr *et al*. 2008). This rapid rise in T_b is a typical component of the "emotional" stress response, and is considered to be a genuine emotion-induced state (Singer *et al*. 1985, Carere & van Oers 2004, Cabanac & Guillemette 2001). Tachycardia, in the absence of any motor or somatic activity, is also often considered another sign of a heightened emotional state (Cabanac & Guillemette 2001).

4.2.7 Heart rate alterations as an effect of capture and handling

The cardiovascular component of the acute stress response involves the increase of heart rate and blood pressure, in which blood flow is diverted to the muscles. Confinement, in particular, is acknowledged to have a marked effect on the heart rate of wild birds, leading to [short term increases? But] long-term decreases of heart rate that are sometimes (but not always) accompanied by loss of body mass. When heart rate becomes very low in such circumstances, arrhythmias may arise (Odum 1941).

In natural conditions, heart rate is higher during the active phase and lower during the resting phase. It is related to other physiological parameters such as: volume of O_2 consumption, muscle tremors (which help to maintain nocturnal body temperature), body temperature, and breathing rate. For this reason heart rate has been suggested to have a very close relationship with metabolic rate. However, Odum (1941) suggests that the conscious centers in the cerebral cortex, may introduce fluctuations in heart rate that sometimes do not produce appreciable increases in heat production. Thus the heart rate may be most useful when used in conjunction with the other physiological variables mentioned above, in order to better understand the response of the organism to stressful conditions (Odum 1941). Heart rate has also been found to vary with age, sex, time of day and year, blood composition, and life habits, but also with factors such as availability of food and mental activity. In this way, for example, long periods of starvation will lead to decreased heart rates (Odum 1941)

4.2.8 Introduction to the work developed in this chapter

In 1997, the European Union for Ringing (EURING) launched an international project “The EURING Swallow project” involving ringers from 25 countries, aiming to capture barn swallows *Hirundo rustica* at roosting sites. This project has encouraged the gathering of essential knowledge about the bird’s migration, allowing us to test optimal migration theories, and served as a symbol of the need for internationally-based conservation efforts and strategies. Despite leading to thousands of swallows being ringed every year, very little is known about the particular effects of capture and handling in this species, other than: i) barn swallows respond to capture and handling stress by increasing the corticosterone levels (Raja-Aho *et al*. 2010); ii) that the most stressed individuals will have lower body temperatures (Møller 2010), and

iii) that they are known to demonstrate tonic immobility upon capture (Møller 2010). Tonic immobility is a condition that reflects the motionless state that some individuals assume upon capture, which sometimes permits the apparently lifeless bird to escape (Møller 2010 and references within).

In the previous chapter I demonstrated how capture and handling did not affect energy regulation in wintering passerines. However it seems pertinent to investigate whether capture and handling affects migrating birds, with higher immediate energetic demands, i.e. maintaining optimal fuel levels to proceed with migration. Also, because in chapter two I discussed how species have different behavioural coping strategies, I have used a second species that also roosts during migration: the Western yellow wagtail *Motacilla flava* in order to investigate whether different behavioural responses are associated with different physiological responses. To separate the two species in terms of their behavioural response to capture, I consider barn swallows as having a “calm” type of behaviour (hence the display of tonic immobility), and yellow wagtails as having an “agitated” type of behaviour. In other respects, the two species are similar in that they are insectivorous long distance diurnal migrants, using similar routes to travel between Western Europe and West Africa (Moreau 1973).

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In this chapter I aimed to investigate how barn swallows and yellow wagtails coped with capture and handling at dusk during migration, in matters of thermal and energy regulation. Because heart-rate is often related to body temperature in birds (Carere & van Oers 2004), being slower during the rest-phase and faster in the active phase, I also investigated whether cardiac changes form part of the overnight energetic response to capture. Specifically I addressed the following questions:

1- Do either of the species (or both) resort to hypothermia, or display “emotional” responses (e.g. fever, Cabanac & Guillemet 2001), following capture at roost sites?
2- Does heart rate vary significantly overnight in either species?
3- Is the amount of body mass lost overnight associated with changes in heart rate and T_b?

4.3 Methods

Data collection was carried out during the summers of 2010 to 2013, at Paúl de Tornada, the location of a constant effort ringing-site in the centre-north of Portugal (GPS coordinates– 39º 26’ 53. 38’’, 9º 07’ 51. 67’’ W).

The capture method used was mist-netting. Either one or two mist-nests of 18 m were erected inside reed bed dominated by common reed (*Phragmites australis*) (figure 4.1), and were opened 30 min before sunset (the number of nets varied according to the number of bird ringers). A recording of either barn swallow or yellow wagtail calls, simulating a roost, was played until darkness, in order to lure individuals into the mist nets.

Figure 4.1: Reed bed line, location of the roosting site of migratory barn swallows and Western yellow wagtails where mist-netting was carried (mist net length = 18 m). Photo by Helder Cardoso.

Following extraction from the net, the birds were handled for ringing, and data was collected regarding: sex, age, weight, muscle and fat scores, presence/absence of brood patch, and body moult. After being handled, barn swallows were kept in groups of a maximum of 10 individuals inside a drawer (18 cm x 47 cm x 15 cm) of a wooden roosting “closet” (80 cm x 50 cm x 50 cm) until sunrise (figure 4.2), when they were released, whilst yellow wagtails were kept in groups of a maximum of 10 individuals inside cardboard boxes (60 cm x 40 cm x 40 cm). There was a separate group of barn swallows that was kept individually inside cotton bird bags, to control for the effects of shared body heat when roosting as a group.

Figure 4.2: Barn swallows being released from the roosting “closet” where they were kept overnight. Photo by Leila Duarte.

The trial consisted of monitoring times of capture, restraint and handling of randomly selected individuals, monitoring changes in their body weight and temperature, and assessing heart rate. The body temperature was measured with a cloacal probe at four time points during the capture and handling process: 1) beginning of handling for ringing; 2) end of handling for ringing (after which the birds were placed in their roosting bags/boxes/closet); 3) middle of the night; and 4) before release at sunrise. The thermometer model used was the TME 2010 model from TM Electronics, Sussex, United Kingdom, and the probe used had a diameter of 1.5mm. The birds’ body mass was measured when ringed (dusk) and before release (dawn), to assess overnight body mass loss with a digital scale to the nearest 0.1g (iBALANCE 1200, My Weigh, Phoenix, Arizona). The heart rate was assessed with a digital stethoscope (Rhythm Digital Stethoscope,model ds32a+, Thinklabs inc., Centennial, Colorado, United States) and recordings were transferred to a computer to be analyzed using Adobe Audition v1.5. Heart rate was assessed at 2 time points: 1) before ringing (dusk), and 2) at release (dawn).

Each year data collection was carried out throughout the peak migration period for each species: for barn swallows from the 15th of July to the end of September (years of 2010 and 2011), and for Western yellow wagtails (simply referred hereforth as yellow wagtails) from the 15th of September to the 15th of October (years of 2011 and 2012). A total of 387 barn swallows and 279 yellow wagtails were sampled across the study period.

The measurements of body temperature and heart rate could increase the stress/disturbance to the birds, potentially biasing the results. To assess the possible effects of the sampling protocol on body temperature three groups of barn swallows were considered: Control (with no extra measurements normal to bird ringing than measuring body mass at dawn), Test group 1 (with temperature measurements 1), 2) and 4) mentioned above), and Test group 2 (with all temperature measurements mentioned above). Additionally one group was sampled for heart rate once at dusk and once at dawn.

In 2010, early in the season (2nd half of July), 116 barn swallows were sampled for body temperature. Of these, 52 birds constituted the test group 1, whilst the remaining 64 birds constituted the test group 2. During 2011, 271 barn swallows, were sampled later in the season (from the end of the 2nd week of August till the 2nd week in September). Of these, 76 birds comprised the control group for which no other measurement was taken other than routine biometrics and putting a metal ring (in case of first captures), 49 had their heart rate measured, 112 constituted the test group 2, and the remaining 34 the test group 1.

To test whether communal roosting had an influence on the response to capture and handling, two different roosting conditions were used for barn swallows in 2010: birds were either kept inside the roosting “closet” (n = 73) or inside bird bags (n = 42).

To test the effect of the sampling protocol on yellow wagtails’ body temperature the same three groups were constituted: Control, Test group 1 and Test group 2. Additionally one group was sampled for heart rate and from one another blood samples were collected.

In 2011, 178 yellow wagtails were captured later in the season, from the 25th of September to the 10th of October, of which 98 remained as controls, 36 constituted test group 2, 25 were sampled for heart rate (one time at dusk, and one time at dawn), and an extra 19 were sampled for blood. A sample of 0,1 ml was collected for each bird according to the corticosterone sampling protocol described in Silverin (1997), at two time points: within three minutes of capture (Romero & Reed, 2005) and after 30 min of capture. The blood sampling protocol served to assess the physiological consequences of the sampling method.

In 2012, 60 yellow wagtails were sampled, of which 46 were sampled for heart rate and temperature (test group 1 protocol), and the remaining 14 served as controls.

In all years, ambient temperatures were similar ranging from 19°C to 25°C at dusk, and 16 °C to 20 °C at dawn. Neither dusk or dawn outdoor temperatures were likely to have had an effect on birds' physiological responses as they were sampled and kept indoors at a constant temperature (20-21 ºC).

The Body Mass Index (BMI) of each bird was calculated following Krams *et al.* (2013), by dividing body mass by the third power of wing length (body mass/wing length^3), and was considered to be a more accurate measurement of the bird's body reserves than body mass *per se*.

The capture and handling of barn swallows and yellow wagtails was carried by a full licensed bird-ringer (equivalent to an A permit in the UK), with the knowledge and consent of the ICNF (Instituto de Conservação da Natureza e das Florestas), the legal authority in Portugal responsible for regulating the bird ringing activities within the country. Legal permits to sample for blood and internal temperature were also obtained from the same institution, under my name, and I was the sole researcher collecting these two parameters.

Data analysis

Data were analysed with the statistical software R 2.12.0 (R Core Development Team 2009). A set of box plots were created to allow for a visual comparison of body masses, temperature variations and heart rate changes of barn swallows and yellow wagtails. All of the box plots show the median (dark line), and the 25th and 75th percentiles (lower and higher ends of box respectively). The ends of the whiskers showed 1.5 times the interquartile range (when outlier circles present) and the furthest value from the median (when no outliers present). Outlier circles represented values outside 1.5 times the interquartile range. References to the mean values of variables include reference to the standard error of the mean, to provide an idea of the accuracy of the mean.

For barn swallows a set of negative binomial Generalized Linear Models (GLMs) were performed to investigate: 1) if the barn swallows sampled in different years belonged to different populations differing in biomentrics (dependent variable (dv) = wing length, independent variable (iv) = year of sampling, family = Gamma, link = identity); 2) if their body condition (BMI) differed between years (dv = BMI, iv = year of sampling, family = Gamma, link = identity); 3) if overnight body mass loss differed between test groups (dv = body mass loss between dusk and dawn, iv= group of sampling, family = Gamma, link = inverse); 4) if body mass losses differed between years and were associated with keeping birds in different devices (dv = body mass loss between dusk and dawn, iv = group of sampling, year of sampling, BMI, keeping device, family = Gamma, link = identity); 5) how individual barn swallow T_b varied during the trial (dv = T_b, iv = sampling time point, family = Gamma, link = identity); 6) if birds measured for heart rate lost body mass similarly to the remaining groups (dv = body mass loss, iv = group of sampling, family = Gamma, link = identity); 7) if heart rate at dusk was different from heart rate at dawn (dv = heart rate, iv = period of sampling, family = Gamma, link= identity); and 8) if heart rate was influenced by the bird's body condition (dv = HR at dusk, iv = BMI, body mass loss, family = Gamma, link= identity).

For yellow wagtails, a set of negative binomial GLMs were also performed but to investigate: 1) if their body condition (BMI) differed between years (dv = BMI, iv = year of sampling, family = Gamma, link = identity); 2) if body mass at dusk differed between years or groups of sampling (dv = body mass at dusk, iv = group of sampling, year of sampling, family = gaussian, link = identity); 3) if body mass loss differed between test groups (dv = body mass loss between dusk and dawn, iv = group of sampling, family = Gamma, link = inverse); 4) how yellow wagtail's T_b varied during the trial (dv = T_b, iv= sampling time point, family = Gamma, link = identity); 5) if their body temperature was influenced by the year of trial or their body mass index (dv = T_b, iv = year of sampling, BMI, family = Gamma, link = identity); 6) if heart rate at dusk was different from heart rate at dawn (dv = heart rate, iv = period of sampling, family = Gamma, link = identity); 7) if the heart rate of yellow wagtails was different from that of barn swallows (dv = heart rate, iv = species, family = Gamma, link = identity) and; 8) if birds measured for heart rate lost body mass similarly to the remaining groups (dv = body mass loss, iv = group of sampling, family = Gamma, link = identity).

Fisher's Exact Tests for Counts were used to assess differences in bird's body condition (i.e. fat and muscle) between years, One Sample t-tests were also used to assess body mass losses, and Pearson's correlation tests were used to correlate T_b and heart rate, as well as heart rate at dusk and dawn and respective BMI, in yellow wagtails.

4.4 Results

4.4.1 Barn swallow case study

In both years, over 90% of the captures were of birds that fledged in that year (i.e. EURING age code 3 – see Appendix A). Birds in 2010 were in a slightly poorer migratory condition than in 2011, as pectoral muscle scores < 2 were observed in 21.6% and 12.2% of birds in 2010 and 2011 respectively, compared to 77.9% and 87.8% with scores ≥ 2 (Fisher's Exact Test for Count Data; *p* = 0.0015). Birds in 2011 carried more fat on average (Fisher's Exact Test for Count Data; *p* < 0.0001) (figure 4.3).

Figure 4.3: Fat score in barn swallows in 2010 and 2011 (see appendix A).

This difference in fat and muscle accumulation could be due to either higher food availability in 2011, or because birds were sampled later in 2011, or had longer to accumulate fat, or they could be coming from different regions (e.g. 2010 birds could come from a more northern distribution, Pilastro *et al*. 1998). The latter possibility was however not confirmed by comparing wing length of both datasets, as statistically they are homogeneous ($F_{1,382}$ = 0.7534, *p* = 0.3860). Likewise, the BMI index was not different between years ($F_{1,333}$ = 0.7336, *p* = 0.3923).

Body mass

Considering all datasets together, the overall mean body mass of barn swallows measured in this trial at dusk was 17.96 ± 0.09 g and at dawn 17.07 ± 0.09 g. In more detail:

Table 4.I: Variation in mean body mass in grams of barn swallows in different treatment groups, at dusk and at dawn (Control = barn swallows with no measurements collected; Test Group 1 = barn swallows with the temperature measurements before handling, after handling and at release; Test Group 2 = the same as test group 1 with an additional measurement half way between end of handling and release).

Weight (g)	***Control***	***Test group 1***	***Test group 2***
Dusk	19.17 ± 0.20	18.50 ± 0.18	17.46 ± 0.11
Dawn	18.51 ± 0.20	17.59 ± 0.18	16.42 ± 0.10
Overnight mass loss	0.66 ± 0.04	0.91 ± 0.04	1.04 ± 0.09

The apparent differences in body mass at dusk across groups (table 4.1) reflect the fact that 2010 birds were significantly lighter than 2011 birds ($F_{1,307} = 10.942$, $p = 0.001$). All three treatment groups lost body mass significantly between dusk and dawn (figure 4.4; 1-sample t-tests, Control: t = 16.037, d.f. = 74, $p < 0.0001$; Test group 1: t = 24.366, d.f. = 80, $p < 0.0001$; Test group 2: t = 10.340, d.f. = 130, $p < 0.0001$).

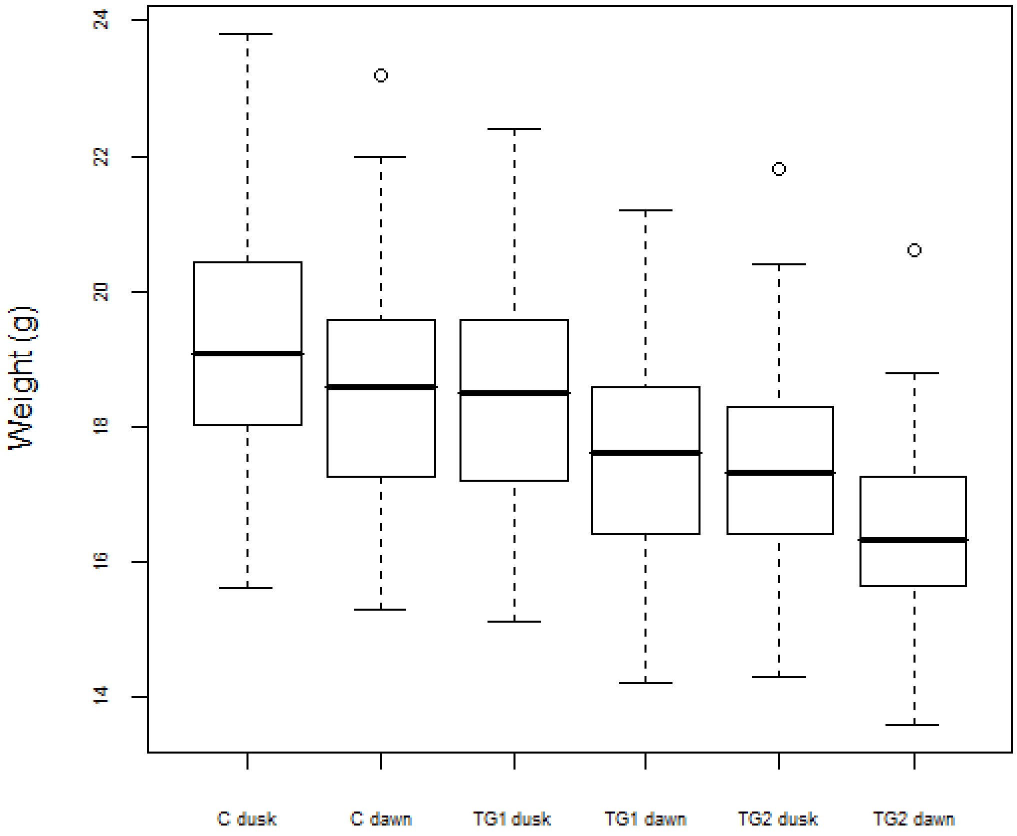

Figure 4.4: Body mass of barn swallows measured at dusk and at dawn in grams for the three groups: Control (C), Test group 1 (TG1) and Test Group 2 (TG2).

The mean body mass loss of control groups was significantly lower than both of the test groups, but mass loss in the two test groups did not differ significantly ($F_{2,320} = 26.83$, $p < 0.0001$; in detail: Control vs Test group 1: t = 4.886, d.f. = 154, $p < 0.0001$, Control vs Test group 2: t = 7.203, d.f. = 239, $p < 0.0001$, Test group 1 vs Test group 2: t = -1.411, d.f. = 247, $p = 0.159$) (table 4.I).

GLM analysis revealed that BMI played a significant role on overnight mass loss, with birds that had larger body reserves losing more mass overnight. Whether the bird was kept in the “closet” or a bag, as well as age, fat and muscle score had no significant influence on overnight mass loss (table 4.II).

Table 4.II: Final model of variables influencing overnight mass loss. Model adjusted $R^2 = 0.2019$, $F_{5, 317} = 17.29$, $p < 0.0001$.

Model Term	**Test statistic F**	**D.F.**	***p***	**Parameter level**	**Coefficient**	**Standard error**
Year	3.1459	1	0.077			
BMI (dusk)	8.6666	1	0.0035			
Trial group	11.4935	2	<0.0001	Control	reference	
				Test group 1	+ 0.2076	0.0602
				Test group 2	+ 0.3904	0.0524

Body temperature

Mean body temperatures ranged from 40.81°C to 41.81°C across the four measurement times (figure 4.5). Based on the values obtained by Prinzinger *et al*. (1991) of body temperature of passerines (rest phase = 36.0-40.8 ºC, active phase = 39.0-44.1 ºC, and high activity phase= 43.1-47.7 ºC) this indicates that birds were active throughout the night.

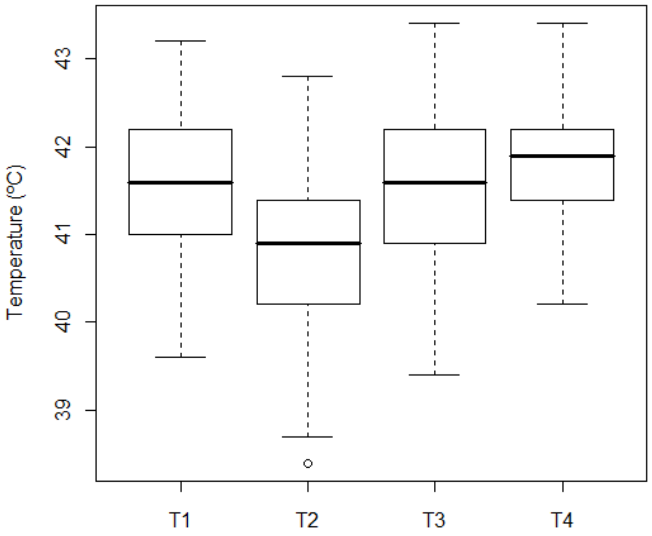

Figure 4.5: Internal cloacal body temperature of barn swallows at four time points: T1 – at start of handling at dusk, T2 – after handling at dusk, T3 – middle of the night (variable according to the time at T2), and T4 – before release at dawn. Mean body temperatures at each time point were: T1= 41.60 ± 0.046 ºC; T2 = 40.81 ± 0.054 ºC; T3 = 41.52 ± 0.065 ºC; and T4 = 41.81 ± 0.04 ºC.

Barn swallows lost temperature between T1 and T2, then progressively increased in temperature from T2 until dawn (T4) ($F_{3,951}$ = 81.59, $p < 0.001$; T1 vs T2: t = -11.658, d.f. = 522, $p < 0.0001$). By dawn (T4), birds had higher body temperatures than at dusk (T1 vs T4: t = 3.124, d.f. = 520, $p = 0.0018$), reflecting the circadian rhythm, in which birds during the day would have a higher body temperature than at night. However, body temperatures were still within the mean interval of temperatures of the active phase of the circadian rhythm.

Heart rate

Heart rate was not significantly different between dusk and dawn ($F_{1,63}$ = 0.2891, $p = 0.5927$) (figure 4.6).

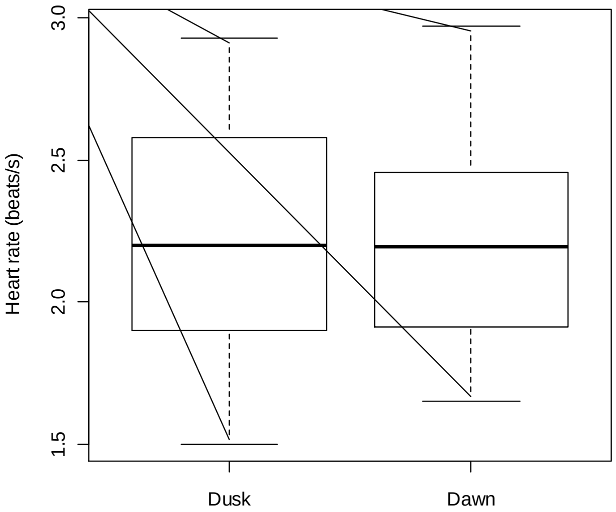

Figure 4.6: Heart rate of barn swallows measured in beats per second at dusk (mean = 2.24 ± 0.064 b/s) and at dawn (mean = 2.19 ± 0.066 b/s) respectively.

Barn swallows that were sampled for heart rate (HR) lost significantly more mass overnight than control birds, but not than test group 1 and 2 ($F_{3,365} = 16.22$, $p < 0.0001$; HR vs Control: t = -3.98, d.f. = 115, $p < 0.0001$; HR vs Test group 1: t = 0.18, d.f. = 127, $p = 0.86$; HR vs Test group 2: t = 1.376, d.f. = 205, $p = 0.17$). It is to be noted, however, that their mean body mass was also very different at dusk: heart-rate sampled birds; mean = 16.92 ± 0.21g and control birds; mean = 19.17 ± 0.20 g respectively. Despite this, BMI was not significantly associated with the heart rate data presented, nor was heart rate associated with overnight mass loss (table 4.III).

Table 4.III: Final model of variables influencing heart rate at dusk. Model adjusted $R^2 = -0.0226$; $F_{2,32} = 0.625$, $p = 0.5417$.

Model Term	**Test statistic F**	**D.F.**	***p***
BMI at dusk	0.1247	1	0.7263
Weight loss	1.1944	1	0.2826

4.4.2 Yellow wagtail's case study

The majority of captures of yellow wagtails in both years corresponded to adults (74.3% in 2011 and 65.6% in 2012). The BMI index was significantly different between years ($F_{1,355} = 8186.2$, $p < 0.0001$), with 2012 birds being in a better body condition than the previous year, as all birds scored muscle ≥ 2, compared to 70% in 2011, and had higher fat accumulations (figure 4.7).

Figure 4.7: Fat accumulation in yellow wagtails on 2011 and 2012

Body mass

The overall mean body mass of yellow wagtails measured in this trial at dusk was 18.22 ± 0.14 g and at dawn 17.19 ± 0.14 g. In more detail:

Table 4.IV: Variation in body mass of yellow wagtails in different treatment groups, at dusk and at dawn (Control = yellow wagtails with no measurements collected; Test Group 1 = yellow wagtails with the temperature measurements before handling, after handling and at release; Test Group 2 = the same as test group 1 with an additional measurement half way between end of handling and release).

Weight (g)	**Control**	**Test group 1**	**Test group 2**	**Blood sample**
Dusk	18.03 ± 0.22	18.58 ± 0.48	17.57 ± 0.33	18.48 ± 0.44
Dawn	17.01 ± 0.22	17.69 ± 0.46	16.51 ± 0.34	17.21 ± 0.43

Overnight mass loss	1.02 ± 0.03	0.89 ± 0.03	1.06 ± 0.05	1.27 ± 0.03

There were no significant differences in body weight at dusk across the groups or years ($F_{4,274} = 1.591$, $p = 0.177$).

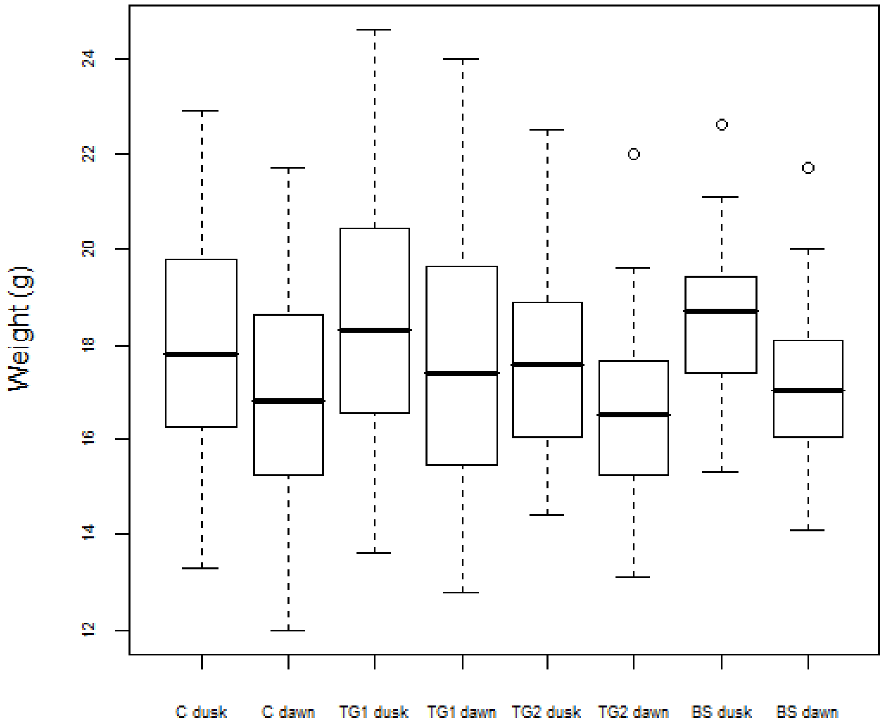

Figure 4.8: Mean body mass of yellow wagtails measured at dusk and at dawn in grams in the four groups: Control (C), Test group 1 (TG1), Test Group 2 (TG2) and Blood sampled (BS).

Birds from all groups lost body mass overnight (One sample t-test, control group: t = 33.108, d.f. = 111, $p < 0.0001$; test group 1: t = 26.526, d.f. = 42, $p < 0.0001$; test group 2: t = 20.982, d.f. = 35, $p < 0.0001$; blood sampled: t = 15.168, d.f. = 18, $p < 0.0001$) (figure 4.8 and table 4.IV).

The blood sampled birds lost significantly more mass compared to the three remaining groups, while control birds lost body mass similarly to test group 2 ($F_{3,204} = 6.2276$, $p = 0.0004$; in detail, Blood Sampled vs Control: t = 2.846, d.f. = 129, $p = 0.0049$; Blood Sampled vs Test group 1: t = -4.046, d.f. =60, $p < 0.0001$, Blood Sampled vs Test group 2: t = -2.200, d.f. = 53, $p = 0.029$) (refer to table 4.IV).

Body temperature

Yellow wagtails had a mean body temperature of 41.34 ± 0.09 ºC, which was significantly higher than that of the barn swallows ($F_{1,338}$ = 7.4017, p = 0.0068), and falls into the Prinziger *et al*. (1991) category of active phase homeothermy. The overnight pattern of temperature variation was similar to the one presented for the barn swallows (figure 4.9).

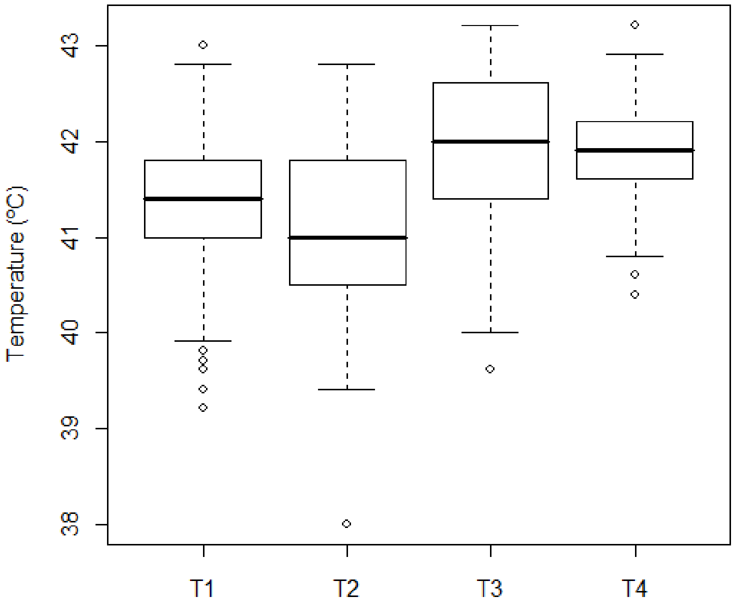

Figure 4.9: Internal cloacal body temperature of yellow wagtails at four time points: T1 – before handling at dusk, T2 – after handling at dusk, T3 – middle of the night (time), and T4 – before release at dawn. Mean body temperatures were: T1 – 41.34 ± 0.088º C, T2 – 41.08 ± 0.10 ºC, T3 – 41.87 ± 0.14 ºC and T4 – 41.92 ± 0.07 ºC.

However, the body temperature at stage T1 was significantly lower than body temperature at both stages T3 and T4, but during handling there was not a significant decrease in body temperature (between time points T1 and T2) (table 4.V).

Table 4.V: Final model of differences in temperature measurements. Model adjusted R^2 = 0.1492, $F_{3,269}$ = 16.9, p < 0.0001.

Model Term	**Test statistic F**	**D.F.**	***p***	**Parameter level**	**coefficient**	**Standard error**
Stage	16.808	3	<0.0001	T1	reference	
				T2	-0.2418	0.1249

				T3	+0.5353	0.1598
				T4	+0.5481	0.1261

The body temperature of yellow wagtails differed significantly between years, being 0.73 °C higher on average in 2012, and was not influenced by the BMI index ($F_{2,76}$ = 20.15, $p < 0.0001$, in detail, body temperature vs. year: t = -4.508, d.f. = 76, $p < 0.0001$, body temperature vs BMI: t = 0.004, d.f. = 76, $p = 0.996$).

Heart rate

Heart rates of yellow wagtails at dusk and dawn (figure 4.10) were not significantly different from one another ($F_{1,125}$ = 0.4757, $p = 0.4917$). However, at dusk yellow wagtails' heart rates were significantly higher than the barn swallows' heart rates ($F_{1,24}$ = 22.723, $p < 0.0001$) (figure 4.6).

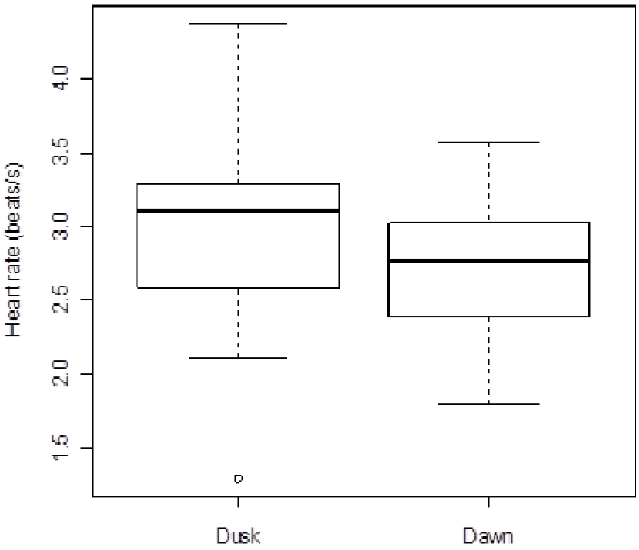

Figure 4.10: Heart rate of yellow wagtail measured in beats per second at dusk (mean = 3.10 ± 0.10 b/s) and at dawn (mean = 3.01 ± 0.10 b/s) respectively.

The mean body mass of yellow wagtails sampled for heart rate was 18.48 ± 0.30 g at dusk and 17.21 ± 0.30 g at dawn and the overnight mass loss of birds whose heart rates were monitored was not significantly different from those of the control group and test group 2 in 2011 ($F_{1,174}$ = 2.5647, $p = 0.056$). Thus heart rate measurement did not influence mass loss ($F_{1,37}$ = 1.3392 $p = 0.255$). Because of these findings, heart rate measurement was repeated in conjunction with temperature measurements in 2012.´

In 2012, heart rates were measured in the same birds as cloacal temperature, in order to assess if birds with a higher heart rate also had substantially higher body temperatures, this was however not supported by the data (Pearson's correlation: r = - 0.083, d.f. = 37, *p* = 0.617).

Unlike barn swallows, the nocturnal heart rates of yellow wagtails showed a significant positive correlation with BMI (both at dusk and dawn) (figure 4.11 a) and b)).

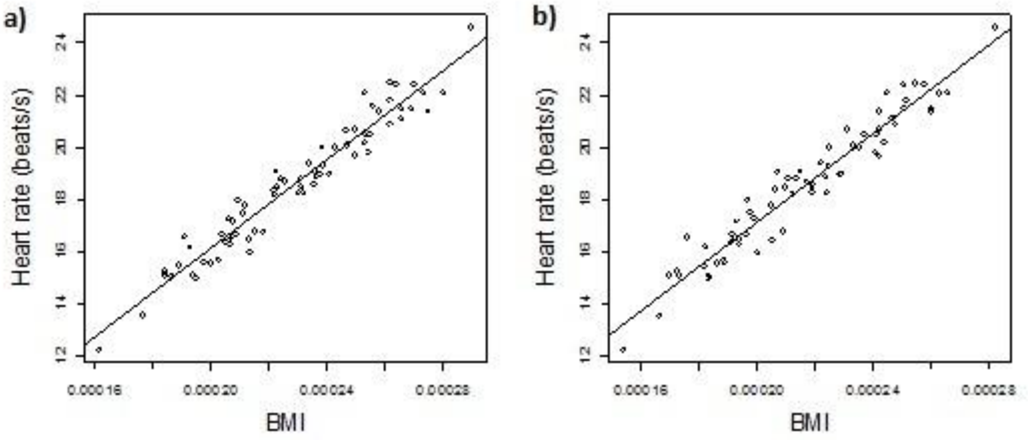

Figure 4.11: The relationship between the BMI and heart rate of yellow wagtails, measured a) at dusk and b) at dawn, demonstrating a positive and significant correlation between the two variables. Pearson's correlation test: a) r = 0.970 (d.f. = 68, *p* < 0.0001) and b) r = 0.972 (d.f. = 67, *p* < 0.0001).

4.5 Discussion

In most bird-ringing contexts, birds are released immediately after ringing and measurement, and thus it is rarely possible to register if any adverse effect occurs in the subsequent hours (but see chapter three). For the purposes of an extended period of monitoring following ringing, the present study of birds roosted in captivity overnight proved to be very useful in measuring the physiological responses to capture stress over a timescale of several hours.

The set up used for this trial was used in order to test how migrant species responded physiologically to capture and handling by a researcher. Prior to carrying out the measurements of body temperature, it was predicted that, like Cabanac & Guillemette's (2001) experiment with eider ducks (*Somateria mollissima*), the birds with calm behaviour would show an increase in body temperature between the first and the second measurement, demonstrating an “emotional fever”, resulting from the fact that they were being handled. However, this was not the case, and both species demonstrated a small decrease in core body temperature. However while barn swallows demonstrated a significant decrease in body temperature of 0.79 ºC during handling, yellow wagtails (0.26 ºC drop) did not. In which case barn swallows could be strategically saving energy for the first opportunity to escape. Alternatively, yellow wagtails were clearly struggling harder to escape, which could explain why their T_b decline was not significant.

Both species demonstrated a significantly higher body temperature in the morning, which could be due to having spent the night in warm roosting conditions, as they were kept indoors, with no shivering thermogenesis (Swanson & King 2013). These increases in temperature started earlier in the evening for wagtails, as compared to barn swallows. It is worth noting that T_b throughout the series of measurements was in the range expected for the birds’ active phase of the circadian rhythm. This is suprising since it would be expected that overnight, the birds should have been resting, thus presenting rest-phase values instead.

Neither species entered torpor (T_b < 20 ºC), as that would have been measurable using the study design. However, test group 2 of the yellow wagtail sample lost more body mass than test group 1, suggesting that the birds in group 2 may have initially decreased their body temperature, and later generated heat metabolically (Fletcher *et al*. 2004) to increase T_b level, and hence the cloacal probe method would not have detected it. In such a scenario, the weight difference in the morning between test groups could be indicative of a higher overnight energetic expenditure.

Furthermore, in barn swallows, birds sampled for temperature lost significantly more weight than control groups, which could be confirmation of the method’s intrusiveness, but could also be due to the fact that birds often defecated after removing the cloacal probe, clearing the gut of undigested food which could than translate into a smaller body weight measurement as birds were sampled for body temperature before being weighed. Nevertheless, such defecation potentially represents loss of gut contents from which energy, nutrients and water could still have been absorbed.

In these trials birds were already under the stress of capture (Angelier *et al*. 2010), thus such observations can highlight yet another point, related to the validity of data using monitoring devices that require the recurrent handling of birds.

Although it is arguable, that if individuals are treated in the same way, then their responses might be comparable, if they are taken within the same species, because as demonstrated in this study and other studies, species may respond differently (Rands *et al*. 2006). Alternatively remote-monitoring methods may be used for monitoring body temperature (e.g. Fiedler 2009).

Also the keeping devices of both species were of different dimensions, i.e. while swallows were kept in confined spaces, and did not have the chance to move much, yellow wagtails, on the other hand were kept inside tall cardboard boxes in which they were constantly moving and “jumping”, after they were put inside (at least during the presence of the researcher inside the room). Although this methodology was selected to suit the biology of each species, it could have influenced the measurements taken, as the remaining birds were disturbed everytime I had to remove a bird for measurement (although each trial group were always kept together and thus only disturbed when necessary and collectively).

Therefore, in the present study, as far as the methodology used allowed impacts to be measured, neither capture nor handling induced subsequent hypothermic states, nor did handling trigger any type of emotional fever, as body temperature tended to drop slightly, but significantly, during handling. Failing to resort to hypothermia could indeed mean that the birds interpreted capture and handling as a continuous threat (Laurila & Hohtola 2005), but it could be as well due to the fact that measurements were intrusive and thus birds were disturbed each time a measurement was taken.

The majority of groups sampled lost mass significantly, except for an odd case of test group 1 in yellow wagtails (in comparison to the control group). The biological implications of this mass loss will remain unknown, as this study was not designed to investigate further stressors other than capture and handling alone.

A good part of the work looking at overnight mass losses in passerines has focused on losses during winter conditions, with longer, colder nights (e.g. Pravosudov & Lucas 2000). According to Dawson *et al*. (1983) the physiological demands of migration and winter acclimatization are not too different. Haftorn (1992) estimated that a small wintering bird of 20g would consume approximately 10% of its dawn body weight overnight. In another study, again with acclimatization to winter conditions, it was found that great tits could survive even losing an average of 12.78% of their evening body mass. In a rare study on autumn migration, Eurasian skylarks (*Alauda arvensis*), a bird of bulkier weight (28g-39g) than barn swallow and yellow wagtail, lost around 7% of their dusk weight overnight (Hegemann *et al*. 2012). Compared to these previous studies, the barn swallow groups in the current study lost: 3.62% (control), 5.40% (Test group 1) and 6.24% (Test group 2) of their body weight, and the yellow wagtails lost: 5.98%, 4.80%, 6.0% and 6.87%, less than typical winter losses and often less than observed among skylarks. Although there are important differences among the studies in time of year, migration length (skylarks carry out shorter migrations than swallows and wagtails) and body mass (swallows and wagtails are normally around half a skylark's weight), these results suggest that the observed body mass decreases following ringing are within the naturally observed range and so the impact may be limited.

Heart rate was similar at dusk and dawn in barn swallows, which would be consistent with an alert state for the first escape opportunity, although the swallows demonstrated tonic immobility. In yellow wagtails, dawn heart rate was slower than at dusk. This could be due to the fact that as yellow wagtails' period without food increased, the birds decreased their heart rate (see Odum, 1941 for similar case examples). These differences in dusk heart rate between the two species may be due to the type of coping behaviour expressed by the two species (Carere & van Oers 2004).

The results of this study may be complicated by an interaction between the different behavioural responses in terms of T_b and heart rate shown by the two species, and the way in which they were held overnight. Swallows were kept in confined spaces, and were not able to move much, whilst yellow wagtails, on the other hand were kept inside tall cardboard boxes in which they were constantly moving, "jumping", and physically active, after they were put inside while I was in the room (I can make no assumption of whether they settled down later). Odum (1941), points out that the amount of feeding prior to capture, the amount of body reserve energy (translated in terms of body mass), and the amount of activity during the first hours of starvation could affect the time taken for the birds to decrease their heart rate. Even though barn swallows sampled for heart rate had the leanest body mass at dusk of 16.50 ± 1.37 g, which was significantly lower than the remaining groups, their dawn heart rate did not reveal any sign of energetic stress (in terms of a decrease in heart rate). Also there was no correlation between the body condition of barn swallows and their heart rate. This contrasts with yellow wagtails, in which body mass at dusk predicted their heart rate, i.e. the higher the body mass, the higher the heart rate.

It also proved more challenging to measure heart rate in barn swallows than in yellow wagtails. While yellow wagtails were more consistent in their heart rate, barn swallows presented short-term fluctuations, such as a series of small variations alternating with larger ones, in which several small "peaks" of heart beats would clump together, whilst there would be periods in which heart beats would be very discernible, and yet others which would be chaotic. According to Odum (1941) this is characteristic of small birds, and might be another cause of unexplained variability in the data, as only discernible beats were counted. Anrep *et al*. (1936) explain this change as a probable "result of reflexes from the lungs acting by way of the respiratory and cardiac centers as well as possibly the "Bainbridge reflex, concurrent with the filling of the right auricle". This effect was indeed noted in a large proportion of the birds sampled. For this reason it is not possible to make definite conclusions regarding heart rate responses in barn swallows.

Two further trials were also carried out: a) comparing keeping devices and b) testing the intrusiveness of blood sampling protocols. Even though cotton bird bags are widely used, in some species, especially in waders and other waterfowl, bird bags are not preferred (Henschel & Louw 1978). This trial was carried out in order to investigate whether the presence of conspecifics could alter the stress response of the individuals, and whether there was any benefit to the bird by its presence, in matters of core temperature regulation ("roosting effect" of shared body heat). However whether the bird was kept in the "closet" or a bag had no influence on T_b. This could be an indicator that roosting, more specifically, the presence of nearby conspecifics, had no influence on the maintenance of temperature or how the birds coped with capture and handling. This result is consistent with the one found by Walsberg (1986), for the phainopepla (*Phainopepla nitens*) (a north-American species of silky flycatcher) during winter, in which thermal air heated by a proximal bird had no thermal benefit to the individual.

Collecting blood samples is very feasible in the field and provides a reliable source of results for numerous fields of work, e.g. endocrinology, parasitology, metabolism, as a source of DNA for population genetics, the study of stable isotopes or even to track infectious diseases; thus it is used widely in ornithological research (Voss *et al.* 2010). Even though there are other alternatives of less intrusive sample collection, such as collection of feces, vomit, feather or nail clips, blood sampling is still preferred by many authors (Hoysak & Weatherhead 1991). There are many studies dedicated to study the impact of blood sampling on reproductive success, behaviour, foraging habits and return rates across taxa (e.g. Angelier *et al*. 2010, Criscuolo 2001, Hoysak & Weatherhead 1991, but for a thorough review see Sheldon *et al*. 2008), and many claim there are no “serious” effects. As the present study of birds held in roost boxes following capture made it possible to keep wild birds for an extended period of time, without much interference in their circadian rhythm, it provided a good opportunity to study how migrant birds reacted energetically to blood sampling. In this particular study, it was possible to measure short term effects on their body mass regulation, demonstrating that there is an immediate effect on the bird’s energetic state. This could be deleterious if the bird has to increase its exposure to predators in order to recoup the additional expended energy (Gosler 2001). However many authors claim even though there can be immediate effects, in the longer term, blood sampling should not have major effects (Sheldon *et al*. 2008), and birds are fairly resilient to blood losses, as they do not exhibit symptoms of shock (Sturkie 1986 *in* Hoysak & Weatherhead 1991). Because this present study has only aimed at measuring short term effects, a condition inherent of working with this type of measurement in wild birds, it is not possible to make assumptions as to whether blood sampling can handicap birds in the longer term. It has, however, revealed the existence of an immediate effect on overnight body mass loss.

Both body temperature and heart rate have been described in the literature (e.g. Cabanac & Guillemette 2001, Cyr *et al*. 2008) as physiological measurements of stress. It was expected at the beginning of this study that both species would cope differently with the acute stress episode triggered by capture and handling. However their responses were similar: a) neither species either resorted to hypothermia or displayed an “emotional fever” with consequently elevated body temperature; b) both lost body mass significantly and accordingly to the intrusiveness of the measurements carried out, except for test group 1 in yellow wagtails, for which there was no measurable physiological explanation; c) a decrease in heart rate between dusk and dawn, consistent with the overnight consumption of energetic reserves in yellow wagtails – a similar effect cannot be ruled out in barn swallows as it could be obscured by fluctuations in the rhythm of heart rate. These findings may suggest that different coping strategies (i.e. tonic immobility in barn swallows and agitation in yellow wagtails) can result in similar physiological responses to capture and handling (depending on the parameters assessed, e.g. Carere *et al*. 2001), although barn swallows seemed to be more susceptible to the impacts of additional measurements (i.e. heart rate and temperature as translated by overnight mass loss) than yellow wagtails.

Chapter 5

Interruption to the incubation routines of nesting passerines

5.1 Abstract

The breeding season is energetically demanding, especially for female passerines, with the incubation of eggs and brooding or feeding of nestlings occupying most of the daylight hours. Bird ringing activities in the vicinity of a nest can result in the capture of a breeding female during an off-bout of incubation. This may cause the nest to be exposed for a more extended period of time than usual to predators, as well as chilling for variable periods of time. To investigate the outcomes of the interruption caused by bird ringing, nests of several species were remotely monitored to record the length of incubation periods and within-nest temperature, across a period of 3 days, in which birds were experimentally captured on day 2. This set-up allowed us to quantify nest attendance disruption, promptness in resuming incubation, and exposure to chilling episodes. On the capture day, birds spent less time incubating, and attended the nest less frequently than on control (pre-capture) days. The time elapsed between release and returning to the nest was highly variable (14 min – 5 h), during which 11 of the 15 sampled nests were exposed to temperatures within 3 ºC of ambient temperature. The effects of capture and handling were only measurable on the day of capture, with an evident return to the normal frequency and incubation effort on the following day. There was no evidence of nest success having been compromised as a result of capture and handling, and effects of chilling episodes on eggs are further discussed.

5.2 Introduction

There is a considerable amount of literature that focuses on the effects that human disturbance has on birds (e.g. Nichols *et al*. 1984, Götmark 1992, Beale & Monaghan 2004), especially assessing the impacts of recreational activities, such as for example, off-road vehicles (ORVs) (e.g. Carney & Sydeman 1999, Smith-Castro & Rodewald 2010). In contrast, studies addressing the impacts that research and monitoring activities have on birds are relatively scarce. Amongst these, a few studies have considered the impacts of bird ringing (e.g. Kania 1992, Olsen & Schmidt 2001, Jennings *et al*. 2009, Spotswood *et al*. 2012) which is of interest given the large number of birds caught and ringed every year. Some of these studies measure impacts at the nest (e.g. clutch survival), behaviour and fitness, and/or compare effects of different capture, handling and marking techniques (Jennings *et al*. 2009, Kania 1992, Lendvai & Chastel 2010).

There are three key studies on the impacts of capture and handling on breeding success, focusing on nest desertion and egg hatching success. The first was carried out by Kania (1992), who reviewed the impacts of capturing adults at the nest in a set of 135 European species (data supplied by 250 ringers across 10 ringing schemes). In this study the author provides information on the probability of nest desertion for detailed stages of the breeding season, i.e.: laying, incubation (1st and 2nd half separately, and then globally), hatching, with nestlings (early, middle late), for each species (when possible). Although this study was published over 20 years ago, it serves as a key reference for investigators, and as a result, capture methodologies have improved since its publication. The second study was carried out by Olsen & Schmidt (2001), and complemented Kania’s review, as it investigated the impacts of capturing hooded crows (*Corvus cornix*) at the nest, a species absent from Kania (1992). The key importance of this study was that it demonstrated that some birds abandoned breeding even in nests where capture failed. The third study was carried out by Jennings *et al*. (2009), looking at the impact of constant-effort mist netting on the breeding success of two species, demonstrating that there is no evidence that mist netting had an effect on their reproductive performance. This is of great importance considering the hundreds of constant-effort ringing sites worldwide and their role in estimating productivity and calculating indexes of reproductive success (Jennings *et al*. 2009).

In chapter one I discussed the trade-offs that birds face during the breeding season, by referring to the “brood value hypothesis”, in which their stress response is modulated according to the value of current reproduction towards the individuals’ survival, also refered to as “cost of reproduction” (Rands *et al*. 2006 and references within). In the context of breeding success, such trade-offs could relate to the parents’ fitness or coping behaviour, by inducing changes in incubation behaviour (in the absence of nest desertion), which is an aspect that has been overlooked in the previous studies mentioned. For example, if a parent bird is kept away from the nest longer than usual by a human (ringer) visiting the nest, the eggs could be chilled, requiring considerable metabolic heat expenditure from the parent to return the nest's temperature back to the optimum level for embryo development (Webb 1987, Spellerberg 1969). Furthermore, there may be sub-lethal effects of these chilling periods on the embryos themselves, including impaired metabolism, growth and development of the embryo, potentially handicapping their overall fitness (Webb 1987, Astheimer 1991, Yalcin & Siegel 2003).

During the breeding season, the elevation of glucocorticoids in the blood will mediate the life-history trade-off between the current value of reproduction relative to the value of future reproduction and survival. If the benefit of current reproduction is higher, then birds will display a mitigated stress response that gives priority to breeding and maximum fitness (Bókony *et al*. 2009). The factors that will influence this trade-off are dependent on the species, bias in parental effort, predictability and availability of suitable habitat and breeding stage (birds at the beginning of a breeding attempt are more likely to desert the nest than birds at a more advanced stage of breeding, Burtt & Tuttle 1983).

In the present chapter, I consider the impacts of routine capture and handling of free-living breeding songbirds upon their subsequent attendance and incubation behaviour at the nest. Generally, I test the hypothesis that capture and handling will lead to an increased time away from the nest, compare the "before" and "after" captivity incubation behaviour, and how it will affect within nest-temperatures. To do so, I utilize two datasets: the first (comprising an analysis of unpublished data collected by RJ Thomas) includes data from five resident species of songbirds nesting both in open and cavity nests, and the second includes data from one migratory species breeding colonially in cavity nests.

The analyses presented in this chapter will thus focus on testing whether capture and routine handling of free-living breeding passerines will influence their subsequent nest attendance and incubation behaviour, and whether such impacts may differ between species.

5.3 Methods

The data utilized for this chapter were collected in two different geographical regions: the United Kingdom and Portugal. In both experiments, standard mist netting and ringing methods for the target species were used (Redfern & Clark 2001) as part of routine licensed ringing under the national ringing schemes of the UK and Portugal. The time required for each step of the ringing process (extraction, transport, ringing and processing), was recorded to the nearest minute. The total incubation duration, was calculated in the same way for both parts of the study. All nests were monitored for a standard period of time (3 days) to allow for standardised data comparisons.

Data set 1

In the United Kingdom, data were collected in the spring of 2004 and 2005 at three sites located near Cardiff consisting of deciduous woodland and nearby hedgerow areas. All of the hole-nesting birds (great tits *Parus major* n = 6 nests, and blue tits *Cyanistes caeruleus* n = 6 nests) were nesting in wooden nestboxes on tree trunks. Nests of open-nesting birds including; common blackbirds *Turdus merula* (n = 4 nests), song thrushes *Turdus philomelos* (n = 2 nests) and European robins *Erithacus rubecula* (n = 2 nests), were found by a combination of searching in suitable nest sites and by following nest-building females.

In each trial, two nests containing completed clutches of the same species, which were matched as closely as possible in terms of clutch size and laying dates, were monitored. Each trial served to sample a control nest and a treatment nest. On day 2, the females of treatment nests were caught in mist-nets (totaling 18-48 m) situated at least 15 m away from their nest and handled as they would normally be in a routine ringing session. After processing the bird was released at the point of capture.

The nests were remotely monitored for within-nest and ambient temperatures over three consecutive days, with temperature data loggers (Hanna Instruments HI141JH). Each temperature data logger consisted of a small (8 cm x 8 cm x 4 cm) data-logging unit, connected to two external temperature probes via 1m cables. To record within-nest temperatures, one probe was placed inside the nest by pushing the 3.5 cm long metal probe through the side of the nest such that the sensor was positioned immediately below the clutch of eggs. To record ambient temperatures the second probe was placed within 10 cm of the nest, shaded from direct sunlight (amongst vegetation). The temperature data loggers were set up at each nest at least 12 hours before the start of a 3-day monitoring period. Because nest temperature is considered a reliable measure of incubation effort (Eikenaar *et al*. 2003) the comparison between the observed within-nest and ambient temperatures allows the patterns of incubation to be measured. The time at which incubation started and stopped was defined as the start of a rapid increase or decrease in nest temperatures respectively, that could not be accounted for by equivalent changes in ambient temperature.

This dataset provides details on the incubation behaviour of five species. In all five species, the incubation effort is carried out solely by the female, and, according to Kania (1992), there are also differences between the species in the risks of nest desertion during incubation (e.g. blackbirds are more prone to nest desertion, and song thrushes are less prone).

In 2004, nest contents were also further checked at 5-7 day intervals following the 3-day trials, to record the numbers of eggs and/or young and incidences of predation of the eggs or unfledged chicks.

Data set 2

A sand martin (*Riparia riparia*) colony was studied in the western region of Portugal (39º30’93.65N, -9º21’60.09W), during the spring of 2013. The western region has typical farming areas where lands are moved seasonally for the production of vegetables, and there are small dams as well as river courses with suitable nesting habitats. As the first records of sand martins occupying territories date to early March, during the course of this month, the area was prospected by car to find suitable sampling areas.

The sand martin is a long-distance migrant, which breeds colonially, preferring to nest in freshly formed river banks or vertical walls of sand or sandy land near lakes or water sources, where insects are abundant and substrate is suitable for tunneling (Szabó & Szép 2009). In this species, the male develops a small brood patch, and even though the male helps in incubating, the females still do most of the incubation (Cramps *et al*. 1994). Perhaps due to the nature of the nesting habitat, Kania (1992) has found that the risk of nest desertion by sand martins fell in the category of “< 2%”, with no reported examples of desertion at all in the data she reviewed during incubation.

To monitor nest attendance, digital video cameras were used to film the colonies. The experimental design was very similar to the one presented in chapter three and consisted of monitoring the colony throughout a course of 3 days, in which day 1 served as a control, day 2 was the capture day, and day 3 the response day. This protocol revealed: a) how many times and for how long the parents were absent from the nest following a capture event, and b) circadian patterns of nest attendance before, during and after capture.

Capture was carried out by mist netting at the entrance of the nest, and the nets were set up quietly before sunrise, with minimal disturbance, following the suggestions in Kania (1992). The colony was composed of 14 nest holes, of which only 8 were occupied. From these, I captured both parents birds in 4 nests (treatment), and the remaining 4 served as controls (with no bird captured). Of the remaining 6 holes, 3 were empty and 3 still had males singing to attract a female. The length of the mist net erected was 12 m. Birds were extracted from the net, ringed and released, as they normally would be in a regular ringing session, in the vicinity of the colony. It was not possible to assess hatching success as the land was subsequently ploughed and thus the nests were destroyed.

Because sand martin males develop a small brood patch, it was not possible to distinguish between male and female. Therefore, even when only a single parent was inside the nest I have considered it to be “incubation time”.

Data analysis

Data were analyzed with the statistical software R 2.12.0 (R Core Development Team 2009). Box plots were performed to allow for a visual comparison between: time duration of absences in the nest; total incubation time per day, and per “before capture” and “after capture” times; number of visits and minutes per visit to the nest across the 3 days. All the box plots created show the median (dark line), and the 25th and 75th percentiles (lower and higher ends of box respectively). The ends of the whiskers showed 1.5 times the interquartile range (when outlier circles present) or the furthest value from the median (when no outliers present). Outlier circles represented values outside 1.5 times the interquartile range. References to the mean values of variables include reference to the standard error of the mean, to provide an idea of the accuracy of the mean.

To investigate the effect of capture and handling on the incubation behaviour of birds a set of Generalized Linear Mixed Models (GLMMs) were performed to assess: a) the effect on total incubation duration using “day”, “species”, “before capture” and “after capture” as fixed effect covariates, in four separate GLMM’s; b) the variation on incubation bouts across days (fixed effect covariate is “day”); and c) the variation in incubation duration per bout across days (fixed effect covariate is “day”). In all GLMM’s individual birds were included as a random factor.

5.4 Results

5.4.1 Effects of capture and handling on the incubation effort of birds

Capture procedures were different in the UK and in Portugal, which was reflected in the total time the bird was kept away from its daily routine. In Portugal, a 12m net was set up immediately in front of the sand martin colony, thus the birds could not be removed immediately after they were seen entering the net. As a result, the birds were held captive in the net for between 22 min and 47 min (mean = 39.75 ± 5.9 min) with additional handling time. In contrast, in the UK birds were either extracted from the net as soon as they were seen entering it, or remained in the net for a period of time, of no longer than 20 min (net round frequency).

The majority of birds did not return to the nest immediately after they were released. The time elapsed between their release and return to incubation varied greatly, ranging from 14 min to 5 h 19 min (mean = 103.7 ± 20.43 min) (figure 5.1). Similarly, there was a wide range of times that birds had been away from the nest prior to capture (range = 0-89 min, mean = 14.0 ± 7.26 min), extending the overall period of absence from the nest. In the extreme case, one great tit was seen to hit the net without getting caught, 22 min after having left the nest, but was not captured until it had hit the net a second time, after 67 min (a total of 89 min after having left the nest). It could have been the case that the brief event in which the bird initially hit the net had put the female off of returning immediately to incubation. Overall, the mean time for all the species to be away from the nest between visits on control days was 18.3 ± 3.49 min, compared to absence periods of 47-333 min on day 2 (mean = 68.04 ± 28.03 min), whilst ringing procedures ranged from 14 min to 47 min (mean = 25 ± 2.42 min).

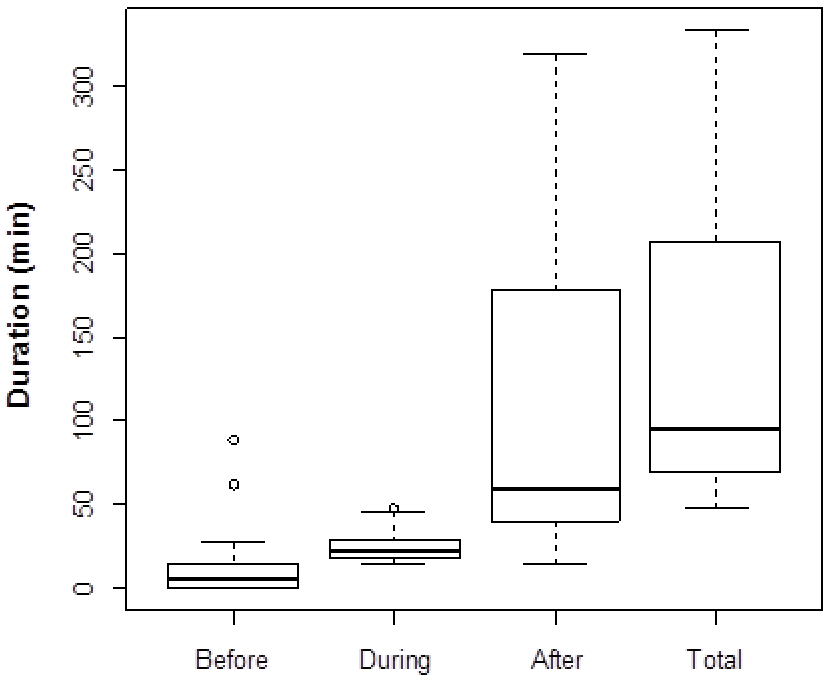

Figure 5.1: Durations of components of the total time absent from the nest resulting from capture and handling. 1) Before = between leaving the nest until capture (mean = 14.58 ± 5.43 min), 2) During = from the moment of capture until release (mean = 25.0 ± 2.42 min), 3) After = from release until returning to the nest (mean = 103.7 ± 20.43 min), and 4) Total = the total time spent away from the nest during capture and handling, including time before capture and after release (i.e. the sum of stages 1, 2 and 3) (mean = 143.3 ± 21.9 min). The data presented here is taken from the nests for which bird ringing procedures were timed (n = 12 nests).

There was significant variation in total daily incubation duration, across the 3-day protocol (figure 5.2, Likelihood ratio = 8.535, d.f. = 2, p = 0.014). Specifically, it appears clear that capture and handling interfered with the incubation routine as the total incubation duration was significantly lower on day 2 (capture day) than on either day 1 or day 3 (pairwise contrasts in GLMM; day 1 vs. day 2, t = 2.052, d.f. = 34, p = 0.048, day 2 vs. day 3, t = 2.940, d.f. = 34, p = 0.006). There was, however, no significant difference in total incubation duration between day 1 and day 3 (t = 0.888, d.f. = 34, p = 0.381), indicating that incubation behaviour had returned to control levels by day 3.

There was no evidence of a “species effect” in the response to capture; specifically, there was no significant interaction between species and day in the GLMM of incubation duration (Likelihood ratio = 8.567, d.f. = 10, p = 0.574), suggesting that different species reacted to capture in a similar way. The failure to detect such a species difference could however be due to the relatively small sample size analysed for each species.

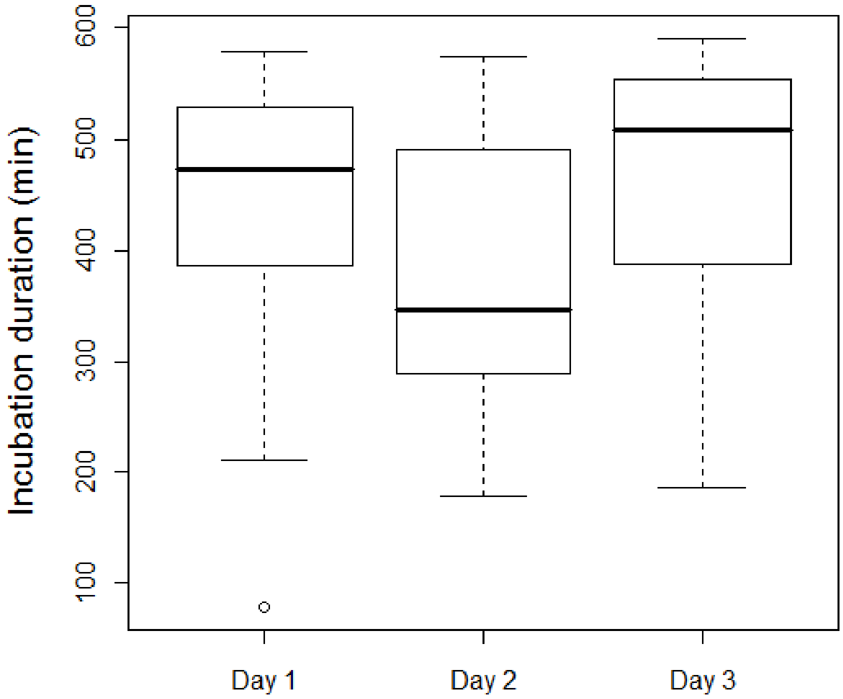

Figure 5.2: Total time the parents spent incubating during the course of day 1 (mean = 434.7 ± 30.05 min), 2 (mean = 368.4 ± 28.03 min) and 3 (mean = 460.8 ± 27.86 min) of the experiment. The boxplot includes nest data from: 4 sand martins, 2 common blackbirds, 5 great tits, 1 European robin, 1 song thrush and 6 blue tits.

This difference in incubation behaviour between days was evident only during the period after the time of capture on day 2. Specifically, the amount of time spent incubating after the time of capture on day 2 was significantly different between days (figure 5.2; Likelihood ratio = 9.5982, d.f. = 2, *p* = 0.0082). In contrast, there was no significant difference between days in incubation during the period before the time of capture on day 2 (Likelihood ratio = 0.543, d.f. = 2, *p*= 0.762) (figure 5.3a).

In both countries the mean time incurred from the bird entering (or simply being "found") in the net was 25 ± 2.42 min (figure 5.1). Based on figure 5.2, on average, the reduction in incubation time (on average, 66.3 min) was much larger than the total time of capture and handling.

Incubation during the period after the time of capture was significantly lower on day 2 than on day 1 (pairwise contrasts in GLMM; day 1 vs. day 2, t = 2.175, d.f. = 34, *p* = 0.037) and was also lower on day 2 than on day 3 (t = 3.147, d.f = 34, *p* = 0.0034). There was no significant difference in incubation during this period after the time of capture, between day 1 and day 3 (t = 0.972, d.f. = 34, *p* = 0.338), again indicating that incubation had returned to control levels by day 3 (figure 5.3b).

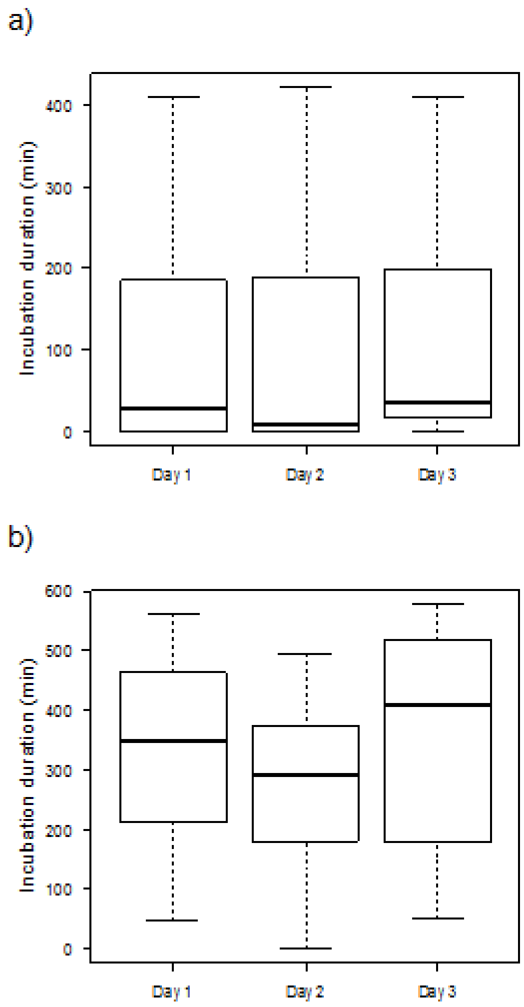

Figure 5.3: Duration of incubation on each day of the study, using each individual's capture time on day 2 as a threshold to separate a) the before capture time and b) the after capture time across the three days. In detail, the incubation duration was calculated as; a) from dawn, to the time on day 2 when the bird was captured, and b) from the time when the bird was captured on day 2, to dusk.

There was significant variation in the number of incubation bouts completed across the three days of the experiment (figure 5.4; Likelihood ratio = 18.03, d.f. = 2, *p* = 0.0001). In agreement with the above results regarding incubation durations, on day 2 birds completed significantly fewer incubation bouts than on day 1 (t = 4.152, d.f. = 34, *p* = 0.0002) or day 3 (t = 3.986, d.f. = 34, *p* = 0.0003) of the experiment. Even though it appears that there is a slight decrease on the mean number of visits on day 3, as compared to day 1, this apparent difference is non-significant (t = -0.166, d.f. = 43, *p* = 0.869).

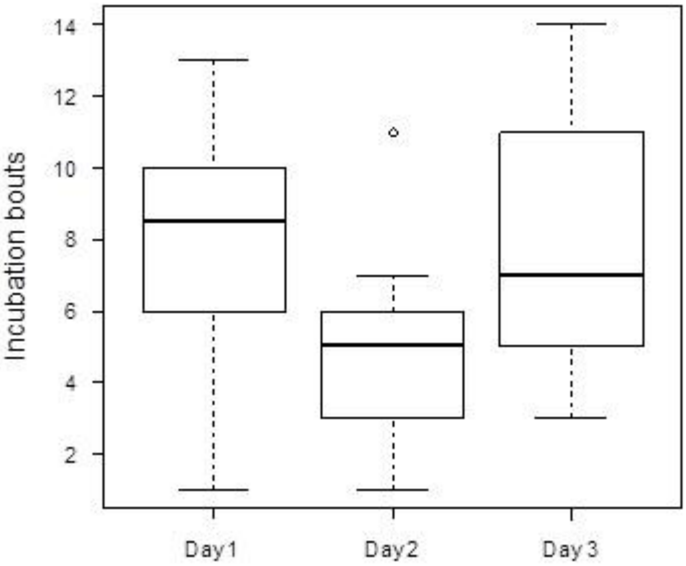

Figure 5.4: Number of incubation bouts (i.e. visits to the nest for incubation) per day, on days 1, 2 and 3 of the experiment.

However, and despite the decrease in the number of “visits” on day 2, the mean incubation time per visit did not vary significantly amongst the 3 days (figure 5.5, GLMM; Likelihood ratio = 5.410, d.f. = 2, *p*= 0.067), and in the majority of cases the decrease in total daily incubation duration on day 2 was explained by the extra time spent outside the nest following capture.

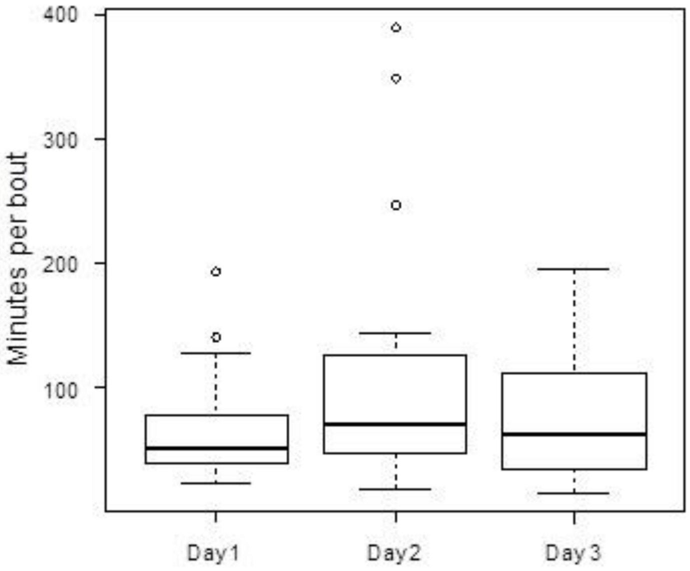

Figure 5.5: Duration of incubation bout, on days 1, 2 and 3 of the experiment. Data plotted are the mean duration (minutes per bout) for each bird on each day.

5.4.2 Within-nest temperature modification

In the nests that were monitored for temperature, it was possible to demonstrate that capturing the females on day 2 coincided with a greater decline of within-nest temperature, than during undisturbed absence on days 1 and 3. In 11 of the 14 treatment nests in 2004, this decline fell to within 3 ºC of ambient levels for a period of time that ranged from 17-277 min (mean = 130.7 ± 27.3 min, n = 14 nests), on day 2. On both day 1 and day 3, periods of chilling within 3 ºC of ambient levels were rare, and occurred only in two nests: the first from the same blue tits mentioned, during day 1 for a total period of 49 min at dawn, and the second nest belonging to great tits, for a period of a further 192 min in day 2 and 137 min in day 3, both bouts at dusk. In figure 5.6 it is visible how capture and handling induces higher absences of the nest, disrupting the normal pattern of incubation, specifically the bird was absent for a period of 65 min, and the nest was exposed to temperatures within 3 ºC of ambient temperature during 22 min.

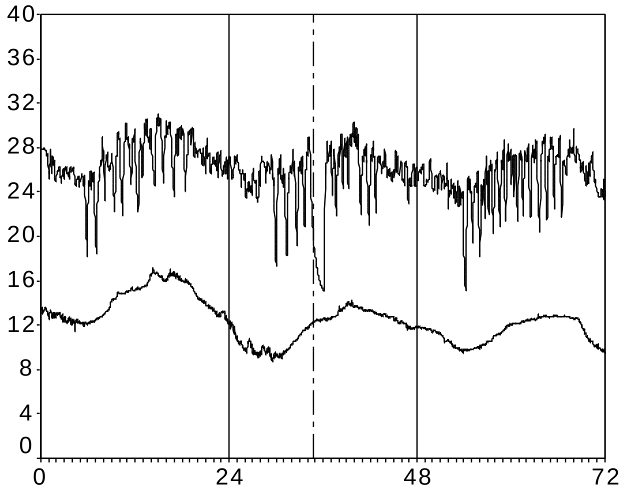

Figure 5.6: Within nest temperature variation for a great tit in a nestbox. The vertical dotted line represents the capture moment, and the bottom line, ambient temperatures across the three days.

5.5 Discussion

Breeding success is central in determining the demography and survival of a population (Fink *et al*. 2010). It depends on many factors, including the successful hatching of eggs, and factors that contribute to the correct development of nestlings. This experiment was designed to assess the effects that capture and handling had on the incubation behaviour of nesting birds, since it not only influences the present generation (the parent birds) but also the future generation (eggs and chicks), and thus the overall demography of bird populations. Demographic monitoring is a major goal of bird ringing (Baillie 2001) and rests on the assumption that the capture and ringing process itself has a negligible impact on demography (Spotswood *et al*. 2012).

In this experiment, capture and handling resulted in a significantly lower incubation time in the nest, consistent with my first hypothesis. For many captured individuals, it clearly was not a priority for the parents to go back to the nest immediately following release (figure 5.4). However, there was considerable variation among individuals, even within a species (e.g. great tits); some individuals resumed incubation rapidly (in two cases, as little as 14min and 17min following release) whereas other individuals took several hours to resume incubation. Periods of incubation before and after capture and handling were not significantly different, suggesting that most birds did not compensate for the extra time they were outside the nest. Because the incubation period after capture and handling was only significantly different between day 1 and day 2 (and not between day 1 and day 3), it is possible to suggest that the birds did not recover immediately from the experience of capture and handling sufficiently to resume normal incubation. However on the following days birds have apparently 'recovered' as incubation time returned to the levels of control day, indicating that capture and handling effects did not last longer than a few minutes or hours (typical duration of an acute stress episode). In quantities, 'recovery time' (i.e. resumption of incubation behaviour similar to that seen prior to capture) was very variable ranging from only a few minutes to a few hours (5 h 19 min was the maximum incubation break registered). Despite the difference in coping behaviour of females of different species, and different priorities (nest success vs individual survival according to the "harm-to-offspring-hypothesis", see below), I did not find any significant species difference in incubation interruption that clearly indicated a different reaction to capture and handling. Future research in this subject should aim to obtain larger sample sizes, matching the laying dates as closely as possible between treatment and control nests.

Capture and handling caused the parents to spend less time incubating than they normally would (figure 5.3), which could partially be explained by the fact that the regular duration of absences is very similar to that of capture and handling (mean = 24 min routine incubation breaks, as compared to 25 min mean duration of capture and handling), adding up to their total absence on the capture moment, if a bird is not to return to the nest immediately. These findings counter a widespread (yet anecdotal) view that adult birds ringed during the breeding season generally quickly return to incubating their eggs or feeding their chicks once they are released.

Nest predation is one of the most important variables affecting nest success (Ricklefs 1969). If the bird is to give preference to the reproductive effort, it is expected to return as soon as possible to the nest, either to promote nest defence (e.g. physical attack or passive scolding) or to conceal the eggs and minimise this possibility (Temple 1989, King *et al*. 1999). However, in the majority of the nests in this study this was not the case, giving preference to the parents' own survival. In reference to encounters with a predator, Dale *et al*. (1996 *in* Listøen *et al*. 2000) suggested the "harm-to-offspring hypothesis", which states that the parents will adjust their behaviour according to the harm the offspring will suffer from the absence of parental care and thus will either resume incubation as quickly as possible or give preference to individual survival, and first recoup from the acute stress episode. Even though predation was later registered (after the sampling) for a robin and a blackbird nest, they both reacted differently when returning to the nest, as the robin took considerably less time to do so (14 min after release, totalling 47 min away) as compared to the blackbird (126 min after release, totalling 141 min away). It could be that these species present different sensitivities to disturbance during nesting time. Because my sample size was limited it was not possible to discern whether factors mentioned previously, such as: type of species, bias in parental effort, predictability and availability of suitable habitat and breeding stage, had any effects on the birds' decision about when to return to the nest.

In the absence of predation or brood parasitism (e.g. by cuckoos *Cuculus canorus*), chilling of otherwise viable eggs could be the main detrimental effect of adult birds staying away from the nest for prolonged periods during the incubation period (Olson *et al*. 2006). In most avian embryo species, periodic cooling occurs when incubating females leave the nest to forage (Olson *et al*. 2006). In this particular experiment it is clear that on day 2, the periods of absence were increased as both a direct and indirect (e.g. coping with stress) result of capture and handling. The nest temperatures within treatment nests fell below the range of 25 ºC and 33 ºC, which are normal values, as this was the temperature measured within-nest, which is not the same as embryo temperature or even egg temperature (e.g. Haftorn 1988). In the present study, 11 of the treatment nests monitored for temperature had a dramatic decline of temperature which fell to within 3 ºC of ambient levels through a period of time that lasted up to 4 h 40 min. The biological significance of this is uncertain at this stage. There was no measurable effect of these chilling episodes on hatching success (and therefore no mortality of embryos) in the current study, but with only 11 nests, the power to detect such effects was limited. It is to be noted, however that a pair of sand martins abandoned the nest on day 3, despite the fact that after capture and handling on day 2, it had made a similar incubation effort as in the previous day (day 1). For this reason it is not possible to infer whether capture and handling was the cause of this abandonment, although the birds were a recently established pair. Also, no nest was predated while the birds were in temporary captivity.

The literature on the effects of chilling presents a mixed picture. Chilling may induce developmental defects, impair embryo metabolism and growth, reduce their post-hatching body condition and viability, or lead to a reduced innate immunity in nestlings (e.g. Olson *et al*. 2006, Ardia *et al*. 2009). Longer-term impacts are also possible, for example, by reducing the lifetime fitness or reproductive success of the hatched chicks (Webb 1987, Astheimer 1991, Yalcin & Siegel 2003). While some studies have shown that small chilling episodes can even promote hatching and also enhance the development of thermoregulation (Webb 1987, Oppenheim & Levin 1975), if chilling episodes become periodic it can have a cost to growth efficiency as well as rate of development (Olson *et al*. 2006). Conversely, some studies support the present findings that embryos are able to survive relatively short periods at sub-optimal temperatures (Webb 1987, Drent 1975, Sockman & Schwabl 1998, Olson *et al*. 2006). In addition to the effects on the chicks, decreases in nest temperature can impose a further energetic constraint on the parent that has to instigate the rapid rise of temperature within the nest (Vleck 1981, Jones 1987).

Although the present study indicated that capture and handling results in higher periods of nest exposure and nest temperature decrease, it was not possible to assess the physiological consequences that such impacts had on the birds (both parents and nestlings). Both parts of the study demonstrated their usefulness to address the question: for sand martins in Portugal it was possible to accurately measure nest absences, whilst for a wider range of species in the UK it was possible to assess nest temperatures and the influence that parental absence had on them. For a further investigation, ideally these two methods should be used in conjunction. Ideally, also, birds should be monitored for longer than the 3-day period used here, to better determine the long term effects that capture can have on birds. However, there could be several factors that could influence nest success, which may not be detectable by digital cameras and temperature loggers. Furthermore, both set ups aimed to catch specific birds, and thus nets were monitored more frequently than usual (birds were generally extracted from the net within a few minutes of being caught), and also, capture and handling times were significantly shorter than in routine ringing studies (e.g. as compared to the ones presented in chapter two), which could potentially mask any other substantial negative impacts.

Chapter 6

Reactions to capture and handling in the seemingly “tame” European storm-petrel (Hydrobates pelagicus)

6.1 Abstract

European storm-petrels (*Hydrobates pelagicus*) can be captured by using sound-recordings of the breeding calls to lure the birds into mist-nets at night. In contrast to most diurnal birds, it has been noted that most storm-petrels do not take off immediately after release, with some individuals taking 30 minutes or more to depart. This behaviour may reflect a temporary disruption to their vision by torchlight or some type of stress response. To investigate whether this results from a disruption of their dark-adaptation visual system, randomly selected birds were handled under white or red light. To test for the role of the physiological stress response in delaying take-off, take-off times were compared between groups subjected to varying handling times, with or without blood sampling. The stress response was assessed using the observed change in the heterophil:lymphocyte ratio. Birds ringed under red-light took an average of 42 seconds less to take off, and although storm-petrels exhibited a substantial change in the heterophil:lymphocyte ratio during capture and handling (indicating a physiological stress response), there was no relation between the magnitude of the immune stress response and the duration of the delay in take-off following release. The use of red-light headtorches when ringing storm-petrels is therefore an effective option to minimize the “dazzling” effect, ensuring that the birds can return to their pelagic environment as quickly as possible following capture and handling for bird-ringing studies.

6.2 Introduction

Since 1990 A Rocha Portugal has carried out an annual ringing project on the European storm-petrel (*Hydrobates pelagicus*) during the species’ northwards migration past the Portuguese coast (Harris *et al*. 1993, Medeiros 2010). This project provides novel insights into the species’ ecology, population dynamics and behavioural responses to climate change (Medeiros 2010), as well as an opportunity to better understand the impacts of capture and handling on a seabird species. Throughout the course of this field work, bird ringers have noticed that storm-petrels rarely take off immediately following release, as opposed to the behaviour of most diurnal bird species, such as most passerines. This lack of urgency to fly away is often interpreted as storm-petrels being “tame”, i.e. unafraid of man. However, another possibility is that storm-petrels are unable or unwilling to take off due to aspects of a physiological stress response, and/or disruption of their dark-adaptation caused by the artificial lights used by the bird-ringers while the birds are being extracted from the mist net and handled for ringing and measurement at night.

There are reasons to expect that the degree of disruption to dark-adaptation depends on the intensity and wavelength spectrum of the light concerned, as brighter and broad-spectrum lights cause bleaching of the light-sensitive rhodopsin molecules in the rod cells that allow for a better night vision, thereby inactivating them (Silman 1969, Lustick 1973). For example, artificially lit structures such as lighthouses and oil and gas platforms at sea are said to cause light-disorientation in birds (McNeil *et al*. 1993, Miles *et al*. 2010), especially if they are illuminated with white light rather than flashing or colored lights. The impacts of bright lights on the avian retina have also been described in the context of nocturnal photography by Olivero and Cohen (2004), who demonstrate that, depending on the conditions, the use of a camera flash can produce a reduction in visual capability (i.e. while the rod cells recover their function) lasting up to 20 minutes.

The time taken for captured birds to take off following release is highly variable both between and within species. Some birds take off immediately, whereas others (including many storm-petrels) may take several minutes or even longer, which could be a result of how stressed the bird is. In chapter one, I described how, in terms of the birds’ physiological response, bird-ringing is considered to be a stressor (Selye 1963) by causing the bird’s adrenal cortex to release corticosterone, which in turn triggers immediate life-saving strategies that allow the bird to cope with the stressor (Sapolsky 1992, Busch 2006). The rise of corticosterone levels in the blood is further associated with a change in immune function, as lymphocyte numbers decline, decreasing the organisms’ defense against viral infections. Simultaneously, heterophil numbers increase (Shapiro & Schechtman 1949, Newman *et al*. 2005, Siegel 1980, Davis *et al*. 2008) enhancing bacterial defense. This prepares and protects the bird for the event of bacterial disease, wounding or infection (Siegel

1980, Dhabhar & McEwen 1997,1999, Martin 2009, Dhabhar 2002, 2009), which could be the result of an acute stressful episode such as capture and handling.

Despite the fact that assessment of corticosterone levels is widely used to quantify the stress response of birds to capture and handling events (Cockrem *et al*. 2008, Cirule *et al*. 2012), some authors such as Vleck *et al*. (2000) and Cirule *et al*. (2012), advocate the use of leukocyte cell counts made from blood smears as a more useful tool (under field circumstances) for stress measurement. Directly assessing baseline levels of corticosterone can be logistically challenging in the field, because the method requires the collection of a blood sample within a short time frame, as changes in corticosterone levels in blood are noticeable as soon as three minutes after being captured (Romero & Reed 2005). In contrast, leukocyte counts take longer to change in response to stress (e.g. 1h), and have the added advantage of assessing immune function directly, as well as avoiding some of the time and logistic constraints that sampling for corticosterone poses under field conditions (Vleck *et al*. 2000, Cirule *et al*. 2011).

For the reasons and rationale presented above, in this chapter I will aim to assess: a) whether the protracted take-off duration commonly observed in mist-netted storm-petrels is at least partly due to disruption of the birds' dark-adapted vision at night; b) assess whether the physiological stress of capture and handling induced an immunological response among captured storm-petrels; and subsequently c) whether the degree of such a stress-response will have any influence on the length of the storm-petrel's take off duration.

6.3 Methods

In June of 2011, 2012 and 2013, European storm-petrels were captured for bird ringing at Ponta de Almádena (37º 04' N, 8º 47' W), on the south west coast of Portugal, a site which storm-petrels pass during their spring migration northwards towards the breeding colonies in NW Europe. The capture method consisted of erecting mist nets at night, and using sound recordings of the male's "purr-call" to lure the storm-petrels onshore, between dusk and dawn. The mist-nets were supervised continuously through the night, and birds were extracted as soon as possible after they were detected as having been caught (in most instances the bird was watched flying into the net).

After being captured, each bird was ringed and handled in order to collect: biometric measurements (wing length, body mass, bill, tarsus), two breast feathers (for DNA analysis of sex and subspecies, and stable isotope analysis of diet), and age (on the basis of the pattern of bleaching of the flight feathers, Bolton & Thomas 2001). After handling, birds were released onto the ground and were supervised until departure to avoid any predator attack. Each step of this process was timed to the nearest second, with a digital chronometer, starting from the time of detection in the net (when extraction from the net began), in the following order: 1) end of extraction from the net (when the bird was placed in a fabric bird-bag), 2) beginning of handling (when the bird was removed from the bird bag), 3) end of handling (when the bird was carried to the release site), and 4) take-off time (from the time when the bird was released onto the ground, to the moment of take-off).

In order to investigate the role of white light disruption on storm-petrel take-off times, in 2010 birds were randomly assigned to be extracted, handled and ringed under either red light (expected to have a minimal impact on the birds' dark adaptation) or white light (expected to have a strong effect on the birds' dark adaptation). Light-emitting diode (LED) headlamps were used with clear or red filters (supplied by www.Tesco.co.uk). White light comprises all wavelengths of the visible spectrum and thus will have more energy than the sole long-wavelength red light, which is at the lowest energy region of the visible spectrum of light.

In June 2012 and 2013, birds were randomly assigned to be captured and handled under white light only, in one of three sampling groups: birds ringed following standard ringing procedures, released immediately after processing (C1), birds held in cotton bags for 1 hour following the end of processing (C2), and birds sampled for blood (BS), to assess the immune effects of capture and handling on storm-petrels. Two blood samples were taken from each bird: blood sample 1, which represented the normal immune profile, was collected within three minutes of the bird being seen to enter the net, and blood sample 2 after 1h to assess capture induced changes (for further details see Davis (2005)). This meant keeping the bird inside a cotton bag for the remaining time between the end of handling until the second blood collection 1 hour later. The group C2 allowed comparison of take-off times between birds that had been blood-sampled, and birds that had not been blood-sampled, but which had been held in a bird bag for an equivalent period of time (1 hour). During this experiment white light was used rather than red light, as a safety procedure for blood collection, as using red light would imply handling the bird in dimmer light, which would have made blood sampling more difficult for the sampler, and thus potentially less safe for the bird.

The blood samples were collected by puncturing the brachial vein with a 23-gauge needle, and collecting drop of blood (< 20 µl) into a standard microhematocrit tube. The blood smear was immediately performed, using the two-slide wedge method (Houwen 2000) and put in a container with silica to air dry. Once dried, the slides were fixed with methanol (100% pure) *in situ*. Samples were later stained with Giemsa stain, and examined under a Novex K-Range microscope, under 1000x. Following Davis (2005), 100 white blood cells were identified as either lymphocytes or heterophylls (to obtain the heterophil:lymphocyte ratio), and the number of white and red blood cells within each field of view was estimated. Each field of view was marked in order not to be counted twice, and only fields of view with a clear view of all cells were examined. This excludes fields of view where red blood cells overlapped, were lysed, or poorly stained (either too much stain or insufficient stain). The measurements allowed the calculation of: a) the total number of white blood cells per 10,000 red blood cells, b) the proportions of lymphocytes and heterophils per sample and c) heterophil:lymphocyte (H:L) ratios with a minimum of influence of the capture event (blood sample 1) and with the potential influence of capture stress (blood sample 2).

For each sampling night, factors determinant of the night's ambient light conditions were registered: moon phase and cloud cover. The cloud cover was recorded in octares; dividing the night sky into 8 parts and counting how many were covered with clouds: 0 cloud cover will thus be a clear sky, and an 8 cloud cover a total cloud covered sky.

Among all birds captured, there was variation in: a) time of capture, b) extraction duration, c) handling duration, d) presence/absence of blood sampling, e) red/white artificial light, and g) take-off time, it was therefore possible to investigate if sampling for blood as well as the color of light and extraction/handling duration each had an effect on take-off time.

The capture and handling of storm-petrels was carried by a group of fully licensed bird-ringers, with the knowledge and consent of the ICNF (Instituto de Conservação da Natureza e das Florestas), the legal authority in Portugal responsible for regulating the bird ringing activities within the country. Blood sampling was solely carried by myself, and I have obtained the legal permits from the same institution, under my name for each year.

Data analysis

Data were analyzed with the statistical software R 2.12.0 (R Core Development Team 2009). Box plots were created to allow for a visual comparison between time duration of stages, take-off times, blood cell counts and H/L ratios. All the box plots performed show the median (either represented by a notch, or a dark line), and the 25th and 75th percentiles (lower and higher ends of box respectively). The ends of the whiskers showed 1.5 times the interquartile range (when outlier circles present) or the furthest value from the median (when no outliers present). Outlier circles represented values outside 1.5 times the interquartile range.

To investigate the difference in take-off times a set of negative binomial generalized linear models (GLMs) were fitted. In these models, the dependent variable was take-off time (i.e. the time between release and take-off) and candidate independent variables examined were: birds handled under white light and red light (family= Gaussian, link= identity), blood cell experiment treatment groups (family= Gaussian, link= identity) and blood cell ratios (family= Gamma, link= log). GLMs were further performed to investigate the variation in leukocyte counts and H/L ration within the time-frame of 1h (both models are family= Gamma, link= identity), and assess differences in handling time between blood sampled individuals and non-blood sampled (family= Gamma, link= identity)

6.4 Results

6.4.1 Duration of take-off relative to different parts of the capture and handling process

Unlike most passerine ringing, storm-petrels were removed from the net as soon as they were seen entering it. Therefore figure 6.1 does not include a “Time in net” equivalent to that in figure 2.1 of chapter two.The extraction stage and time in a bag were much shorter than the equivalent stages for passerine mist netting in chapter two, whilst processing (handling) took (on average) longer. Handling was the longest stage of the bird ringing process for this particular species (figure 6.1).

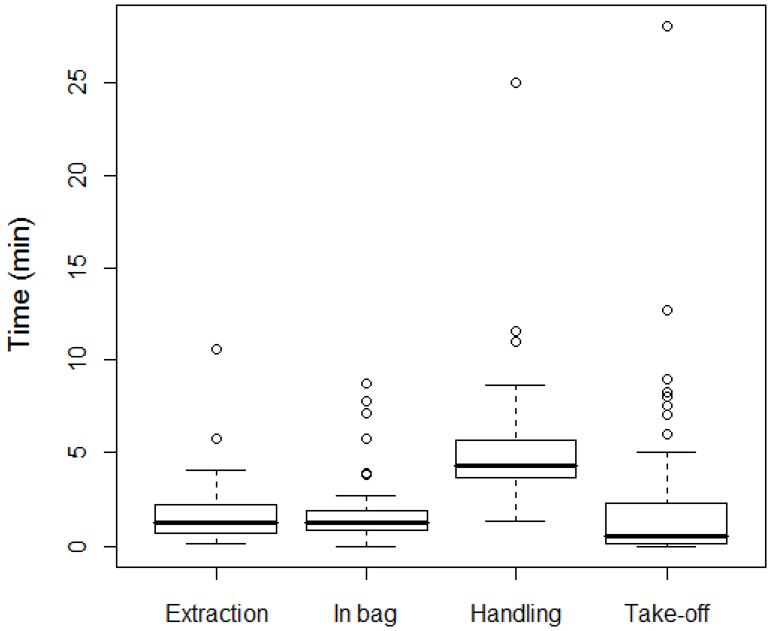

Figure 6.1: Duration in seconds of the bird ringing stages: Extraction (mean = 1 min 44 ± 14 s); Inside the bag (mean = 1 min 47 ± 15 s); Handling (mean = 5 min 10 ± 14 s) i.e. putting an individual metal ring and collection of samples and biometrics; and Take-off (mean = 1 min 55 ± 23 s) i.e. the time between release, and the bird flying away (n = 90). The data presented include only birds sampled in June 2010, when no blood sample measurements were collected, nor were the birds restrained for an extra 1 h period.

Take-off duration was very variable, ranging from immediate take-off (i.e. 0 s) to as much as 28 min (1681 s; figure 6.2). There was a marked difference in take-off times between birds handled under red light and white light (figure 6.2). Specifically, the mean take-off time with red light was: 34 ± 20 s, and with white light was: 1 min 16 ± 40 s. This difference was significant ($F_{1,89}$ = 6.4577, p = 0.0127). Nevertheless handling time was similar for birds ringed under white light (mean = 4.56 ± 0.25 min) than red light (mean = 5.26 ± 0.41 min) (t = -1.45, d.f. = 50, p = 0.153).

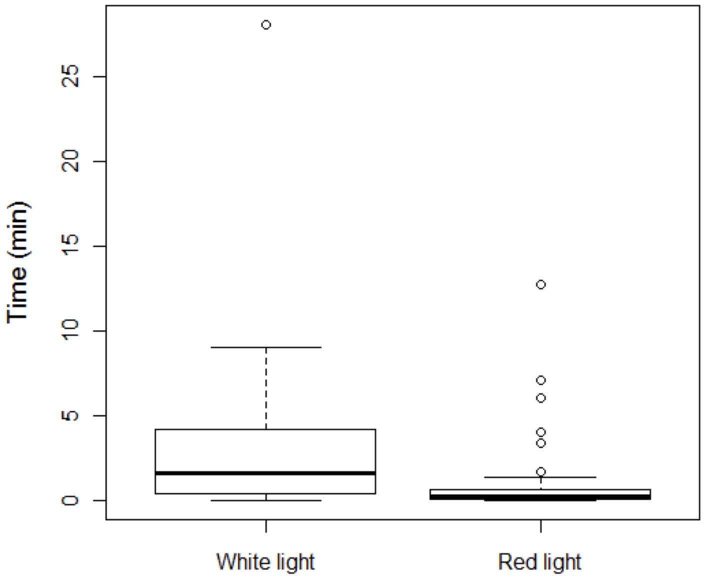

Figure 6.2: Mean time of take off of storm-petrels handled either with red light or white light. (n = 90 birds, of which 45 were ringed under each light condition).

6.4.2 Measuring the immune stress response during capture and handling

Total handling time was not significantly different between birds that were sampled for blood and birds not sampled for blood ($F_{1,49}$= 0.7829, p= 0.3806). However, there was significant difference in mean take-off time between the three treatment groups ($F_{2,396}$ = 64.493, p < 0.0001) (figure 6.3). Specifically, birds that were sampled for blood took significantly more time to take-off than birds not sampled but held for the same amount of time (Blood sampled birds vs. control group C2: t = -3.237, d.f. = 104, p = 0.001) or simply not sampled (Blood sampled birds vs. control group C1: t = -5.269, d.f. = 350, p < 0.0001). In contrast, take-off times did not differ significantly between the two (non-blood-sampled) control groups (C1 vs. C2: t = 1.765, d.f. = 296, p = 0.0783).

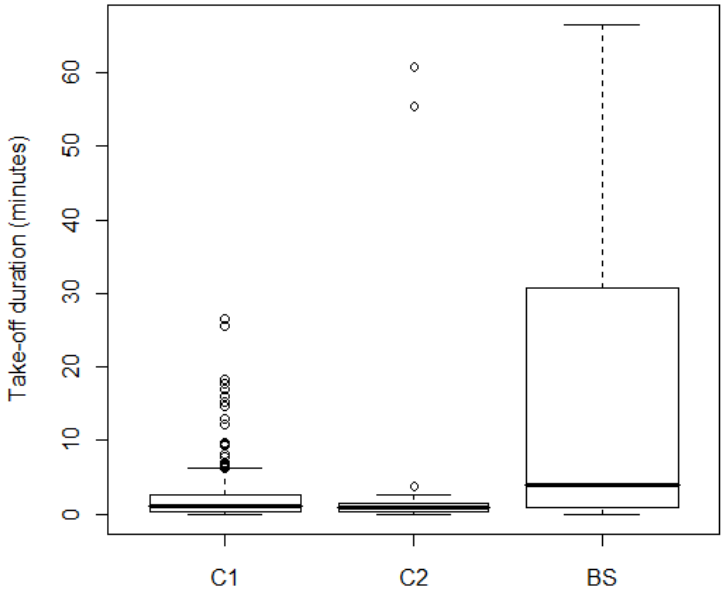

Figure 6.3: Take off time intervals for group the control group (C1) (n = 265), control in bag for 1h (C2) (n = 26), and blood sampled (BS) (n = 80).

As predicted, the analysis of blood smears revealed a significant decrease in leukocyte abundance across the 1 hour interval between blood samples ($F_{1,58}$ = 4.014, p = 0.04979) (figure 6.4). The numbers of lymphocytes decreased and heterophils increased (figure 6.5 a) and b)), which resulted in a significant increase in the H:L ratios ($F_{1,56}$ = 21.27, $p < 0.0001$) (figure 6.6).

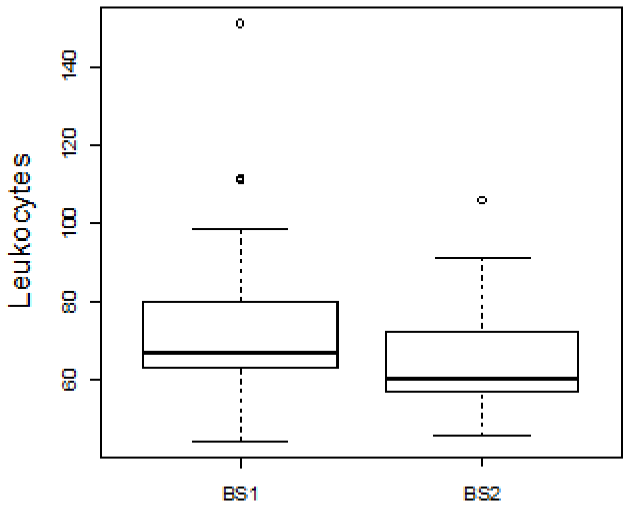

Figure 6.4: Total number of leukocytes per 10,000 red blood cells in the first (BS1) and the second (BS2) and second blood smear samples.

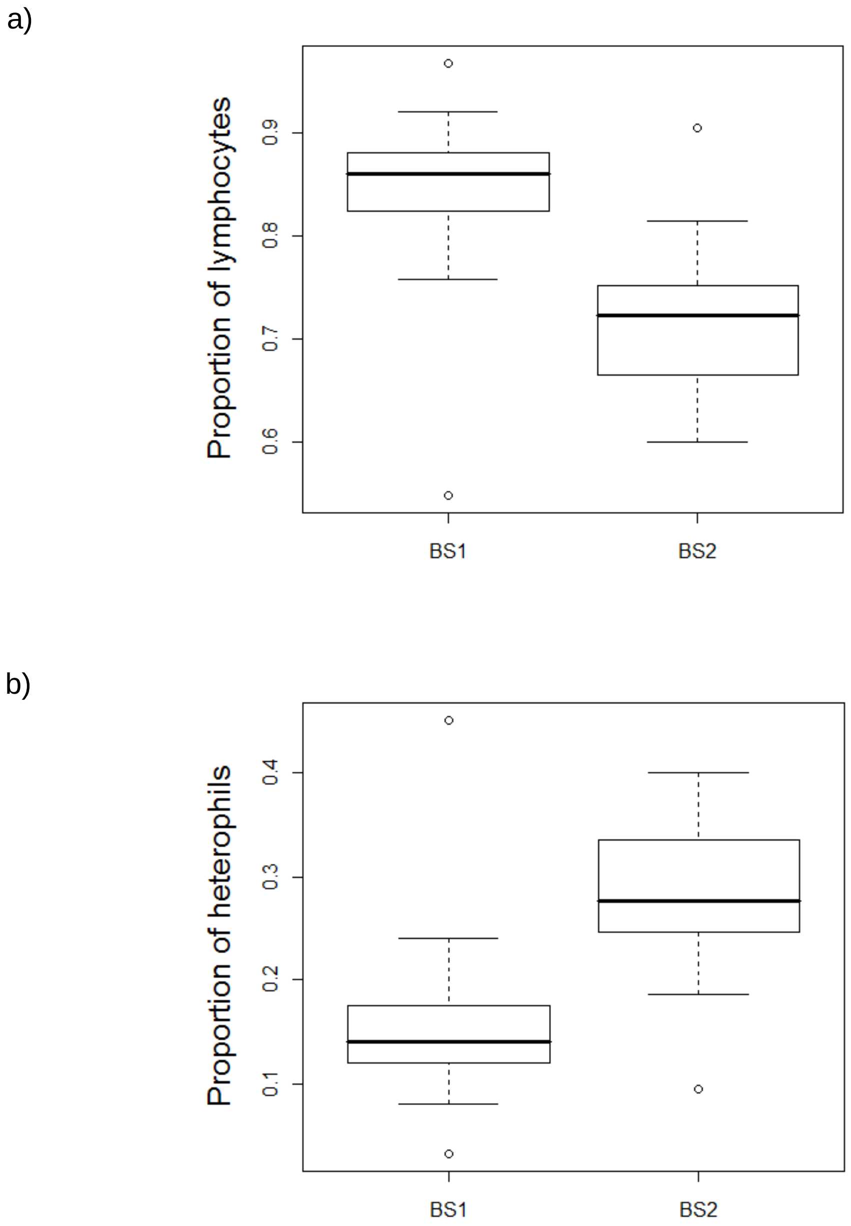

Figure 6.5: a) Proportion of lymphocytes and b) heterophils in relation to red blood cells. BS1 corresponds to the first blood sample and BS2 to the second blood sample.

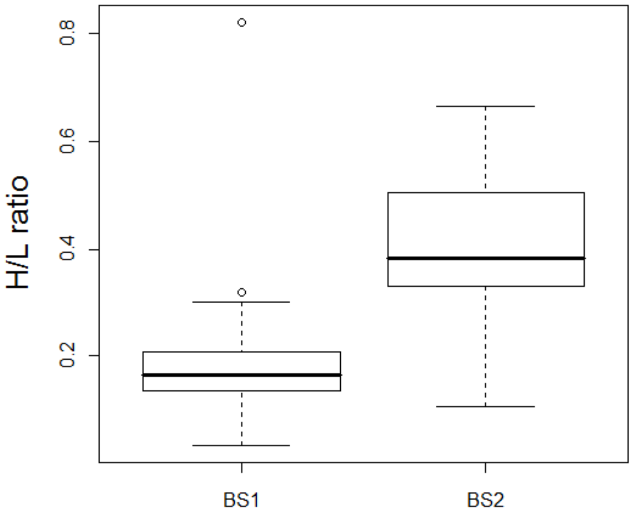

Figure 6.6: Heterophil/lymphocyte ratios in the first (BS1) and second (BS2) blood samples.

Despite the immune response being different in its magnitude among individuals, there was no significant association between the duration of the delay in take-off and the heterophil:lymphocyte ratio (H/L) of the blood-sampled individuals ($F_{1,26} = 0.2427$, $p = 0.6264$).

6.5 Discussion

Tameness, i.e. low wariness (Rodl *et al*. 2007) is often associated with island animals or domesticated animals, and whenever an animal does not demonstrate that it is directly affected by human presence, it is normal to assume it to be “tamed”. The results of this study suggest that the reluctance of storm-petrels to take off immediately after release does not seem to be a result of tameness, as there is evidence of a stress response and different behaviour under different lighting conditions. Anecdotal evidence also appears to support this conclusion, as ringers have noted that storm-petrels sometimes struggle to escape, or even “sing” (vocalizing which could be interpreted as alarm-calling), whilst being processed.

The results obtained here clearly demonstrate that birds ringed under red light take less time to fly off after release than birds ringed under white light. The eye of nocturnal seabirds is often larger than diurnal species of equivalent body size and have a preponderance of rod photoreceptor cells in their retinas, for night and motion sensory information (Olivero & Cohen 2004). In bright light, the rod cells’ pigment, rhodopsin, is “photobleached” thus inactivating the cells (Olivero & Cohen 2004, Weller *et al*. 1975, McNeil *et al*. 1993 and references within), and causing dazzling. Once released, birds need a period of adaptation to darkness once again, during which the beached rod cells gradually “re-charge” and become functional, hence the birds do not take-off immediately. This “re-charging” period is variable, and will depend on the degree of bleaching and amongst individuals (Olivero & Cohen 2004), which could be associated with the variance in take-off times observed.

Rod cells are more sensitive to the blue-green (403nm – 550nm) region of the light spectrum (Washington *et al*. 2007, Grimm *et al*. 2000), and are less sensitive to longer wavelengths such as red light, which are captured by cone cells (responsible for colour vision in bright daylight) (Berg *et al*. 2002). Because of this, the rhodopsin in rod cells is depleted more slowly under red light, and the rods take less time to readjust as compared to white light exposure. This difference in the relative rate of re-charge following red or white light exposure is probably the main reason for the differences in take off time (figure 6.2).

Artificial lighting at night has been considered a form of ecological pollution that can affect ecosystems throughout the world (Aubrecht *et al*. 2010). As a result of nocturnal artificial lighting, many migrating birds die or lose a large amount of their energy reserves during migration (Poot *et al*. 2008, Jones & Francis 2003). There is evidence that the bright lights cause them to get disorientated and interfere physiologically with their circadian rhythm. Also for birds that forage at night, the effects of artificial light (including bleaching of the photoreceptors) can cause them to delay their nightly activities, as biological rhythms are light controlled. The use of head torches in this experiment cannot be directly compared to the extent of artificial lighting used in major structures such as buildings and shipping, however one can question whether these short periods of light exposure will cause birds to become disorientated or even interfere in their foraging ability.

The intrusiveness of blood sampling was evident as birds sampled for blood took significantly longer to take-off following release, than the control birds. This result was observed both where control birds had been kept inside a cotton bag for 1h after processing, simulating the handling conditions the BS group was under, and where they were released immediately. The effects of blood sampling have been studied in the literature with differential outcomes. For example, Sheldon *et al*. (2008) found that blood collection had no major negative effects on developing and adult birds, or on birds’ survival, whereas Brown & Brown (2009) found it to have detrimental effects. Although the present study did not assess long-term bird survival, it is clear that blood sampling had a more detrimental effect (as measured by the impact on delayed take-off) on birds than simple handling. For every storm-petrel sampled for blood, the recommended procedure was followed whereby after removing the blood collection needle, pressure was applied to help minimize the development of haematomas (Fair *et al*. 2010). The bird was only handed to a ringer, or the person who would release it, after 10-20 seconds once the hemorrhage had stopped.

When the second sample was going to be collected, it was not uncommon to find haematomas caused by the first sample. During the season of 2013 one of the birds sampled for blood was recaptured 2 nights after the collection and still presented haematomas on both wings in the place where blood was collected. Because storm-petrels rely heavily on flight, wing haematomas can be especially detrimental or even affect survival negatively (Brown & Brown 2009). Also, this particular bird lost 1.7 grams, nearly 7% of its initial weight, between the capture days. While it is not possible to associate this loss with either the capture event or the blood sampling, it is possible that the haematomas could have handicapped the bird’s foraging activities or even that the blood sampling had induced anaemia, although the blood volume collected was well under the maximum advised of 130µl (calculated from Hoysak & Weatherhead 1991). It is possible that haematomas in the wing could have a significant role in the take-off time of birds. However comprehensive data describing the prevalence of haematomas were not collected, preventing any conclusions being drawn.

Consistent with Davis (2005), I found a significant decrease in the leukocyte number over a time frame of 1h. Leukocyte numbers are expected to decrease when stress conditions (e.g. capture and handling) activate the hypothalamic-pituitary-adrenal axis (HPA axis), although later in the stages of the stress response, as a result of their redistribution in the on the organism (Welsh *et al*. 2007). This decrease was coincidental with the decrease in lymphocytes proportion, as lymphocytes represent the majority of the leukocyte cells (e.g. 70% of the leukocytes are lymphocytes in house finches *Haemorhous mexicanus*, Davis *et al*. 2004), and an increase in heterophils. Thus, like Cirule *et al*. (2011, experiment with great tits *Parus major*), I found that the immune response of storm-petrels to capture and handling stress was evident within 1h, unlike previous studies of other taxa (e.g. Davis 2005, Davis *et al*. 2008, Krams *et al*. 2011) which only detected an effect after a period of 1h.

It is well established that birds release corticosterone (their principal glucocorticoid) in response to a wide range of stressful events (Romero & Reed 2005). This release will depress immune activity, in which lymphocytes take part in varied functions, to decrease inflammation and suppress the organism’s normal immune defense (Davis *et al*. 2008). At the same time, heterophils, which are the main phagocytic leukocyte, are released into the blood from the bone marrow to cope with possible infections, inflammation and “stress” resulting from the stressful event (Cirule *et al*. 2011 and references within). Increased levels of corticosterone also prevent the migration of heterophils to other parts of the body and force the circulating lymphocytes to migrate from the blood to other tissues (e.g. skin) to isolate them (Cirule *et al*. 2011 and references within). The shift in concentrations of leukocytes are believed to be a way of ensuring that the different type of cells are distributed to the compartments they are most need during the stress response, for example, redistributing leukocytes to the skin will enhance the skin immunity (Dhabhar *et al*. 2000). This process may explain the changes in the proportions of leukocytes on the blood over the 1h sampling period in this experiment.

In conclusion, storm-petrels showed substantial changes in leukocyte ratios over the hour following capture, consistent with an acute stress response to capture and handling. Despite the intrinsic role that the immune system has on the acute stress response, I have found no evidence that the leukocyte profile was associated with take-off duration in storm-petrels, thereby excluding the hypothesis that the stress response had any influence in the take-off time of these birds. In contrast, take-off duration was significantly associated with the lighting conditions under which the storm-petrels were handled, indicating that delayed take off is at least partly caused by disruption of the storm petrels’ visual capabilities.

Chapter 7

Analysing the safety of capture and handling: what are the injuries and mortality risks to the bird?

7.1 Abstract

Bird-ringing is an essential tool for field ornithologists, providing important data in a wide range of research fields. The methods used to catch and ring birds have been continuously developed over the last 100 years in order to standardize the methodology and to minimize the effects on birds. Nevertheless, injuries and fatalities may still occur, and because mist netting and bird ringing are so widely used, there is a need to get a comprehensive understanding of the nature and extent of possible impacts. To better understand the scale of impact, two complementary studies were carried out. In the first, questionnaire surveys directed at bird ringers were carried out, recording their perceptions of injury and fatality rates, causes and types of injuries, and the methodological details about how they capture birds (including mist netting and other capture techniques) Secondly, empirical data on mortality and injury were collected from >7500 captures at two bird-ringing stations, whose primary method of capture was mist-netting. The predicted mortality and injury rates from the questionnaires (both <1%) were consistent with than the values recorded at the two ringing stations (0.27% mortality and 0.89% injury rate). The main cause of mortality identified by both studies was predation, whilst injuries were mainly related to cuts and wing related injuries. In conjunction, the two studies highlighted Common blackbirds (*Turdus merula*), European greenfinches (*Chloris chloris*), common chiffchaffs (*Phylloscopus collybita*), long-tailed tits (*Aegithalos caudatus*) and common kingfishers (*Alcedo atthis*). Birds with an agitated/ aggressive behaviour were more likely to suffer from injuries, however visual cues of apathy and lethargy, are useful to evaluate the birds' condition. This study confirms that injury/mortality rates are generally low and vary in predictable ways amongst species/habitats.

7.2 Introduction

Bird-ringing (or banding) is an essential tool for field ornithologists, providing important datasets in a wide range of research fields, including avian ecology, conservation biology, and climate change biology (e.g. Clark *et al*. 2009). Bird ringing has been continuously developed over the 100 years since its initiation, to standardize the methodology and to minimize the effects on birds. Currently, several million birds are ringed worldwide each year, yet relatively little information about injury and mortality rates as a consequence of bird ringing is available. Prior to 2011, sparse information was available, scattered across relatively little-known publications, some in the native language of the researcher and often providing only very limited data or referring to very specific methods (e.g. Petronilho, 2002, Recher *et al*. 1985, Poole & Brown 2007, Collins 2007), (see table 1.I).

Increasing ethical concerns, especially regarding the use of animals in scientific research, and the bias it can introduce into data collection, have recently increased interest in the possible impacts of mist netting (now the most common method of capture) and bird ringing in general. Spotswood *et al*. (2012) published a major study into the safety of mist-netting, providing detailed information about observed mortality and injury levels, as well as highlighting potentially vulnerable species and traits. They collated data from 22 banding organizations in the United States and Canada about mortality and injuries rates, and from five organizations to assess the types of incidents, including leg injuries, broken legs, internal bleeding and cuts, tangling-related injuries and wing strain. Injury and mortality rates of 0.59% and 0.23% respectively were found (Spotswood *et al*. 2012). Whilst combined injury and mortality rates were <1%, it is hard to make any inference about the impacts on populations. Also, in the same year, a publication by Saraux *et al*. (2011), revealed that flipper banded king penguins *Aptenodytes patagonicus* had fewer chicks and lower survival rate than unbanded individuals over a 10 year study. Even though the latter study refers to the long-term effects of marking (beyond the scope of this PhD project), it highlights the risk that bird ringing could to some extent jeopardize the welfare and/or conservation of the study species, and in so doing undermine the validity of the conclusions drawn from such studies (e.g. by biasing survival rates).

Although mist netting of passerines and near passerines is the most widespread basis for bird ringing, groups such as wildfowl, marine birds or raptors are usually monitored using other sampling methods. Examples of such methods are Heligoland traps, cannon-netting, noose carpets and capturing birds at their nests. Unlike for mist netting (Spotswood *et al*. 2012), there are few data describing injury and mortality rates for these other trapping methods (refer to table1.I for more detail).

Heligoland traps consist of a large, static, tapering wire-netting enclosure, with a wide opening, narrowing towards the end that consists of a collection box with a transparent back, into which the birds can be flushed once they have entered the mouth of the trap (for further details see Woodford 1959, Woodford & Hussel 1961, Brownlow 1952, or NABC 2001). Heligoland traps are especially efficient for catching large numbers of birds where they occur in high densities (e.g. at migration stopover sites). Although they have been largely replaced by mist nets (Williamson 1957), Heligoland traps are still in use at some field centers and bird observatories. Despite the numbers of birds that are still captured in heligoland traps, I have not found data regarding mortality for this capture method.

Cannon netting relies on birds roosting or gathering at a specific site, and consists of projecting a large mesh net attached to projectiles and propelled by explosive charges over a flock of birds (Bub 1995). Because of its risk of injury or death to birds and humans, it is only performed by highly trained individuals and involves a big logistical operation, such as finding a flocking place, monitoring tides and the behaviour of the flocks, setting explosives at a specific angle to minimize the probability that the birds would be hit by the projectiles carrying the net over the flock, as well as calculating the precise site where all the flock should be in order to safely shoot the net. Birds are removed as quickly as possible after capture to avoid wing strain or capture myopathy incidents among the captured birds (NABC 2004). Cannon netting has been claimed to be one of the safest techniques to catch wildfowl, waders and seabirds, especially when compared to general capture techniques such as mist netting (Minton 1993). However when incidents do occur they are likely to be fatal, with mortality rates in individual catches as high as 6.63% (table 7.I). In addition to mortality, there are other risks associated with this capture method, including capture myopathy (refer to chapter one for details) which is mostly a consequence of cannon-netting activities.

Table 7.I: Capture mortality rates for shorebirds, sucks and seabirds found in the literature. The number of deaths includes deaths during handling as well as deaths incurred during the capture event itself. Total number caught includes all individuals captured and handled, whether they survived or not (i.e. including deaths).

Taxonomic group	Capture method	Deaths/ Number caught	Mortality rate	Reference
Shorebirds	Rocket nets	0-28/1350	0-2.1%	Jurek 1974
Ducks	Rocket nets	12/1116	1%	Cox Jr & Afton 1994
	Rocket nets	23/347	6.63%	Cox Jr & Afton 1998
	4 different trap methods: swim-in bait traps, swim-in bait traps with decoys, floating bait traps, and decoy traps.	30/661	4.5%	Evrard & Bacon 1998
	Benning II, cloverleaf, oval and star trap	127/10.966	1.16%	Dieter *et al*. 2009
	Benning II, cloverleaf and oval with lead traps	35/10,849	0.32%	Dieter *et al*. 2009
	Benning II, oval with and without lead traps	20/11,737	0.17%	Dieter *et al*. 2009
Seabirds	Mist nets	4/6000	0.06%	R.J. Thomas, unpublished data

Many ecological studies require that ringers handle breeding birds. The most straightforward method to do so is sometimes (e.g. for box-nesting species) to capture them at the nest. The methods of successfully catching a bird at the nest are well covered in Bub (1995) and because it is such a sensitive period of a bird's life cycle, the impacts that such a technique has on the nest desertion of species has also been thoroughly described elsewhere (for 135 European species in Kania, 1992; see chapter one). Although there is some literature on the effects that capturing at the nest has on the breeding success of larger birds (e.g. Hill & Talent 1990, Olsen & Schmidt 2001), very little information is available concerning the impacts that capture and handling has on the survival or injury rate of parent birds.

Other less frequently used capture methods include “Bal-chatri” or “noose carpet” traps, which were originally used to capture raptors, but can be adapted to trap birds that walk on the ground (e.g. sandgrouses). A noose carpet consists in a flat piece of wire mesh to which have been tied numerous monofilament nooses (Doerr *et al.* 1998), and is placed along favored feeding, roosting or nest sites.

The present chapter aims to quantify the perceptions that bird ringers have towards bird ringing injuries and mortality rates, by collecting information through questionnaire surveys. This is contrasted with detailed data collection of the rates and circumstances of injuries and fatalities at two major ringing stations (totaling >7500 captures). In addition, smaller data sets for four other capture methods; Heligoland traps, cannon netting, noose carpets and capture at nest, are collated to provide additional estimates to injury/fatality rates.

7.3 Methods

7.3.1 Bird Ringing Questionnaire Survey

To obtain a more comprehensive idea of the main effects of capture and handling on wild birds, a questionnaire (previously designed by Thomas, RJ and Rosenfeld, E) was circulated internationally through the online bird ringing group on the web platform Facebook (Appendix B). The questionnaire focused primarily on mist netting activities, as this is the most widespread and frequently used method of capturing wild birds. The questionnaire aimed to obtain estimates of injury and fatality rates, examine the types and causes of injuries, and the perception of bird ringers regarding rates that they considered to be acceptable. It consisted of three groups of questions, both short-answer and multiple choice, that provided a description of the ringing scheme and the ringer, their mist-netting methods (e.g. length of mist net, frequency of net rounds, modulation of decisions), and the effects that their activity had on the wild birds, specifically types and rates of injuries and mortalities encountered, perception of susceptible species and conditions affecting rates of incidents (Appendix B).

An introductory text to the questionnaire informed the recipients of the anonymous nature of the questionnaire as well as its integration in a PhD program, with the knowledge of the national bird-ringing scheme (i.e. UK), although it was also shared with bird ringers from different countries in which bird-ringing schemes can vary enormously. Additionally it emphasized that it was not aiming at discrediting bird ringing or defending any ethical point of view (Appendix B).

The inter-relationships between aspects of ringers' activities/behaviour were analysed using Generalised Linear Models (GLMs), using the statistical software R 2.12.0 (R Core Development Team 2009). The length of the interval between checking the nets for birds ('net-rounds') was regressed onto: i) the length of netting used per ringer, to test whether ringers actively attempted to adjust the frequency of net rounds in response to the number of nets set, ii) experience of the ringer, to test whether intended net round frequency varied between 'intermediate' and 'experienced' ringers, and iii) country, to look for evidence of differences among ringing schemes. The threshold levels of injury and mortality of which bird ringers claimed to cease their activity were regressed onto the levels of mortality and injury they claimed to have, to test the association between these vales.

7.3.2 *Incident Report Form Survey*

To obtain estimates of the actual numbers of injuries and deaths incurred by mist-netting of birds, and whether they matched the ones provided in the questionnaires, an "incident report form" was developed (Appendix B). The form was disseminated amongst bird-ringers in Portugal through the Portuguese bird ringer's association (APAA – Associação Portuguesa de Anilhadores de Aves). The following information was recorded every time an incident occurred during mist netting/bird ringing: ring number, species, the type of incident (either mortality or injury), part of the bird-ringing procedure in which the incident occurred (Extraction, Handling or Release), and if possible in the "Notes" column, indicate the type of behaviour ("agitated" or "calm"), e.g.:

	Ring	**Species**		**Process**	**Incident**	**Notes**
(...)	*F000000*	*TURMER*	(...)	*(E)xtraction*	*Tongue caught*	*Agitated. Struggle in net, apathetic on release*
	O00000	*PHYCOL*		*(H)andling*	*Fracture*	*Calm. Fracture on right metatarsus when ringing*
	A000000	*CARCAR*		*(E)xtraction*	*Death*	*Found dead in net*

Figure 7.1: Example of data to be collected included in the instructions sheet of the "Incident Report Form" (Appendix B). An "Agitated" type of behaviour is an active response in which the bird shows aggression and territorial control, whilst the "Calm" is characterized by immobility and low levels of aggression.

Whenever a bird died during the ringing-sessions in Portugal or the UK, if possible, bird ringers kept them to later perform visual necropsies. Whenever a bird was inspected a form was completed describing biometrics, external and internal condition (adapted of Work 2000, in Appendix B).

Two field stations in Portugal recorded detailed information for all incidents observed between the 1st of August 2010 and the 31st of July 2013. The resulting data comprised >7500 captures of both resident and migratory passerines and near-passerines using mist nets. The ringing stations were: A Rocha in the Algarve (GPS coordinates= 39º26’53.38’’Ne, 9º07’51.67’’W) and Paúl de Tornada in Estremadura (West Portugal) (GPS coordinates= 37º07’17.94"N - 8º32´52.06’’W).

7.3.3 *Other methodologies*

Data describing incidence rates for trapping methods other than mist netting were obtained from anonymous ringers in Portugal and the UK. The data sets for the four trapping methods were:

1. For Heligoland traps, data on 178 birds caught in Portugal (data withheld for anonymity).

2. For cannon netting, data from 7243 birds provided by two bird ringers, each of whom holds a permit to carry out cannon-netting to capture waders, wildfowl and gulls in the UK. Both databases include only the captures in which the ringer was the sole cannon-netting license holder present (data withheld for anonymity).

3. For captures at the nest, 508 Cory’s shearwater (*Calonectris diomedea*) (data withheld for anonymity).

4. Noose carpet data were collected from 68 sandgrouse of four species (Crowned Sandgrouse, *Pterocles coronatus*; Spotted Sandgrouse, *P. senegallus*; Chestnut-bellied Sandgrouse, *P. exustus*; and Lichtenstein’s Sandgrouse, *P. lichtensteinii*), by an anonymous bird ringer. Additional data recorded regarding the behaviour of each bird (i.e. aggressive or calm) (data withheld for anonymity).

7.4 Results

7.4.1 *General Bird Ringing Questionnaire Survey*

Twenty four individuals responded to the questionnaire. Most were male (82%) and in the age class of 21-34 (figure 7.2). Ringers from seven countries responded:

Portugal, Spain, UK, Denmark, Netherlands, Finland, Germany and Canada, with half of the replies from Portugal.

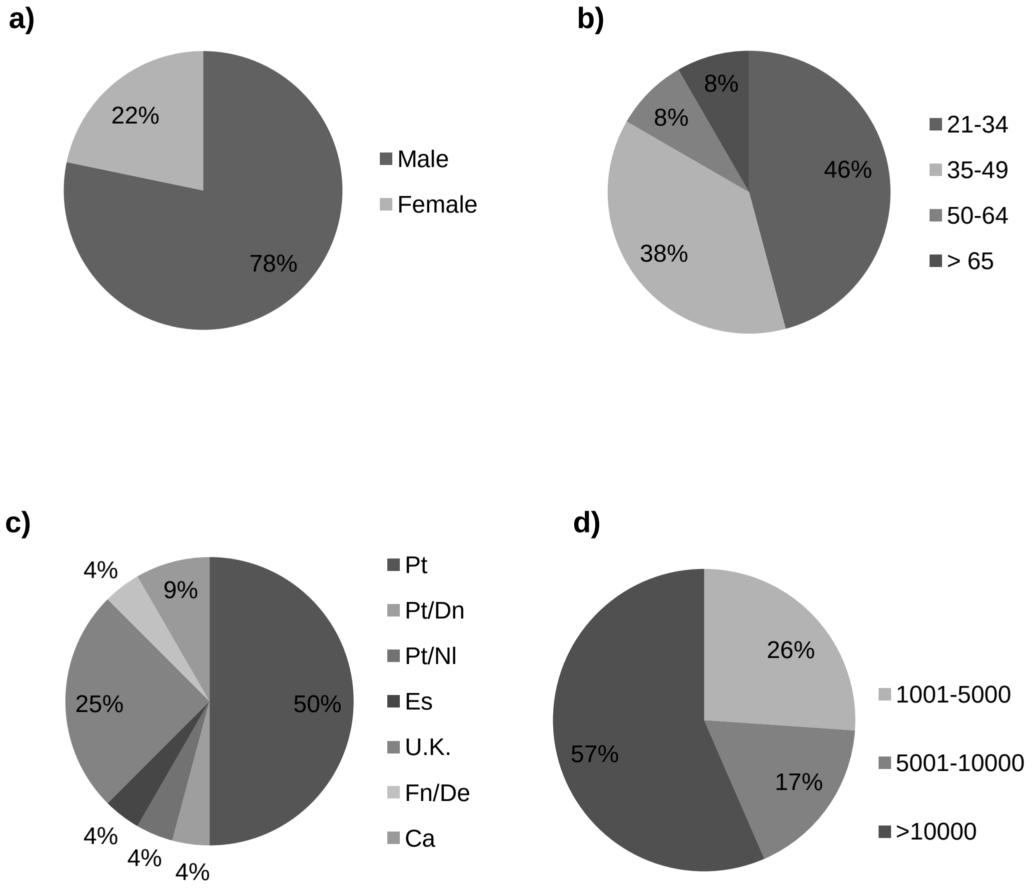

Figure 7.2: Characterization of the surveyed bird ringers, by: a) gender; b) age class, c) country in which they have permits to ring birds, and d) average quantity of birds ringed and seen being ringed (Appendix 1).

The majority of the participants were classified as “Experienced” ringers (54%), claiming to have seen more than 10000 birds being ringed, and 77% of those individuals held an UK A permit or the equivalent (e.g. Master, Evaluator). The remaining 46% were “Intermediate” ringers with the equivalent to UK C permit.

Bird-ringing methodologies

The majority of bird ringers reported operating a mean of 115 ± 15.9 m of mist-net per ringer ([9-350 m]), aiming for net-rounds at a mean interval of 47 ± 3.1 min. The most frequent intervals were 60, 30 and 45 min (41.7%, 20.8% and 12.5% respectively) (figure 7.3). Net round frequency showed only a weak relationship with the length of mist netting used, that was not significant ($F_{1,20} = 2.201$, $p = 0.1535$; figure 7.3). Net round durations differed significantly between countries, but were not related to the experience of the ringer (country difference; $F_{7,12} = 10.3547$, $p = 0.0002$; experience difference; $F_{3,12} = 0.8239$, $p = 0.506$).

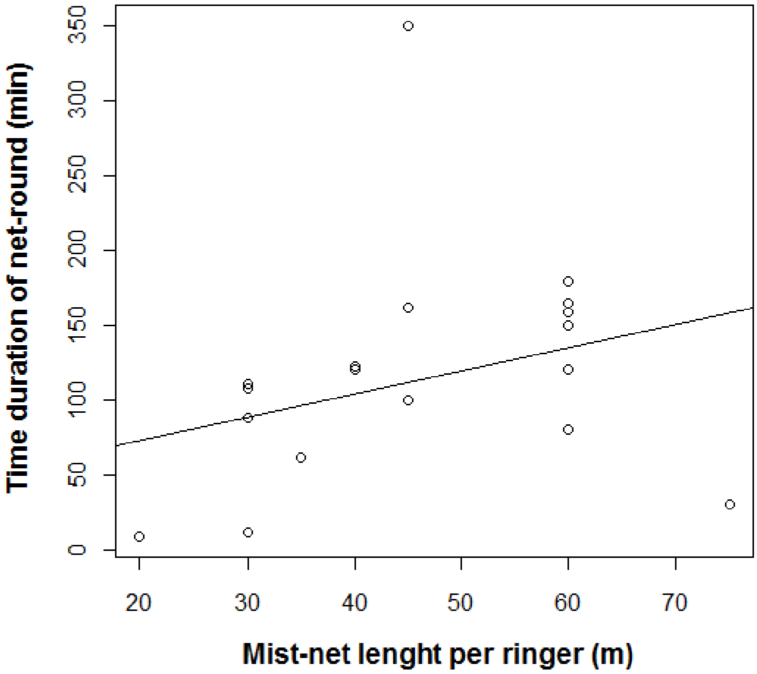

Figure 7.3: Effects of mist-net length used per ringer in the time duration of net-rounds.

In response to unexpectedly busy periods when relatively large numbers of birds are caught, participants reported a variety of strategies. In decreasing order of frequency, these were: to not alter the interval between net rounds (37.5%); increase the intervals (25%); decrease them (20.8%, only bird ringers that do rounds shorter than 60 minutes claimed to do this); and lastly close the nets (16.7%).

There are no published guidelines about the order in which birds should be ringed. While 33% of the participants claimed to ring birds in the order in which they were extracted from the nets, the remainder prioritised birds according to one of four strategies (figure 7.4): i): the species judged to be most sensitive first; ii) condition (i.e. distressed or physically injured birds first); iii) smaller birds first; and iv) type (i.e. fledgings and incubating females first, and rarities last). The list of species considered to be more sensitive and most susceptible to injuries and death as a direct consequence of capture and handling are shown in figure 7.5 b); European greenfinches (*Chloris chloris*) were highlighted as the most susceptible species, followed by common blackbirds (*Turdus merula*) and common chiffchaffs (*Phylloscopus collybita*).

Regardless of the perceived sensitivities of different species, bird ringers identified particular circumstances which can enhance the risk of injuries and fatalities. The most frequently cited factor was extremes of temperature such as cold or heat. Altogether weather conditions comprised over 70% of the risky circumstances identified by the ringers. Considering these circumstances, 70.8% of bird ringers claimed to adjust their ringing practice to cope with these specific conditions, of which 12.5% would only do so in 'extreme' conditions (non-specified). The remaining 29.2% did not provide an answer. The adjustments suggested were: reducing ringing effort (e.g. close some nets), carrying out more frequent net rounds and taking fewer biometrics.

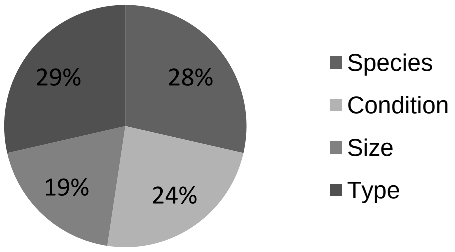

Figure 7.4: Categories in which birds ringers claim to prioritize birds. "Species" = particular species perceived as sensitive, "Condition" = birds that are either distressed or injured, "Size" = smaller species first, and "Type" = incubating females, fledglings first and rare birds last.

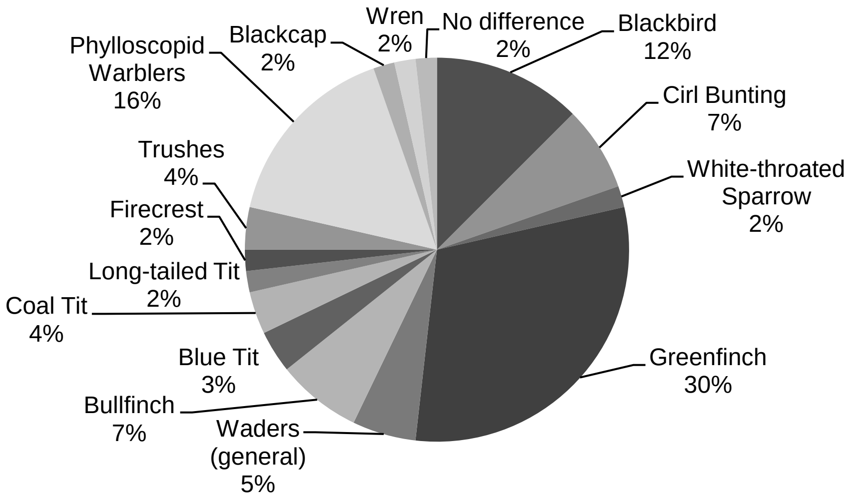

Figure 7.5: Species that bird ringers considered as being the most sensitive in the questionnaire.

The majority (85%) of participants estimated that mortality rates were usually under 1%, with only 5% of the participants claiming it to be 2-2.5%, and the remaining 14% of participants estimating between 1-2%. According to their experience the majority of mortalities occurred when birds were either trapped in the net, or during handling (figure 7.6). The other steps of bird ringing highlighted as possible times when mortalities occur were release (with the possibility of birds flying into windows or dying due to stress) and during transportation from the net to the location of ringing (e.g. accidents).

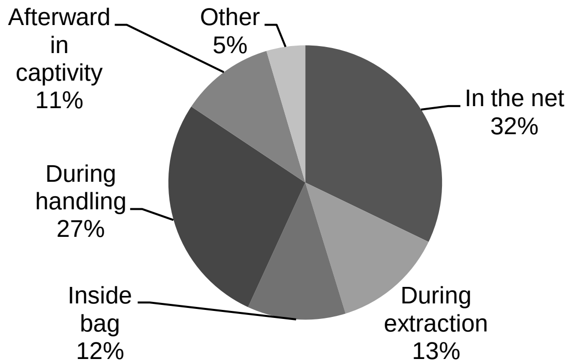

Figure 7.6: Stages in which mortality is perceived to be higher by bird ringers.

Amongst the causes of mortality identified by the ringers (figure 7.7), 54.3% were attributed directly to the capture stage, specifically: due to injuries incurred in the net, predation, wing injuries, and hyper or hypothermia. Only 9% could be considered a direct result of handling (i.e. poor handling), and another 3% of the bird's previous body condition (i.e. weakness) (figure 7.7).

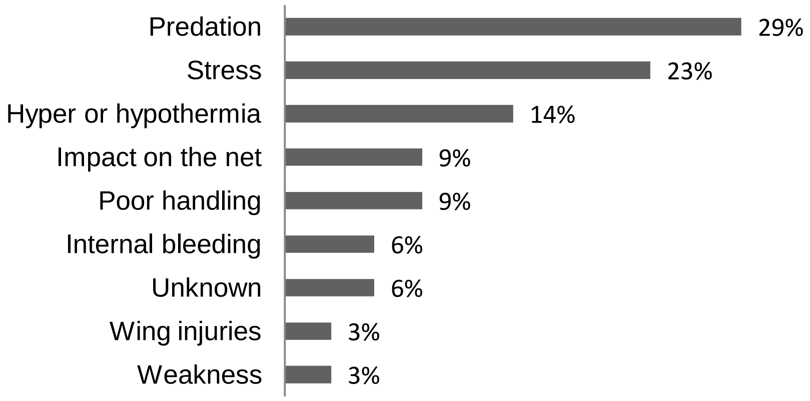

Figure 7.7: Main causes of mortality on birds during capture and handling, that bird ringers claimed to encounter, and respective frequency.

More than 50% of bird ringers estimated that the rates of injury they have experienced were below 1%, with 30% estimating it to have been larger than 2%. One participant estimated an injury rate of up to 20% including minor injuries (e.g. feather loss, breakage). Bird ringers identified 11 types of injury observed during bird ringing sessions, some of which were judged to be of "natural" occurrence (presented on table 7.II). Four injuries could have been either natural or mist netting/bird ringing induced. Nearly 50% of the participants claimed to have found birds with healed broken legs, and 21% with "scars" of wounds (considered under "Cuts" type of injury in table 7.II) or with what were assumed to be deleterious natural injuries such as "broken bill/ mandible deformation" (see figure 7.8). Although loss of tail feathers was only mentioned by one participant, further in the questionnaire, when asked how many incidents of feather loss had observed, 91.7% claimed that they encountered this situation, and that from 0.05% to as much as 25% of the birds ringed would lose their tail feathers. Most of the participants believed that birds with wing strain will recover after a few hours (44%) or within minutes (22%), with a smaller proportion claiming the birds will most likely die (11%). The remaining 33% claimed to not know. In addition to natural injuries, 5% of the bird ringers also mentioned that some birds had ectoparasites.

Table 7.II: Types and frequency of injuries incurred during bird ringing activities, and natural injuries encountered.

Type of injury	Bird Ringing	Natural
Dislocated joint	6%	
Wing strain / injury	23%	
Broken wing	4%	
Broken leg	21%	28%
Broken/ Missing toes of legs		15%
Broken bills /Mandible deformation		13%
Cuts	25%	13%
Tongue injury	17%	3%
Injured eyes		3%
Tumours / Skin disease		10%
Loss of tail feathers	2%	5%
No injuries observed	2%	5%

Figure 7.8: Deformation on the maxillary of a Eurasian reed warbler. Photo by Leila Duarte.

When bird ringers encountered a bird that was judged to be seriously injured (e.g.broken leg), the majority of participants (69.6%) preferred to release it without ringing, while 13% would first attempt to treat the bird, either releasing it immediately afterwards or awaiting for its recovery. Only one participant claimed that their response was modulated by the species of bird.

There was a range of signs that bird ringers claimed helped them to recognise when a birds was suffering from harmful distress. The main indicators were judged to be the birds' body behaviour, especially if a bird was very quiet (i.e. apathetic, fluffing) or demonstrated any sensory difference (i.e. eyes closing, panting or gasping, shivering). Physical injury (i.e. dragging wing, limpness) and signs of agitation were some of the least frequently reported symptoms (figure 7.9).

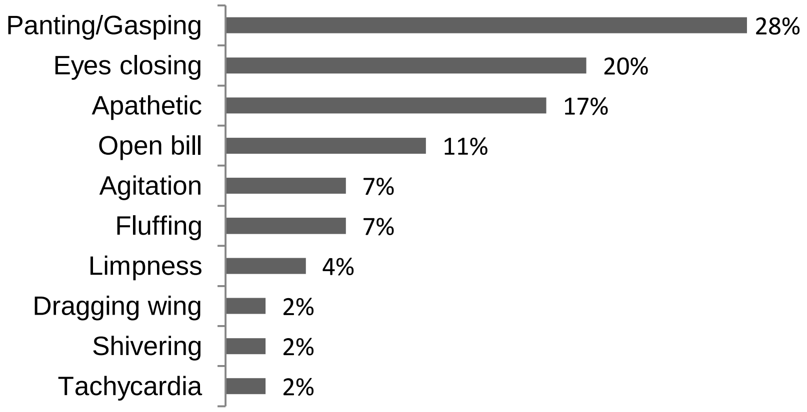

Figure 7.9: Main visual cues that bird ringers use to identify if a bird is under "stress" or unwell, and respective frequency of use.

Generally, when asked how many incidents would cause them to stop ringing, bird ringers claim that only higher mortality and injury rates than those experienced would cause them to cease bird ringing activities. The threshold at which ringing would stop was significantly higher than the observed rates, for both mortality and injury (mortality; $F_{1,37} = 16.758$, $p= 0.0002$; and injury; $F_{1,37} = 7.032$, $p = 0.012$). The mean threshold of the maximum acceptable mortality and injury rate were: 2.6% [0.1%, 10%] and 4.5% [0.1%, 20%] respectively.

7.4.2 Incident Report Form Survey

Between August 2010 and July 2013 incident forms were filled in during ringing sessions at Paul de Tornada and A Rocha ringing stations. A total of 7,598 birds of 74 species (see Appendix C) were captured during the data collection sessions. The species most susceptible to suffer incidents in these particular ringing stations (amongst those species with a sample size > 100 birds) were long-tailed tits, kingfishers, blackbirds and greenfinches (table 7.III). There is a particular case of a little bittern (*Ixobrychus minutus*), counted as capture zero, as it was found dead in the net.

Table 7.III: List of species for which incidents (i.e. mortality or injury) were registered, with corresponding numbers of incidents and capture times in both sampling sites.

Species	**Incidents**	**Total catch**
Common blackbird *Turdus merula*	21	593
European greenfinch *Chloris chloris*	9	305
European chiffchaff *Phylloscopus collybita*	9	447
Eurasian blackcap *Sylvia atricapilla*	7	1419
House sparrow *Passer domesticus*	6	809
Common kingsfisher *Alcedo atthis*	5	109
European robin *Erithacus rubecula*	5	351
Eurasian reed warbler *Acrocephalus scirpaceus*	4	263
Cetti's warbler *Cettia cetti*	3	135
Sardinian warbler *Sylvia melanocephala*	3	137
Long-tailed tit *Aegithalos caudatus*	2	19
Western yellow wagtail *Motacilla flava*	2	187
Black-headed weaver *Ploceus melanocephalus*	2	41
Iberian Magpie *Cyanopica cooki*	1	101
Common waxbill *Estrilda astrild*	1	229
Savi's warbler *Locustella luscinioides*	1	18
European stonechat *Saxicola rubicola*	1	27
European serin *Serinus serinus*	1	97
Garden warbler *Sylvia borin*	1	175
Eurasian wren *Troglodytes troglodytes*	1	31
Song thrush *Turdus philomelos*	1	58
Eurasian hoopoe *Upupa epops*	1	51
Little bittern *Ixobrychus minutus*	1	0
Total	88	5602

From these 7,598 birds captured, 0.27% birds died during capture or handling. The majority of fatalities occurred during the period birds spent unattended in the net (Table 7.IV).

Table 7.IV: Confirmed or inferred causes of mortality of wild birds during from capture and handling, per stage of the process (n= 18 individual birds). *Refers to birds whose cause of death was assessed via a necropsy (post-mortem dissection).

	In the net	Extraction	Bag	Handling	Release
Predation	3				
Internal bleeding				1*	
Internal parasite load					1*
Oxygen depletion			1*		
Overheating	1	1			
Neck fracture	1*		1*		
Exhaustion	1			2	1
Accident				1*	
Unknown	1	1	2		1
Total	**7**	**2**	**4**	**4**	**3**

For these particular bird-ringing stations, feral cats (*Felis silvestris catus*) were responsible for the predation fatalities in table 7.IV. Although Eurasian sparrowhawks (*Accipiter nisus*) were seen attacking birds caught in the nets on several occasions, they were always unsuccessful. Only one of the 15 fatalities for which the cause of death was identified had a direct human cause: a yellow wagtail, incorrectly sampled for blood from the jugular vein (chapter four). From the two birds that died with a neck fracture, the first was a little bittern that was found in the net with an exposed neck fracture, perhaps due to struggling, and the second corresponded to a chiffchaff in a bird bag that fell to the floor from the hook where it was waiting to be processed. Two other notable fatalities were a blackcap with a severe parasite load in the stomach, and a yellow wagtail with diseased lungs, both considered unlikely to have survived for long in the wild, even in the absence of capture.

The combined injury rate for both stations was below 1% (0.89%) (table 7.V).

Table 7.V: Types of injuries incurred in each ringing stage, and respective number of birds affected (n = 68 individual birds).

Type of injury	In the net	Extraction	Bag	Handling	Release
Damaged pin feather	2	5			
Extreme feather loss		1			
Wing superficial cut	1	7			
Wing strain	1	1			4
Wing dislocation					1
Fail to take-off					16
Hindlimb superficial cut	2	8		3	
Broken leg				1	
Tongue superficial cut	4	1			
Tongued		2			
Heat exhaustion	2				
Internal bleeding		3		3	
Total	12	28	0	7	21

Although 21 injuries were recorded at release, these must have been caused during the previous stages, but only noticed when birds failed to take-off, and hence put under this category. In five of these birds it was possible to distinguish that it was due to wing related injuries (i.e. wing strain or dislocation). The majority of birds that presented difficulties in taking-off demonstrated a calm and apathetic behaviour, even though some species such as blackbirds are more commonly associated with restlessness.

Extraction seems to be the most sensitive stage with the most injuries seeming to be caused by the mesh of the net, which due to a conjunction of factors (e.g. wind, struggling) can make a skintear that will bleed. Those reported on the Incident Report forms were mainly to the feet, and in the majority of cases were either due to birds having feet tumors (which can get caught on the mesh) or cold winter mornings which appear to make the skin more prone to being cut. The other cases of “cuts” during extraction were also most commonly found on less robust parts of the body: the patagial membrane (the elastic fold of skin connecting the shoulder to the carpal joint) and the spot where flight feathers insert into the wing. The net mesh can also be damaging to growing feathers that are “in pin” (i.e. growing feathers, recently erupted from the skin surface and still enclosed in a waxy sheath). The three birds that bled from the beak during extraction were: a house sparrow, a blackbird and an azure-winged magpie.

Handling seems to be a stage in which relatively few incidents occur, and apart from internal bleeding, the direct cause of these injuries was mostly related to a poor closing of the ring around the leg. Internal bleeding during handling was registered for three greenfinches. This species, as emphasized in the questionnaire results, is regarded to be particularly prone to this type of injury.

Whenever possible, when injuries were recorded, bird ringers made a note of the behaviour the bird demonstrated through the entire process, i.e. whether they were “agitated” or “calm” (figure 7.10). An “agitated” bird is one that struggles, demonstrates aggression towards the ringer, and utters fear/ alarm calls frequently (e.g. song thrush), whilst a “calm” bird will do the contrary (e.g. barn swallow).

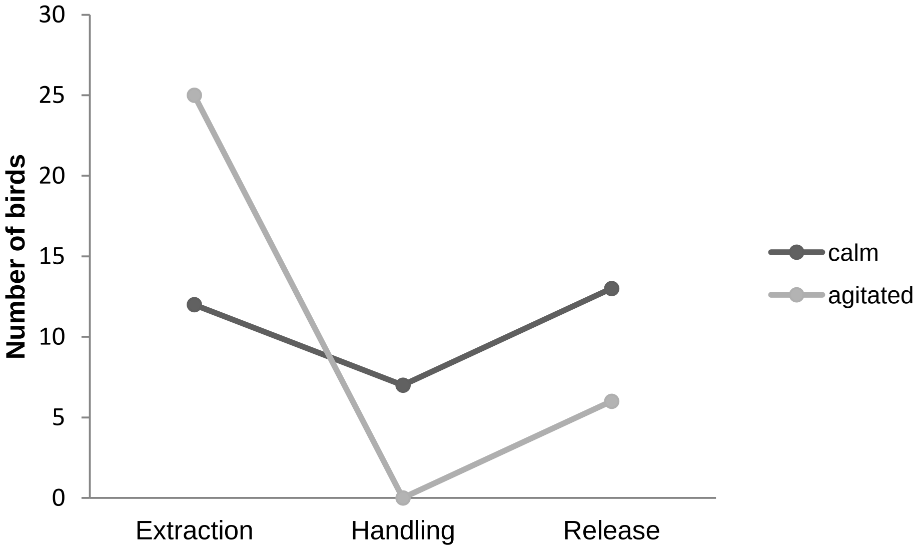

Figure 7.10: Type of behaviour exhibited by each bird, per stage at which the injury was registered (n = 64 injured individuals). “In the net” injuries were subscribed to the “Extraction” stages.

Overall, birds were most prone to injury during the extraction stage (figure 7.10), mostly by superficial cuts or strain caused by struggling on the net mesh. There was significant variation between stages of the ringing process, in the likelihood of injury (Fisher's Exact Test for Count Data, $p = 0.0004$). The chart presented in figure 7.10 follows the same rationale as the previous results in regards to definition of the “Release” stage.

7.4.3 Other methodologies

7.4.3.1 Heligoland traps

During the period studied 178 birds were trapped and 2 incidents were recorded (1.12%). These two incidents corresponded to great tits getting severe scratching in their foreheads, in which most of the feathers were scraped off, trying to escape from the metallic mesh that walls the trap.

7.4.3.2 Cannon-netting

The total numbers of birds captured by both the bird ringers was: 3189 gulls, 248 wildfowl and 3806 waders, of which 0.19%, 0.4% and 1.18%, respectively, died during the capture and handling event.

Some risk to the birds from cannon-netting seems inevitable, as it is impossible to control whether a bird decides to take-off at the same time as firing the net, which was the main cause of mortality and injuries in gulls.

7.4.3.3 Capturing at the nest

During the study on 508 breeding Cory's shearwater, two were injured (0.4% of captures) and one died (0.2%) as a result of trapping activities. Both injuries were broken legs, and one of the birds fully recovered. The fatality resulted from an accidental rock spill of top of the bird and no details on the trapping method were provided.

7.4.3.4 Noose carpet

The majority of sandgrouses (76%) caught using noose carpets displayed a calm behaviour whilst handled, departing with no behavioural stress response, with the remaining 24% showing aggressive behaviours. However from the total of 68 birds captured and ringed, 6 birds (8.8%) suffered scratches from the trap. From these, two demonstrated aggressive behaviour whilst for the remaining four no behavioural data were collected. This suggests that aggressive birds might be more likely to suffer injuries (Fisher's Exact Test for Count Data, $p = 0.056$), although an increased sample size would be needed to clarify this result.

7.5 Discussion

In order to quantify and evaluate the risks associated with capture and handling, there is a need first to evaluate the method’s injury and mortality rates (Spotswood *et al*. 2012, Wilson & McMahon 2006). This may also reveal whether there is a potential bias in the data collected introduced by the stress response (e.g. Saraux *et al*. 2011), and suggest how the method may be developed to reduce its impacts.

There was a perception amongst the bird ringers that responded to the questionnaire that blackbirds, chiffchaffs and greenfinches are the species most prone to injuries and death during mist netting and bird ringing. Similarly, at the two ringing stations where incident reports were completed, the majority of birds injured belonged to these species. However, these three species were amongst the most frequently caught: considering the incident rates within each species (controlling for the total numbers caught) two other species were highlighted as being more susceptible: long-tailed tits (the most sensitive) and kingfishers. Most ringers aimed to handle ‘sensitive species’ first, but opinions differed between individual ringers and between ringing stations as to which were the “most susceptible” species.

The perception of fatality rates from the questionnaires matched those found in practice (< 1% and 0.27% respectively), and were under the mean level that bird ringers considered to be acceptable (2.6%). The level of mortality was very similar to that found by Spotswood *et al*. (2012) of 0.23% and the acceptable level suggested by Ralph *et al*. (1993) of < 1%. Also the perception of which stage was the most sensitive matched that observed at the two ringing stations (i.e. “In the net” and “handling”), both for the rate of mortality and the reasons, with predation being one of the biggest causes.

In this study, “stress” was not used as a generic cause for mortality as, when possible, I performed necropsies on the birds with the aim of identifying specific causes. In a few cases such a tool was helpful in distinguishing what may have been described under the category “stress” or “unknown”. Spotswood *et al*. (2012) concluded that the two major causes of mortality are stress and predation (matching the bird ringers’ opinion), however I believe that stress is too general a term, as it comprises many physiological responses that can only be properly assessed through necropsies with tissue analysis (e.g. Bradley *et al*. 1980). This would also allow us to understand if bird ringing can be acting as a “natural selection factor” in a population, by selecting birds that are “less equipped” to cope with the event or if it is a merely random incident. To support the rationale of the selection hypothesis, Brown & Brown (2013), have found that road kills presented a selective pressure on the population of nesting cliff swallows (*Petrochelidon pyrrhonota*) on highway overpasses in Nebraska, so that the wing length of cliff swallows found dead in the road became shorter over time, favoring rapid evasion in response to the spotting of an approaching car. Similarly bird ringing of resident populations could be inducing similar selection pressures on the birds to withstand and survive capture-stress.

It is evident from the results of both the current study and Spotswood *et al*., (2011) that predation whilst birds are in the net is a major contributor to mist-netting mortality, although some ringing schemes have precise rules designed to minimise it (e.g. UK, Redfern & Clark 2001). While it is not possible to tend constantly the nets to monitor for the presence of predators, especially wandering feral cats (although Churchwell and Barton (2006) have found that carrying out patrols to the nets would decrease predation but not frequency of captures) it is possible to minimize predation risk by constantly watching for avian predators and mammal tracks, placing the mist-nets to avoid predator routes, and increasing the frequency of net rounds. Shorter net rounds would also minimize the risks of death by overheating if nets are in direct sunlight. In Portugal, as mentioned in the questionnaires, the intervals between net rounds are usually 45-60 min, whereas the BTO advises shorter intervals of around 20-30 min (Redfern & Clark 2001). Both of the ringing stations that completed the Incident Report Forms consistently operated 45-60 min net round intervals, preventing an assessment of whether net round spacing had any influence on mortality and injury rates. Although it might seem straightforward that longer net rounds will incur more accidents because birds will be defenseless (e.g. against predators) and exposed for corresponding longer periods of time, this needs to be tested empirically. Some bird ringing guidelines advocate that a 60 min interval is “tolerable” under specific circumstances, but there is limited data on which to base such recommendations. Factors such as mist net-length per ringer, and ambient conditions (with Portugal being significantly milder in climate than central and northern European countries), could also be taken into account.

Regarding injuries, the rate observed at the two ringing stations (0.89%) again matched the one perceived by the participants in the questionnaire (< 1%), and was similar to that of Spotswood *et al*. (2012) (0.59%, $\chi^2 = 0.0608$, d.f. = 1, $p = 0.8052$). Despite the perception of ringers that handling is a more sensitive stage (considering both injuries and mortality), the data presented here indicates that the extraction stage was the most susceptible, with “cuts” (of the wing, hindlimb, tongue and pin feathers) being the most frequently reported. Spotswood et *al*. (2012) on the other hand reported “wing injuries”, and again, “stress” as the most recurrent types of injury. In part, this difference may be explained by differences in the classifications of injuries. Spotswood *et al*.’s (2012) “wing” category not only included birds that presented strain or dislocation, but also those that failed to take-off: combining these categories in the current study would encompass 35.4% of the injured birds. However I decided not to combine them as failure to take off could also be due to previous ill state, cold, or “exhaustion” in which the bird would get “traumatized” or too stressed and hence need time to recover. Although the “release” does not involve handling, some birds, if injured or “traumatized”, may not display ill effects during handling, and hence it will only be visible that something is wrong with them if they do not fly away properly. However wing injuries can also occur as a result of damaging handling during the ringing stage itself, for example, by overlapping of the wings across the back of the bird or bringing the wings forward below the line of the body (NABC 2001). Out of the 21 birds that did not fly away, it was only possible to identify specifically wing related injuries in five of them.

There is a general perception that birds with wing strain will take a few hours to recover, or may die in some instances. However there is evidence that birds recover within few hours, or in a worst case scenario, days (NABC 2001). Wing strain in birds is an important issue, as if a bird that is unable to fly properly it can become more susceptible to predation. According to Spotswood *et al*. (2012) birds of smaller size are most prone to wing injuries.

A special case of the injury category “cut” presented in this study is that of growing blood feathers. These usually grow in a “pin” (i.e. follicle), which is irrigated by blood, thus when damaged, will bleed. The damaging of a pin feather may be potentially dangerous in terms of “traumatizing” the feather insertion, which might disable it from generating further feathers (EFSA Panel on AHAW 2010). This could be particularly serious if it affected a primary flight feather.

Internal bleeding was reported for a few birds (4.6% of the recorded injuries, and in the questionnaires it was perceived as 6% of the injuries) and is usually detected when a bird is bleeding from the mouth. This condition usually indicates serious internal injury in organs or the brain (Fair *et al*. 2010, Veltri & Klem 2005), that will potentially lead to death. When a bird was seen to be bleeding it was released immediately in a sheltered place to promote recovery. There is a belief that internal bleeding is mainly caused by rupture of air sacs (e.g. Spotswood *et al*. 2012, and personal conversations with bird ringers), but this normally leads to the leakage of air from the sac and its accumulation under the bird’s skin (subcutaneous emphysema). Instead in the present study it was linked to internal organs bleeding, as air sacs were not ruptured in the birds that were examined post mortem.

Logistically it is not feasible to assess the true handicap that injuries may pose to birds. However, bird ringers encounter healed injuries that could have been caused by previous bird ringing, and birds seem to survive and adjust to these (e.g. broken legs and cuts mostly). Also Spotswood *et al*. (2012), through capture and re-capture data, concluded that injured birds survived in similar numbers to those that were released uninjured.

Across techniques, while capturing the same type of species, and although sample sizes are relatively small, it appears that the rate of injuries in mist-nets is not significantly different from that in Heligoland Traps ($\chi^2 = 0.0263$, d.f. = 1, $p = 0.871$). The injuries recorded in Heligoland traps, according to the North American Bander's Manual For Banding Passerines and Near Passerines (2001), are likely to occur when a metal mesh is used to build the trap, which was the case in the present study. Also, when capturing with noose carpets, aggressive sandgrouses seem to be more prone to getting injured in the same manner as agitated birds are more prone to getting injured in mist-nets (although separate distinctive species are captured in each method). It is likely that a coping behaviour favoring escape can increase the risk of injury to the individual bird, which can range from simple scratches (e.g. sandgrouses) or death by strangling in the net, and exposed fracture (e.g. little bittern).

Although it allows for the capture of a large number of individuals, because of the nature of the cpture method, cannon-netting is probably the technique presented in this chapter that can potentially cause the most harmful incidents. However, in the current study, the mortality rates resulting from cannon-netting were within the levels presented in the literature (refer to table 7.I).

Despite the injury and fatality rates observed in this study being considered "acceptable" (e.g. Ralph *et al*. 1993), low (e.g. Spotswood *et al*. 2012), or not enough to stop bird-ringing (questionnaire replies), bird-ringers should always strive to minimize these levels by continuously re-evaluating their methodologies, to assure that their work is beyond reproach and to minimize the potential impact of the procedures on bird populations. I suggest that bird ringers should be strongly encouraged (or even obliged) to record and report the types of injury and mortality, so they can continuously be aware of their effects and improve their methodologies. Although the mortality and injury rates assessed are far lower than those widely considered to be acceptable, ethical concerns on the welfare of the bird should always be a priority. The Bird Bander's Code of Ethics (*in* NABC 2001) summarizes this by saying: "Banders should not consider that some mortality is inevitable or acceptable in banding. Every injury or mortality should result in a reassessment of your operation. Action is then needed to minimize the chance of repetition."

Bird ringers claim to be able to recognize that a bird is unwell through visual cues such as difficulties in breathing (panting, open bill, tachycardia) and lethargic appearance (eyes closing, apathetic, fluffing of feathers) (figure 7.9). While it was not quantified whether this correlated with real injuries, it may provide indications of when bird ringers should act quickly to prevent mortality, or at least to minimize the physiological stress to which the bird is submitted (Spotswood *et al*. 2012, Holberton 1999).

In chapter two, I have demonstrated how the majority of birds demonstrate an agitated coping response towards bird ringing, favoring a "flight" behaviour, thus it is of no surprise that the majority of incidents occur during extraction or even in the net before the arrival of the ringer, and that agitated birds will be more prone to injuries in this stage, as chances of entanglement and net mesh related injuries will be increased. Also, I have demonstrated how birds tend to demonstrate an agitated behaviour, which some authors generally refer to as the fight-or-flight response. Whether a bird is agitated or not, the fight-or-flight physiological response will be triggered and some manifestations are: increases in blood pressure, muscle tone and respiration rate (Siegel 1980). Increased respiration rate is also referred to as a visual cue of the bird being "unwell". The resultant increase in blood pressure can increase the risk of haemorrhage. Also cardiac arrest might result from increased heart rate, leading to death. This could have happened in the present study, among birds for which there was an "unknown" cause of death. Also in chapter three I have demonstrated how birds could perceive capture and handling as a predation event, for which tail loss is a strategy for escaping (Møller *et al*. 2006). Indeed, in the questionnaire, over 90% of bird ringers claimed to have encountered such response in birds.

According to the Bird Bander's Code of Ethics (*in* NABC 2001), the safety of bird ringing "depends on the use of proper techniques and equipment and on the expertise, alertness and thoughtfulness of the bander [ringer]". Indeed, there are situations in which the bird ringer needs to adjust their methodology. In such situations the majority of bird ringers claims to adjust their methodologies to favor the bird's welfare, in situations such as unexpectedly big numbers of birds being captured in a net round (e.g. a flock of waxbills) worsening of weather conditions or even when particularly “stress-sensitive” species or individuals are captured.

Chapter 8

General Discussion

Bird-ringing has often been fiercely criticized by its detractors, and equally fiercely defended by its practitioners, yet these debates have largely been carried out in the absence of a meaningful evidence-based analysis of the nature and magnitude of impacts of capture and handling on wild birds. The data presented in this thesis illustrate the wide range of ways in which capture and handling has measurable impacts on birds, either as direct consequence of procedures or via physiological changes forming part of the birds' behavioural, physiological and energetic response to capture stress. Such datasets and analyses allow an objective assessment of the impacts of capture and handling on wild birds, and move the debate on from the anecdote- and assertion-based discussions that have often led to polarized views on both sides.

Briefly, in chapter one I provided a literature compilation of the consequences of bird ringing on birds themselves. Specifically, chapter one comprised a review of the avian stress response, defining it generally as an "animal's defence mechanism in the presence of a stimulus (stressor)". It's manifestations are primarily at an hormonal level, in which the adrenal system sends signals to the various parts of the body triggering the subsequent physiological modifications. These however will depend on how the bird perceives capture and handling, which reflects both internal state (e.g. body mass, life-stage period) and external conditions (e.g. predator abundance, food availability). Such factors will determine the energy-regulation decisions of the bird (body mass, thermal regulation), which can have measurable demographic effects (e.g. handicapping reproductive effort). Chapter one also reviews the various capture techniques used and the potential impacts that they can have on individual birds, which depending on the ringing effort or species involved, might also have detrimental demographic effects.

Chapter one identified some gaps in the literature, including a need to better understand the interruption that capture and handling has on the birds' daily behavioural routine. Therefore, chapter two focused mainly on describing the temporal disruption. This study revealed that the total duration of capture and handling (from entering the net or trap, to departure following release) can sometimes last nearly 2h, and several aspects influenced the duration of this period of time: namely the bird ringer's experience, the species and their coping strategies. During the capture and handling period, some birds lost body mass, and incubating females lost incubation time leading to a reduction in nest temperature, and some birds uttered distress calls.

In chapter three and four, energy regulation questions were addressed. Both chapters were aimed at understanding the physiological basis of how birds regulate their body mass. In chapter three birds did indeed decrease their foraging rate following capture, but with no significant measurable mass loss, and in chapter four birds did not resort to hypothermia following capture in order to save energy. However the experimental designs were partly intrusive, which could limit the interpretation of these results. This then raises the question of whether some studies using bird ringing as a tool will also be biased.

Chapter five focused on the priorities of incubating females, and whether the nest survival was a priority after capture and handling. For most females this was not the case, as capture led to them being absent from the nest for a variable duration following release, even though it meant that eggs would be exposed for longer periods to predators or "chilling", which could have subsequent negative effects on the chicks growth, compromising the captured bird's lifetime inclusive fitness.

In chapter six I have focused mainly on the causes of the storm-petrel's (*Hydrobates pelagicus*) delayed take-off after capture and handling. In this chapter I demonstrated that their delayed take-off was not primarily related to capture stress (assessed through their immune response), or the method itself, but rather with the type of light used to handle birds, as their night vision was being disrupted, impairing their ability to take-off.

Lastly in chapter seven, I addressed the direct consequences of capture and handling through assessing the perceived risks of injury or mortality estimated by bird-ringers, and compared these estimates with the injury and mortality rates actually encountered. Bird ringing related mortality and injuries are usually below 1%, at the places where the focal data was collected, in line with the general perception of bird-ringers. The main cause of mortality is predation and the most common injuries are wing related.

All of the physiological aspects of the stress response measured individually in this thesis are inter-related, mediated by the initial release of stress-hormones. Together, these physiological responses determine how a bird copes with the event and the amount of "harm" it sustains, in terms of impacts (or potential impacts) on its fitness. Specifically in chapters one and three I have described how birds appear to perceive capture and handling similarly to an encounter with a predator.

The main limitations of this project were primarily related to experimental design and sampling size. For example, when evaluating immediate changes in body mass (hypothesis six, chapter two), the bird's immediate capture weight should have been measured, as it could be argued that birds may lose the largest part of their capture-induced weight change during the first moments of capture (as defecation or regurgitation, Clark 1979 and Fair *et al*. 2010). Furthermore, the analysis did not account for the time spent in the net but only during handling. Additionally, in chapter three, although the experimental set up allowed the remote-weighing of the birds, the distinction between the birds' behavioural response to capture and handling, and the birds' association between being fed and captured (i.e. clap traps being set too near the feeder) might have introduced bias to the interpretation of the results, such that clear conclusions could not be drawn. Regarding changes in body temperature, although comparisons between birds were meaningful as the set up was identical between experimental treatments, the fact that measurements were taken intrusively might have masked any effect that capture and handling could have generated. Arguably, corticosterone levels are likely to peak during migration, hence bird stress responses at this part of the annual cycle are usually more tolerant of the acute stressor (Falsone *et al*. 2009). In chapter six, I demonstrated how simple adjustments to the capture and handling protocol, resulted in a lower impact to the bird (in this particular case, by lowering the time disruption of daily routines of storm-petrels). However, one important disadvantage of processing storm-petrels under red light is that it is very hard to reliably determining the bird's age from the pattern of feather abrasion. One solution could be to cover the bird's head, and switch to a brighter (white) light while assessing the bird's age.

Evolutionarily, only animals that are able to maintain or maximize their fitness by keeping themselves alive and by reproducing, will be the ones successfully passing on their genes to the next generation. In the wild, birds are frequently faced with life-threatening events, and the only way to survive these is to develop physiological strategies that will help them cope effectively and escape from dangerous situations. Overall, although capture and handling caused immediate changes in behaviour, in all experiments birds seemed to cope effectively at the individual level, generally (i.e. excluding deaths and serious physical injuries) with no measurable detrimental effects on individual survival in the longer term. This was done by either modulating their foraging behaviour with no detrimental long-term impact on their body mass (chapter three), avoiding going into states of energetic savings to maintain themselves alert for an escape opportunity (chapter four) or prioritizing their own survival instead of the survival of their eggs or progeny (chapter five). Arguably, birds were not sampled for “longer-term” changes, for example, impacts on hatching or fledging success following capture during the breeding season. Capture seemed to not have a major effect on the breeding effort as females did not immediately desert the nest, but it is possible that a chilling period might have caused a detrimental developmental handicap on the chicks, which could potentially handicap their ability to survive or to reproduce in the future. Additionally to this experimental design, it would have been of interest to compare body condition of non-captured females with that of captured females, by the end of the breeding season. Although logistically demanding (as the total sample size would have needed to be higher), this could be achieved by putting a digital scale with a perch in front of the nest (so when females left the nest they would be remotely-weighed, c.f. Rands *et al.* 2006).

Sample sizes were often limited in the experiments, observational studies and questionnaire surveys presented in this thesis. To some extent, this is a limitation inherent to the nature of the individual-focused repeated-measures experimental designs, the reticence of bird-ringers to report bird deaths and injuries, and the limited time duration of the project. For example, in chapter seven, the data collected point to “predation” as one of the most recurrent causes of mortality in the net. Although this was true for those specific bird ringing stations, most likely it is not illustrative of the reality in other bird ringing stations (e.g. in the UK, the BTO has specific guidelines to avoid predation in the net, hence it is expected to be lower in this country). Similarly, Spotswood *et al*. (2012) obtained data from 22 bird-ringing stations collected over periods of 10-20 years, and still regretted “sample size limitations”. Chapter seven, which was the chapter with potential to answer the recurrent question of “what are the impacts that capture and handling has on populations?”, did not provide an unequivocal answer, partly due to its limited timescale. Thus in order to better understand the potential impacts that changes in physiology can have at an individual scale, that could be then translated into a significant population impact, longer term studies should be carried out. An example of such a study, is that of Saraux *et al*. (2011) which is related not with capture and handling, but rather with the use of flipper bands on king penguins (*Aptenodytes patagonicus*), over a period of time of 10 years. Interestingly, the impacts of capture and handling on charismatic, large and long-lived birds such as penguins may be perceived to be different from the impacts on other birds. Arguably, every life should be equal, but clearly the conservation value of king penguins is perceived to be different to those of, for example, a common blackbird (*Turdus merula*). There is no threshold of an acceptable number of incidents for wildlife research, and arguably, bird ringers should consider that any mortality or injuries are unacceptable (NABC 2001).

Ideally, every bird ringer should critically evaluate the implications of their methodology. For example, even though mist-netting, is widely used, the way in which this capture method is implemented can vary widely, with consequences for the likelihood of deaths and injury (chapter seven), the physiological stress response (chapter three, four and six) and the impact on the normal time-budget of the captured birds (chapter two). Specifically, the duration of net rounds appears to have a major impact on the total amount of time that the bird is exposed to the risk of injury or predation, and on the total amount of time that it is removed from its environment, preventing it from feeding (chapter three) or incubating (chapter six).

In this thesis I have emphasized recurrently how bird-ringing is an invaluable tool for scientific investigations of individual wild birds and their populations. However, it is exactly as tool that it should treated, rather than as an end in itself. Specifically, given the range of impacts that capture and handling can have on individual birds, bird ringing should be used to answer specific research questions or to fulfill specific purposes (e.g. for conservation monitoring or as an environmental education tool), rather than being carried out in an unfocussed manner with no clear aim.

The question of whether bird ringing has impacts on birds, is recurrent. Probably no bird-ringer that carries out public ringing demonstrations can claim he (or she) has never heard the question “Does it harm birds?” It is most likely that none of them will reply “yes it does” or “to some extent, yes”. Indeed, public pressure can be an element that deters bird-ringers from “admitting” that sometimes harmful incidents might occur. In this sense it is of outmost importance that rigorous and objective studies of such impacts are conducted, such as those previously presented in this thesis. For example, it is important to quantify the real number of harmful incidents affecting birds, so that instead of assumptions or assertions about the “safety of the technique”, bird ringers can be acquainted with real data. Naturally, more data than is currently available would be useful, but taken together with that of Spotswood *et al*. (2012) it emphasizes the importance of this type of data collection despite the current difficulties in obtaining it. The questionnaires generally demonstrated that bird ringers have an accurate perception of mist-netting risks (as their estimated death and injury rates generally matched those of the incident report forms). However, not all bird ringers appear to modify their methods in a way that would minimize the impacts of capture and handling on birds under specific circumstances, such as particularly busy net rounds, or especially detrimental conditions.

An important addition that this thesis brought to the study of Spotswood *et al*. (2012) is that it assessed the intrusion caused by the experience of the bird ringer, in terms of prolonged handling duration. However, the dataset did not allow for a test of the potential correlation between the ringer's experience, and the number and type of injuries. It is arguable that, if exhaustion is a major cause of mortality as well as predation (chapter seven), that death or injury could be a consequence of poor handling methods, as well as prolonged net rounds.

Most of the data collection I have carried out relied heavily on the co-operation of bird ringers. While there was no obligation to participate in the project, I have encountered reluctance and skepticism towards the project. For example, despite the extensive circulation of the questionnaires, only a very small portion of individuals replied to these. Also many researchers openly expressed their discontent towards the project, justifying themselves with reasons such as "*it is unnecessary*", "*it is completely safe, it's a waste of time*", or more straightforwardly "*I already have problems enough in getting permits, let alone having someone measuring the impact that I cause to birds*". I believe that these are the attitudes that explain the surprising lack of studies on the impacts of mist-netting, and reluctance in self-assessment and reporting of impacts. My personal experience is that bird-ringing is a tool to achieve important results, and like any other research method involving the use of animals it can, and it should be exposed to critical appraisal and, wherever necessary, improved to the point that a bird ringer can say that their work "is beyond reproach". One of the outcomes of this thesis, is that it demonstrates how collective results, much more than individual self-reporting, can be used to improve methodologies and if researchers would join their efforts in the transparent and blame-free reporting and evaluation of impacts, then the situation of the methodology becoming "beyond reproach" could be ensured.

Despite weaknesses in some of the experimental designs chosen, this thesis highlights that possibly the most detrimental effects on birds are not so much physiological or behavioural, but rather related to possible injuries and mortality. It is important that bird ringers critically evaluate their activity so as to ensure the welfare of animals, and to continuously assess their work with a view to changing their methodology where necessary. From my personal experience, I suggest that ringing training schemes should supplement the existing training in bird-ringing, with specific training in the birds' physiology and health. For example, the Portuguese Association of Bird Ringers (APAA) is unusual among national ringing schemes in providing a "First aid to wild birds" training as part of its training of bird ringers, covering aspects of the birds physiology and teaching techniques of recovery, so that the bird ringer is better equipped to decide whether to treat an injured bird, or to release it. For example, I have encountered bird ringers who would open their nets earlier in the morning so they could catch migrants as soon as they arrived in the territory. Ethically this may be debatable, as birds may be exhausted from the long flight and would need to recoup energy rather than being held in captivity for periods of up to nearly 2h (chapter two). A solution presented was to supply saline solution to any birds that appeared to be exhausted or emaciated. However, through personal conversations with bird-ringers, most were opposed to implementing this, explaining that "it wasn't natural", they "would be helping the bird and intruding on natural selection". In this context, it can be noted that bird ringing is in itself a human intrusion.

Altogether the results of this thesis suggest that bird-ringing durations can in exceptional cases remove birds from their daily routines for periods of nearly 2 hours. This corresponds not only to a period in which the bird is under stress of capture, and thus corticosterone levels will be increased for an extended period of time, but also to periods at which the bird will either not be foraging, causing longer declines in body mass as suggested by Refsnider (1993), or attending the nest. In chapter five, breeding females were targeted and although the frequency of net checks did not exceed 20 minutes, nevertheless some females took extended periods of time to return to the nest and as a result a substantial number of nests were exposed to temperatures close to ambient temperature. Although no inference can yet be made on the effects of these "chilling" periods, it is to be expected that the duration of such interruptions to the birds' regular incubation routines could have a significant effect on reproductive success. Because the specific hypothesis in chapter five focused on the short-term disruption of incubation, rather than the issue of eventual reproductive success, data collection was limited to make any longer-term assessment. Further data collection could assess whether factors such as body condition, time of day and age had any significant effect on return durations among incubating females of the same species, and could monitor the subsequent impacts of these durations on ultimate reproductive success. The long absence from incubation of some individuals contradicts the frequently-heard assertion that breeding birds return quickly to incubation (or chick-provisioning) following capture and release. Bearing these revelations in mind, investigators should always weigh the costs and benefits of sampling birds during the breeding season, and adjust their methodology to this period (as already recommended by the British Trust for Ornithology), by:

1 – prioritising the extraction, processing and release of any incubating females that may be captured (i.e. those exhibiting a fully-vascularised brood patch) (Redfern & Clark 2001);

2 – carrying out more frequent net rounds (at <20 minute intervals), and where appropriate shortening the total length of the ringing session, even if this means catching fewer birds (Redfern & Clark 2001);

3 – assessing whether nesting is at a critical stage (eg. species known to be susceptible to nest-desertion or prolonged absences following handling, or birds at the beginning of the breeding attempt) and avoiding capture in that period (Redfern & Clark 2001);

4 – reassess the position of nets to avoid known nests, or even not setting them up in areas of potentially higher risk of nest disturbance (Redfern & Clark 2001); and,

5 – when applicable, choose from capture methods that have proven to be less intrusive even if it means catching fewer birds, or even to substitute capture and handling with visual monitoring techniques.

Whether during the breeding season or not, it is important that bird ringers use short net round durations, so as to minimize the impacts of capture on the daily time-budgets of the captured birds. Where necessary (e.g. where large numbers of birds may be captured together), ringers should also consider reducing the length of mist nets used, to cope with limited logistic resources (e.g. number of experienced bird ringers available to rapidly process and release birds).

This thesis contains examples of the types of impacts that capture and handling may have on wild birds. However, many other fields of impact remain to be explored, such as: horizontal transmission of parasites through bird bags, and effects on migratory orientation of birds (e.g. utilising Busse’s flat cages to assess stress effects on migratory direction and activity of birds).

To conclude, bird-ringing has contributed greatly to our understanding of birds, and will continue to provide valuable data in the future. However, this thesis highlights that bird-ringing is not always “cost-free” for the birds concerned, having impacts that are sometimes immediate and obvious (e.g. accidental injury and death) but sometimes subtle, delayed (e.g. interruption of foraging and incubation routines) or harder to evaluate in terms of their impacts on overall fitness. Therefore, researchers need to evaluate and (when necessary) minimize these impacts by adjusting their methodologies, rather than assuming that such impacts do not exist.

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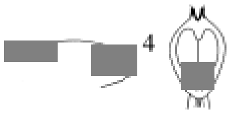
Appendix A: Bird ringing codes for age, fat and pectoral muscle.

EURING age codes

EURING NUMBER	Definition
0	Age unknown - only to be used if data have been lost and the ringer has no idea if a birdwas a nestling or full-grown
1	Pullus (Use P not 1 for pullus on handwritten schedules as a series of 1s looks like a ditto line)
1J	Passerines only - fledged, but flying so weakly that it is obviously incapable of having flownfar from the nest (include as pullus for annual totals)
2	Fully grown, year of hatching quite unknown (including current year)
2J	Fully grown, year of hatching quite unknown (including current year), still partly or completely in juvenile body plumage (rarely used)
3	Definitely hatched during current calendar year (eg first-years in autumn)
3J	Passerines only - definitely hatched this calendar year and still partly or completely in juvenile body plumage*
4	Hatched before current calendar year - exact year unknown (eg many adults in autumn)
4I	Hatched before current calendar year - exact year unknown but definitely not full adult
5	Definitely hatched during previous calendar year (eg first-years in early spring)
5J	Definitely hatched during previous calendar year (eg first-years in early spring) still partly or completely in juvenile body plumage rarely used)
6	Hatched before last calendar year - exact year unknown (eg many adults in Spring)
6I	Hatched before last calendar year - exact year unknown but definitely not full adult
7	Definitely hatched in calendar year before last
8	Hatched three or more years ago - exact year unknown
8I	Hatched three or more years ago - exact year unknown but definitely not full adult
9	Definitely hatched three years ago
10	Hatched four or more years ago - exact year unknown
11	Definitely hatched four years ago
12	Hatched five or more years ago - exact year unknown
13	Definitely hatched five years ago
14	Hatched six or more years ago - exact year unknown
15	Definitely hatched six years ago
16	Hatched seven or more years ago - exact year unknown

*Because juvenile plumage - ie the feathers grown by the birds in the nest - is more easily recognised than the plumage which succeeds it, the analyst can assume a high degree of accuracy in birds aged 3J, whereas those aged 3 might possibly include a few individuals which were in fact older.

Fat Scores

a) ESF System			**b) BWG System**	
Score	**Description**		**Score**	**Description**
0	0 no visible fat. Dark red		0	no visible fat. Dark red
1	F: wide wedge of fat. A: trace of fat. Light red		1	F: trace of fat. (~E0.5) Light red/pink
2	F: completely covered but deeply concave. A: slips of fat.		2	F: base of tracheal pit ob scured by fat to about one third full. (~E1.0) Yellow-pink
3	Light yellow F: moderate fat reserves cover ends of inter-clavicles but concave. A: flat or slightly bulging pad. Light yellow		3	F: tracheal pit about twothirds full. Muscle within tracheal pit visible between fat and clavicles. (E~1.5) Yellow-pink
4	F: filled up to far end of clavicles. A: covered by clearly bulging pad of fat. Yellow		4	F: completely filled up to far end of clavicles but still concave (not bulging). (~E3.0) Pale yellow
5	F: convex bulge, perhaps overlapping breast muscles. A: extreme convex bulge. Yellow		5	*As ESF*
6	F and A: fat covering breast muscles by several mm.		6	*As ESF*
7	F and A: 3/4 of breast muscles covered. Yellow		7	*As ESF*
8	F and A: breast muscles not visible. Yellow		8	*As ESF*

(F=Furcular region or tracheal pit; A=Abdomen)

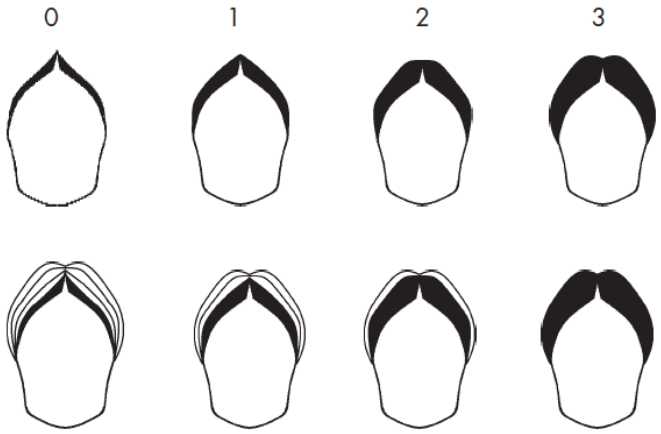
Under the BWG scale, ESF scale equivalents are given (eg E0.5) eg a score of 3 on the BWG scale is equivalent to about 1.5 on the ESF scale.

From *Ringers' Manual* BTO, Thetford

Pectoral Muscle Score

Score class	*Prominence of sternum*	*Pectoral muscle shape*
0	Sternum sharp	Muscle depressed
1	Sternum easy to distinguish	Muscle neither depressed, sharp but not sharp nor rounded
2	Sternum still distinguishable	Muscle slightly rounded
3	Sternum difficult to distinguish	Muscle rounded (full)

Muscle scores



The black areas are de muscles
The withe areas on the bottom row show the differences between scores.
From *Ringers' Manual* BTO, Thetford

Appendix B: Forms used in Chapter 7: Questionnaire Survey, Incident Report Form and Necropsy Form.

Bird-Ringing Questionnaire

Thank you for taking the time to complete this questionnaire.
***** All responses will be treated as anonymous & strictly confidential *****

Definitions:
Fatalities *= birds that die during capture or handling*
Injuries *= birds that do not die but suffer damage or harm during capture or handling.*

Ringer details:

Country in which you are a licensed bird ringer:

Ringing license type:

(e.g. in the UK –T/C/B/A permit, and any endorsements & restrictions)

Bird catching methods:

What is the length of mist-net (metres) per ringer that you usually use?

How frequently (e.g. every 30 minutes) do you normally check your nets?

Have you used catching methods other than mist nets over the last 12 months? (Please describe).

Ringing data:

Number of birds ringed during the last 12 months:

Are you willing to provide basic ringing data for these birds? (i.e. age, sex, mass, wing length, date, time & location of capture).

Effects of capture & handling:

Of the total number of birds ringed over the last 12 months, how many fatalities have you seen?

Of these fatalities, how many were:
a) found dead in the net…………………………☐
b) died during extraction……………………….. ☐
c) died in the bag……………………………….. ☐
d) died during handling/processing…………… ☐
e) died in captivity following a ringing accident ☐

f) other (please explain)…………………………☐

Please provide breakdown by species, if appropriate.

What were the causes of death? (where this could be identified)

What kinds of <u>injuries</u> resulting from capture & handling have you observed?

Of these injuries, how many were:

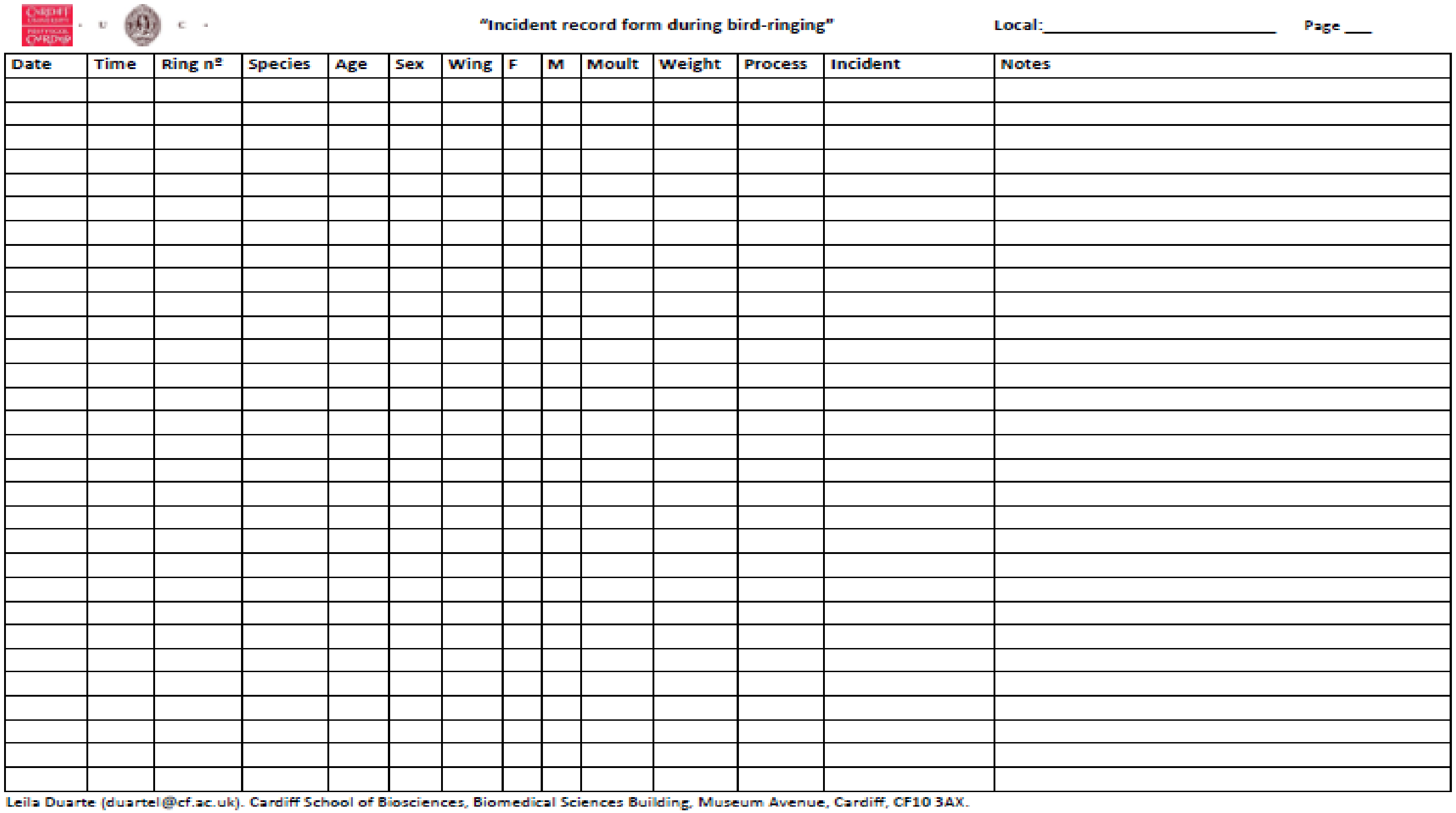
a) injured in the net…………..……….….………..☐
b) injured during extraction………………………..☐
c) injured in the bag………………………………..☐
d) injured during handling/processing…..…………☐
f) other (please explain)………………...…………☐

***Please provide breakdown by species, if appropriate.**

<u>Additional comments</u>:

Do you have any other observations or comments about the effects of capture & handling on wild birds? (Please continue on a separate sheet if necessary.)

Incident Report Form



"Incident record form during bird-ringing"

Local:__________________________ Page ___

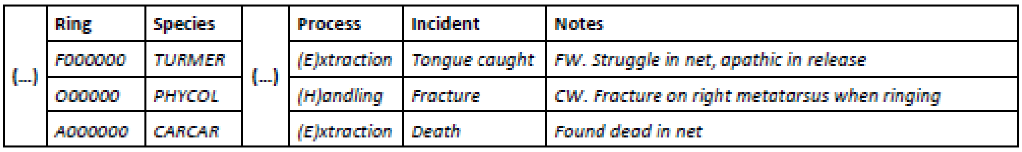
Date	Time	Ring nº	Species	Age	Sex	Wing	F	M	Moult	Weight	Process	Incident	Notes

Leila Duarte (duartel@cf.ac.uk). Cardiff School of Biosciences, Biomedical Sciences Building, Museum Avenue, Cardiff, CF10 3AX.

Completion Instructions:

The registration form is very similar to the ringing sheets, and include data about the biometrics and that is indicative of the condition of the individual. The novelty lies only in the fields: "Process" which states what stage of the capture and handling the incident occurred, ie capture, retention, handling and release (terms that can and should be abbreviated according with the first letter); and "Incident", in which we should briefly indicate the type of injury. The "Notes" section serve as a brief description of the parameters and nature of the incident, or any other information that the ringer considers relevant.

Example:



(...)	**Ring**	**Species**	(...)	**Process**	**Incident**	**Notes**
	F000000	*TURMER*		*(E)xtraction*	*Tongue caught*	*FW. Struggle in net, apathic in release*
	O00000	*PHYCOL*		*(H)andling*	*Fracture*	*CW. Fracture on right metatarsus when ringing*
	A000000	*CARCAR*		*(E)xtraction*	*Death*	*Found dead in net*

In the "Notes" in the example shown, there are two acronyms FW (Flight or Fight) and CW (Conservation Withdrawal), which are optional, but indicate the kind of behaviour that the bird displayed: - FF (Fight or Flight) active response in which the bird shows aggression and territorial control. - CW (Conservation Withdrawal): characterized by immobility and low levels of aggression.

To understand the species’ susceptibility and the general pattern, the capture values of the day are also needed, as well as the number of species and individuals ringed by species. If by chance the bird is to perish in the net, the registration of age and biometrics is also important.

Finally, the project includes performing necropsies, to: a) identify the main causes of death, and b) to establish relations of cause and consequence. For this reason it is asked, optionally, to preserve the bodies in the freezer as soon as possible. For your collection, I address myself to the site at the time that is convenient for both parties.

NECROPSY DATA SHEET

(all measurements are metric)

Species____________ ID#__________ Date Collected_________ Necropsied __________
Mmddyy Mmddyy

Collection site____ Weight (kg/g)__________

History ____SEX (M/F/U) AGE _______

(Circle most appropriate term(s)). Add notes as you see fit.

BODY CONDITION: (Good, fair, poor)

POST-MORTEM CONDITION: (Fresh dead, ~1 day old, >2 days old)

EXTERNAL EXAM (Skin, mouth, eyes, nostrils, cloaca)

MUSCULOSKELETAL: (*Pectoral muscle atrophy*-None, moderate, severe*; Fat*: firm, soft, jelly-like; *body cavity*-Lots of fluid, small amounts of fluid, no fluid)

LIVER: (*Surface*: smooth, rough, granular, wrinkled*; Consistency*: firm, friable*; Color*: homogenous/mottled, red, black, brown, purple, tan, yellow.)

HEART: (*Surface*: smooth, rough, granular, wrinkled*; Consistency*: firm, friable*; Color*: homogenous/mottled, red, pink, black, brown, purple, tan, yellow.)

LUNGS: (*Surface*: smooth, rough, granular, wrinkled*; Consistency*: firm, friable, spongy*; Color*: homogenous/mottled, pink, tan, yellow, grey, red, brown.)

TRACHEA-*Lumen*: smooth, rough*; Color*: homogenous/Mottled, tan, white, red, brown, green, pink.)

SPLEEN: (*Surface*: smooth, rough, granular, wrinkled*; Consistency*: firm, soft*; Color*: homogenous/mottled, pink, brown, tan, red, black, yellow.)

KIDNEY: (*Surface*: smooth, rough*; Consistency*: firm, soft*; Color*: homogenous/mottled, brown, tan, red, black, brown, yellow.)

GONAD: (*Surface*: smooth, rough*; Consistency*: firm, friable*; Color*: homogenous/mottled, red, black, brown, purple, tan, yellow.)

THYROID: (*Surface*: smooth, rough*;* *Consistency*: firm, friable*;* *Color*: Translucent/mottled, orange, red, tan, yellow.)

ORAL: (Mucosa: smooth, rough, granular, pitted*;* *Color*: homogenous/mottled, pink, tan, yellow, grey, red, brown) Any contents?

ESOPHAGUS-*Mucosa*: smooth, rough*;* *Serosa*: smooth, rough*;* *Color*: homogenous/Mottled, tan, white, red, pink.) Contents?

PROVENTRICULUS (*Mucosa*: smooth, rough*;* *Serosa*: smooth, rough; *Color*: homogenous/mottled, tan, brown, red, yellow, black) Contents?

VENTRICULUS: (*Mucosa*: smooth, rough*;* *Serosa*: smooth, rough*;* *Color*: homogenous/mottled, tan, brown, red, yellow, black) Contents?

SMALL INTESTINES: (*Mucosa*: smooth, rough*;* *Color*: homogenous/mottled, tan, brown, red, yellow, black) Contents?

LARGE INTESTINES: (*Mucosa*: smooth, rough*;* *Serosa*: smooth, rough*;* *Color*: homogenous/mottled, tan, brown, red, yellow, black, brown) Contents:

PANCREAS: (*Surface*: smooth, rough*;* *Consist*: Firm, friable; *Color*: homogenous/mottled, pink, tan, red, yellow, black, brown)

CAECUM: (*Mucosa*: smooth, rough***; Serosa*:** smooth, rough***; Color*:** homogenous/mottled**,** tan, brown, red, yellow, black) Contents?

SAMPLES:

Formalin:____

Frozen:____

ADDITIONAL NOTES:

From: Avian necropsy Manual for biologists in remote refuges, U.S. Geological survey National wildlife health centre, Hawaii field station.

Appendix C: Total ringing numbers at A Rocha and Paúl de Tornada, between August 2010 and July 2013.

Appendix c: Numbers of captured and recaptured birds by species at A Rocha and Paúl de Tornada, between August 2010 and July 2013 (numbers do not distinguish captured from recaptured birds).

Species		Total
Eurasian sparrowhawk	*Accipiter nisus*	1
Great reed warbler	*Acrocephalus arundinaceus*	16
Sedge warbler	*Acrocephalus schoenobaenus*	16
Eurasian reed warbler	*Acrocephalus scirpaceus*	263
Long-tailed tit	*Aegithalus caudatus*	19
Common kingfisher	*Alcedo atthis*	109
Eurasian teal	*Anas crecca*	1
Tree Pipit	*Anthus trivialis*	4
European bee eater	*Apus melba*	1
Little owl	*Athene noctua*	12
Red-necked nightjar	*Caprimulgus ruficollis*	2
Common linnet	*Carduelis cannabina*	2
European goldfinch	*Carduelis carduelis*	121
Red-rumped swallow	*Cecropis daurica*	2
Short-toed treecreeper	*Certhia brachydactyla*	17
Cetti’s warbler	*Cettia cetti*	135
European greenfinch	*Chloris chloris*	305
Zitting cisticola	*Cisticola juncidis*	8
Hawfinch	*Coccothraustes coccothraustes*	1
Blue Tit	*Cyanistes caeruleus*	73
Iberian azure-winged magpie	*Cyanopica cooki*	101
Common house martin	*Delichon urbicum*	2
Great spotted woodpecker	*Dendrocopos major*	2
Lesser spotted woodpecker	*Dendrocopos minor*	2
Corn bunting	*Emberiza calandra*	2
Common reed bunting	*Emberiza schoeniclus*	4
European robin	*Erithacus rubecula*	351
Waxbill	*Estrilda astrild*	229
Common kestrel	*Falco tinnunculus*	1
European pied-flycatcher	*Ficedula hypoleuca*	278
European chaffinch	*Fringilla coelebs*	67
Melodious warbler	*Hippolais polyglotta*	69
Barn swallow	*Hirundo rustica*	418
Wryneck	*Jynx torquilla*	2
Savi’s warbler	*Locustella luscinioides*	18
Common grasshopper warbler	*Locustella naevia*	9
Common nightingale	*Luscinia megarhynchos*	47
White wagtail	*Motacilla alba*	6

Appendix c: (continued)

Species		Total
Grey wagtail	*Motacilla cinerea*	2
Western yellow wagtail	*Motacilla flava*	187
Spotted flycatcher	*Muscicapa striata*	12
Northern wheatear	*Oenanthe oenanthe*	2
Eurasian golden oriole	*Oriolus oriolus*	36
Scops owl	*Otus scops*	1
Great tit	*Parus major*	327
House sparrow	*Passer domesticus*	809
Spanish sparrow	*Passer hispaniolensis*	7
Eurasian tree sparrow	*Passer montanus*	1
Black redstart	*Phoenicurus ochruros*	16
Common redstart	*Phoenicurus phoenicurus*	26
Bonneli's warbler	*Phylloscopus bonelli*	11
Common chiffchaff	*Phylloscopus collybita*	447
Iberian chiffchaff	*Phylloscopus ibericus*	11
Willow warbler	*Phylloscopus trochilus*	250
Yellow backed weaver	*Ploceus melanocephalus*	41
Penduline tit	*Remiz pendulinus*	1
Sand martin	*Riparia riparia*	18
European stonechat	*Saxicola rubicola*	27
European serin	*Serinus serinus*	97
Eurasian siskin	*Spinus spinus*	18
Eurasian collared dove	*Streptopelia decaocto*	6
Tawny owl	*Strix aluco*	1
Spotless starling	*Sturnus unicolor*	4
Eurasian blackcap	*Sylvia atricapilla*	1419
Garden warbler	*Sylvia borin*	175
Subalpine warbler	*Sylvia cantillans*	16
European whitethroat	*Sylvia communis*	42
Sardinian warbler	*Sylvia melanocephala*	137
Eurasian wren	*Troglodytes troglodytes*	31
Common blackbird	*Turdus merula*	593
Song thrush	*Turdus philomelos*	58
Barn owl	*Tyto alba*	2
Hoopoe	*Upupa epops*	51
Total		7598